GRADUATE STUDIES TEXAS TECH UNIVERSITY

Evolution of Vertebrate Endocrine Systems

Edited by
P. K. T. Pang and A. Epple

TEXAS TECH UNIVERSITY

Lauro F. Cavazos, President

Regents.—Robert L. Pfluger (Chairman), J. Fred Bucy, Clint Formby, Roy K. Furr, Nathan C. Galloway, B. Joe Pevehouse, James L. Snyder, Lee Stafford, and Don R. Workman.

Academic Publications Policy Committee.—J. Knox Jones, Jr. (Chairperson), Delford C. Carter (Executive Director and Managing Editor), Robert J. Baker, David K. Davies, Harold E. Dregne, Leslie C. Drew, Arnold J. Gully, Charles S. Hardwick, Ray C. Janeway, Daniel R. Krieg, Walter R. McDonald, and Charles W. Sargent.

Graduate Studies No. 21

404 pp.

23 May 1980

Paper, \$25.00

Cloth, \$35.00

Graduate Studies are numbered separately and published on an irregular basis under the auspices of the Dean of the Graduate School and Director of Academic Publications, and in cooperation with the International Center for Arid and Semi-Arid Land Studies. Copies may be purchased from Texas Tech Press, Sales Office, Texas Tech University Library, Lubbock, Texas 79409, U.S.A. Institutions interested in exchanging publications should address the Exchange Librarian at Texas Tech University.

ISSN 0082-3198

ISBN 0-89672-076-4 paper

ISBN 0-89672-077-2 cloth

Texas Tech Press, Lubbock, Texas

1980

**GRADUATE STUDIES
TEXAS TECH UNIVERSITY**

Evolution of Vertebrate Endocrine Systems

Edited by
P. K. T. Pang and A. Epplé

No. 21

May 1980

TEXAS TECH UNIVERSITY

Lauro F. Cavazos, President

Regents.—Robert L. Pfluger (Chairman), J. Fred Bucy, Clint Formby, Roy K. Furr, Nathan C. Galloway, B. Joe Pevehouse, James L. Snyder, Lee Stafford, and Don R. Workman.

Academic Publications Policy Committee.—J. Knox Jones, Jr. (Chairperson), Dildford C. Carter (Executive Director and Managing Editor), Robert J. Baker, David K. Davies, Harold E. Dregne, Leslie C. Drew, Arnold J. Gully, Charles S. Hardwick, Ray C. Janeway, Daniel R. Krieg, Walter R. McDonald, and Charles W. Sargent.

Graduate Studies No. 21

404 pp.

23 May 1980

Paper, \$25.00

Cloth, \$35.00

Graduate Studies are numbered separately and published on an irregular basis under the auspices of the Dean of the Graduate School and Director of Academic Publications, and in cooperation with the International Center for Arid and Semi-Arid Land Studies. Copies may be purchased from Texas Tech Press, Sales Office, Texas Tech University Library, Lubbock, Texas 79409, U.S.A. Institutions interested in exchanging publications should address the Exchange Librarian at Texas Tech University.

ISSN 0082-3198

ISBN 0-89672-076-4 paper

ISBN 0-89672-077-2 cloth

Texas Tech Press, Lubbock, Texas

1980

CONTENTS

INTRODUCTION	5
COMPARATIVE PHYSIOLOGY, SYSTEMATICS, AND THE HISTORY OF LIFE	7
<i>James W. Atz, August Epple, and Peter K. T. Pang, American Museum of Natural History, New York, New York 10024; Daniel Baugh Institute of Anatomy, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania 19107; Department of Pharmacology, Texas Tech University School of Medicine, Lub- bock, 79409.</i>	
ENVIRONMENTAL FACTORS IN THE EVOLUTION OF VERTEBRATE ENDOCRINE SYSTEMS	17
<i>Keith Stewart Thomson, Peabody Museum of Natural History and Department of Biology, Yale University, New Haven, Connecticut 06520.</i>	
EVOLUTION OF THE ENDOCRINE PINEAL ORGAN	33
<i>Herbert A. Underwood, Department of Zoology, North Carolina State University, Raleigh, 27607.</i>	
HYPOTHALAMO-ADENOHYPOPHYSIAL RELATIONSHIPS AMONG VERTEBRATES	59
<i>Milton H. Stetson and E. Gordon Grau, Physiology Section, School of Life and Health Sciences, University of Delaware, Newark, 19711; Department of Zoology, University of California, Berke- ley, 94720.</i>	
EVOLUTIONARY ASPECTS OF ESTROGEN-SENSITIVE STRUCTURES IN THE VERTEBRATE FOREBRAIN	85
<i>Walter E. Stumpf, Madhabananda Sar, Ye Soon Kim, Donald A. Keefer, and Marie Christine Martinez-Vargas, Departments of Anat- omy and Pharmacology, University of North Carolina, Chapel Hill, 27514. Kim: Center for Biomedical Education, The City College of New York, 137th Street and Convent Avenue, New York, 10031.</i>	
EVOLUTION OF NEUROHYPOPHYSIAL PEPTIDE FUNCTIONS	95
<i>P. J. Bentley, Department of Pharmacology and Ophthalmology, Mt. Sinai School of Medicine of The City University of New York, 100th Street and Fifth Avenue, New York, 10029.</i>	
ADENOHYPOPHYSIS: STRUCTURE AND FUNCTION	107
<i>Martin P. Schreibman, Department of Biology, Brooklyn College of The City University of New York, Brooklyn, 11210.</i>	

MECHANISM OF ACTION OF SOME POLYPEPTIDE HORMONES IN AN EVOLUTIONARY SERIES OF Vertebrates.....	133
<i>Kenneth V. Honn and Walter Chavin, Department of Radiology and Biology, Wayne State University, Detroit, Michigan 48202.</i>	
PHYLOGENETIC TRENDS IN HORMONAL CONTROL OF GONADAL STEROIDOGENESIS	167
<i>Valentine Lance and Ian P. Callard, Department of Biology, Boston University, Boston, Massachusetts 02215.</i>	
ROLE OF THE THYROID IN VERTEBRATE EVOLUTION	233
<i>William Etkin and Young So Kim, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York 10461; Center for Biomedical Education, The City College of New York, 137th Street and Convent Avenue, New York, 10031.</i>	
EVOLUTION OF THE GASTROINTESTINAL ENDOCRINE SYSTEM	247
<i>Susan Van Noorden, Julia M. Polak, and A. G. E. Pearse, Histo- chemistry Department, Royal Postgraduate Medical School, London W12 OHS, England.</i>	
EVOLUTION OF PANCREATIC ISLET FUNCTIONS	269
<i>August Epple, Jack E. Brinn, and Jean B. Young, Daniel Baugh Institute of Anatomy, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania 19107; Department of Anat- omy, School of Medicine, East Carolina University, Greenville, North Carolina 27834; Daniel Baugh Institute of Anatomy, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania 19107.</i>	
EVOLUTION OF ENDOCRINE CONTROL OF CALCIUM REGULATION	323
<i>Peter K. T. Pang, Alexander D. Kenny, and Chitaru Oguro, Depart- ment of Pharmacology and Therapeutics, Texas Tech University School of Medicine; Department of Pharmacology and Therapeutics, Texas Tech University School of Medicine, Lubbock, 79409; Department of Biology, Toyama University, Toyama, Japan.</i>	
PHYLOGENY OF VERTEBRATE ADRENAL CORTICOSTEROIDS	357
<i>D. R. Idler and B. Truscott, Marine Sciences Research Laboratory, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7.</i>	
EVOLUTION OF THE RENIN-ANGIOTENSIN SYSTEM	373
<i>Hiroko Nishimura, Department of Physiology and Biophysics, Uni- versity of Tennessee Center for the Health Sciences, Memphis, 38163.</i>	

INTRODUCTION

This volume contains the proceedings of a regional Symposium on "Evolution of Vertebrate Endocrine Systems," held on 3-4 June 1977, on the campus of Thomas Jefferson University in Philadelphia, Pennsylvania. The Symposium was sponsored by the Division of Comparative Endocrinology of the American Society of Zoologists and by the College of Graduate Studies, Thomas Jefferson University.

The organizers gratefully acknowledge the financial support provided by an NSF grant (#PCM 7709877) to the American Society of Zoologists, and by additional funds from the American Society of Zoologists. They furthermore wish to thank Mary Wiley (ASZ) and Barbara J. Nibbio (TJU) for invaluable help in the preparation of this highly successful meeting. Our thanks are due also to R. C. Baldrige, Dean of the College of Graduate Studies (TJU), and the following officers and members of the ASZ who assisted at various stages of organizing the symposium: G. T. Bagnara, N. B. Clark, Aubrey Gorbman, and W. B. Vernberg.

Contributions in this book are based on papers presented at the Symposium, which have since been revised and edited with the assistance of Dr. R. Pang. The editors hope that this work will provide some glimpses at the state of the art, and that it will further cooperation among laboratories of the northeastern USA and adjacent provinces of Canada.

P. K. T. Pang
A. Eppler

COMPARATIVE PHYSIOLOGY, SYSTEMATICS, AND THE HISTORY OF LIFE

JAMES W. ATZ, AUGUST EPPLE, AND PETER K. T. PANG

The notion that life on Earth evolved has had a long history, but not until the middle of the nineteenth century did it begin to receive general attention. Since then, biologists have come to recognize that one of the foremost challenges they face is to describe the history of life. This task was first taken up by investigators concerned with plant and animal classification, comparative anatomy, embryology, and paleontology; eventually these workers were joined by physiologists and other experimentalists.

Perhaps the single greatest contribution that the idea of organic evolution has made to Biology has been to provide it with a unifying explanation for the order that appears to pervade all manifestations of life. This became particularly apparent to systematists when they realized that their pre-evolutionary classifications had turned out to be surprisingly good representations of evolutionary relationships. Such classifications were considered to be *natural*—in contrast to artificial ones for which the overriding purpose was to facilitate the cataloging and identification of living or once-living organisms. These two antagonistic views of biological classification are not the only ones that have been upheld by biologists, however, and sharp controversy still exists as to what constitutes an ideal classification and exactly what a biological classification can, or should, accomplish (Hull, 1970). From the viewpoint of the comparative biologist, that is, the biologist interested in the history of life (Nelson, 1970; Atz, 1973), the most important development in the field of systematics is the growing conviction that zoological and botanical classifications can and should precisely represent the lines of descent of living things, in other words, their phylogeny (Nelson, 1973). If this is indeed the case, systematists and all other comparative biologists ought to be the closest of collaborators. On the one hand, the systematist erects his classification, using all available pertinent information. In turn, the comparative physiologist, for example, sets up his own hypotheses of evolutionary relationships that can be tested against the established classification. If their conclusions as to phylogenetic history do not agree, both systematist and physiologist then may be able to reexamine their data and resolve the incongruity. This cooperative effort ought to improve the physiologist's understanding of his data as well as the prevailing classification.

In the past, however, cooperation between systematists and other comparative biologists has been sporadic at best. Most experimental biologists have ignored taxonomy and systematics, some even to the extent of not bothering to provide their animals with proper identifications or scientific names. For

their part, systematists have been unable to explain the principles and practices of systematics, at least in terms meaningful to other biologists. Experimentalists generally have considered systematics and systematists irrelevant, and systematists have considered experimentalists naive. Fortunately, recent changes in the methods of classifying animals and plants have provided the science of systematics with a rigorous methodology, the principal operations of which are not too difficult to explain. For the first time, the systematist is in a strong position to enlist the aid of other biologists in the great task of describing the history of life. The furtherance of that recruitment is the main purpose of the present article.

The new systematics that is bringing the systematist onto firmer ground with other biologists is called phylogenetics or cladistics. Although there is a rapidly growing literature about it, no introductory text exists, and the interested student might find the most comprehensive treatment, written by its founder Willi Hennig (1966), too involved. More directly accessible are the summaries of Hennig (1965) and Griffiths (1972:5-28). We shall confine our exposition to those aspects directly concerned with the applications of phylogenetics to the data of physiology, but the principles are the same throughout comparative biology.

The basic tool of the phylogenetic or cladistic approach to systematics is the cladogram, which is a graphic representation of the evolutionary relationship among recognizable groups of animals (or plants). The groups may be as circumscribed as species or populations, or as inclusive as classes or phyla. Membership in them is rigidly defined: all the members of a group must be genealogically related, that is, all must share somewhere in their past a single common ancestor. Moreover, no individual that is so related can exist outside the group. The systematist calls such uniform and unique groups monophyletic. Fig. 1a is a simple cladogram. It states that groups *A*, *B*, and *C* are descended from a common ancestor and that *B* and *C* are more closely related to each other than to *A*. In this diagram, node *x* may be considered the common ancestor of *A*, *B*, and *C* and node *y* the common ancestor of *B* and *C*. In this manner, it is possible to consider the cladogram as an abstracted depiction of the course of evolution, that is, an arrangement evolutionists often call a family tree.

In the strictest sense, however, a cladogram is simply an operational device. Given three monophyletic groups of animals, the comparative zoologist wishes to determine which two are most closely related. When he has done so, he draws a cladogram to describe the hypothesis of relationships that he has erected. Although the dichotomous nature of the branches of the cladogram might be a reflection of real events in evolution—for example, the process of speciation—these do not concern us here inasmuch as we are not, at this time, dealing with an evolutionary tree. The dichotomy at each node of the cladogram is an expression of an unequivocal decision as to what group is related to what. If, in fact, we could not decide whether *A* was more closely related to *B*, *A* to *C*, or *B* to *C*, our diagram perforce

FIG. 1.—Permutations of a three-taxon cladogram: a, taxa B and C are more closely related to each other than to A; b, taxa A and C are more closely related to each other than to B; c, Taxa A and B are more closely related to each other than to C. Note that reversing the positions of the two terminal taxa, as shown in each cladogram on the right, does not alter the relationship.

would take the form of a trichotomy (Fig. 2). The succession of nodes in a cladogram is an expression of hierarchical classification. In Fig. 3, for example, all five groups must share a certain identifying character, or constellation of characters, C, D, and E must share another, D and E still another, and A and B yet another. As indicated above the cladogram, this hierarchy consists of four levels and easily could be translated into a Linnaean classification, such as family I (consisting of subfamilies IIa and IIb), subfamily IIa (consisting of genera A and B), subfamily IIb (consisting of tribe IIIb with its genera D and E and tribe IIIa with a single genus C).

But hierarchical arrangement is not what distinguishes cladistics from other methods of biological classification; since Linnaeus, practically all biologists have classified animals and plants hierarchically. Rather, it is the kind of characters used to relate one group to another that is critical and that sets cladistics apart. Two groups of organisms can be considered each other's closest relative if, and only if, they share at least one unique and derived character between them, because only such characters provide unequivocal evidence of common ancestry. If, in Fig. 3, we let A represent the hagfishes, B the lampreys, C the cartilaginous elasmobranchs, D the bony ray-finned fishes, and E the bony fringe-finned ones; then all are united by the presence of a backbone; C, D, and E are united by the presence of

FIG. 2.—An unresolved three-taxon cladogram, in which it is not known whether *A* is more closely related to *B* than to *C*, *B* is more closely related to *C* than to *A*, or *C* is more closely related to *A* than to *B*.

jaws whereas *A* and *B* lack them; *C* lacks bone but *D* and *E* are united by the presence of this tissue. It is the unique and derived nature of each of these characters (shared-derived characters) that makes them decisive in defining the groups. For example, the presence of a backbone is a character that defines the vertebrates and, moreover, is evidence of the monophyletic nature of the Vertebrata, inasmuch as all vertebrates exhibit a backbone and no nonvertebrate has one. The presence of a backbone, however, does not help classify any particular vertebrate group with relation to another. Although the vertebral column proclaims hagfishes, elasmobranchs, and bony fishes all to be vertebrates, it provides no information, for instance, as to whether elasmobranchs are more closely related to lampreys or to bony fishes. At this level of the classification, the presence of a backbone is not a derived (apomorphic) character; it is a primitive (plesiomorphic) one. The distinction seems obvious in this case, but confusion between such character states, as they are called, has been the bane of systematics since its inception. It has brought about the erection of innumerable taxonomic groups of animals and plants that have been classified together on the basis of the common possession of primitive characters, frequently quite a number of them. One of the principal tasks of present-day systematists is to weed out such paraphyletic groups, and the double jeopardy that comparative physiologists continually face is especially apparent here: not only must they be ever-vigilant in their own evaluation of character states, but they should realize that the accepted classification of the animals (or plants) with which they are working might not be the best possible one, even in the light of presently available information.

A related aspect of character appraisal concerns the significance of the absence of a particular character as compared with its presence. If the presence of a backbone defines the vertebrates, does the lack of this structure similarly define the invertebrates? Does the absence of a backbone in, say,

FIG. 3.—The hierarchical relationship described by a cladogram. Three levels of taxa are represented by the successively circumscribed figures with A—E occupying the fourth and lowest level.

the insects, the molluscs, and the sponges genealogically relate these animals one to the other in any way? The common ancestor of the Vertebrata must have had a backbone; what must the common ancestor of these three invertebrate groups have had? The question is, in one sense, meaningless, since the answer to it is "nothing." A survey of all the disparate groups of animals without backbones leads to the conclusion that although absence of a backbone might be a character useful in a key to separate vertebrates from invertebrates, it is devoid of informational content as far as phylogenetic relationship is concerned. It should be pointed out, however, that not all characters involving the absence of a certain structure or function are useless in classification. For example, one of the characteristics of the snakes is a lack of limbs, but this situation represents a loss of the two pairs of appendages present in some lizardlike ancestor. In contrast, none of the invertebrates ever had a backbone.

The evaluation of characters, including their apomorphous and plesiomorphous states, plays an absolutely critical role in the phylogenetic analyses of all comparative biologists. The process calls for the broadest possible knowledge, because one cannot predict from where a decisive bit of information could come. In general, however, two problems must be faced: the identification and definition of the character in question and its distribution among all the various animal (or plant) forms. Cutting across both of these is ontogeny, because characters do not remain the same during the development of the individual and because these developmental changes can be related to evolutionary ones (Nelson, 1978). Functions, by their very nature,

that is their intangibility, are more difficult to define and compare than are structures. Moreover, comparative anatomy has had a much longer and fuller history than comparative physiology and thus can draw upon a much greater body of knowledge. Examples are not hard to find: the indubitable identity of the thyroid gland throughout the vertebrates is not paralleled by any as yet known common function—beyond the production of hormones of the same, or very similar, chemical composition (thyroxine and triiodothyronine). As soon as structure, be it morphological or biochemical, is left behind, any comparison of the physiology and behavior of animals that are not very similar, and therefore not closely related, becomes difficult indeed (Atz, 1970).

This situation also makes it difficult to survey the distribution and expression of a given physiological character in as many different kinds of animals as possible. This process is known as outgroup comparison and it serves to put the character into phylogenetic perspective, as it were. With occasional help from ontogeny, it is the only way to obtain information as to whether a particular character should be considered apomorphous (advanced, derived) or plesiomorphous (primitive, ancestral). For example, the faculty of bringing forth living young, instead of laying eggs, is confined to 11 very different families of ray-finned bony fishes among some 400 that are currently recognized. These viviparous fishes are all advanced types, that is, they exhibit a considerable preponderance of apomorphous characters relative to the gamut shown by the group as a whole. As far as the ray-finned bony fishes are concerned, viviparity clearly looks like an advanced character, independently acquired by most of the different livebearing families. But what about the livebearing coelacanths, an ancient group of bony fringe-finned fishes the one living species of which (*Latimeria chalumnae*) closely resembles the coelacanths that lived 300 million years ago? The lungfishes, usually considered to be the coelacanths' closest living relatives, lay eggs. Most living elasmobranchs give birth to living young, and those that lay eggs belong to the less advanced families. As with the ray-finned bony fishes, viviparity appears to be an apomorphous condition among the elasmobranchs. Because of our limited information about the fossil fringe-finned fishes, however, we cannot relate the viviparity of the coelacanths to the mode of reproduction of any other group of fishes, fringe-finned or otherwise; in particular, viviparity among coelacanths cannot be considered as evidence that the early (most primitive) bony fishes brought forth their young alive (Smith *et al.*, 1975). Even in this apparently straightforward case, it can be seen that outgroup comparison is not at all a simple matter and that the conclusions reached are often limited and always provisional.

One aspect of the identification and definition of characters used to classify animals and plants involves the phenomenon generally called convergence, that is, the appearance of what seem to be the same characters in genealogically unrelated forms. The most frequently offered explanation for this situation is that unrelated organisms have responded to similar envi-

ronmental conditions or constraints: fishes, whales, and ichthyosaurs to the physical properties of water, for instance. Because of their incorporeal nature, functions are less easy to break down into their components for analysis than are structures. The fins and streamlined bodies of fishes, whales, and ichthyosaurs are well recognized as being different in morphology; viviparity, on the other hand, is often treated as a single condition—as we ourselves have done earlier in this paper. Even preliminary studies on livebearing fishes, however, reveal fundamental differences in the timing of various phases of the gravidity cycle, the nurturing of the unborn young, their state of development at birth, and other features. Convergence, then, represents a statement of ignorance as regards phylogeny, inasmuch as the likelihood of genetically unrelated forms producing identical responses (adaptations) to any particular environment is in effect nil.

Convergence, as well as other misidentifications of characters, is often the reason for lack of agreement among the characters used in constructing cladograms. Another cause of disagreement is the misinterpretation of character states, most frequently the failure to recognize plesiomorphous characters. Consequently, for these and other reasons, situations arise in which some characters support one cladogram whereas others support a different one. In such cases, the more parsimonious solution should be accepted. One of the great virtues of cladistics is that it brings all incongruencies to the fore and that it also calls attention to lacunae in the definition of any groups. By forcing the phylogeneticist to make decisions about hypothesized genealogical relationships in accordance with clear-cut rules, cladistics has produced consistent classifications that are an accurate representation of phylogenetic relationships so far as they are known. Moreover, these phylogenetic schemata can be tested in a uniform, logical way when new, pertinent items of information come to light. Some of these new items will be, of course, physiological.

In the reconstruction of phylogenies, the treatment of ancestors has proven a difficult problem and one that has been fraught with controversy. It has been pointed out repeatedly that no extant animal (or group of animals) could be the ancestor of another. According to the fossil record, for example, the sharks and their relatives and the bony fishes and theirs (including the tetrapods) have existed separately for at least the past 330 million years, so the naivete of regarding the dogfish as some kind of ancestral jawed vertebrate is obvious. The fragmentary nature of the fossil record precludes any reasonable hope of actually finding a fossilized ancestor, and its entirely plesiomorphous appearance would make it unrecognizable if it were unearthed (Nelson, 1969, 1970). For these and other reasons, biostratigraphy, that is, the location of a given form in the chronology of the fossil record, can provide little or no critical information about specific ancestors (Schaeffer *et al.*, 1972). The fact that phylogenetic trees derive their validity from the cladograms on which they are based means that the same constraints govern them both; one of these is that the common ancestors

(represented by the nodes of the trees) are entirely theoretical constructs and exist only as a result of the erection of the cladogram. Finally, an analysis of cladistic and phylogenetic relationships, as scientifically testable hypotheses, has shown that ancestor—descendant relationships cannot be tested and therefore are unscientific (Engelmann and Wiley, 1977; Platnick, 1977). Thus, both logical deduction and empirical evidence clearly show that the search for ancestors, either among animals living today or in the fossil record, is a futile one, even though there is not the slightest doubt that such ancestors did at one time exist.

Fossils, however, seldom provide any information, even of a collateral nature, about physiological functions. Nevertheless, it is precisely for this reason that the place of ancestors in the cladistic scheme of things should be of concern to comparative physiologists. The characteristics of a particular common ancestor in a phylogenetic tree are defined by the characters held in common (that is, the plesiomorphous characters) among the groups subsumed by the two phyletic lines the connection of which gives rise to the node (common ancestor) in question. If we assume that the tree (and, of course, the cladogram on which it is based) is correct, we can then hypothesize what that particular common ancestor must have been like. What we are doing is constructing a morphotype or physiotype. Because few, if any, of the data used to erect cladograms are physiological in nature, any physiotypes we construct will not be trivial and might even prove unpredictable to a significant extent. Such a physiotype might provide clues about the course of evolution of certain functions, or insights that could lead to the reinterpretation of functions or structures of these and other animals. A search for common physiological denominators, so to speak, among the lungfishes, the coelacanth, the sturgeons and paddlefishes, and the bichirs and reedfish might present a revealing picture of the common ancestor of all the bony fishes, a fish that probably existed about 400 million years ago.

We have touched on a few of the more obvious interactions that ought to be taking place between comparative physiologists and systematists. Any biologist who wishes to put his work into an evolutionary context ignores systematics at his own risk. Now that systematists have begun to put their own house in order and are better able to provide assistance to their fellow comparative biologists, it behooves all of us to seek that help. In doing so we shall be helping the systematist in return. Such cooperative effort cannot fail to promote the great task of producing the best possible history of life.

ACKNOWLEDGMENTS

Most of the ideas expressed here were discussed on numerous occasions with colleagues at the American Museum of Natural History, most importantly Dr. Gareth J. Nelson, but also Drs. Niles Eldredge, Eugene S. Gaffney, Norman I. Platnick, and Donn E. Rosen. We thank them for this vital help and for reading the manuscript and commenting on it. This work was supported in part by a grant from The Griffis Foundation, Inc.

LITERATURE CITED

- ATZ, J. W. 1970. The application of the idea of homology to behavior. Pp. 53-74, in *Development and evolution of behavior: essays in memory of T. C. Schneirla* (L. R. Aronson, E. Tobach, D. S. Lehrman, and J. S. Rosenblatt, eds.), W. H. Freeman, San Francisco, xviii+656 pp.
- . 1973. Comparative endocrinology and systematics. *Amer. Zool.*, 13:933-936.
- ENGELMANN, G. F., AND E. O. WILEY. 1977. The place of ancestor—descendant relationships in phylogeny reconstruction. *Syst. Zool.*, 26:1-11.
- GRIFFITHS, G. C. D. 1972. The phylogenetic classification of Diptera Cyclorrhapha. W. Junk, The Hague, 340 pp.
- HENNIG, W. 1965. Phylogenetic systematics. *Ann. Rev. Entomol.*, 10:97-116.
- . 1966. Phylogenetic systematics. Univ. Illinois Press, Urbana, 263 pp.
- HULL, D. L. 1970. Contemporary systematic philosophies. *Ann. Rev. Ecol. Syst.*, 1:19-54.
- NELSON, G. J. 1969. Origin and diversification of teleostean fishes. *Ann. New York Acad. Sci.* 167:18-30.
- . 1970. Outline of a theory of comparative biology. *Syst. Zool.*, 19:373-384.
- . 1973. Classification as an expression of phylogenetic relationships. *Syst. Zool.*, 22: 344-359.
- . 1978. Ontogeny, phylogeny, paleontology, and the biogenetic law. *Syst. Zool.*, 27: 324-345.
- PLATNICK, N. I. 1977. Cladograms, phylogenetic trees, and hypothesis testing. *Syst. Zool.*, 26:438-442.
- SCHAEFFER, B., M. K. HECHT, AND N. ELDREDGE. 1972. Phylogeny and paleontology. *Evol. Biol.*, 6:31-46.
- SMITH, C. L., C. S. RAND, B. SCHAEFFER, AND J. W. ATZ. 1975. *Latimeria*, the living coelacanth, is ovoviviparous. *Science*, 190:1105-1106.

ENVIRONMENTAL FACTORS IN THE EVOLUTION OF VERTEBRATE ENDOCRINE SYSTEMS

KEITH STEWART THOMSON

It is one of the beauties of biological science that the paleontologist and evolutionary biologist can have a great deal in common with someone working in such a seemingly unrelated field as endocrinology. What brings us together is a two-fold common ground. First, the science of modern biology is unified by a single generalising principle, Darwin's theory of evolution by natural selection. This theoretical framework allows the evolutionary biologist to organise and interpret his data on genetics, morphology, patterns of distribution in space and time, genotypic and phenotypic variability, and life history phenomena. Equally, this theory allows the comparative endocrinologist to interpret his data on the nature of control systems in organisms through his comparisons among organisms. The comparative method is itself the second common ground of all biologists. It is as fundamental as a mode of organising one's investigation and data as the theory of evolution is fundamental to interpreting it. The comparative method has an even more honorable history, reaching back to Aristotle and beyond.

I see my own role in this symposium as an attempt to synthesize some paleontological and comparative physiological data in order to establish the background of environmental history that is vital to placing diverse physiological mechanisms in their correct phylogenetic context. The situation is far from clear. In many cases, the paleontological data on the environments in which different groups lived is ambiguous and confusing, and often the phylogenetic relationships between groups are inconclusive. But at least some basic questions can be identified and the outlines of a scheme of environmental and evolutionary theory may be drawn up. The problem is simple: in view of the fact that so much of the result of endocrinological function in the vertebrates is related directly to environmental adaptations of long and short term, what is the environmental context in which different groups of organisms and their different adaptations have evolved over time? I will concentrate on one of the most vexing problems of all, the environment of early fishes, marine or freshwater, for these particular environmental constraints can be shown to have far-reaching significance in the history of early vertebrate evolution.

The available evidence is limited. From the morphological point of view, there is direct evidence from the structure of kidneys (a favorite subject some 40 years ago), the presence and nature or absence of lungs, the basic construction of the skeleton, evidence of modification of the skeleton during life history, and, more remotely, reconstruction of mode of life, feeding, swimming, and the like. There is a certain amount of evidence from the fine

structure of the endocrine organs themselves that allows us to study the relationships of groups (Epple and Brinn, 1975; Atz *et al.*, this volume). On the physiological side, the principle evidence is the data (newly added to in certain key groups) concerning the ionic composition and total ionic concentration of the plasma and the general nature of osmoregulatory mechanisms.

This is, perhaps, an unpromising set of data to work with. My own introduction to the exigencies and challenges of the data came from work with the living coelacanth *Latimeria chalumnae*. As is now familiar to all, prior to 1966 it was thought that there was a fundamental difference between cartilaginous fishes (elasmobranchs and chimaeroids) and bony fishes (the teleosts and surviving chondrosteans and holosteans plus the living lungfish) in the matter of osmoregulatory mechanisms. The former were thought to be alone in having a plasma that, in terms of ionic composition, was hypoosmotic to seawater and that was balanced by the retention of urea in large volumes, together with other major organic compounds. Also considered unique was the synthesis of urea through the ornithine-urea cycle. Bony fishes, on the other hand, also had a hypoosmotic plasma but were seawater drinkers and selectively secreted salts through the gut and gills. This tidy scheme was disturbed by the finding (see Goldstein and Forster, 1970, for review) that lungfish had a high level of activity of all five enzymes of the ornithine-urea cycle and were highly uremic during aestivation, apparently in parallel, at least, with cartilaginous fishes. It was a small comfort that no living agnathan fish, either hagfish or lamprey, showed uremia or the presence of the ornithine-urea cycle enzymes (see, for example, Read, 1968, 1975). The scheme was thrown into total disarray when Professor Grace E. Pickford took some blood from the first coelacanth ever transported frozen from the Comores (see Thomson, 1966) and discovered within hours of the dissection that it had a slightly hyperosmotic plasma and large volumes of urea (Pickford and Grant, 1967; Griffith, *et al.*, 1974; Lutz and Robertson, 1971). Later, Brown and Brown (1967) and Goldstein *et al.* (1973) confirmed the presence and high activity of the full consort of ornithine-urea cycle enzymes.

The new hypothesis devised to incorporate these findings had two alternatives: either the ornithine-urea cycle and uremia as an osmoregulatory device were primitive features held in common by all early gnathostome fishes (but not agnathans), or they had evolved independently in parallel at least three times (elasmobranchs and chimaeroids, dipnoans, and coelacanths). The latter alternative was rendered less likely by the subsequent discovery that certain osteichthyans showed several, if not all, of the ornithine-urea cycle enzymes, and at least one teleost, *Opsanus tau*, had all five with a reasonable level of activity (Read, 1971; see also Huggins *et al.*, 1969). This would seem to indicate that physiological uremia and the presence of the full ornithine-urea cycle must have been selectively lost in bony fishes, possibly as the result of a long period of evolution in freshwater where the maintenance of a plasma of high osmotic pressure was unnecessary. But there are

difficulties with this view also. If this were the case, then a long period of freshwater history should be readily observable in the fossil record. It is not. Similarly, there is one group of living fishes, the hagfishes (for example, *Myxine*, *Eptatretus*), in which the ionic concentration of the plasma is essentially equal to that of seawater. Although the exact phylogenetic relationship of hagfish to other fishes is not completely clear, it generally is assumed that they arose from a very ancient group of jawless vertebrates (as did the lampreys) and that the ionic composition of their plasma represents the sole example among living fishes of the primitive vertebrate condition. If this is correct, there ought to be a long history of freshwater occupation between the ancestors of hagfish and the ancestral gnathostomes, to account for the drop in ionic concentration, but again there is not. Lastly, the characteristic adaptation by which bony fishes like teleosts take care of the problems raised by drinking salt water must be part of an extremely ancient rather than very new adaptation, for members of all the Actinopterygii, including the surviving chondrosteans and holosteans, have chloride cells (apparently homologous) in the gills.

MAJOR GROUPS OF FISHES

It is necessary, therefore, to bring some order into this chaos. The first step is a coherent review of the evidence concerning the major groups of fishes. I will not repeat the data on endocrine organs summarised by Epple and Brinn (1975). The ionic data are summarised in Table 1.

The hypothetical vertebrate ancestor.—The vertebrates probably evolved sometime during the mid to late Cambrian and the first fossil record, probably coinciding roughly with the evolution of a true bony skeleton, occurs in the early Ordovician. All of the direct geological evidence points to a marine origin of the vertebrates (Robertson, 1957; Denison, 1956; White, 1958; *pace* Romer, 1955). The modern view (Robertson, 1957; *pace* Smith, 1932; Marshall and Smith, 1930) is that early vertebrates in salt water evolved the glomerular kidney, functional as an ionic but not an osmotic regulator.

Agnathan fishes.—The first known fossil vertebrates are agnathans. Modern hagfishes (*Myxine* and relatives) are the descendants of unknown groups (there is much argument about the origin of modern forms from different fossil agnathan groups, see Moy-Thomas and Miles, 1971, for review). Modern lampreys can be traced through the fossil record back to the Carboniferous (Bardach and Zangerl, 1968). There is no fossil record of myxinoids, but they are considered to be extremely primitive because they, alone among vertebrates, have a plasma roughly isoosmotic with sea water (Robertson, 1976). Actually, it may be slightly hyperosmotic. Lampreys are primarily euryhaline and have a plasma with a reduced ionic content (review in Morris, 1972). When in sea water, they also osmoregulate by drinking and have chloride cells in the gills (Conte, 1969). The chloride secreting cells of modern agnathans are not considered homologous with those of gnathostomes (Conte, 1969; Morris and Pickering, 1975). Agnathans have no lungs.

TABLE 1.—Summary of data on ionic composition (in mM) and total osmolality (in mOsm/l) of the plasma and maximum geologic age (in millions of years) of derivation of groups of fishes. The ionic values given are maxima for the groups. As noted in the text, various members may show values lower than these. Data principally from Urist and van de Putte (1967), Urist et al. (1972), and Holmes and Donaldsen (1969).

Taxon or group	Na	Ca	Cl	Total Ions	Total Plasma	Geol. Age
Myxinioids	485	6.0	5000	1100	1100	550
Petromyzontoids	105		200	275	600	300
Chimaeroids	270	6.0		600	1070	450
Marine elasmobranchs	280	5.0	300	525	1100	450
Freshwater sharks	245	3.5	200	400	550	100?
Freshwater potamotrygonids	164	3.0	152	332		100?
Coelacanthini	180		200	425	110	400
Lungfish	110	2.0	90	225	240	400
Polypterids	105	2.3	90	200	200	300
Sturgeons	130	1.8	110	255	340	210
<i>Polyodon</i>	130	2.2	105	250		210
<i>Lepisosteus</i>	155	2.6	140	320		100
<i>Amia</i>	140	2.9	120	275		210
Marine Teleosts	180	3.2	200	355	475	150
Freshwater Teleosts	140	2.6	110	275	300	110

Gnathostome fishes.—The first gnathostome fossils are late Silurian in age. A point of fundamental importance in modern vertebrate phylogeny is that the agnathan and gnathostome fishes can no longer be considered to have an ancestor-descendent relationship. There is no evidence that gnathostomes evolved from any known agnathan, and the two groups are best regarded as having diverged separately from some unknown common ancestor (see Moy-Thomas and Miles, 1971).

The major groups of living fishes are Elasmobranchii, Chimaeroidea, Sarcopterygii (lobe-finned fishes including lungfishes and *Latimeria*) and Actinopterygii (ray-finned fishes). There also is an array of extinct gnathostomes (principally those known as Placodermi and Acanthodii), the relationship of which to living groups is entirely enigmatic. The placoderms and acanthodians appeared first in marine deposits (Denison, 1956) and only later members entered freshwaters.

Elasmobranchii and Chimaeroidea.—The cartilaginous fishes (Chondrichthyes) consist of two distinct groups that are characterized by different osmotic adaptations. Both have a plasma with reduced ionic concentration when compared with myxinioids, and both have shown uremia. However, the plasma ionic concentration of the chimaeroids is significantly higher than is that of elasmobranchs. Both groups lack lungs and a bony skeleton, although traces of the latter may be found in the fossil record. Both trace their origin back at least to the late Silurian, and geological evidence suggests a marine ancestry for these fishes. There are at least two periods during which cartilaginous fishes entered freshwater. In the late Palaeozoic, there was a distinct group, the Pleuracanthodii, which were freshwater in habit

and distinguished by a unique skeletal morphology. Their existence offers the only possible hint of a freshwater connection for early elasmobranchs. However, their origin is unknown and they became extinct in the early Triassic. Members of various modern shark and ray groups have invaded freshwater in Recent time. In each case, the total ionic concentration of the plasma is reduced from the marine level, and the degree of uremia is greatly reduced. Freshwater stingrays (*Potamotrygonidae*) are different from other forms entering freshwater and show active sodium uptake (see Pang *et al.*, 1977, for review).

Osteichthyes.—The bony fishes present a curious problem. Their earliest records, particularly of the lobe-finned fishes, are marine and yet they have lungs that have always been thought to be freshwater adaptations. Packard (1974) may have provided a solution to this dilemma in a study that attempted to show that lungs might easily have evolved in marine or brackish conditions, in which case a major line of argument for freshwater origins and history is removed. The presence of a true bony skeleton with cellular bone capable of remodelling must be highly significant, particularly with respect to calcium metabolism. A most important point is that all marine osteichthyans show a total ionic concentration of the plasma that seems similar to, but significantly lower than, the cartilaginous fishes, the levels in *Latimeria* being higher than in marine teleosts.

Sarcopterygii.—The Dipnoi (lungfishes) and Crossopterygii (extinct Rhipidistia, ancestors of tetrapods, plus the Coelacanthini including the living *Latimeria*) are quite different groups of fishes, only very distantly related. It is a pity that the lungfishes, which show a completely freshwater history from the Upper Devonian, are not more closely related to the tetrapods, because the only living crossopterygian belongs to a lineage essentially continuously marine from the Devonian (Thomson, 1969a, 1969b). However, both major groups are principally marine in habit during the very earliest part of their history—the early Devonian. The history of the coelacanth shows both marine and freshwater phases, but, at the family level, there is no clear cut evidence of a long period of exclusively freshwater history, and most genera seem to be found in a variety of environments. The same is true of many Devonian Rhipidistia. The Rhipidistia also are interesting because of extensive evidence of resorption and redeposition of the dentinous part of the exoskeleton—cosmine (Thomson, 1976, 1977), which must be of considerable interest in terms of calcium metabolism and may well indicate a special adaptation to euryhaline biology and an anadromous life history.

Actinopterygii.—The vast majority of living bony fishes belong to the advanced group Teleostei. The surviving chondrosteans belonging to the Polypteridae are all freshwater in habit with no evidence of a saltwater ancestry (Lutz, 1975). *Polyodon* is a freshwater form. The sturgeons are all euryhaline. The surviving holosteans *Amia* and *Lepisosteus* also are freshwater (although *Lepisosteus* has euryhaline connections). However, the majority of fossil chondrosteans and holosteans are marine or mixed in

environment, and there is, as noted above, no indication of a long fresh-water period in their history. The Teleostei evolved in the Jurassic from marine ancestors. Freshwater teleosts occur at all stages in the history of the group but at no time in the history of osteichthyan fishes, except for the lungfish, is there fossil evidence for a very long period of exclusively fresh-water existence. The Actinopterygii is the only major group of fishes for which the geological evidence can support a freshwater or brackish water ancestry (Denison, 1956).

DISCUSSION

A scheme of the phyletic relationships of these major groups of fishes and some of the constituent groups that will be important in the discussion are shown in Fig. 1. This scheme is based on the available morphological and palaeontological data, but it is interesting that it agrees in all important points with the pattern of relationships that is indicated by the data on endocrine organ histology that Epple and Brinn (1975) recently discussed. A critical point is the relationship among lungfishes, coelacanth, and the rhipidistian-tetrapod assemblage. There are important points of similarity between lungfishes and tetrapods, but these points are attributable to the retention of primitive characters and are not real synapomorphies in the cladistic sense. We should not let these similarities blind us to the evidence relating all crossopterygian fishes (coelacanth plus rhipidistians) to one another and setting them collectively apart from the lungfishes. *Latimeria* itself retains some primitive characters, giving an apparent relationship to the elasmobranchs (uremia, rectal gland salt secretion, and the like), but this is the retention of a different suite of primitive characters.

It long has been believed (for example, Baldwin, 1964; Robertson, 1957) that the pattern of ionic composition of the plasma and the total ionic concentration of the plasma must in some direct way reflect the environmental history of the various ancestors of modern fishes. Thus, the lower total ionic concentration of the plasma in freshwater versus saltwater teleosts, or freshwater versus saltwater elasmobranchs and the condition in lampreys, are reasonably thought to reflect the differential osmotic stresses of the freshwater and marine environments. For comparisons between fishes of different environments within single lineages, this explanation seems to hold true today. However, broader scale extrapolations also have been considered, and these, it is submitted, fall very far short of support. There is no evidence for a long freshwater phase in the ancestry of the cartilaginous fishes (elasmobranchs and chimaeroids) to explain their lower total ionic concentration compared with marine agnathans. Nor is there evidence to support such an explanation for the differences in elasmobranchs and chimaeroids. Furthermore, if this explanation were to hold true, the differences between the total ionic concentrations of the plasma between marine osteichthyans and chondrichthyans also should reflect a long history of freshwater existence, the values

FIG. 1.—Summary of phyletic relationships among the major groups of fishes discussed in this paper. Note that purely fossil groups are excluded. The bold black lines indicate sections of the evolutionary history during which marine existence occurred. The narrow lines indicate freshwater adaptations. An oblique stroke across a line indicates that within the main lineage one or more freshwater groups may have evolved.

for the former being lower than for the latter. But there is, as we have seen, no direct evidence for such a period in the ancestry of osteichthyan fishes.

In an effort to gain information about the possible history of the principle groups of fishes, the evidence on ionic composition, concentration, and regulation must be reviewed once more, for it is the only broadly comparable evidence that we have for all the fishes.

A summary of the ionic data is shown in Table 1, and some broad geological time scale data have been added so that the ionic data can be compared in a time frame. It will be noted that the only geological dates that can be given are the approximate date of the first appearance of a group in the record. This is not necessarily the date at which any given physiological adaptation appeared. Thus, modern lungfishes have a low total ionic concentration of the plasma. The Dipnoi can be traced back to the early Devonian through a sequence of genera that look remarkably like the living ones and all of which are freshwater in environment, with important marine

FIG. 2.—Summary of the distribution of different total ionic concentrations (mM/l) of fish plasma in time. The geological age given is the point at which the group first differentiated in the fossil record and is not necessarily the time at which the ionic patterns measured in the living groups actually arose (see text). Marine fishes are placed in circular boxes, freshwater fishes in squares, and euryhaline fishes in rhomboids. The dashed line shows the rough trend of lower ionic concentrations with age for freshwater fishes. The dotted line loosely connects data points for marine fishes. Abbreviations used in Fig. 2 and 3 are: Ac, sturgeons; Am, *Amia*; Ch, chimaeroids; Co, Chondrichthyes; D, lungfishes; Fc, freshwater Chondrichthyes; L, Lepisosteus; M, myxinoidei; P, Petomyzontoids; Pn, Polyodon; Ps, polypterids; T, teleosts.

exceptions from the middle and early Devonian. This is not the same thing as saying that the modern pattern evolved in the Middle Devonian, but it gives a maximum time span during which the adaptations could have evolved. The values given in Table 1 are all maximal. Within each group, there is considerable variation, presumably according to individual adaptations the scope of which well may be time related.

If we examine the data for freshwater fishes first (Fig. 2), we see that there is a consistent trend: the more recently differentiated groups have a higher ionic concentration than do the more ancient groups, following essentially a

FIG. 3.—Summary of reduction in total ionic concentration in freshwater groups arising at different times in the geological record (see legend for Fig. 2, and text). The data are based on the assumption that the ancestral condition for all these groups was 425 mM/l.

straightline relationship. However, when we examine the saltwater fishes, the pattern is far less clear. If we try to draw a "curve" connecting the points (Fig. 2, dotted line) we find that a very abrupt change must have occurred between the Ordovician and Silurian but that once the gnathostomes were differentiated, the curve flattens out, with modern teleosts lower in total ionic concentration of the plasma than are coelacanths or cartilaginous fishes, but not in such a proportion that anything like a straight line relationship could hold.

The data for saltwater fishes may be interpreted as showing that for all marine gnathostome groups there is a selective advantage in having a plasma hypoosmotic to seawater in terms of ionic composition and therefore the evolution of differing mechanisms to avoid catastrophic osmotic water loss has been a "price worth paying" in return for a physiological advantage of lower individual and total ionic concentrations. The data on freshwater fishes (Fig. 2, dashed line; Fig. 3) show that when a group enters freshwater from saltwater, there is a selective advantage in further reduction of the total ionic concentration, but the range of reduction is rather small,

and reduction has been achieved relatively slowly in geological time. These two phenomena must be distinguished very carefully.

In the data for saltwater fishes, the evidence provided by the living coelacanth *Latimeria* is critical. Its ionic plasma composition is listed in Table 1. The values are significantly lower than for elasmobranch fishes, even though both use the same urea retention method of osmotic balance. Furthermore, the elasmobranch values are lower than are those of chimaeroids. It is tempting to assume that the coelacanth values are the same as are those that would have been found in Devonian coelacanths and all other Devonian marine Osteichthyes. In this case, we could assume that the early osteichthyan fishes had a total ionic concentration of approximately 425 mM/L, not very much greater than that of modern marine teleosts (355 mM/l.) and the holostean *Lepisosteus* (320 mM/l.). I assume that the difference in value between the maxima for the coelacanth (425 mM/l.) and marine teleosts (about 350-375 mM/l.) has evolved over time as a difference of degree rather than kind. On balance, I am tempted to assume that the basic late Silurian-Devonian marine osteichthyan had a total ionic concentration in the plasma of no less than 400 mM/l. and no more than 450.

On this basis, as Fig. 3 shows, the reductions of ionic concentrations in the plasma of freshwater fishes are apparently more or less linearly related to time elapsed since the origin of the constituent group. Curiously, if we examine chloride alone (Table 1), the trend is less clear, but there seems to be an upper limit on reduction of chloride ion concentration of about 100 mM. Perhaps the most important observation from the comparative data is that no group shows any increase in total ionic concentration of the plasma compared with its nearest, more recent relatives. Decrease in ionic concentration seems to have been a consistent feature of all vertebrates. Equally, there seems to be a lower limit to ionic concentration. Only certain euryhaline fishes such as migrating salmon show a total concentration of less than 100 mM. The lowest values for most marine or euryhaline fishes is in the region of 275 mM., and this is found in teleosts, elasmobranchs, and lampreys.

In comparison of Figs. 2 and 3, the most striking fact is the rapid change in total ionic concentration that must have occurred between the evolution of chordates as a whole and the origins of gnathostomes. We are probably on safe grounds in assuming that the near total ionic isoosmicity of myxinooid plasma and sea water is an ancient feature from which the condition in gnathostome fishes, through whatever phylogenetic agencies, must have evolved. I would like to suggest that we should consider seriously the possibility that the presumed change between the early isoosmotic agnathans and the gnathostome ancestor of elasmobranchs, chimaeroids, and osteichthyans was too abrupt to be accounted for in terms of a gradual adjustment to particular environmental osmotic challenges (as in the case, for example, of the lowering of total ionic concentration in the various freshwater lineages).

If we were to assume, for the sake of argument, that the difference in total ionic concentration of plasma of coelacanths and chondrichthyans (which in

general have similar osmoregulatory mechanisms) were due to the ancestors of coelacanth (and all Osteichthyes) having a significant cumulative history in freshwater environments, how much longer a period in freshwater would have to be hypothesized in order to account for the differences between an early isoosmotic ancestor and chondrichthyans? In fact, there is not time in the geological record for such a reduction. When we add the fact that there is essentially no direct evidence for a freshwater period in early gnathostome history, then we must be open to other suggestions to explain the differences in total ionic concentration of agnathans and gnathostomes.

I believe it may be instructive to take the position that reduced total ionic concentration of the plasma is not in itself sufficiently valid evidence to establish that the particular group of fishes evolved in freshwater. In order to make a safe prediction of this sort, it is necessary for there to be confirmation in the form of direct evidence that the fossils of the earliest members of the group are in fact found in freshwater deposits. I find that most categorical statements in the literature to the effect that this or that group evolved in freshwater are actually merely inferences based on the physiological evidence alone. Given Packard's conclusions about the origin of the lungs (1974, see above), even the evidence of presence of lungs cannot be taken as direct evidence of freshwater ancestry, and, of course, the presence of a glomerular kidney has long since been abandoned as a safe reference point. Too much therefore rests upon the ionic data alone.

According to the simple criterion of requiring confirmatory geological evidence, we must conclude that a freshwater ancestry for agnathans, acanthodians, placoderms, chondrichthyans, and probably the osteichthyans as an entity, is not proven. On the other hand, there is ample reason to suppose that the ionic patterns of lampreys, freshwater and euryhaline chondrichthyans (and probably the pleuracanth were the same), modern dipnoans, and many living actinopterygians are a direct function of life in reduced salinities at an early stage of the evolution of their particular (sub) group.

This leads us therefore to formulate an alternative model to the simple scheme in which reduced total ionic concentration of the plasma has been thought to be a direct result of adaptation to life in freshwater. The attraction of the "freshwater ancestry" model is that it does not require the evolution of complex osmoregulatory mechanisms until groups reenter salt or brackish water. In freshwater, the plasma is hyperosmotic, there is a heavy water influx that can be eliminated via the kidneys, and renal function is necessary to avoid salt loss. In fact, an active salt uptake may be adopted also. If we were to assume that the major groups of fishes lived in saltwater, or even if we were to follow the most likely explanation that they were in a variety of environments in which the reduced plasma content would be variously hyper and hypoosmotic, then a different model must be proposed. Under these conditions, reduction of ionic concentration of the plasma (for whatever reason) must be accompanied by some mechanism of osmoregulation, allowing osmotic parity in the given range of environments.

At this point, it is worth adding a note about ionic regulation. For it is not the case that once gnathostomes achieved an isoosmotic or nearly isoosmotic plasma (myxinoids, chimaeroids, elasmobranchs, and coelacanth are actually slightly hyperosmotic), all ionic problems were solved. The reduced concentration of individual ions compared with seawater will lead to an influx of ions, particularly sodium and chloride, across the gills. Therefore, all these fishes have needed, and apparently have very early acquired, mechanisms of active salt secretion in addition to normal kidney function. In the case of the agnathans, there are chloride cells in the gills, but these apparently are not homologous with those of gnathostomes. In chimaeroids and elasmobranchs, there are chloride cells (review in Conte, 1969; see also Payan and Maetz, 1973), but the major site of salt (sodium chloride) excretion is the rectal gland. In the marine teleosts, there are, of course, chloride cells in the gills and also elimination of ions through the gut wall. However, these adaptations are not confined to teleosts. The chondrosteian sturgeons and the holostean *Lepisosteus* also have chloride cells in the gills (see for example, Pen'kova, 1974; Krayushkina and Dyubin, 1974; Harb, 1969). Finally, the coelacanth is another fish that is slightly hyperosmotic and has rectal gland salt elimination for ionic regulation in addition to renal function.

In consideration of all of the above, I am inclined to present the following account as an alternative to the traditionally accepted explanation of reduced total ionic concentrations in the blood of major groups of fishes. I am inclined to the view that the four major lines of gnathostome vertebrates, acanthodians, placoderms, chondrichthyans, and osteichthyans evolved in conditions of high salinity. Furthermore, I propose the hypothesis that the reduction of the total ionic concentration in the plasma of these lines compared with ancestral forms isoosmotic with seawater had a positive physiological advantage in terms of general cell physiology, particularly transmembrane functions, quite independent of the requirements of life in fresh or brackish water. Although it might be that there can be quite significant adjustments of ionic concentration of the plasma during evolution within a given group, and according to environmental constraints, nevertheless, I suggest that the general ionic level is an *independent* adaptation of fundamental cell physiology. In these four lines, I suggest, an adaptation concomittant with reduction of ionic plasma concentration was the adoption of uremia as a mechanism of osmoregulation, with urea being synthesized via the ornithine-urea cycle. At the same time, mechanisms of extrarenal salt excretion also evolved, possibly involving both gills and rectal gland from the beginning. This common gnathostome pattern allowed subsequent entry into conditions of varying salinity, as is shown by the radiation of all groups (even the chondrichthyans) into freshwater lines as well as marine. The same adaptations persisted into the ancestors of modern chondrichthyans and osteichthyans, the latter being demonstrated by the living coelacanth and lungfishes. But the living lungfishes, never entering salt

waters and having a very long history continuously in freshwater, have lost extrarenal excretory mechanisms and the rectal gland. The Actinopterygii (ray-finned fishes) started with the same set of adaptations but progressively, at unknown stages (early) in their evolution, replaced osmoregulation by means of uremia with a pattern of salt water drinking and massive extrarenal salt excretion via the gills. Using the criterion of supporting geological evidence, only this last set of evolutionary changes may have occurred in nonmarine fishes. But even in the Actinopterygii, there is no really long sequence of totally freshwater existence and the chances are that this set of adaptations occurred in euryhaline fishes: if individual species did not live in a variety of environments, certainly at the family level there is a wide scope beyond pure freshwater.

Interestingly, Krogh (for example, 1939), who also had not been persuaded by the apparent evidence for a freshwater origin of gnathostomes, and particularly of elasmobranchs, suggested that a specific nonosmoregulatory correlate of reduced total ionic composition of the plasma may exist in terms of nerve-cell physiology. Possibly formation of higher association centers is not possible without such reduction.

Finally, what seems to be needed in order to make any more sense out of the available data is more detailed information about the environment of deposition of the earliest members of the major gnathostome lineages and consideration of what sorts of adaptations of cell physiology, perhaps including particularly the metabolism of calcium and the evolution of the bony skeleton, might require a lowering of individual and total ionic concentrations in the plasma, regardless of environmental challenges.

CONCLUSIONS

The evidence of different ionic concentration and composition of the plasma among groups of fishes seems genuinely to indicate adaptations related directly to the physiology of osmoregulation. Particularly, all groups entering freshwater from marine conditions undergo a reduction in total ionic concentration of the plasma, with a limit of change of about 110 mM/l. To this extent, therefore, the ionic concentration of the plasma may be used as an index to environmental conditions.

This relationship, however, seems only to apply *within* the four major known vertebrate lineages—agnathans, elasmobranchs, actinopterygians and lungfish, crossopterygians and tetrapods.

The reduced total ionic concentration of the plasma of chondrichthyans and osteichthyans, when compared with *Myxine* (which is presumed to represent primitive conditions in this regard) cannot readily be explained by a long period of existence of early gnathostomes in freshwater conditions.

All major groups of fishes (Agnatha, Chondrichthyes, Sarcopterygii, and Actinopterygii) show adaptations of ionic regulation involving selective excretion of salts. A primitive gnathostome pattern is for sodium chloride to be excreted through the rectal gland. The branchial excretion of chloride

through special cells was apparently independently evolved in agnathans and gnathostomes, but may be an extremely ancient feature of the latter.

It is hypothesized that the ancestral condition for all Osteichthyes was a total ionic plasma concentration of about 400-450 mM/l.

Physiological uremia and the synthesis of urea through the ornithine-urea cycle was a primitive feature of the gnathostomes and is retained in coelacanth, lungfish, and tetrapods, but mostly has been lost in the Actinopterygii.

The hypothesis that reduced total ionic concentration of the plasma is an adaptation that evolved in freshwater is not readily supported by the geological data. Particularly, the geological evidence summarised by Denison (1956) indicates that placoderms, acanthodians, chondrichthyans, and osteichthyans all evolved in marine conditions and only radiated later into more freshwater environments.

The suggestion is made that the patterns of ionic composition, mechanisms of ionic regulation, and osmoregulation of gnathostomes evolved together in saltwater conditions and that their origin must be traced to adaptations of basic cell physiology rather than to environmental constraints.

ACKNOWLEDGMENTS

I am grateful to Dan E. Simanek for assistance during this study. The illustrations were prepared by Linda Price Thomson. Financial support from the National Science Foundation (grant number BMS 74-07759) is gratefully acknowledged.

LITERATURE CITED

- BALDWIN, E. 1964. An introduction to comparative biochemistry. Cambridge Univ. Press, xix+179 pp.
- BARDACH, D., AND R. ZANGERL. 1968. First fossil lamprey: a record from the Pennsylvanian of Illinois. *Science*, 102:1265-1267.
- BROWN, G. W., AND S. G. BROWN. 1967. Urea and its formation in coelacanth liver. *Science*, 155:570-573.
- CONTE, F. P. 1969. Salt secretion. Pp. 241-291, in *The physiology of fishes* (W. S. Hoar and D. J. Randall, eds.), Academic Press, New York, 1:xiv+1-465 pp.
- DENISON, B. 1956. A review of the habitat of the earliest vertebrates. *Fieldiana, Geol.*, 11: 359-457.
- EPPLE, A., AND J. E. BRINN. 1975. Islet histophysiology: evolutionary correlations. *Gen. Comp. Endocrinol.*, 27:320-349.
- GOLDSTEIN, L., AND R. P. FORSTER. 1970. Nitrogen metabolism in fishes. Pp. 496-518, in *Comparative biochemistry of nitrogen metabolism* (J. W. Campbell, ed.), Academic Press, New York, 2:xi+1-916+I+lv.
- GOLDSTEIN, L., S. HARLEY-DEWITT, AND R. P. FORSTER. 1973. Activities of ornithine-urea cycle enzymes and of trimethylamine oxidase in the coelacanth, *Latimeria chalumnae*. *Comp. Biochem. Physiol.*, 44B:357-362.
- GRIFFITH, R. W., B. L. UMMINGER, F. B. GRANT, P. K. T. PANG, AND G. E. PICKFORD. 1974. Serum composition of the coelacanth, *Latimeria chalumnae* Smith. *J. Exp. Zool.*, 187:87-102.

- HARB, J. M. 1969. Comparative fine structure of the acidophil cell of the pseudobranch and gill of three teleosts. Unpublished Ph.D. dissertation, Tulane Univ., 193 pp.
- HOLMES, W. N., AND E. M. DONALDSON. 1969. The body compartments and the distribution of electrolytes. Pp. 1-89, in *Fish physiology* (W. S. Hoar and D. J. Randall, eds.), Academic Press, New York, 1:xiv+1-465 pp.
- HUGGINS, A. K., G. SKUTSCH, AND E. BALDWIN. 1969. Ornithine-urea cycle enzymes in teleostean fish. *Comp. Biochem. Physiol.*, 28B:587-602.
- KRAYUSHKINA, L. G., AND V. P. DYUBIN. 1974. The reaction of juvenile sturgeon to alteration of environmental salinity. *J. Ichthyol.*, 14:971-976.
- KROGH, A. 1939. Osmotic regulation in aquatic animals. Cambridge Univ. Press., vii+242 pp.
- LUTZ, P. L. 1975. Osmotic and ionic composition of the polypterid *Erpetoichthys calabaricus*. *Copeia*, 1975:119-123.
- LUTZ, P. L., AND J. D. ROBERTSON. 1971. Osmotic constituents of the coelacanth *Latimeria chalumnae* Smith. *Biol. Bull. Woods Hole*, 141:553-560.
- MARSHALL, E. K., AND H. W. SMITH. 1930. The glomerular development of the kidney in relation to habitat. *Biol. Bull. Woods Hole*, 59:135-153.
- MORRIS, R. 1972. Osmoregulation. Pp. 193-240, in *The biology of lampreys* (M. W. Hardisty and I. C. Potter, eds.), Academic Press, New York, 2:xiv+1-466 pp.
- MORRIS, R., AND A. D. PICKERING. 1975. Ultrastructure of the presumed ion-transferring cells in the gills of ammocoete lampreys, *Lampetra fluviatilis* (L) and *Lampetra planeri* (Bloch). *Cell. Tiss. Res.*, 163:327-341.
- MOY-THOMAS, J. A., AND R. S. MILES. 1971. Palaeozoic fishes. Saunders, Philadelphia, xi+259 pp.
- PACKARD, G. C. 1974. The evolution of air-breathing in Paleozoic gnathostome fishes. *Evolution*, 28:320-325.
- PANG, P. K. T., R. W. GRIFFITH, AND J. W. ATZ. 1977. Osmoregulation in elasmobranchs. *Amer. Zool.*, 17:365-378.
- PAYAN, P., AND J. MAETZ. 1973. Branchial sodium transport mechanisms in *Scyliorhinus canicula*: evidence for $\text{Na}^+/\text{NH}_4^+$ and Na^+/H^+ exchanges and for a role of carbonic anhydrase. *J. Exp. Biol.*, 58:487-502.
- PEN'KOVA, Y. A. 1974. Functional changes in the internal tissue of adult sturgeon [*Acipenser guldenstadti* (Brandt)] in the course of the life cycle. *J. Ichthyol.*, 14:114-121.
- PICKFORD, G. E., AND F. B. GRANT. 1967. Serum osmolality in the coelacanth *Latimeria chalumnae*: urea retention and ion regulation. *Science* 155:568-570.
- READ, L. J. 1968. A study of ammonia and urea production and excretion in the freshwater-adapted form of the Pacific lamprey *Entosphenus tridentatus*. *Comp. Biochem. Physiol.*, 26B:455-466.
- . 1971. The presence of high ornithine-urea cycle enzyme activity in the teleost *Osiphanus tau*. *Comp. Biochem. Physiol.*, 39B:409-413.
- . 1975. Absence of ureogenic pathways in liver of the hagfish *Bdellostoma cirrhatum*. *Comp. Biochem. Physiol.*, 51B:139-141.
- ROBERTSON, J. D. 1957. The habitat of early vertebrates. *Biol. Rev.*, 32:156-187.
- . 1976. Chemical composition of the body fluids and muscle of the hagfish *Myxine glutinosa* and the rabbitfish *Chimaera monstrosa*. *J. Zool.*, 178:261-277.
- ROMER, A. S. 1955. Fish origins—fresh or salt water? *Deep Sea Res.*, 3(suppl.):261-280.
- SMITH, H. W. 1932. Water regulation and its evolution in fishes. *Quart. Rev. Biol.*, 7:1-26.
- THOMSON, K. S. 1966. Intracranial mobility in the coelacanth. *Science*, 153:999-1000.
- . 1969a. The biology of lobe-finned fishes. *Biol. Rev.*, 44:91-154.
- . 1969b. The environment and distribution of Palaeozoic sarcopterygian fishes. *Amer. J. Sci.*, 267:457-464.
- . 1976. The biology of cosmine. *Bull. Peabody Mus. Nat. Hist.*, 40:1-59.
- . 1977. On the individual history of cosmine and a possible electroreceptive function of the pore-canal in fossil fishes. Pp. 247-270, in *Problems in vertebrate evolution*.

- (S. M. Andrews, R. S. Miles, and A. D. Walker, eds.), Academic Press, New York, Linnean Soc. Symp. Ser., 4:xi+1-411 pp.
- URIST, M. R., AND K. A. VAN DEPUTTE. 1967. Comparative biochemistry of the blood of fishes. Pp. 271-288, in *Sharks, skates and rays* (L. G. Gilbert, R. F. Mathewson, and D. P. Rall, eds.), Johns Hopkins Press, Baltimore, xv+624 pp.
- URIST, M. R., S. UYENO, E. KING M. OKADA, AND S. APPEGATE. 1972. Calcium and phosphorous in the skeleton and blood of the lungfish *Lepidosiren paradoxa*, with comment on humoral factors in calcium homeostasis in the Osteichthyes. *Comp. Biochem. Physiol.*, 42A:393-408.
- WHITE, E. I. 1958. Original environment of the craniates. Pp. 212-234, in *Essays on fossil vertebrates* (T. S. Westoll, ed.), Athlone Press, London, xii+263 pp.

EVOLUTION OF THE ENDOCRINE PINEAL ORGAN

HERBERT A. UNDERWOOD

All pineal organs are derived embryologically as evaginations from the roof of the diencephalon (Wurtman *et al.*, 1968). Although the pineal system of mammals and birds consists of a single pineal organ, or *epiphysis cerebri*, the pineal systems of lower vertebrates are often more complex. In lower vertebrates, the pineal system may be comprised of two distinct organs; the intracranial pineal organ, which is attached to the roof of the diencephalon, and a more superficial parapineal organ (often called the frontal organ in anuran amphibians or the parietal eye in lizards). The superficial component originates either as an outpouching from the pineal organ or as a separate diverticulum from the diencephalon.

A purely endocrine role has been ascribed to the pineal organs of birds and mammals whereas a photosensory role has been emphasized for the pineal organs of lower vertebrates. However, a clear delimitation between the functions of the pineal organs of the lower vertebrates and those of the birds and mammals is probably unwarranted. There is good evidence that the pineal organs of lower vertebrates are capable of considerable secretory activity. On the other hand, the pineal organs of young birds and mammals may be directly photosensitive.

PINEAL ORGANS OF FISH, AMPHIBIANS, AND REPTILES

Photosensitivity

Neurophysiological investigations have shown that the pineal and parapineal organs of fish, the frontal and pineal organs of amphibians and the pineal organ and parietal eyes of lizards are directly photosensitive (Dodt and Heerd, 1962; Dodt, 1963; Dodt and Jacobson, 1963; Dodt and Scherer, 1968; Hamasaki and Dodt, 1969; Hamasaki, 1969; Hamasaki and Streck, 1971). In general, the pineal organ exhibits achromatic responses to light whereas the parapineal, frontal, or parietal organs show chromatic responses. Achromatic responses typically involve inhibition of on-going spike activity by all wavelengths of light, and chromatic responses usually show inhibition of electrical activity by shorter wavelengths and excitation by longer wavelengths of visible light. In some cases, both graded (slow) potentials as well as action potentials are observed; the graded potentials are most noticeable in the frontal organs of frogs and lizard parietal eyes. For example, in the lizard's parietal eye a graded potential is seen that is similar to the electroretinogram (ERG) of the lateral eyes (Dodt and Scherer, 1968; Hamasaki, 1969).

The ultrastructure and innervation of the pineal organs of lower vertebrates has been studied in some detail, particularly by researchers in Western

Europe. This work has revealed that the pineal organs of lower vertebrates contain several kinds of cells: photosensory cells, supporting cells, and neurons (Kappers, 1967; Collin, 1971; Oksche, 1971). The morphology and number of photosensory cells, as well as the number of neurons present, varies among species. Pineal photoreceptive cells have outer segments that are composed of regular discs, each of which consists of a double membrane; the outer segment is derived from an axial centriole and contains a 9+0 fibrillar pattern. An inner segment, cell body, and synaptic pedicle also are present. The outer and inner segments protrude into the lumen of the pineal organ and the cell pedicle is directed toward the basement membrane. The synaptic pedicle in fish and amphibians makes synaptic contact with intrapineal nerve cells, and these nerve cells, which are analogous to the ganglion cells of the lateral eyes, send their axons toward the epithalamic region of the brain. Synaptic pedicles typically contain synaptic vesicles and synaptic ribbons—presumably the structural elements containing the neurotransmitter.

Although the above description applies to pineal photosensory cells in general there can be considerable differences in the morphology of the sensory cell, the number of intrapineal neurons present, and in the termination of the pinealo-fugal (afferent) nerves. Photoreceptor cells with outer segments that resemble the cones of the lateral eyes have been demonstrated in the pineal organs of cyclostomes, fish, and amphibians (Kelly, 1965; Meiniel and Collin, 1971; Bergmann, 1971; Rdeberg, 1971; Korf, 1976). However, in lizards and turtles these photoreceptive cells often show a more degenerate appearance and have been termed "secretory rudimentary photoreceptors (SRP)" (Oksche and Kirschstein, 1968; Vivien-Roels, 1970; Collin and Meiniel, 1971; Oksche, 1971; Collin, 1971). Both lizards and turtles rarely possess sensory cells with a normally developed outer segment; the outer segments do not show regular stacks of discs but are disorganized or concentrically arranged. Often the pedicle is asynaptic and not associated with a neuron. It must be kept in mind, however, that in spite of the more disorganized appearance of the photoreceptors and the paucity of neurons present electrical responses to illumination can still be recorded. A pineal nerve is often present although there is a reduction in the number of axons. The snake pineal contrasts with pineals of other lower vertebrates in that photoreceptive cells are absent in the adult (Petit, 1971). A direct photosensitivity of the ophidian pineal has not been demonstrated.

In the majority of the lower vertebrates, the exact termination(s) of the pinealo-fugal nerve fibers, which course to the epithalamus, are unknown. A detailed study by Hafeez and Zerihun (1974) on the rainbow trout, *Salmo gairdneri*, however, shows that the pineal nerve gives off branches to the parapineal organ, the lateral habenular nucleus, the pretectal area, the diencephalic and mesencephalic periventricular grey, the dorsomedial and dorsolateral thalamus, the nucleus of Darkschewitsch, the dorsal tegmentum, and possibly cells of the medial longitudinal fasciculus and the preop-

tic nucleus. The pineal tract, therefore, consists of pinealo-fugal fibers that project centrally over an extensive sensorimotor area of the brain. In lampreys, amphibians, and reptiles the pineal tract courses in the direction of the posterior commissure, and rarely are nerve fibers seen to enter the habenular commissure (Kappers, 1967; Oksche and Kirschstein, 1968; Paul, 1972; Korf, 1976).

Pinealo-petal (efferent) nerve fibers have been observed in the pineal organs of fish, amphibians, and reptiles and the fiber endings contain vesicles characteristic of noradrenergic nerve terminals (Oksche, 1971; Collin, 1971). The origin of these nerve fibers is unknown, but it has been postulated that this system is homologous to the autonomic sympathetic system that innervates the pineal organs of birds and mammals.

In those lower vertebrates possessing a parapineal as well as a pineal organ, the potential for functional interaction is present. Anurans show a reciprocal innervation between the frontal organ and the pineal (Ueck *et al.*, 1971; Zimmerman and Paul, 1972). In the collared lizard, *Crotaphytus collaris*, the parietal eye nerve contains both afferent and efferent fibers (Engbretson and Lent, 1976). The afferent fibers carry photic information to the habenular area of the brain and probably also to the pineal organ. The efferent fibers originate in the pineal organ and modify the parietal eye's response to light. The efferent nerves are not photosensitive but they are chemosensitive; norepinephrine and serotonin when applied to the pineal organ will initiate activity in these nerves.

Secretion

In 1958 Lerner and his co-workers isolated the substance from bovine pineals regulating aggregation of pigment granules in amphibian melanophores and identified it as 5-methoxy-N-acetyltryptamine (melatonin) (Lerner *et al.*, 1958). Many of the ensuing investigations into the synthesis of the biochemicals by pineal organs have focused on melatonin and related indoleamines. Fig. 1 shows the biosynthetic pathways for indoleamines that have been elucidated for mammalian pineals but this figure will serve as a basis for the discussion of those indoleamines in other vertebrate classes as well.

Several indoleamines, including 5-hydroxytryptophan, 5-hydroxytryptamine (serotonin) and melatonin, have been identified in fish pineals (Hafeez and Quay, 1969; Fenwick, 1970a). Quay (1965) detected hydroxyindole-0-methyl transferase (HIOMT) activity in the pineal organ as well as in the retinas of fish. This enzyme is responsible for the 0-methylation of N-acetylserotonin in the biosynthesis of melatonin. Under artificial 24 hr. light-dark cycles, higher levels of HIOMT activity occur during the dark portion of the cycle in the steelhead trout, *Salmo gairdneri*, as well as during the night under natural lighting conditions (Smith and Weber, 1974, 1976). However, in the rainbow trout, a non-migratory form of *Salmo gairdneri*, Hafeez and Quay (1970) were unable to detect a light dependent

FIG. 2.—Melatonin production by HIOMT in the pineal complex (pineal + parapineal + habenular ganglion) of ammocoetes of the lamprey (figure redrawn from Joss, 1977).

lizard *Lacerta vivipara*, and the labels are always most concentrated in the regions of secretory granules (Collin and Meiniel, 1973a,b). HIOMT activity has been demonstrated in the pineal, parietal eyes, and retinas of a variety of reptiles (Quay, 1965; Quay *et al.*, 1971). The serotonin content of the pineal of the lizard *Lacerta muralis* and the turtle *Testudo hermanni* (Fig. 3) exhibits a diurnal variation under daily light-dark cycles with the highest serotonin levels early in the night (Vivien-Roels *et al.*, 1971). An annual study with *T. hermanni* showed, in addition, an annual variation in the amplitude of the daily serotonin rhythm (Vivien-Roels and Petit, 1975).

Function

Attempts to determine pineal function usually have involved the classical procedures of removal of the organ or injecting or implanting a putative pineal hormone (usually melatonin) and assaying the effects of these procedures on specific target organs. The participation of the pineal organ in the control of dermal melanophores in amphibians provides the clearest demonstration that the pineal organs of lower vertebrates can act as endocrine organs (Bagnara and Hadley, 1970). Fairly early in development, larval amphibians will respond to illumination by remaining dark due to the dispersal of melanin granules within dermal melanophores but will blanch when placed in the dark due to an aggregation of these granules. A variety

FIG. 3.—Pineal serotonin content of turtles exposed to daily light cycles (figure from Vivien-Roels *et al.*, 1971).

of experiments have indicated that this response is mediated by the pineal; darkness stimulates the pineal to release melatonin, which acts to inhibit melanocyte stimulating hormone at the level of the dermal melanophores thereby causing a concentration of melanin granules and turning the animal pale (Bagnara and Hadley, 1970). Light inhibits melatonin release by the pineal so that the melanophores are expanded under conditions of illumination. In general, melatonin does not have much blanching effect on adult frogs. The loss of sensitivity to melatonin in adult amphibians appears to be a developmental phenomenon and coincides with the change from a pineal to a retinal control of melanophore responses. Comparative studies have shown that the pineal organ is involved in color responses in other vertebrate groups as well. Removal of the pineal and parapineal organ abolishes a daily rhythm of color change in the larval lamprey *Lampetra planeri* (Young, 1935). Blinding adults but leaving the pineal system intact abolished the color response to light-dark cycles. Similar results were obtained from larvae of the Australian lamprey, *Geotria australis*, by Eddy and Strahan (1968). In another lamprey *Mordacia mordax*, which lacks a parapineal organ and has a degenerate pineal, color rhythm is not affected by pinealectomy (Eddy and Strahan, 1968). In some but not all fish, melatonin affects melanophores by causing aggregation of melanosomes in some cases and expansion in others (Reed *et al.*, 1969; Hafeez, 1970; Wilson and Dodd, 1973).

Effects of pinealectomy also have been noted on the reproductive systems of lampreys, fish, and lizards. In most of these studies, effects of pinealectomy could be noted only at certain times of year and under certain temperature and photoperiodic regimens (Fenwick, 1970b; Joss, 1973; Urasaki,

1973; Levey, 1973; De Vlaming, 1975). Depending upon the phase of the reproductive cycle and upon the thermal and photoperiodic conditions under which the animals are held, pinealectomy can elicit either no effect, a progonadal, or an antigonadal effect. Melatonin injections have an antigonadal effect in fish, amphibians, and lizards (Fenwick, 1970a; Urasaki, 1972; Levey, 1973; De Vlaming *et al.*, 1974; Sundararaj and Keshavanath, 1976).

Among the lower vertebrates, numerous studies have shown that extraretinal photoreceptors (ERRs) can participate in a wide variety of responses to light including phototaxis and photokinesis, photoperiodism, orientation, and entrainment of the biological clock (Adler, 1976; Underwood and Menaker, 1976). Because of the obvious photoreceptive capacity of the pineal system, these organs have been suggested as sites for this extraretinal photoreception. However, definitive localization of these extraretinal photoreceptors to the pineal system has been accomplished in only a few cases. It is abundantly clear that light can affect some of these responses (for example, entrainment of the lizard's biological clock) even after the lateral eyes and pineal system have been removed (Underwood and Menaker, 1976). Localization experiments have shown that extrapineal photoreceptors located in the brain can mediate some of these responses.

Neither the pineal organ nor the parietal eye is necessarily a photoreceptor mediating entrainment of the lizard's biological clock but the pineal organ is an important component of circadian organization in lizards (Underwood, 1977). Removal of the lizard's pineal organ under conditions of continuous illumination causes the circadian locomotor activity rhythm to either split into two circadian components or to become arrhythmic, indicating that the lizard's pineal is a coupling device between circadian oscillators.

In summary, neurophysiological, cytological, and biochemical studies of the pineal organs of lower vertebrates demonstrate secretory as well as photoreceptive capacities. Although the biosynthetic pathways for indoleamines have not been as completely elucidated in the lower vertebrates as they have in mammals, the presence of various derivatives of tryptophan such as serotonin and melatonin within the pineals and the presence of enzymes such as HIOMT suggest that the pineals of the lower vertebrates may have as much indoleamine synthesizing capacity as mammalian pineals. It is likely that the pineals of all lower vertebrates are capable of producing melatonin, which is probably a major pineal hormone. In those animals in which the cellular sites of indoleamine synthesis have been identified, the photosensory or SRP cells are usually the exclusive sites of indoleamine synthesis. Studies involving pinealectomy or injection of melatonin have implicated the pineal organ of lower vertebrates as endocrine organs participating in several functions, including reproduction and color changes. The lizard pineal is also an important component of circadian organization. In some cases, daily changes in the levels of serotonin or HIOMT activities were noted in response to daily light cycles. Light might directly influence the synthesis

FIG 4.—Pathways of light to the pineal organ of lower vertebrates. Solid arrows indicate established pathways whereas dashed arrows indicate possible pathways. ERR, extraretinal photoreceptors.

and secretion of pineal hormones or it might act via various other photoreceptors (Fig. 4). As Fig. 4 demonstrates, the potential for interaction among different kinds of photoreceptors in the control of pineal function is enormous. Afferent nerves leaving the pineal organ and projecting centrally are undoubtedly conveying photic information to the brain, but the function of this innervation is, essentially, unknown.

AVIAN PINEAL

Structure and Innervation

The dual pineal system present in many of the lower vertebrates is absent in birds and mammals. Within the avian and mammalian pineals, the principle cell type is the pinealocyte or pineal parenchymal cell. Pinealocytes are thought to belong to the same cell line as the photosensory cells of the lower vertebrates because certain cellular elements in the pinealocytes are characteristic of the pineal photoreceptive cells of lower vertebrates. In many avian species such as the chicken, pigeon, duck, white-crowned sparrow,

Japanese quail, and house sparrow, typical photoreceptive cells are absent but pinealocytes are often found with very rudimentary outer segments. These outer segments vary from simple bulbous ciliary derivatives to irregular whorls of lamellae (Oksche and Vaupel-von Harnack, 1966; Bischoff, 1969; Oksche and Kirschstein, 1969; Ueck, 1970, 1973; Ueck and Kobayashi, 1972; Oksche *et al.*, 1972; Boya and Zamorano, 1975). These cells have been termed secretory rudimentary photoreceptors (SRP) by some workers to emphasize their similarities with the photosensory cells of lower vertebrates. However, the pineal organ in adult birds is apparently not directly photosensitive, as determined by neurophysiological methods (Morita, 1966; Ralph and Dawson, 1968). The avian pineal is innervated primarily by pinealo-petal postganglionic sympathetic fibers from the superior cervical ganglia (Hedlund and Nalbandov, 1969). Light perceived by the eyes inhibits spontaneous multiple-unit neuronal discharge in the avian pineal (Herbuté and Baylé, 1974). Occasional neurons are seen within the avian pineal and in some cases acetylcholinesterase-positive nerve fibers have been traced leaving the pineal organ via its stalk and coursing toward the habenular area of the brain (Oksche and Kirschstein, 1969; Ueck, 1970; Ueck and Kobayashi, 1972). In the stalk, the axons intermingle with pinealocyte processes containing synaptic ribbons as well as conventional synapses. Vesicles and dense-core granules are present also in these pinealocyte processes showing that sensory and secretory structures are present in the same cell.

Indoleamines

HIOMT activity in both birds and mammals was thought to be confined solely to the pineal organ. Consequently, the O-methylated derivatives of serotonin, especially melatonin and to a lesser extent 5-methoxy-tryptophol, have received the greatest attention as possible pineal hormones. Recently, HIOMT activity has been found in the retinas and Harderian glands of higher vertebrates, but these areas apparently do not contribute much, if any, melatonin to the blood stream for plasma melatonin content in pinealectomized birds and mammals is either non-existent or at very low levels (Cardinali and Wurtman, 1972; Cardinali and Rosner, 1972; Ralph, 1976; Ozaki and Lynch, 1976).

The uptake of radioactive 5-hydroxytryptophan (H^3 -5-HTP) and serotonin (H^3 -5-HT) is confined to the SRP cells in parakeets and ducks; no labeling is seen in the supportive (ependymal) cell type (Collin *et al.*, 1976). A secretory activity is evident in SRP cells; dense-cored vesicles originate in the Golgi complex of the "myoid" region of the inner segment and are conveyed to the perinuclear areas and especially to the basal processes of the cell. These basal processes often contact the basement membrane and are considered "asynaptic pedicles" because of the apparent absence of contact with nerves. Significantly, most labeling occurs over the regions with dense secretory granules and is usually super-imposed on these granules. The dense core is considered to consist mainly of protein, suggesting a protein-

indoleamine link at the level of these granules. These results are similar to those previously described in the lizard SRP cells after injection of H^3 -5-HTP. Inasmuch as decarboxylation of 5-HTP is relatively fast, the radioactivity probably reflects the presence of 5-HT as well. Theoretically, therefore, the 5-HT may be metabolized to melatonin or deaminated by monoamine oxidase (MAO) to form compounds such as 5-methoxyindole acetic acid or 5-methoxytryptophol (Fig. 1).

A striking feature of avian pineals is the presence of daily rhythms in various indoleamines and enzymes. For example, under daily light-dark cycles daily rhythms in pineal serotonin and melatonin content are seen (Meyer *et al.*, 1973; Binkley, 1976a, b; Binkley *et al.*, 1973). A diurnal rhythm in melatonin content is seen also in the blood and brain of chickens with the highest amounts occurring at night; this rhythm is in phase with the rhythm in pineal melatonin content (Ralph, 1976). At midnight, the melatonin concentration in whole brain tissue is five times higher than serum melatonin levels, suggesting that the brain concentrates melatonin from the blood with the hypothalamus containing the highest melatonin concentrations. No melatonin was detected in blood and brain following pinealectomy.

A small amplitude rhythm in HIOMT activity has been observed by some but not by others (Binkley *et al.*, 1973; Binkley, 1976a). However, a large amplitude rhythm in N-acetyltransferase is seen (Binkley *et al.*, 1973; Binkley, 1976a, b). N-acetyltransferase activity appears to be the rate limiting enzyme in the formation of melatonin from serotonin (Fig. 5). Significantly, the rhythms in N-acetyltransferase activity as well as pineal melatonin content persist under conditions of continuous darkness (DD) showing that these rhythms are driven by an endogenous "biological clock" (Binkley and Geller, 1975).

There are two important aspects of avian pineal systems that also deserve attention: the location of the photoreceptors influencing pineal rhythms, and the locus of the endogenous clock. In young chicks, the pineal organ may be directly photosensitive; neither blinding nor sympathetic denervation of the pineal prevents a light-induced elevation in HIOMT activity in chicks (Lauber *et al.*, 1968). A pineal melatonin rhythm also persists in chicks exposed to light-dark cycles even after interruption of nervous input from the superior cervical ganglia (Ralph, 1976). Light also can influence N-acetyltransferase activity in blind chicks, but the eyes must be involved because the decrease in N-acetyltransferase activity caused by exposure to light during the normal dark time is greater in sighted than in blind chicks (Binkley *et al.*, 1975; Ralph *et al.*, 1975). A direct photosensitivity of duck pineals *in vivo* as well as *in vitro* has been suggested by the studies of Rosner and colleagues (Rosner *et al.*, 1972; Hisano *et al.*, 1972). For example, pineals taken from 10-month-old ducks and maintained for 24 hrs. in organ culture under continuous light showed significant increases in pineal indole metabolism and incorporation of H^3 uridine by RNA over pineals cultured in continuous darkness.

FIG. 5.—Probable pathway for melatonin synthesis in the pineal gland (left) and graphs of N-acetyltransferase activity, HIOMT activity, and melatonin content in chicken pineal glands (right) (figure from Binkley *et al.*, 1973; Copyright 1973 by the American Association for the Advancement of Science).

The above studies strongly support the contention that the avian pineal is directly photosensitive. This photosensitivity may be a developmental phenomenon that is lost in older birds inasmuch as the pineals of at least some species of adult birds do not respond directly to light as determined by neurophysiological methods. Clearly, however, more research is needed on this important question in view of the studies of Rosner and colleagues suggesting a direct pineal photosensitivity even in 10-month-old ducks. Other photoreceptors that may be involved in regulating pineal function include the eyes and extrapineal brain photoreceptors. Extrapineal brain photoreceptors in birds can mediate entrainment of circadian activity rhythms and photoperiodism and it is possible that these receptors may have an input into the pineal (Menaker and Underwood, 1976). Any photic information reaching the pineal indirectly would have to travel via the superior cervical ganglia.

The studies of Gaston and Menaker (1968), Zimmerman and Menaker (1975), and Menaker and Zimmerman (1976) suggest that the avian pineal is the site of an endogenous clock. These studies indicate that the avian pineal

is the site of a "master oscillator" that drives the overt circadian rhythm of locomotor activity because: 1) pinealectomy in the house sparrow, *Passer domesticus*, abolishes circadian rhythmicity; pinealectomized house sparrows are arrhythmic in DD; and 2) birds rendered arrhythmic by pinealectomy in DD can be made immediately rhythmic by transplantation of a pineal organ into the anterior chamber of the eye. The fact that rhythmicity can be restored immediately by a pineal transplant and that interruption of the nervous input and output of the house sparrow's pineal organ does not abolish rhythmicity shows that the pineal is hormonally, rather than neurally, coupled to other components of the circadian system. Melatonin implants in the house sparrow cause a shortening of the period of the circadian activity rhythm or arrhythmicity raising the possibility that melatonin is the hormone produced by the pineal that couples it to other elements of the circadian system (Turek *et al.*, 1976a).

Reproduction

In birds, reports on the endocrine function of the pineal are not extensive and are sometimes contradictory. Effects of pinealectomy or superior cervical ganglionectomy on gonadal function have been observed but depend on age, sex, species, and lighting conditions. For example, in ducks under natural conditions, pinealectomy at the beginning of spring interfered with normal increase in testicular size and also led to a decreased testosterone content and *in vitro* biosynthesis of steroids; however, no differences were observed between pinealectomized and control ducks in the subsequent seasonal involution of the reproductive system nor in the recrudescence of the gonads during the following breeding season (Cardinali *et al.*, 1971). In contrast, pinealectomy in the Indian weaver bird, *Ploceus philippinus*, caused precocious testicular recrudescence (Balasubramanian and Saxena, 1973). In the chicken, pinealectomy of young cockerels caused testicular atrophy (Shellabarger, 1952) whereas pinealectomy in older cockerels caused testicular hypertrophy (Shellabarger, 1953). In Japanese quail, some investigators have noted transient effects of pinealectomy or superior cervical ganglionectomy on the reproductive system, especially in females, but others have not observed any effects (Homma *et al.*, 1967; Sayler and Wolfson, 1968; McFarland *et al.*, 1968; Arrington *et al.*, 1969; Ralph *et al.*, 1970). Despite the observation that the pineal is progonadal in the duck (Cardinali *et al.*, 1971), melatonin is inhibitory to the production of steroids by duck testicular homogenates (Cardinali and Rosner, 1971). Melatonin injections or implants, however, are usually without effect on the reproductive systems of Japanese quail (Sayler and Wolfson, 1968; Oishi and Lauber, 1974), although Homma *et al.* (1967) observed that small but not large doses of melatonin retarded ovarian and oviducal weights in Japanese quail. The possibility of gonadal feedback on pineal activity was shown by Preslock (1976); castration decreased pineal HIOMT activity in Japanese quail and administration of gonadal steroids restored HIOMT activity.

FIG. 6.—Composite diagram of a typical mammalian pinealocyte (figure from Quay, 1974; courtesy of Charles C. Thomas, Publisher, Springfield, Ill.).

MAMMALIAN PINEAL

Structure, Innervation, and Indoleamines

The principle cell of the mammalian pineal, the pinealocyte or pineal parenchymal cell, comprises the majority of cells within the pineal and is the site of the synthesis of indoleamines and, probably, polypeptides (Fig. 6). This cell apparently is derived from the sensory cells of lower vertebrates. This conclusion stems from the observation that indoleamine metabolism is confined to the SRP cells of lower vertebrates and from the cytological differentiation of the pinealocyte during maturation. In neonatal rats between the ages of 4 to 12 days, the developing pinealocytes form structures similar to those observed in photoreceptors—the cells become elongated and polarized with the nuclei located at one pole and at the opposite pole the cells develop elongated cell processes from which extend cilia with a 9+0 arrangement of microtubules (Zimmerman and Tso, 1975). Lamellated and vesicular membranes are seen at the tips of the cilia. These morphological features are transient and cannot be observed in rats older than 17 days.

In the rat, innervation of the pineal is entirely via sympathetic postganglionic fibers from the superior cervical ganglia (Quay, 1974). No afferent nerves leave the pineal of rats. In other mammals, however, innervation may be more complex. Rabbits, for example, have both adrenergic and cholinergic postganglionic nerve endings as well as intrapineal neurons (Romijn, 1975). Romijn (1975) concludes that the cholinergic parasympathetic nerves arise from ganglion cells in the greater petrosal nerve as well as from ganglion cells within the pineal itself. Romijn also hypothesized that the intrapineal parasympathetic neurons are innervated by both preganglionic parasympathetics and postganglionic sympathetics. The ferret's pineal also receives extensive innervation from the superior cervical ganglia, but within the caudal part of the pineal a collection of acetylcholinesterase containing nerve cells called the "pineal ganglion" receives an input from the habenula (David and Herbert, 1973). Intrapineal neurons also are observed in various kinds of monkeys and related lower primates (Quay, 1974).

The activities and concentrations of various pineal enzymes (such as MAO, decarboxylase, N-acetyltransferase, HIOMT) undergo daily fluctuations (Quay, 1974). For example, a daily rhythm in the activity of the enzyme HIOMT is seen in light-dark cycles but it does not persist in DD. On the other hand, some of these daily rhythms are truly circadian; that is, they persist even under constant conditions. The enzyme N-acetyltransferase, which is the rate-limiting enzyme in the conversion of serotonin to melatonin illustrates this point for it undergoes a daily rhythm in activity that persists in DD. Consequently, the concentration of melatonin in the pineal also shows a circadian rhythm (Ralph, 1976). Melatonin is secreted into the blood and plasma melatonin levels fluctuate in phase with pineal melatonin levels (Ralph, 1976). On diurnal light-dark cycles, both avian and mammalian pineals exhibit maximal melatonin concentrations at night (Fig. 7).

DIURNAL VARIATION IN PINEAL MELATONIN CONTENT

FIG. 7.—Diurnal variation in pineal melatonin content. Shaded areas represent the dark phase of the diurnal light cycle (figure from Lynch, 1971).

Pineal circadian rhythms are driven by a clock or clocks located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Moore and Eichler, 1976). This clock receives photic information by a direct retino-hypothalamic tract and communicates with the pineal by a neural pathway that includes the medial forebrain bundle, preganglionic fibers from the cervical spinal cord to the superior cervical ganglia, and postganglionic sympathetic fibers from the superior cervical ganglia to the pineal (Fig. 8). The pathway mediating "tonic responses" to light, such as that of HIOMT activity, is partially separate from that just described. Photic information regulating HIOMT activity is transmitted via the inferior accessory optic tract to the medial forebrain bundle and apparently bypasses the SCN.

Light regulates pineal rhythms by the release of norepinephrine from sympathetic nerves. Light inhibits nervous activity in pineal sympathetic nerves, but darkness stimulates activity. As a result, there is a diurnal cycle in norepinephrine turnover in the pineal with the turnover being higher during the night. Norepinephrine interacts with β -adrenergic receptors on the pinealocytes, which activate adenylyl cyclase; the adenylyl cyclase subsequently stimulates an increase in cyclic 3', 5'-AMP. Among other functions, cyclic 3', 5'-AMP causes the induction of N-acetyltransferase, which,

FIG. 8.—Pathway of light to the mammalian pineal.

in turn, causes the production of melatonin. Because the rhythm of N-acetyltransferase is driven by a clock in the SCN, the primary action of light is entrainment of this clock. In the absence of light, there must be a circadian rhythm in nervous impulses to the pineal via the superior cervical ganglia.

Lighting information reaches the pineal of adult rats only via the eyes for blinding abolishes the effects of light on pineal rhythms. In juvenile rats, however, an extraretinal receptor has been implicated in the control of the daily rhythms in pineal serotonin content and HIOMT activity. Machado *et al.* (1969a, 1969b) demonstrated that the rhythm in serotonin content persists in DD in intact juvenile rats but is abolished in DD after interruption of the sympathetic innervation. Significantly, however, the serotonin rhythm persists under light-dark cycles after interruption of the sympathetic innervation. The HIOMT rhythm also continues under LD cycles in blinded juvenile rats (Wetterberg *et al.*, 1970a, 1970b). Wetterberg *et al.* (1970a, 1970b) suggest that the Harderian gland may be the extraretinal receptor involved because its removal eliminates the effect of light on serotonin or HIOMT activity in blinded juvenile rats. If the Harderian gland is involved,

however, it must influence the pineal via hormonal rather than neural routes. A direct photosensitivity of the juvenile rat pineal cannot be ruled out and is consistent with the photoreceptive appearance of pinealocytes in neonatal rats.

Reproduction

The pineal organ has been implicated in the control of a number of mammalian target organs including the brain, thyroid, adrenals, and gonads. A comprehensive review of these effects is beyond the scope of the present discussion but a number of reviews can be consulted for additional information (Wurtman *et al.*, 1968; Wolstenholme and Knight, 1971; Quay, 1974; Reiter, 1974; Relkin, 1976). Effects of the pineal on reproductive systems have received the greatest amount of attention and some progress has been made in identifying pineal products that influence reproduction. In the rat, progonadal effects can be seen after removal of the pineal, including accelerated pubertal onset, hypertrophy of the gonads and accessory sex organs, and increased LH synthesis and release. The most dramatic effects of pinealectomy, however, are observed in a strongly photoperiodic mammal, the golden hamster. Pinealectomized hamsters can maintain a fully functional testis in the presence of short photoperiods or even after blinding but unoperated hamsters will undergo rapid testicular involution under these conditions (Reiter, 1974). The antigonadotropic action of mammalian pineals requires an intact sympathetic innervation. Interruption of this innervation by, for example, superior cervical ganglionectomy, is as effective as pinealectomy in preventing gonadal atrophy.

Two classes of pineal compounds have been implicated in reproduction: methoxyindoles, particularly melatonin, and polypeptides (Ebels, 1975; Benson *et al.*, 1976; Vaughan *et al.*, 1976). A central nervous system action of both classes of compounds is indicated. Among the observations suggesting a central nervous system action of melatonin are: 1) labeled melatonin is more highly concentrated in the hypothalamus than in other brain areas; 2) melatonin implants in the median eminence and midbrain reduce pituitary stores of LH; 3) melatonin administered intraventricularly reduces LH stores; 4) endogenous melatonin is present in the cerebrospinal fluid of the lateral brain ventricle; and 5) melatonin does not prevent stimulation of the gonads by exogenous gonadotropins, suggesting an extragonadal site of action of melatonin. It seems likely that melatonin acts at hypothalamic or hypophysial levels through the inhibition of synthesis or release of gonadotropin releasing hormone(s). A more direct action of melatonin on gonads also has been observed—the addition of melatonin to incubations of testicular tissue inhibits androgen biosynthesis (Peat and Kinson, 1971).

Pineal extracts, which do not contain methoxyindoles, also have antigonadal properties; these extracts are polypeptides of low molecular weight (<5000) (Ebels, 1975; Benson *et al.*, 1976). A peptide, arginine vasotocin (AVT), has been isolated from mammalian pineals and exhibits antigonadal

properties. However, if AVT is a pineal antigonadotropin, others must exist because a comparison of AVT with other partially purified pineal antigonadotropic polypeptides indicates some significant differences (Benson *et al.*, 1976).

Recent studies indicate that indoleamines may interact with pineal antigonadal polypeptides and inhibit their activities. Orts *et al.* (1975) isolated two extracts from bovine pineal glands that were believed to be small peptides and that inhibited compensatory ovarian hypertrophy in mice. When the extracts were tested for antigonadotropic activity in the presence of melatonin, only one fraction was found to be effective. Melatonin, therefore, blocked the antigonadal properties of at least one of the pineal polypeptides. These results may, at least partially, explain the observations that melatonin can have either an antigonadal or a progonadal effect in some mammals, such as the golden hamster (Turek *et al.*, 1976b; Reiter *et al.*, 1976).

A number of studies have demonstrated that a negative feedback system seems to operate between the gonads and the pineal organs of male and female rats (Fig. 9). HIOMT activity in male or female rats decreases after castration but physiological doses of testosterone or estradiol increase HIOMT activity (Nagle *et al.*, 1974). Estradiol and testosterone also increase pineal protein synthesis, which may include antigonadal peptides (Nagle *et al.*, 1975). Receptors for sex steroids are present within pineal cells and they are controlled via β -adrenergic receptors by norepinephrine released from the postganglionic nerve fibers of the superior cervical ganglia (Cardinali *et al.*, 1975). Superior cervical ganglionectomy decreases the pineal uptake of estradiol and testosterone, modifies the metabolism pathways of testosterone within the pineal, and abolishes testosterone-dependent stimulation of protein synthesis.

SUMMARY

Pineal organs of lower vertebrates are directly photosensitive. The pinealo-petal nerves noted in the pineal organs of lower vertebrates may be a route by which the eyes, parapineal organs, or other brain extraretinal photoreceptors can transmit photic information to the pineal. Light could either control pineal biochemical activities or be coded into nervous impulses that are transmitted by afferent nerves to the epithalamus of the brain; however, the function of this afferent innervation is unknown. Although the bird pineal has cells with a rudimentary photoreceptive appearance, the pineal organ does not respond electrically to light in the adult. In adult birds, photic information reaches the pineal only via the eyes or, possibly, via extraretinal photoreceptors in the brain. Extraretinal photoreception is absent in adult mammals, and only light perceived by the eyes can influence pineal activity. However, experiments with both young birds and young mammals suggest the pineal is probably directly photosensitive. Interestingly, light has an inhibitory role on nervous activity in all pineal organs: in lower vertebrates visible light directly inhibits spontaneous action

FIG. 9.—Feedback system between the gonads and pineal organs of rats.

potentials arising from intrapineal neurons and in birds and mammals light perceived by the retina inhibits spontaneous activity in the sympathetic nerves projecting to the pineal.

The selective pressures causing a shift from direct pineal photosensitivity in lower vertebrates to an indirect photosensitivity via the eyes in higher vertebrates are uncertain. The shift may have been a developmental phenomenon dictated by the extensive reorganization that occurred in the brain during the evolution of the higher vertebrates. Or, perhaps, the increased opacity of mammalian skulls compared to other vertebrates made direct light detection difficult and required a shift to retinal photoreception.

In addition to the obvious photoreceptive capacity of lower vertebrate pineals, these organs are capable also of considerable secretory activity. For example, the pineal photosensory cells have melatonin synthesizing capabilities in some lower vertebrates. The fact that indoleamine biosynthesis is confined to the pinealocytes of birds and mammals, combined with the rudimentary photoreceptive appearance of some pinealocytes, indicates that the pinealocyte is derived from the sensory cell line of lower vertebrates. A striking feature of avian and mammalian pineals is the large multiple daily rhythms seen in various indoleamines and enzymes. Although the data are sparse, the demonstration of daily rhythms in HIOMT activity and serotonin levels in fish and reptiles respectively shows that rhythmic pineal activity is not restricted to the obviously endocrine pineals of birds and mammals.

The control of these daily rhythms may have undergone extensive changes during evolution. In mammals, a circadian oscillator in the SCN controls many pineal rhythms but the pineal organ may not contain a circadian oscillator itself. In birds, the role of the SCN has not been determined, but it is likely that the pineal contains an oscillator that drives overt circadian

rhythms, possibly via the rhythmic secretion of melatonin. In lizards, the pineal is also an important component of circadian organization and may either be the locus of a circadian oscillator or may act as a coupling device between other circadian oscillators. Potentially, pineal rhythms in lower vertebrates could be either driven directly by light or indirectly by the entraining action of light on circadian oscillators.

The mammalian pineal satisfies all of the criteria of an endocrine organ: it secretes substances such as melatonin into the blood; these substances affect a number of target organs; and the target organs (such as the gonads) can modulate pineal activity via feedback mechanisms. Less work has been done on the endocrine function of the avian pineal but it, too, can secrete substances (for example, melatonin) that can affect target organs such as regions of the central nervous system responsible for the generation of overt circadian rhythmicity or regions involved in the control of reproduction, as well as directly affecting the gonads. A gonadal feedback mechanism may exist between gonadal hormones and indoleamine synthesis.

Past studies on the pineals of lower vertebrates have concentrated on the photoreceptive nature of these structures but interest in the endocrine role of these organs is increasing. Melatonin is a pineal hormone that mediates the effects of lighting stimuli on dermal melanophores in lower vertebrates. Effects of pinealectomy and melatonin injection on reproductive systems also have been noted; however, the interpretation of the effects of pinealectomy in lower vertebrates must be made with caution because the pineal organ can influence physiological processes by either a sensory or a hormonal route (or both).

ACKNOWLEDGMENT

Part of the work described in this paper was supported by NSF grant PCM 76-06842.

LITERATURE CITED

- ADLER, K. 1976. Extraocular photoreception in amphibians. *Photochem. Photobiol.*, 23: 275-298.
- ARRINGTON, L. C., R. K. RINGER, AND J. H. WOLFORD. 1969. Effect of pinealectomy of *Coturnix* quail reared under non-stimulatory photoperiods. *Poultry Sci.*, 48:454-459.
- BAGNARA, J. T., AND M. E. HADLEY. 1970. Endocrinology of the amphibian pineal. *Amer. Zool.*, 10:201-216.
- BAKER, P. C. 1969. Melatonin levels in developing *Xenopus laevis*. *Comp. Biochem. Physiol.*, 28:1387-1393.
- BALASUBRAMANIAN, K. S., AND R. N. SAXENA. 1973. Effect of pinealectomy and photoperiodism in the reproduction of Indian weaver birds, *Ploceus philippinus*. *J. Exp. Zool.*, 185:333-340.
- BENSON, B., M. J. MATTHEWS, AND V. J. HRUBY. 1976. Characterization and effects of a bovine pineal antigonadotropic peptide. *Amer. Zool.*, 16:17-24.
- BERGMANN, G. 1971. Elektronenmikroskopische Untersuchungen an Pinealorgan von *Pterophyllum scalare* Cuv. et Val. (Cichlidae, Teleostei). *Z. Zellforsch. Mikrosk. Anat.*, 119:257-288.

- BINKLEY, S. 1976a. Pineal gland biorhythms: N-acetyltransferase in chickens and rats. *Fed. Proc.*, 35:2347-2352.
- . 1976b. Comparative biochemistry of the pineal glands of birds and mammals. *Amer. Zool.*, 16:57-65.
- BINKLEY, S., AND E. B. GELLER. 1975. Pineal N-acetyltransferase in chickens: rhythm persists in constant darkness. *J. Comp. Physiol.*, 99:67-70.
- BINKLEY, S., S. E. MACBRIDE, D. C. KLEIN, AND C. L. RALPH. 1973. Pineal enzymes: regulation of avian melatonin synthesis. *Science*, 181: 273-275.
- . 1975. Regulation of pineal rhythms in chickens: refractory period and nonvisual light perception. *Endocrinology*, 96:848-853.
- BISCHOFF, M. B. 1969. Photoreceptor and secretory structures in the avian pineal organ. *J. Ultrastruct. Res.*, 28:16-26.
- BOYA, J., AND L. ZAMORANO. 1975. Ultrastructural study of the pineal gland of the chicken. *Acta Anat.*, 92:202-226.
- CARDINALI, D. P., AND J. M. ROSNER. 1971. Effects of melatonin, serotonin and N-acetylserotonin on the production of steroids by duck testicular homogenates. *Steroids*, 18: 25-37.
- . 1972. Ocular distribution of hydroxyindole-O-methyltransferase (HIOMT) in the duck (*Anas platyrhynchos*). *Gen. Comp. Endocrinol.*, 18:407-409.
- CARDINALI, D. P., AND R. J. WURTMAN. 1972. Hydroxyindole-O-methyltransferase in rat pineal, retina and hardierian gland. *Endocrinology*, 91:247-252.
- CARDINALI, D. P., A. E. CUELLO, J. H. TRAMEZZANI, AND J. M. ROSNER. 1971. Effects of pinealectomy on the testicular function of the adult male duck. *Endocrinology*, 89: 1082-1093.
- CARDINALI, D. P., C. A. NAGLE, AND J. M. ROSNER. 1975. Control of estrogen and androgen receptors in the rat pineal gland by catecholamine transmitter. *Life Sci.*, 16:93-106.
- COLLIN, J. P. 1971. Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. Pp. 79-125, in *The pineal gland* (G. E. W. Wolstenholme and J. Knight, eds.), Churchill Livingstone, Edinburgh and London, xi+401 pp.
- COLLIN, J. P., AND A. MEINIEL. 1971. L'organe pinéal. Études combinées ultrastructurales, cytochimiques (monoamines) et expérimentales, chez *Testudo mauritanica*. Grains denses des cellules de la lignée «sensorielle» chez les vertébrés. *Arch. Anat. Microscop.*, 60:269-304.
- . 1973a. Métabolisme des indolamines dans l'organe pinéal de *Lacerta* (Reptiles, Lacertiliens). I. Intégration sélective de 5-HTP-³H (5-hydroxytryptophane-³H) et rétention de ses dérivés dans les photorécepteurs rudimentaires sécrétoires. *Z. Zellforsch., Mikrosk. Anat.*, 142:549-570.
- . 1973b. Métabolisme des indolamines dans l'organe pinéal de *Lacerta* (Reptiles, Lacertiliens). II. L'activité MAO et l'incorporation de 5-HTP-³H et de 5-HT-³H, dans les conditions normales et expérimentales. *Z. Zellforsch. Mikrosk. Anat.*, 145:331-361.
- COLLIN, J. P., A. CALAS, AND M. T. JUILLARD. 1976. The avian pineal organ. Distribution of exogenous indoleamines: a qualitative study of the rudimentary photoreceptor cells by electron microscopic radioautography. *Exp. Brain Res.*, 25:15-33.
- DAVID, G. F. X., AND J. HERBERT. 1973. Experimental evidence for a synaptic connection between habenula and pineal ganglion in the ferret. *Brain Res.*, 64:327-343.
- DE VLAMING, V. L. 1975. Effects of pinealectomy on gonadal activity in the cyprinid teleost, *Notemigonus crysoleucas*. *Gen. Comp. Endocrinol.*, 26:36-49.
- DE VLAMING, V. L., M. SAGE, AND C. B. CHARLTON. 1974. The effects of melatonin treatment on gonosomatic index in the teleost, *Fundulus similis*, and the tree frog, *Hyla cinerea*. *Gen. Comp. Endocrinol.*, 22:433-438.
- DODT, E. 1963. Photosensitivity of the pineal organ in the teleost, *Salmo irideus* (Gibbons). *Experientia*, 19:642-643.
- DODT, E., AND E. HEERD. 1962. Mode of action of pineal nerve fibers in frogs. *J. Neurophysiol.*, 25:405-429.

- DODT, E., AND M. JACOBSON. 1963. Photosensitivity of a localized region of the frog dien-cephalon. *J. Neurophysiol.*, 26:752-759.
- DODT, E., AND E. SCHERER. 1968. The electroretinogram of the third eye. Pp. 231-237, in *Advances in electrophysiology and pathology of the visual system*, 6th ISCERG Symposium. VEB G. Thieme, Leipzig, vii+401 pp.
- EBELS, I. 1975. Pineal factors other than melatonin. *Gen. Comp. Endocrinol.*, 25:189-198.
- EDDY, J. M., AND R. STRAHAN. 1968. The role of the pineal complex in the pigmentary effector system of the lamprey, *Mordacia mordax* (Richardson) and *Geotria australis* (Gray). *Gen. Comp. Endocrinol.*, 11:528-534.
- EICHLER, V. B., AND R. Y. MOORE. 1975. Studies on hydroxyindole-O-methyltransferase in frog brain and retina: enzymology, regional distribution and environmental control of enzyme levels. *Comp. Biochem. Physiol.*, 50C:89-95.
- ENGBRETSON, G. A., AND C. M. LENT. 1976. Parietal eye of the lizard: neuronal photo-responses and feedback from the pineal gland. *Proc. Nat. Acad. Sci.*, 73:654-657.
- FENWICK, J. C. 1970a. Demonstration and effect of melatonin in fish. *Gen. Comp. Endocrinol.*, 14:86-97.
- . 1970b. The pineal organ: photoperiod and reproductive cycles in the goldfish, *Carassius auratus* L. *J. Endocrinol.* 46:101-111.
- GASTON, S., AND M. MENAKER. 1968. Pineal function: the biological clock in the sparrow? *Science*, 160:1125-1127.
- HAFAEEZ, M. A. 1970. Effect of melatonin on body coloration and spontaneous swimming activity in rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.*, 36:639-656.
- HAFAEEZ, M. A., AND W. B. QUAY. 1969. Histochemical and experimental studies of 5-hydroxy-tryptamine in pineal organs of teleosts (*Salmo gairdneri* and *Atherinopsis californiensis*). *Gen. Comp. Endocrinol.*, 13:211-217.
- . 1970. Pineal acetylserotonin methyltransferase activity in the teleost fishes, *Hesperoleucus symmetricus* and *Salmo gairdneri*, with evidence for lack of effect of constant light and darkness. *Comp. Gen. Pharmacol.*, 1:257-262.
- HAFAEEZ, M. A., AND L. ZERHUN. 1974. Studies on central projections of the pineal nerve tract in rainbow trout, *Salmo gairdneri* Richardson, using cobalt chloride iontophoresis. *Cell Tiss. Res.*, 154:485-510.
- HAMASAKI, D. I. 1969. Spectral sensitivity of the parietal eye of the green iguana. *Vision Res.*, 9:515-523.
- HAMASAKI, D. I., AND E. DODT. 1969. Light sensitivity of the lizard's epiphysis cerebri. *Pflugers Arch.*, 313:19-29.
- HAMASAKI, D. I., AND P. STRECK. 1971. Properties of the epiphysis cerebri of the small-spotted dogfish shark, *Scyliorhinus caniculus*, L. *Vision Res.*, 11:189-198.
- HEDLUND, L., AND A. V. NALBANDOV. 1969. Innervation of the avian pineal body. *Amer. Zool.*, 9:1090.
- HERBUTÉ, S., AND J. D. BAYLÉ. 1974. Multiple-unit activity in the pineal gland of the Japanese quail: spontaneous firing and responses to photic stimulations. *Neuroendocrinology*, 16:52-64.
- HISANO, N., D. P. CARDINALI, J. M. ROSNER, C. A. NAGLE, AND J. H. TRAMEZZANI. 1972. Pineal role in the duck extraretinal photoreception. *Endocrinology*, 91:1318-1322.
- HOMMA, K., L. Z. MCFARLAND, AND W. O. WILSON. 1967. Response of the reproductive organs of the Japanese quail to pinealectomy and melatonin injections. *Poultry Sci.*, 46:314-319.
- JOSS, J. M. P. 1973. Pineal-gonad relationships in the lamprey *Lamprisma fluviatilis*. *Gen. Comp. Endocrinol.*, 21:118-122.
- . 1977. Hydroxyindole-O-methyltransferase (HIOMT) activity and the uptake of ³H-melatonin in the lamprey, *Geotria australis* Gray. *Gen. Comp. Endocrinol.*, 31:270-275.
- KAPPERS, J. A. 1967. The sensory innervation of the pineal organ in the lizard, *Lacerta viridis*, with remarks on its position in the trend of pineal phylogenetic structural and functional evolution. *Z. Zellforsch. Mikrosk. Anat.*, 81:581-618.

- KELLY, D. E. 1965. Ultrastructure and development of amphibian pineal organs. *Progr. Brain Res.*, 10:270-287.
- KORF, H. W. 1976. Histological, histochemical and electron microscopical studies on the nervous apparatus of the pineal organ in the tiger salamander, *Ambystoma tigrinum*. *Cell Tiss. Res.*, 174:475-497.
- LAUBER, J. K., J. E. BOYD, AND J. AXELROD. 1968. Enzymatic synthesis of melatonin in avian pineal body: extraretinal response to light. *Science*, 161:489-490.
- LERNER, A. B., J. D. CASE, Y. TAKAHASHI, T. H. LEE, AND W. MORI. 1958. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J. Amer. Chem. Soc.*, 80:2587.
- LEVEY, I. L. 1973. Effects of pinealectomy and melatonin injections at different seasons on ovarian activity in the lizard *Anolis carolinensis*. *J. Exp. Zool.*, 185:169-174.
- LYNCH, H. J. 1971. Diurnal oscillations in pineal melatonin content. *Life Sci.*, 10:791-795.
- MACHADO, C. R. S., A. B. M. MACHADO, AND L. E. WRAGG. 1969a. Circadian serotonin rhythm control: sympathetic and nonsympathetic pathways in rat pineals of different ages. *Endocrinology*, 85:846-848.
- MACHADO, C. R. S., L. E. WRAGG, AND A. B. M. MACHADO. 1969b. Circadian rhythm of serotonin in the pineal body of immunosympathectomized immature rats. *Science*, 164:442-443.
- McFARLAND, L. Z., K. HOMMA, AND W. O. WILSON. 1968. Superior cervical ganglionectomy in the Japanese quail. *Experientia*, 24:245-247.
- MEINIEL, A., AND J. P. COLLIN. 1971. Le complexe pinéal de l'ammocète (*Lampetra planeri*, Bl.). *Z. Zellforsch. Mikrosk. Anat.*, 117:354-380.
- MENAKER, M., AND H. UNDERWOOD. 1976. Extraretinal photoreception in birds. *Photochem. Photobiol.*, 23:299-306.
- MENAKER, M., AND N. ZIMMERMAN. 1976. Role of the pineal in the circadian system of birds. *Amer. Zool.*, 16:45-55.
- MEYER, D. C., P. D. STURKIE, AND K. GROSS. 1973. Diurnal rhythm in serotonin of blood and pineals of chickens. *Comp. Biochem. Physiol.*, 46A:619-623.
- MOORE, R. Y., AND V. B. EICHLER. 1976. Central neural mechanisms in diurnal rhythm regulation and neuroendocrine responses to light. *Psychoneuroendocrinology*, 1: 265-279.
- MORITA, Y. 1966. Absence of electrical activity of the pigeon's pineal organ in response to light. *Experientia*, 22:402.
- NAGLE, C. A., D. P. CARDINALI, AND J. M. ROSNER. 1974. Effects of castration and testosterone administration on pineal and retinal hydroxyindole-O-methyltransferases of male rats. *Neuroendocrinology*, 14:14-23.
- . 1975. Testosterone effects on protein synthesis in the rat pineal gland. Modulation by the sympathetic nervous system. *Life Sci.*, 16:81-91.
- OISHI, T., AND J. K. LAUBER. 1974. Pituitary-pineal-endocrine relationships in Japanese quail. *Endocrinology*, 94:1735-1738.
- OKSCHE, A. 1971. Sensory and glandular elements of the pineal organ. Pp. 127-146, in *The pineal gland* (G. E. W. Wolstenholme and J. Knight, eds.), Churchill Livingstone, Edinburgh and London, xi+401 pp.
- OKSCHE, A., AND H. KIRSCHSTEIN. 1968. Unterschiedlicher elektronenmikroskopischer Feinbau der Sinneszellen im Parietallauge und im Pinealorgan (Epiphysis cerebri) der Lacertilia. *Z. Zellforsch. Mikrosk. Anat.*, 87:159-192.
- . 1969. Elektronenmikroskopische Untersuchungen am Pinealorgan von *Passer domesticus*. *Z. Zellforsch. Mikrosk. Anat.*, 102:214-241.
- OKSCHE, A., AND M. VAUPEL-VON HARNACK. 1966. Elektronenmikroskopische Untersuchungen zur Frage der Sinneszellen im Pinealorgan der Vögel. *Z. Zellforsch. Mikrosk. Anat.*, 69:41-60.
- OKSCHE, A., H. KIRSCHSTEIN, H. KOBAYASHI, AND D. S. FARNER. 1972. Electron microscopic and experimental studies of the pineal organ in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Z. Zellforsch. Mikrosk. Anat.*, 124:247-274.

- ORTS, R. J., K. M. KOCAN, AND I. B. WILSON. 1975. Inhibitory action of melatonin on a pineal antigonadotropin. *Life Sci.*, 17:845-850.
- OZAKI, Y., AND H. J. LYNCH. 1976. Presence of melatonin in plasma and urine of pineal-ectomized rats. *Endocrinology*, 99:641-644.
- PAUL, E. 1972. Innervation und zentralnervöse Verbindungen des Frontalorgans von *Rana temporaria* und *Rana esculenta*. *Z. Zellforsch. Mikrosk. Anat.*, 128:504-511.
- PEAT, F., AND G. A. KINSON. 1971. Testicular steroidogenesis *in vitro* in the rat in response to blinding, pinealectomy and to the addition of melatonin. *Steroids*, 17:251-263.
- PETIT, A. 1971. L'épiphyse d'un serpent: *Tropidonotus natrix* L. I. Étude structurale et ultrastructurale. *Z. Zellforsch. Mikrosk. Anat.*, 120:94-119.
- PRESLOCK, J. P. 1976. Regulation of pineal enzymes by photoperiod, gonadal hormones and melatonin in *Coturnix* quail. *Hormone Res.*, 7:108-117.
- QUAY, W. B. 1965. Retinal and pineal hydroxyindole-O-methyltransferase activity in vertebrates. *Life Sci.*, 4:983-991.
- . 1974. Pineal chemistry. Charles C. Thomas, Springfield, Ill. xv+430 pp.
- QUAY, W. B., R. C. STEBBINS, T. D. KELLEY, AND N. W. COHEN. 1971. Effects of environmental and physiological factors on pineal acetylserotonin methyltransferase activity in the lizard *Sceloporus occidentalis*. *Physiol. Zool.*, 44:241-248.
- RALPH, C. L. 1976. Correlations of melatonin content in pineal gland, blood, and brain of some birds and mammals. *Amer. Zool.*, 16:35-43.
- RALPH, C. L., AND D. C. DAWSON. 1968. Failure of the pineal body of two species of birds (*Coturnix coturnix japonica* and *Passer domesticus*) to show electrical responses to illumination. *Experientia*, 24:147-148.
- RALPH, C. L., H. J. LYNCH, G. C. GUNDY, AND L. HEDLUND. 1970. Pineal function and oviposition in Japanese quail: superior cervical ganglionectomy and photoperiod. *Science*, 170:995-997.
- RALPH, C. L., S. BINKLEY, S. MACBRIDE, AND D. KLEIN. 1975. Regulation of pineal rhythms in chickens: effects of blinding, constant light, constant dark, and superior cervical ganglionectomy. *Endocrinology*, 97:1373-1378.
- REED, B. L., B. C. FINNIN, AND N. E. RUFFIN. 1969. The effects of melatonin and epinephrine on the melanophores of freshwater teleosts. *Life Sci.*, 8:113-120.
- REITER, R. J. 1974. Pineal regulation of hypothalamicopituitary axis: gonadotrophins. Pp. 519-550, in *Handbook of physiology*, Section 7, Endocrinology (R. O. Greep and E. B. Astwood, section eds.), The pituitary gland and its neuroendocrine control, Pt. 1 (E. Knobil and W. H. Sawyer, volume eds.), Amer. Physiol. Soc., Washington, D. C., 4:vii+1-576 pp.
- REITER, R. J., M. K. VAUGHAN, P. K. RUDEEN, AND R. C. PHILO. 1976. Melatonin induction of testicular recrudescence in hamsters and its subsequent inhibitory action on the antigonadotrophic influence of darkness on the pituitary-gonadal axis. *Amer. J. Anat.*, 147:235-242.
- RELKIN, R. 1976. The pineal. Eden Press, Montreal, 187 pp.
- ROMIJN, H. J. 1975. Structure and innervation of the pineal gland of the rabbit, *Oryctolagus cuniculus* (L.). *Cell Tiss. Res.*, 157:25-51.
- ROSNER, J. M., J. H. DENARI, C. A. NAGLE, D. P. CARDINALI, G. D. P. BEDÉS, AND L. ORSI. 1972. Direct action of light on serotonin metabolism and RNA biosynthesis in duck pineal explants. *Life Sci.*, 11:829-836.
- RÜDEBERG, C. 1971. Structure of the pineal organs of *Anguilla anguilla* and *Lebistes reticulatus* Peters (Teleostei). *Z. Zellforsch. Mikrosk. Anat.*, 122:227-243.
- SAYLER, A., AND A. WOLFSON. 1968. Influence of the pineal gland on gonadal maturation in the Japanese quail. *Endocrinology*, 83:1237-1246.
- SHELLABARGER, C. J. 1952. Pinealectomy vs. pineal injections in the young cockerel. *Endocrinology*, 51:151-154.
- . 1953. Observations of the pineal in the white leghorn capon and cockerel. *Poultry Sci.*, 32:189-197.

- SMITH, J. R., AND L. J. WEBER. 1974. Diurnal fluctuations in acetylserotonin methyltransferase (ASMT) activity in the pineal gland of the steelhead trout (*Salmo gairdneri*). *Proc. Soc. Exp. Biol. Med.*, 147:441-443.
- . 1976. The regulation of day-night changes in hydroxyindole-O-methyltransferase activity in the pineal gland of steelhead trout. *Canadian J. Zool.*, 54:1530-1534.
- SUNDARARAJ, B. I., AND P. KESHAVANATH. 1976. Effects of melatonin and prolactin treatment on the hypophysial-ovarian system in the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endocrinol.*, 29:84-96.
- TUREK, F. W., J. P. McMILLAN, AND M. MENAKER. 1976a. Melatonin: effects on the circadian locomotor rhythm of sparrows. *Science*, 194:1441-1443.
- TUREK, F. W., C. DESJARDINS, AND M. MENAKER. 1976b. Melatonin: antigonadal and progonadal effects in male golden hamsters. *Science*, 190:280-282.
- UECK, M. 1970. Weitere Untersuchungen zur Feinstruktur und Innervation des Pinealorgans von *Passer domesticus* L. *Z. Zellforsch. Mikrosk. Anat.*, 105:276-302.
- . 1973. Fluoreszenzmikroskopische und elektronenmikroskopische Untersuchungen am Pinealorgan verschiedener Vogelarten. *Z. Zellforsch. Mikrosk. Anat.*, 137:37-62.
- UECK, M., AND H. KOBAYASHI. 1972. Vergleichende Untersuchungen über acetylcholinesterase-haltige Neurone im Pinealorgan der Vögel. *Z. Zellforsch. Mikrosk. Anat.*, 129:140-160.
- UECK, M., M. VAUPEL-VON HARNACK, AND Y. MORITA. 1971. Weitere experimentelle und neuroanatomische Untersuchungen an den Nervenbahnen des Pinealkomplexes der anuren. *Z. Zellforsch. Mikrosk. Anat.*, 116:250-274.
- UNDERWOOD, H. 1977. Circadian organization in lizards: the role of the pineal organ. *Science*, 195:587-589.
- UNDERWOOD, H., AND M. MENAKER. 1976. Extraretinal photoreception in lizards. *Photochem. Photobiol.*, 23:227-243.
- URASAKI, H. 1972. Effects of restricted photoperiod and melatonin administration on gonadal weight in the Japanese killifish. *J. Endocrinol.*, 55:619-620.
- . 1973. Effect of pinealectomy and photoperiod on oviposition and gonadal development in the fish, *Oryzias latipes*. *J. Exp. Zool.*, 185:241-246.
- VAN DE VEERDONK, F. C. G. 1967. Demonstration of melatonin in amphibians. *Curr. Mod. Biol.*, 1:175-177.
- VAUGHAN, M. K., G. M. VAUGHAN, D. E. BLASK, M. P. BARNETT, AND R. J. REITER. 1976. Arginine vasotocin: structure-activity relationships and influence on gonadal growth and function. *Amer. Zool.*, 16:25-34.
- VIVIEN-ROELS, B. 1970. Ultrastructure, innervation et fonction de l'épiphyse chez les Chéloniens. *Z. Zellforsch. Mikrosk. Anat.*, 104:429-448.
- VIVIEN-ROELS, B., AND M. A. PETIT. 1975. Dosage spectrofluorimétrique de la sérotonine (5-hydroxytryptamine=5-HT) épiphysaire chez les reptiles. Etude des variations du taux de 5-HT en fonction du cycle saisonnier chez *Testudo hermanni*. *C. R. Acad. Sci., ser. D (Paris)*, 280:467-470.
- VIVIEN-ROELS, B., M. A. PETIT, AND C. LICHTÉ. 1971. Dosage spectrofluorimétrique de la sérotonine épiphysaire chez les reptiles. Etude des variations du taux de sérotonine en fonction de la période d'éclairement. *C. R. Acad. Sci., Ser. D (Paris)*, 272:459-461.
- WETTERBERG, L., E. GELLER, AND A. YUWILER. 1970a. Harderian gland: an extraretinal photoreceptor influencing the pineal gland in neonatal rats. *Science*, 167:884-885.
- WETTERBERG, L., A. YUWILER, R. ULRICH, E. GELLER, AND R. WALLACE. 1970b. Harderian gland: influence on pineal hydroxyindole-O-methyltransferase activity in neonatal rats. *Science*, 170:194-196.
- WILSON, J. F., AND J. M. DODD. 1973. The role of the pineal complex and lateral eyes in the colour change response of the dogfish, *Scyliorhinus canicula* L. *J. Endocrinol.*, 58: 591-598.
- WOLSTENHOLME, G. E. W., AND J. KNIGHT, EDs. 1971. The pineal gland. Churchill Livingstone, Edinburgh and London, xi+401 pp.

- WURTMAN, R. J., J. AXELROD, AND D. E. KELLY. 1968. The Pineal. Academic Press, New York, x+199 pp.
- YOUNG, J. Z. 1935. The photoreceptors of lampreys. II. The function of the pineal complex. J. Exp. Biol., 12:254-270.
- ZIMMERMAN, N. H., AND M. MENAKER. 1975. Neural connections of sparrow pineal: role in circadian control of activity. Science, 190:477-479.
- ZIMMERMANN, P., AND E. PAUL. 1972. Reaktionsmuster verschiedener Mittel- und Zwischenhirnzentren von *Rana temporaria* L. nach Unterbrechung der Nervenbahnen des Pinealkomplexes. Z. Zellforsch. Mikrosk. Anat., 128:512-537.
- ZIMMERMAN, B. L., AND M. O. M. Tso. 1975. Morphologic evidence of photoreceptor differentiation of pinealocytes in the neonatal rat. J. Cell Biol., 66:60-75.

HYPOTHALAMO-ADENOHYPOPHYSIAL RELATIONSHIPS AMONG VERTEBRATES

MILTON H. STETSON AND E. GORDON GRAU

In discussing the endocrine control mechanisms of the vertebrate hypothalamus, we will focus on a few topics that we feel will be areas of active research in the future. In introducing this discussion we therefore find it necessary to begin by stating what we have omitted. For information on the comparative morphology, cytology, and cytochemistry of the vertebrate hypothalamus, readers are referred to the extensive reviews by Kobayashi *et al.* (1970) and Dodd *et al.* (1971). Additionally, reviews on single vertebrate classes are also available: teleosts (Peter, 1973; Ball and Baker, 1969; Ball *et al.* 1972), amphibians (Jørgensen, 1970), reptiles (Saint Girons, 1970), birds (Oksche and Farner, 1974; Assenmacher, 1973; Follett, 1973; Kobayashi and Wada, 1973; Epple and Stetson, 1976), mammals (Nauta and Haymaker, 1969; Raisman, 1970; Raisman and Field, 1971; DeRoos and Hommes 1974; Lammers and Lohman, 1974; Daniel and Prichard, 1975). The anatomy of the hypothalamo-hypophyseal portal system in those vertebrates that possess one has not been described here nor has a discussion of the preoptico (supraoptic and paraventricular nuclei in tetrapods) neurohypophyseal system been included because these topics are adequately covered in many of the reviews cited above. Information on hypothalamic endocrine control mechanisms regulating food intake, osmotic balance, and body temperature can be found in the monographs and reviews by Stevenson (1969), Myers (1969), Balagura (1970), Whittow (1970, 1971, 1973), Epstein *et al.* (1973), Calder and King (1974), and Novin *et al.* (1976).

Our intent is to examine specifically the role of the hypothalamus in regulating hormone release from the pars distalis. The nature of hypothalamic regulation of anterior pituitary hormone release in vertebrates has been investigated *in vivo* by hypophysectomy and ectopic hypophyseal transplantation, ablation of specific hypothalamic regions, hypothalamic implants of target hormones, administration of hypothalamic hormones and/or extracts, and partial or complete neural isolation of the hypothalamus followed by gravimetric and microscopic examination of pituitary target organs, or, more recently, measurement of target organ hormones. *In vitro* analysis of this phenomenon involves such techniques as pituitary incubation in the presence or absence of hypothalamic extracts followed by direct or indirect assay for release of pituitary hormones, radioimmunoassay for hypothalamic hormones, and immunohistofluorescence identification of cells and/or fibers in the hypothalamus containing neurohormones.

CYCLOSTOMES

In cyclostomes, where no neural or neurovascular communication appears to exist between hypothalamus and adenohypophysis (Fernholm, 1972; Gorbman, 1965; Gorbman *et al.*, 1963), no direct evidence exists for hypothalamic regulation of pituitary hormone secretion. However, Matty *et al.* (1976) have demonstrated an increase in circulating thyroxin in hypophysectomized hagfish (*Eptatretus stouti*) bearing 1-10 pituitaries transplanted in the subcutaneous sinus of the head. These data suggest that pituitary thyrotropin may normally be under inhibitory control, and that in its absence the pituitary gland secretes high levels of thyrotropin. Aside from these data, the results of hypophysectomy in adult cyclostomes generally are reported as without effect on thyroid function in hagfish (Matty *et al.*, 1976; Henderson, 1976; Gorbman and Tsuneki, 1975; Henderson and Lorscheider, 1975; Falkmer and Matty, 1966) and lampreys (Pickering, 1972, 1976; Larsen and Rosenkilde, 1971). Hypophysectomy apparently also interferes with gonadal function, including gametogenesis and steroidogenesis, in hagfish (Matty *et al.*, 1976), and in lampreys it greatly retards oogenesis (Dodd *et al.*, 1960; Evennett and Dodd, 1963; Larsen, 1965, 1969; Dodd, 1975). Results of hypophysectomy on adrenal function in the lamprey (*Lampetra fluviatilis*) are somewhat paradoxical (Hardisty, 1972), reportedly being interrenal hyperplasia accompanied by decreased nuclear diameter.

ELASMOBRANCHS

Our understanding of hypothalamic regulation of anterior pituitary function in the elasmobranchs is as restricted as that in cyclostomes. Although various tropic functions have been assigned to the rostral and proximal pars distalis and the ventral lobe (of Selachians) of the anterior pituitary (see Holmes and Ball, 1974), direct evidence of hypothalamic control of tropic hormone release is lacking (see Dodd, 1972a, 1972b, 1975). However, the presence of a well-developed median eminence and hypophysial portal system (Meurling, 1960, 1967; Kobayashi *et al.*, 1970; Jasinski, 1969; Jasinski and Gorbman, 1966) at least provides the anatomical basis for hypothalamic control of anterior pituitary hormone release. Indeed, Deery and Jones (1975) have reported recently activation of adenylyl cyclase activity of the entire pituitary of the dogfish (*Scyliorhinus canicula*) following incubation with neutralized acid extracts of dogfish median eminence. Extracts of other areas of the dogfish brain were without effect. Incubation of dogfish pituitary with synthetic TRH and GnRH resulted in enzyme activation only in the ventral lobe, that region of the anterior pituitary the extracts of which reportedly display thyrotropic (Jackson and Sage, 1973) and gonadotropic (Firth and Vollrath, 1973; Scanes *et al.*, 1972) activities. These data suggest that the hypothalamus of the dogfish may influence hormone release from the pituitary, but clearly further investigations are necessary to establish the role of the elasmobranch hypothalamus in pituitary hormone release.

TELEOSTS

Teleost fish represent an exception to the typical vertebrate pattern in that the portal system and perivascular spaces of the median eminence are replaced by axons extending from the hypothalamus and terminating in direct or proximal contact with glandular cells of the rostral and proximal adenohypophysis (Zambrano, *et al.*, 1972). Innervation of the teleost pars distalis has been investigated in detail (Knowles and Vollrath, 1965; Kobayashi, *et al.*, 1970; Zambrano, *et al.*, 1972). Secretory cells in this region of the adenohypophysis are innervated directly by unmyelinated type "B" fibers (according to the nomenclature of Knowles, 1965). The fibers contain large granulated vesicles (900-1000Å in diameter) and appear to be aminergic in nature because treatment with 6-HODA (6-Hydroxydopamine), a "false" neurotransmitter that has effect only in adrenergic systems, selectively destroys these fibers. Other investigations have noted the presence of "A" type peptidergic fiber terminals in the pars distalis of several species (for discussion, see Kaul and Vollrath, 1974). Thus on a morphological basis there exists the potential for a dual hypothalamic control mechanism regulating adenohypophysial function in teleosts. In addition, various histological studies (reviewed by Zambrano, 1970; and Zambrano *et al.*, 1972) strongly contend that these type "B" fibers (aminergic) arise from the *nucleus lateralis tuberis* (NLT) of the hypothalamic tuberal region and project into the adenohypophysis.

On the basis of available adenohypophysial transplant studies, Peter (1973) tentatively concluded that secretion of ACTH by the teleost pituitary was dependent on hypothalamic stimulation. However, Nagahama *et al.* (1974) found that ACTH cells demonstrate no clear histological alterations after pituitary transplantation in the goby, *Gillichthys mirabilis*. These results are in agreement with those from earlier *in vitro* experiments in which goldfish pituitaries secreted ACTH spontaneously in the absence of hypothalamic influence (Sage, 1968; Sage and Purbott, 1969). In the latter experiment, however, incubation of pituitaries with a hypothalamic homogenate increased ACTH secretion which suggests the existence of a CRF. Redgate (1974) demonstrated that hypothalamic stimulation (electrical) in carp (*Cyprinus carpio*) resulted in a significant increase in plasma cortisol, an effect presumably mediated by ACTH. The identity of the putative teleost CRF is still in doubt, although intracisternal injections of 6-HODA failed to alter the ultrastructure of ACTH cells in pituitaries of *Tilapia mossambica* (Zambrano *et al.*, 1973/74). This finding tends to rule out the involvement of "B" (aminergic) axons in controlling ACTH secretion. Although our knowledge concerning hypothalamic regulation of ACTH release remains limited, the preponderance of recent evidence indicates that the teleost pituitary is capable of some spontaneous secretion but that full secretory activity is modulated by a hypothalamic CRF.

Tonic inhibition, first identified by Ball *et al.* (1963) is well established as the primary mechanism of control by the hypothalamus over pituitary thy-

rotropin release in most teleosts. This subject has been amply reviewed by Peter (1973) and Ball *et al.* (1972) so only the more recent studies will be highlighted here. Earlier studies showed that pituitary thyrotropin cells are clearly activated following pituitary transplantation. This activation is similarly reflected by enhanced thyroid activity (Higgins and Ball, 1970; Peter, 1972; Grau and Stetson, 1977). In addition, lesion experiments (Peter, 1970) provide direct evidence of both the site and nature of hypothalamic control in the goldfish. The presence of a factor with TIF activity also has been demonstrated in hypothalamic extracts of goldfish (see Peter, 1973). Other studies have focused on establishing the identity of the TIF. Mammalian TRH (thyrotropin releasing hormone) has been identified by radioimmunoassay in the hypothalamus of salmon in concentrations comparable to those found in rats (Jackson and Reichlin, 1974), but its possible role in regulating thyrotropin release in the bony fish is far from resolved. TRH produced no change in radioiodine uptake by the thyroid of the African lungfish, *Protopterus ethiopicus* (Gorbman and Hyder, 1973). Similarly TRH had no effect on the adenyl cyclase activity of the pars distalis of the goldfish (Deery, 1975). On the other hand, intraperitoneal injections of TRH induced histological changes indicative of hypertrophy in both thyrotropic cells and thyroidal follicular epithelium of the teleost, *Chasmichthys dolichognathus* (Tsuneki and Fernholm, 1975), and the thyrotropic cells of the goldfish pituitary (Kaul and Vollrath, 1974b). Conversely, Bromage (1975) showed that injections of TRH into male guppies reduced thyrotropic cell activity as well as thyroid activity. Also, thyroid tissue preincubated with pituitaries *in vitro* show several distinct signs of activation, including a significant reduction in follicular colloid. When TRH was added to the incubation medium, these changes were prevented and thyroid colloid remained at control levels. Thyroid tissue incubated without pituitary preincubation was unaltered by TRH treatment. These last results were interpreted as indicating that TRH effects on thyroid activity are pituitary mediated. Peter (see Peter and McKeown, 1975) has shown that TRH causes a dose dependent suppression of radioiodine uptake in goldfish. TRH, however, also caused a suppression of serum thyroxin even in the presence of exogenous TSH, indicating that the molecule may be acting at the thyroid. Treatment of goldfish with melanocyte-stimulating hormone-inhibiting factor (MIF) produced similar results. In these studies, only somatostatin (SRIF) appeared to inhibit pituitary thyrotropin release in that its effects on thyroid metabolism occurred only in the absence of exogenous TSH. The discrepancy between the conclusions of Bromage (1975) and those of Peter and McKeown (1975) with respect to the site of action of TRH in limiting thyroid activity may represent species differences or possibly a difference in assay sensitivity. As Peter measured thyroid hormone levels, he may be able to detect more subtle changes in thyroid activity than are possible using histological or interferometric criteria, as employed by Bromage (1975). In any event, in view of the obvious conflict in the results and conclusions pre-

sented to date, resolution of the role, if any, that TRH may play in regulating pituitary thyrotropin release in teleosts awaits further investigation. The finding that somatostatin may act as a TIF in teleosts is especially intriguing because this molecule is known to block TRH-mediated TSH release *in vivo* and *in vitro* in mammals (Vale *et al.*, 1975). In addition, the demonstration by immunohistofluorescence of "somatostatin" in the neurons innervating the teleost pars distalis, and in the gland itself, lends further credence to the possibility that this tetradecapeptide may be the teleost TIF (Dubois *et al.*, 1974).

Pituitary transplant studies first indicated that prolactin release in teleosts is chronically inhibited by the hypothalamus (see Peter, 1973; Ball *et al.*, 1972); this relationship has been substantiated since by more direct methods. Most importantly, Peter and McKeown (1974) have identified the nucleus lateralis tuberis pars lateralis as the site of control of prolactin release in goldfish. Bilateral lesions in this area caused a significant increase in serum prolactin as measured by a heterologous RIA. On the other hand, these investigators also found that both thalamic lesions and total deafferentiation of the anterior hypothalamus (Peter and McKeown, 1975) caused a significant decrease in serum prolactin. They interpreted these results as indicating that afferent pathways entering the anterior hypothalamus stimulate prolactin secretion by inhibiting PIF activity. The identity of PIF has received considerable attention from several laboratories. Histological studies (for example, Zambrano, 1970; Nagahama *et al.*, 1974) have shown that "B" type aminergic fibers from the hypothalamus terminate in direct contact with prolactin secreting cells in the adenohypophysis of *Gillichthys mirabilis*. Also Deery (1975) found that pituitary adenyl cyclase activity increased in the pars distalis of goldfish (*Carassius auratus*) following *in vitro* treatment with norepinephrine, epinephrine (10^{-6} M), and dopamine (10^{-4} M). These studies suggest that catecholamines might act directly in controlling the release of anterior pituitary hormones, including prolactin. In line with this, Nagahama *et al.* (1975) found that both reserpine, an aminergic blocker, and 6-HODA, which causes the degeneration of "B" fibers, activate prolactin cells, as judged by histological and functional criteria. Treatment with 6-HODA also enhanced prolactin secretion in *Tilapia mossambica* (Zambrano *et al.*, 1973/1974). Conversely, after treatment with L-DOPA (an immediate precursor of dopamine) the prolactin cells of *G. mirabilis* appeared less active (Nagahama *et al.*, 1975). In similar fashion, *in vitro* incubation of pituitaries from *Tilapia mossambica* with dopamine caused a significant decrease in the secretion of newly synthesized radiolabeled prolactin when compared with controls, leading these investigators to conclude that "B" fibers containing catecholamines are involved in controlling prolactin secretion, though it is as yet uncertain as to whether this control is direct or mediated by a PIF elaborated by other cells. The involvement of catecholamines in regulating teleost prolactin secretion has been investigated further by Olivereau (1975) and Olivereau and Lemoine (1973 a, b)

who found that treatment with L-DOPA or ergocryptine, which stimulates dopamine receptors, inhibited prolactin secretion in freshwater eels (*A. anguilla*), whereas injections of 6-HODA stimulated prolactin cell activity.

Prolactin is well known for its ability to promote freshwater survival in hypophysectomized euryhaline teleosts (for review, see Ball, 1969; Ensor and Ball, 1972). This freshwater survival provides good evidence of the presence of prolactin. In two studies (Kramer *et al.*, 1973; McKeown, 1972a), ergocryptine prevented freshwater survival in killifish (*Fundulus heteroclitus*), swordtails (*Xiphophorus helleri*), and mollies (*Poecilia latipinna*).

Peter (1973) has discussed the effect of pituitary transplantation on gonadotropic cell activity. In every case where the receptor fish was hypophysectomized gonads regressed in spite of the pituitary graft. Direct evidence of the existence and site of hypothalamic regulation in one teleost, the goldfish, has been provided by the lesion experiments of Peter (1970). Lesions in the *nucleus lateralis tuberis* resulted in gonadal regression. Direct evidence of hypothalamic stimulation of gonadal activity in teleosts also has been provided by the incubation of hypothalamic extracts *in vitro* with pituitaries of carp. These extracts caused a dose dependent increase in gonadotropin secretion. Similar extracts from both the carp and trout stimulated LH secretion from pituitaries of sheep, and sheep hypothalamic extracts stimulated gonadotropin secretion by carp pituitaries (Breton *et al.*, 1971; Breton *et al.*, 1972). *In vivo* application of hypothalamic extracts and synthetic mammalian GnRH were likewise effective in raising plasma gonadotropin levels in the carp and the goldfish (Breton and Weil, 1973; Crim *et al.*, 1976). This gonadotropic "releasing factor" has been partially characterized as having a molecular weight of less than 5000. It is apparently distinct from neurohypophysial octapeptides as well as from the neuroamines epinephrine, nor-epinephrine, serotonin, and dopamine, all of which possess no GnRH activity *in vitro* (Breton *et al.*, 1975). Synthetic GnRH was found to stimulate ovulation, as well as the release of secretory granules from the pituitary gonadotropes of goldfish (Lam, Pandey and Hoar, 1975; Lam *et al.*, 1976). Doses below the ovulatory threshold stimulate gonadal development.

Taken together, the above investigations clearly point to the existence of a gonadotropic releasing factor elaborated by the teleost hypothalamus. It is possible that this factor is a molecule similar to mammalian GnRH. Attempts to identify such a molecule by radioimmunoassay using an antibody to synthetic GnRH, however, were unsuccessful (Deery, 1974). Failure to demonstrate immunological binding between teleost GnRH and antisynthetic GnRH does not preclude similarities between the biologically active sites of the two hormones.

Very little is known about hypothalamic regulation of growth hormone release in teleosts. Pituitary transplant studies suggest that growth hormone release may be inhibited by a hypothalamic factor(s) in some species (Peter, 1973). More recently, *in vitro* incubation of eel and trout pituitaries in the absence of hypothalamic extracts was characterized by continuous synthesis

and secretion (eel) or secretion (trout) of growth hormone as measured by polyacrylamide gel electrophoresis (Baker and Ingleton, 1975).

In summary, the functional relationship between the teleost hypothalamus and adenohypophysis is most clearly characterized for prolactin (PIF), thyrotropin (TIF), and gonadotropin (GnRF). Although less well established, normal ACTH secretion apparently depends on hypothalamic stimulation (CRF), whereas growth hormone secretion may be inhibited by the hypothalamus.

AMPHIBIANS

Considerable data exist dealing with control of tropic hormone release by the amphibian hypothalamus. Corticotropin release (as measured by circulating corticosteroids) from the pituitary of *Bufo bufo* ceased following pituitary transplantation to an eye muscle and following transection of the hypothalamus between the median eminence and optic chiasma (Buchmann *et al.*, 1972). Corticotropin release in *Rana pipiens* probably is regulated by negative feedback of cortical steroids. Hypothalamic implants of corticosterone, aldosterone, or betamethazone significantly depressed circulating corticosterone levels from those of controls bearing cholesterol implants and also blocked stress-induced ACTH release as measured by increased serum corticosterone (Laub *et al.*, 1975). Dierickx and Goossens (1970), using interrenal karyometric measurements as an assay for corticotropic function in *Rana temporaria*, found decreased corticotropic activity following interruption of all neural pathways to the median eminence. The situation in urodeles is far from clear.

Thyrotropic activity of the amphibian anterior pituitary has been claimed by some to be under stimulatory control by the hypothalamus and by others to be inhibited by hypothalamic neurohormones (see Rosenkilde, 1972). Surgical extirpation of the pars magnocellularis of the newt *Notophthalmus viridescens* significantly reduced thyroid turnover of ^{131}I three weeks after surgery (Compher and Dent, 1973), indicating that the magnocellular neurosecretory system may regulate TSH release from the anterior pituitary. Direct evidence for the presence within the hypothalamus of *Rana temporaria* of a "TRH" capable of stimulating thyrotropin release and a "TIH" capable of depressing thyrotropin release in estrogen-primed male rats has been presented by Kuhn and Engelen (1976). Jackson and Reichlin (1974) found in the hypothalamus of adult and larval *Rana pipiens* high concentrations of a substance that immunologically resembles mammalian TRH (see also Taurog *et al.*, 1974; Jackson *et al.*, 1977). These findings are intriguing when one considers the failure of TRH to evoke increased iodine uptake in adult *Rana temporaria* (Vandesande and Aspeslagh, 1974), to hasten metamorphosis in *R. catesbeiana* tadpoles (Etkin and Gona, 1968), to induce metamorphosis in the mexican axolotl (Taurog *et al.*, 1974), or to stimulate thyrotropin secretion *in vitro* by the pituitaries of *Ambystoma mexicanum*, *Ambystoma tigrinum*, and *Rana pipiens* (Taurog *et al.*, 1974).

Prolactin release from the amphibian pituitary apparently is inhibited by a hypothalamic "PIH" (Etkin *et al.*, 1969; McKeown, 1972b), although in addition to PIH activity, Kuhn and Engelen (1976) also demonstrated the presence of a substance in the hypothalamus of *Rana temporaria* that stimulated prolactin release in estrogen-primed male rats. Inhibition of prolactin release by ergocornine resulted in acceleration of metamorphosis in neotenic *Ambystoma tigrinum* (Platt, 1976), suggesting that neurohormonal control of prolactin release in this salamander is similar to that in mammals (PIH) in which ergocornine also prevents prolactin release (Floss *et al.*, 1973).

The release of pituitary gonadotropins in amphibians has received extensive investigation, primarily by Dierickx and his colleagues (see Dierickx, 1964, 1965, 1966, 1967a, 1967b; Dierickx *et al.*, 1972, 1973a, 1973b) and Jørgensen (1968, 1970). Their findings indicate a gonadotropic center in the tuber cinereum of the ventral hypothalamus. Cells of this center appear to manufacture and tonically release into the hypophysial portal vasculature gonadotropin releasing hormone(s). The *in vitro* demonstration of GnRH activity in the hypothalamus of *Rana pipiens* (Thornton and Geschwind, 1974) capable of stimulating gonadotropin release from *R. pipiens* pituitaries provides direct evidence for neurohormonal regulation of pituitary gonadotropin release in Amphibia. Ectopic pituitary transplants are incapable of maintaining gonadal function in *Rana temporaria* (Dierickx, 1964) but in *Bufo bufo* they maintain the ovaries even after extirpation of the hypothalamic gonadotropic center (Vijayakumar *et al.*, 1971). In addition to a tonic "GnRH" center in the ventromedial hypothalamus, the anterior hypothalamus apparently functions in the regulation of ovulation for transections behind the optic chiasma are without effect on ovarian and testicular development but inhibit ovulation in *Rana temporaria* (Dierickx, 1967a, 1967b) and *Bufo bufo* (Jørgensen, 1968, 1970). Such a dual control system for gonadotropin release in females is remarkably similar to that described in mammals. The immunohistochemical identification within the anterior hypothalamus and in extrahypothalamic tissues of nerve cells containing immunologically active GnRH in *Rana pipiens* and *R. catesbeiana* (Alpert *et al.*, 1976), *Rana esculenta* (Goos *et al.*, 1976), and *Xenopus* (Deery, 1974) supports this hypothesis. Interestingly, these investigators failed to find any GnRH in perikaria of the ventromedial hypothalamus.

In summary, strong evidence exists for the presence in the amphibian hypothalamus of a CRH, a GnRH, and a PIH. Evidence for the presence of a TRH is less convincing, and virtually nothing is known about hypothalamic regulation of growth hormone release in amphibians.

REPTILES

Of vertebrate classes, with the possible exception of the cyclostomes, our knowledge of hypothalamic regulation of anterior pituitary tropic hormone release is most lacking in the reptiles. Direct evidence for hypothalamic regulation exists only for gonadotropin and corticotropin release. Intrahypo-

thalamic implants, localized to the median eminence, of testosterone or estradiol inhibited seasonal maturation of the gonads in the iguana *Dipsosaurus dorsalis dorsalis*. Implants elsewhere in the hypothalamus were without effect (Lisk 1967). Similarly, progesterone (Callard and Doolittle, 1973) and estradiol (Callard *et al.*, 1972) implants blocked ovarian development in *Sceloporus cyanogenys*.

Corticotropin release from the anterior pituitary, as assessed by circulating levels of corticosterone in *Sceloporus cyanogenys*, was curtailed by anterior hypothalamic and median eminence lesions (Daugherty and Callard, 1972). Lesions elsewhere in the brain were without effect. Similar lesions also depressed adrenal growth and prevented metopirone-induced adrenal hypertrophy in the same species (Callard and Chester Jones, 1971). Corticoid sensitivity of the hypothalamus of male *S. cyanogenys* was demonstrated by Callard and Willard (1969). Intrahypothalamic implants of betamethazone blocked metopirone-induced adrenal hypertrophy but implants in other neural regions were without effect. Additionally, anterior hypothalamic and median eminence implants of corticosterone or aldosterone resulted in a significant suppression of circulating corticosterone in female *Dipsosaurus dorsalis* (Callard *et al.*, 1975).

The presence of a substance immunologically similar to TRH has been demonstrated recently in the hypothalamus of the snake, *Thamnophis sirtalis* (Jackson and Reichlin, 1974). It is clear that investigations in reptiles of hypothalamic regulation of the release of other pituitary hormones are sorely needed.

BIRDS

Except for the mammals, we know more about regulatory input from the hypothalamus on the release of adeno-hypophysial hormones of birds than of any other class of vertebrates. Even here, however, research has been limited for the most part to domestic species like fowl, quail, ducks, and pigeons. A pleasant exception to this rule is the continued output from Farner's laboratory of research on the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). Much of the data on hypothalamic-hypophysial relationships in birds have been summarized in the reviews of Assenmacher (1973), Follett (1973), Kobayashi and Wada (1973), Oksche and Farner (1974), and Epple and Stetson (1976) and will not be repeated here. The major distinguishing characteristic of avian hypothalamo-hypophysial control mechanisms is the presence in the avian hypothalamus of a putative PRH (prolactin releasing hormone) (Kragt and Meites, 1965; Nicoll, 1965; Gourdji and Tixier-Vidal, 1966; Gourdji, 1970; Tixier-Vidal and Gourdji, 1972). This was determined by stimulation of prolactin release from the adeno-hypophysis upon the addition of hypothalamic extracts *in vitro*, or by the *in vivo* response of the pigeon crop sac to injected hypothalamic extracts (Knight and Chadwick, 1975) or to pituitary transplants in hypophysectomized birds (Baylé, 1969).

The mode of hypothalamic control of the release of all other pituitary tropic hormones is also stimulatory. Growth hormone release from rat pituitaries *in vitro* was enhanced by hypothalamic extracts, but not cortical extracts, from a number of mammalian species, a frog, and a pigeon (Muller *et al.*, 1967).

Thyrotropin release from the avian adenohypophysis has been investigated only scantily. Data derived primarily from experiments in which the effects of hypophysectomy or hypophysial portal vein transection were performed (see for example, Rosenberg *et al.*, 1967; Baylé and Assenmacher, 1967), suggested a fair degree of autonomy from hypothalamic influence of pituitary thyrotropin secretion. Similarly, the avian thyroid was found to function somewhat independently in the absence of the adenohypophysis. In later studies, Baylé and Assenmacher (1969) and Baylé (1970) reexamined these effects in ducks, quail, and pigeons. Their results were for the most part confirmatory: hypophysectomy greatly reduced thyroid activity (as measured by radioiodine labelled plasma protein (PBI) and radioiodine uptake by the thyroid) in all three species with the exception of birds bearing an ectopic adenohypophysial graft, in which case thyroid activity did not differ appreciably from controls. This latter result was found to be time dependent, however. Birds with pituitary grafts sampled at two weeks had values of PBI and iodine uptake similar to those of hypophysectomized animals, whereas if sampled at two months following receipt of the transplant, thyroid function was near normal. Interestingly, Baylé (1970) found no effect of median eminence lesions in ducks on thyroid weight, thyroid radioiodine uptake, and PBI when compared with controls or lesioned animals bearing hypophysial grafts. Other investigators have, however, demonstrated an effect of hypothalamic lesions on thyroid activity. Severe destruction of the anterior region of the avian hypothalamus reduced thyroid activity in chickens (Egge and Chiasson, 1963; Kanematsu and Mikami, 1970) and Japanese quail (McFarland *et al.*, 1966). More recently, Egge *et al.* (1975) examined the effect of hypothalamic lesions on TSH content of the cephalic and caudal lobes of the chicken adenohypophysis. Lesions in the septomesencephalic tract and/or lateral forebrain bundle were correlated with a significant elevation of TSH in the cephalic lobe. Lesions in the supraoptic or ventrolateral nuclei, with which other authors have correlated altered thyroid function (see references above), had no effect on TSH content of either lobe of the adenohypophysis. In summary, although the avian hypothalamus appears to possess TRH activity, the hypophysial-thyroid axis can function independently. Whether this ever occurs *in vivo* in any avian species is both unlikely and unknown.

Corticotropin release from the avian adenohypophysis is also under stimulatory control by the hypothalamus. Lesions in the ventromedial hypothalamus of chickens caused a significant reduction in adrenal corticosteroids in adrenal vein plasma (Frankel *et al.*, 1967a). Similarly, in this species, dexamethasone, which is thought to exert its effect via hypothalamic loci, com-

pletely suppressed corticosterone output a few hours after injection (Frankel *et al.*, 1967b). Also, ablation of the posterior medial and lateral nuclei of the pigeon hypothalamus caused a significant decrease in circulating corticosterone and blocked stress-induced (restraint) release of ACTH from the adenohypophysis (Baylé and Bouillé, 1971; Bouillé and Baylé, 1973a). Electrical stimulation of the same regions of the hypothalamus dramatically increased plasma corticosterone (Bouillé and Baylé, 1973b). Hypothalamic CRH activity in pigeons is apparently modulated by the limbic system (Bouillé and Baylé, 1973/1974) and septal areas (Bouillé and Baylé, 1975b). Hippocampal and septal lesions or stimulation significantly elevated or depressed, respectively, plasma corticosterone titers but had little effect on neurogenic (restraint) stress-induced ACTH release. Similarly, removal of the cerebral hemispheres of pigeons abolished the diurnal rhythm of plasma corticosterone but had no effect on stress-induced ACTH release (Baylé, 1976). Total or partial isolation of the pigeon hypothalamus from neural input from other brain regions has differing effects on circulating corticosterone. Diurnal fluctuations of plasma corticosterone were abolished by total hypothalamic deafferentation (Bouillé *et al.*, 1973) as was ACTH release induced by neurogenic (restraint) but not humoral (ether) stress. Partial deafferentation posteriorly in the hypothalamus blocked neurogenic stress-induced release of ACTH but did not influence diurnal corticosterone fluctuations (Bouillé *et al.*, 1975). The converse occurred in pigeons with anterior hypothalamic deafferentations. No operations affected ACTH release after ether stress.

Demonstration of CRH activity in the hypothalamus of fowl (Salem *et al.*, 1970a, 1970b), pigeons (Péczy and Zboray, 1967; Péczy, 1972a, 1972b; Péczy *et al.*, 1970; Sato and George, 1973a, 1973b), and ducks (Stainer and Holmes, 1969) and the demonstration of selected accumulation of tritiated corticosterone, dexamethasone, or triamcinolone in cells of the anterior hypothalamus (paraventricular nuclei), hippocampus, and septal nuclei of ducks (Abel *et al.*, 1975) lends further support for hypothalamic control of avian ACTH release.

Considerable data exist attesting to the relative independence of the adenohypophysial-adrenal axis from the hypothalamus in certain avian species. For example, numerous reports have suggested that duck and pigeon adrenals function normally in hypophysectomized birds bearing an ectopic pituitary graft (see for example Baylé, 1968; Baylé *et al.*, 1967; Baylé *et al.*, 1971; Bouillé and Baylé, 1975a; Boissin, 1967; Daniel and Assenmacher, 1969; Péczy *et al.*, 1970). Contrary to the situation in ducks and pigeons, however, circulating levels of corticosterone in hypophysectomized fowl bearing ectopic adenohypophysial transplants (Resko *et al.*, 1964) did not differ from levels in hypophysectomized controls. The degree of functional autonomy possessed by the avian adrenal cortical tissue has not been determined precisely. Hypophysectomy results in a significant depression in the levels of circulating corticosterone in all species investigated. Whether the detectable levels of corticosterone in the plasma of the hypophysectomized

bird represent a definite, albeit greatly suppressed, degree of adrenal cortical secretory activity or instead is the result of a greatly increased half-life of corticosterone (Holmes *et al.*, 1970) has not been elucidated.

The release of LH and FSH from the avian anterior pituitary is under stimulatory hypothalamic control. This topic has been reviewed extensively (see Follett, 1973 for reference prior to 1971) by others. We will therefore attempt to review only those more recently published communications dealing with this aspect of avian neuroendocrinology. As the gonad is the one target organ that requires the release of two pituitary tropic hormones for normal function and as it has only been quite recently that specific assays were developed for avian LH (Follett, *et al.*, 1972) and FSH (Croix *et al.*, 1974; Follett, 1976), the analyses of most research in this field have relied primarily on changes in morphology and function of the gonads or on relatively nonspecific bioassays for the gonadotropins. Nevertheless, significant progress has been made from the latter half of the decade to the present in an attempt to elucidate the neuroendocrinology of reproduction in birds.

Of all the avian target endocrines, the gonads are by far the most dependent on the hypothalamo-hypophysial axis. Hypophysectomy leads to immediate cessation of gametogenic and steroidogenic activity. This marked dependence of the gonad on a functional adenohypophysis has allowed significant advances to be made in determining hypothalamic-pituitary-gonadal relationships.

Several areas of the avian hypothalamus have been identified as "gonadotropic." The median eminence is essential for normal gonadal activity in every species investigated, although some confusion remains as to whether in each species the entire median eminence, the anterior division, or the posterior division is involved in neurohumoral communication between GnRH cells of the hypothalamus and gonadotropin cells of the adenohypophysis. For example, in ducks destruction of the anterior median eminence blocks testicular growth (Assenmacher, 1970; Stetson and Farner, unpublished data), in white-crowned sparrows testicular growth ceases upon destruction of the posterior median eminence (Stetson, 1969), and in Japanese quail the entire median eminence must be destroyed to block testicular growth (Sharp and Follett, 1969a; Stetson, 1972a, 1972b, 1973). These apparent species differences may rest on the degree of point-to-point communication via hypophysial portal vessels between either division of the avian eminence and gonadotropes in the cephalic and caudal lobes of the adenohypophysis (see Epple and Stetson, 1976). In the white-crowned sparrow, for instance, portal veins arising from the primary capillary plexus of the posterior median eminence vascularize *only* the caudal lobe of the adenohypophysis (Vitums *et al.*, 1964). A similar relationship exists in this species between vascular drainage from the anterior median eminence and the cephalic lobe of the adenohypophysis. After meticulous examination of the vascular supply of the adenohypophysis of the Japanese quail, Sharp and Follett (1969b) came to the same conclusion. Other species have not been examined as thoroughly,

but in these two species at least a point-to-point communication between gonadotropes in the cephalic or caudal lobe of the adenohypophysis and neuroendocrine (GnRH) cells in the hypothalamus via pituitary portal vessels is an exciting possibility that warrants further investigation. The demonstration of immunoreactive GnRH substance(s) in the palisade layer of both divisions of the median eminence of the fowl (de Reviers and Dubois, 1974), duck (Calas *et al.*, 1973; McNeill *et al.*, 1976), and greenfinch (Sharp, Haase and Fraser, 1975) further documents the role of the median eminence in regulating gonadal activity. These studies, however, do not negate the results of investigations in which ablation of one division of the median eminence apparently blocks testicular development.

A considerable number of investigations have documented the presence within the avian hypothalamus of gonadotropic hormone releasing activity. Neutralized acid extracts of quail hypothalamus induce a dose-dependent release of gonadotropins from fowl pituitaries (Follett, 1970) and quail pituitaries (Smith and Follett, 1972) *in vitro* and from the pituitaries of estrogen-progesterone primed ovariectomized rats *in vivo* (Casey *et al.*, 1971). Similar results were found by Erickson (1975), using hypothalamic extracts from white-crowned sparrows in an *in vitro* system employing chicken pituitaries. In the domestic fowl, a number of investigations have demonstrated gonadotropin releasing activity in hypothalamic extracts (Jackson and Nalbandov, 1969; Tanaka *et al.*, 1969; Harrison *et al.*, 1974; Tanaka *et al.*, 1974a, 1974b), tested in *in vitro* systems using avian pituitaries or in *in vivo* systems using rats (see above). Opel and Lepore (1972) found that direct infusion of chicken hypothalamic extract into the adenohypophysis, but not the hypothalamus, of laying fowl caused premature ovulation. Infusion of similar doses into the jugular vein were without effect. Fowl GnRH has been partially purified by Jackson (1971a, 1971b, 1972): avian LRF and FRF activities migrated similarly on Sephadex and ion exchange columns, and in the latter system were shown to differ from LRF and FRF activities from extracts of rat hypothalami. Synthetic GnRH effectively causes the release of LH from the adenohypophysis of domestic fowl (Furr *et al.*, 1973; Bonney *et al.*, 1974; Wilson and Sharp, 1975), causes premature ovulation in hens (Reeves *et al.*, 1973; Tanaka and Kamiyoshi, 1976), and produces ultrastructural modifications (increased size and granulation) in pituitary gonadotropes of Japanese quail similar to those produced by photostimulation (Wada, 1975).

Thus, there is little doubt that the avian hypothalamus regulates, via a neurohormone(s), LH and FSH release from the adenohypophysis. The source of this neurohormone remains unknown, but a multitude of studies have been performed in an attempt to identify the cells of origin. McNeill *et al.* (1976) identified a few cells in dorsal regions of the arcuate nucleus of the duck that reacted immunologically with antibodies to synthetic GnRH. Hypothalamic lesions in many avian species point to the ventral infundibular region of the hypothalamus (=arcuate region) as a potential source of

avian GnRH: fowl (Graber *et al.*, 1967; Ravona *et al.*, 1973a, 1973b), Japanese quail (Sharp and Follett, 1969a; Stetson, 1972a, 1972b; Davies and Follett, 1975a; see also Wada, 1974a), ducks (Stetson and Farner, unpublished data), and white-crowned sparrows (Wilson, 1967; Stetson, 1969). In addition, gonadotropin release also is blocked by lesions in the anterior hypothalamus of Japanese quail (Stetson, 1972b; Davies and Follett, 1975b). Conversely, electrical stimulation of the ventral infundibular region (posterodorsal portion) or the anterior (preoptic) region of the quail's hypothalamus evoked a rapid release of LH from the adenohypophysis (Davies and Follett, 1975c). These regions of the hypothalamus are also loci for gonadal steroid hormone feedback in quail (Wada, 1972; Stetson, 1972c, 1972d) and ducks (Gogan, 1968). Whether the "gonadotropic" region in the anterior hypothalamus of birds is involved in the manufacture of GnRH or in some other ways effects GnRH release from axon terminals in the median eminence remains to be determined.

Considerable effort has been expended attempting to determine whether the avian hypothalamus, isolated from all other regions of the brain, can support gonadal activity. Removal of the cerebral hemispheres of Japanese quail reportedly is without effect on photoperiodic testicular growth (Baylé *et al.*, 1975). Although some differences exist, most authors agree that the isolated infundibular nuclear complex is not capable of supporting gonadal activity in pigeons (Bouille *et al.*, 1973), quail (Wada, 1974b; Davies and Follett, 1975a), and ducks (Stetson and Farner, unpublished data). Davies and Follett (1975a, 1975b), in an extensive series of investigations, determined that a diffuse neural input to the ventral infundibular region from anterior and lateral directions is essential for normal gonadal function in Japanese quail. Their results suggest that the preoptic region of the hypothalamus may be the source of these fibers, but their precise function(s) in regulating gonadotropin release remains unknown. These fibers may arise from extraretinal photoreceptors and stimulate GnRH release from cells in the tuberal hypothalamus. They may arise from an endogenous pacemaker (biological clock) and transmit temporal information to other neurons. They may arise from cells that produce GnRH and transmit this hormone to the median eminence. All of these possibilities can be tested experimentally.

In summary, the release of all of the pituitary tropic hormones appears to be under stimulatory regulation by hypothalamic neurohormones. The cellular sources of some of these neurohormones are under active investigation. Other aspects of avian neuroendocrinology in need of investigation include the mode and mechanisms regulating neurohormone secretion, mechanism(s) of action of avian neurohormones on pituitary tropic cells, and the isolation and characterization of the hormones themselves.

MAMMALS

Our knowledge of hypothalamic-pituitary control mechanisms is by far most extensive in the mammals. Interested readers are directed to the many books and reviews cited below. The availability of large numbers of hypothalami of commercial mammals such as sheep, pigs, and cattle have allowed the isolation and characterization of a number of hypothalamic neurohormones (see Blackwell and Guillemin, 1973; Motta *et al.*, 1975; and Reichlin *et al.*, 1976, for review). Distribution of the hypothalamic releasing and inhibiting hormones within and without the hypothalamus also has received considerable attention (see Brownstein *et al.*, 1976; Zimmerman, 1976). Three mammalian hypothalamic hormones have been fully characterized and their chemical composition determined. All are peptides: TRH(3aa); GnRH(10aa); SRIF (14aa). The other neurohormones regulating growth hormone release (GRF), corticotropin release (CRF), and prolactin release (PIF, PRF) have not been characterized. The discovery, isolation, and characterization within the past few years of the hypothalamic tetradecapeptide somatostatin (SRIF) (see Vale *et al.*, 1975) is a major achievement in hypothalamic hormone research. This neurohormone has been shown to depress growth hormone release (hence SRIF), to depress TRH-directed TSH release, and to depress spontaneous prolactin release but not TRH-directed prolactin release in a number of mammals (see Vale *et al.*, 1975; Reichlin *et al.*, 1976). In addition, the neurohormone depresses glucagon and, to a lesser extent, insulin release from pancreatic islet cells. A somatostatin-like substance(s) has been demonstrated by immunohistofluorescence in the median eminence of several mammals, a bird, an amphibian, and in fibers directly innervating the pars distalis of a teleost (Dubois *et al.*, 1974), which suggests wide phylogenetic distribution of this hypothalamic hormone. Its function in submammalian vertebrates remains for the most part a mystery.

LITERATURE CITED

- ABEL, J. H., U. TAKEMOTO, D. HOFFMAN, T. MCNEILL, G. P. KOZLOWSKI, J. F. MASKEN, AND P. SHERIDAN. 1975. Corticoid uptake by the paraventricular nucleus in the hypothalamus of the duck, *Anas platyrhynchos*. *Cell. Tissue Res.*, 161:285-291.
- ALPERT, L. C., J. R. BRAWER, I. M. D. JACKSON, AND S. REICHLIN. 1976. Localization of LHRH in neurons in frog brain (*Rana pipiens* and *Rana catesbeiana*). *Endocrinology*, 98:910-921.
- ASSENMACHER, I. 1970. Importance de la liaison hypothalamo-hypophysaire dans le contrôle nerveux de la reproduction chez les oiseaux. Pp. 167-192, in *La photorégulation de la reproduction chez les oiseaux et les mammifères* (J. Benoit, and I. Assenmacher, eds.), C.N.R.S., Paris, xi+397 pp.
- . 1973. Reproductive endocrinology: the hypothalamo-axis. Pp. 158-191, in *Breeding biology of birds* (D. S. Farner, ed.), Nat. Acad. Sci., Washington, D.C., xi+ 515 pp.
- BAKER, B. I., AND P. M. INGLETON. 1975. Secretion of prolactin and growth hormone by teleost pituitaries *in vitro*. II. Effect of salt concentration during long-term organ culture. *J. Comp. Physiol.*, 100:269-282.

- BALAGURA, S. 1970. Neurochemical regulation of food intake. Pp. 181-193, in *The hypothalamus* (L. Martini, M. Motta, and F. Fraschini, eds.), Academic Press, New York and London, xi+705 pp.
- BALL, J. N. 1969. Prolactin and osmoregulation in teleost fishes: a review. *Gen. Comp. Endocrinol.*, suppl. 2:10-25.
- BALL, J. N., AND B. I. BAKER. 1969. The pituitary gland: anatomy and histophysiology. Pp. 1-110, in *Fish physiology* (W. S. Hoar, and D. J. Randall, eds.), Academic Press, New York, 2:xiv+1-446 pp.
- BALL, J. N., M. OLIVEREAU, AND K. D. KALLMAN. 1963. Secretion of thyrotrophic hormone by pituitary transplants in a teleost fish. *Nature*, 199:618-620.
- BALL, J. N., B. I. BAKER, M. OLIVEREAU, AND R. E. PETER. 1972. Investigations on hypothalamic control of adeno-hypophysial functions in teleost fishes. *Gen. Comp. Endocrinol.*, suppl. 3:11-21.
- BAYLÉ, J. D. 1968. Evolution post-opératoire de la corticostéronémie après hypophysectomie ou déconnexion hypothalamo-hypophysaire chez le canard. *J. Physiol. (Paris)*, 60:397-398.
- . 1969. Régulation hypothalamique de la sécrétion de la prolactine chez les oiseaux. *Arch. Anat. Microscop. Morphol. Exp.*, 58:375-386.
- . 1970. Importance respective des principaux niveaux de commande des régulations hypothalamo-thyreotropes chez les oiseaux. *J. Physiol. (Paris)*, 62:51-79.
- . 1976. Basal and stress-induced adrenal cortical activities in the chronic thalamic pigeon. *Neuroendocrinology*, 20:14-20.
- BAYLÉ, J., AND I. ASSENMACHER. 1967. Contrôle hypothalamo-hypophysaire du fonctionnement thyroïdien chez la caille. *C. R. Acad. Sci., ser. D (Paris)*, 264:125-128.
- . 1969. Les régulations hypothalamo-hypophysaires des fonctionnements corticosurénalien et thyroïdien chez la caille. *Bull. Biol.*, 103:245-260.
- BAYLÉ, J. D., AND C. BOUILLÉ. 1971. Variations de la corticostéronémie après lésions de l'hypothalamus chez le pigeon. *Ann. Endocrinol. (Paris)*, 32:265-266.
- BAYLÉ, J. D., J. BOISSIN, AND I. ASSENMACHER. 1967. Le taux plasmatique de la corticostérone liée chez l'oiseau, après hypophysectomie ou déconnexion hypothalamo-hypophysaire. *C. R. Acad. Sci., ser. D (Paris)*, 265:1524-1526.
- BAYLÉ, J. D., J. BOISSIN, J. Y. DANIEL, AND I. ASSENMACHER. 1971. Hypothalamic-hypophysial control of adrenal function in birds. *Neuroendocrinology*, 7:308-321.
- BAYLÉ, J., C. BOUILLÉ, AND J. OLIVER. 1975. Repercussions de l'hémisphérectomie neuroendocrinotens chez les oiseaux. *C. R. Acad. Sci., ser. D (Paris)*, 281:1501-1504.
- BLACKWELL, R. E., AND R. GUILLEMIN. 1973. Hypothalamic control of adeno-hypophysial secretions. *Ann. Rev. Physiol.*, 35:357-390.
- BOISSIN, J. 1967. Le contrôle hypothalamo-hypophysaire de la fonction corticosurénalienne chez le canard. *J. Physiol. (Paris)*, 59:423-444.
- BONNEY, R. C., F. J. CUNNINGHAM, AND B. J. A. FURR. 1974. Effect of synthetic luteinizing hormone releasing hormone on plasma luteinizing hormone in the female domestic fowl, *Gallus domesticus*. *J. Endocrinol.*, 63:539-547.
- BOUILLÉ, C., AND J. D. BAYLÉ. 1973a. Experimental studies on the adrenocorticotrophic area in the pigeon hypothalamus. *Neuroendocrinology*, 11:73-91.
- . 1973b. Effects of hypothalamic stimulation on pituitary-adrenocortical activity in conscious unrestrained pigeons. *Neuroendocrinology*, 12:284-294.
- . 1973/1974. Effects of limbic stimulations or lesions on basal and stress-induced hypothalamic-pituitary adrenocortical activity in the pigeon. *Neuroendocrinology*, 13:264-277.
- . 1975a. Adrenal cortical activation induced by hypothalamic stimulation and stress in pigeons bearing ectopic pituitary autografts. *Neuroendocrinology*, 18:35-41.
- . 1975b. Influence of septal nuclei on basal pituitary-adrenocortical function in birds. *Neuroendocrinology*, 18:281-289.

- BOUILLÉ, C., S. HERBUTE, AND J. D. BAYLÉ. 1973. Modulatory influences of central nervous structures on pituitary-adrenocortical activity: effect of chronic deafferentation on the hypothalamus in the pigeon. *J. Physiol. (Paris)*, 66:437-446.
- . 1975. Effects of hypothalamic deafferentation on basal and stress-induced adrenocortical activity in the pigeon. *J. Endocrinol.*, 66:413-419.
- BRETON, B., AND C. WEIL. 1973. Effets du LH/FSH-RH synthétique et d'extraits hypothalamiques de Carpe sur la sécrétion d'hormone gonadotrope *in vivo* chez la Carpe (*Cyprinus carpio* L.). *C. R. Acad. Sci., ser. D (Paris)*, 277: 2061-2064.
- BRETON, B., B. JALABERT, R. BILLARD, AND C. WEIL. 1971. Stimulation *in vitro* de la libération d'hormone gonadotrope hypophysaire par un facteur hypo-thalamique chez la Carpe (*Cyprinus carpio*). *C. R. Acad. Sci., ser D (Paris)*, 273: 2591-2594.
- BRETON, B., C. WEIL, B. JALABERT, AND R. BILLARD. 1972. Activité réciproque des facteurs hypothalamiques de Béliér (*Ovis aries*) et de poissons téléostéens sur la sécrétion *in vitro* des hormones gonadotropes c-HG et LH respectivement par des hypophyses de carpe et de Béliér. *C. R. Acad. Sci., ser D (Paris)*, 274:2530-2533.
- BRETON, B., B. JALABERT, AND C. WEIL. 1975. Caractérisation partielle d'un facteur hypothalamique de libération des hormones gonadotropes chez la Carpe (*Cyprinus carpio*). Etude *in vitro*. *Gen. Comp. Endocrinol.*, 25:405-415.
- BROMAGE, N. R. 1975. The effects of mammalian thyrotropin-releasing hormone on the pituitary axis of teleost fish. *Gen. Comp. Endocrinol.*, 25:292-297.
- BROWNSTEIN, M. J., M. PLAKOVITS, M. SAAVEDRA, AND J. S. KISER. 1976. Distribution of hypothalamic hormones and neurotransmitters within the diencephalon. Pp. 1-23, in *Frontiers in neuroendocrinology* (L. Martini and W. F. Ganong, eds.), Raven Press, New York, xii+419 pp.
- BÜCHMANN, N. B., I. SPIES, AND S. VIJAYAKUMAR. 1972. Hypophysial corticotropin function and its hypothalamic control in *Bufo bufo* (L.) evaluated by the plasma concentration of corticosterone. *Gen. Comp. Endocrinol.*, 18:306-314.
- CALAS, A., B. KERDELHUÉ, I. ASSENMACHER, AND M. JUTISZ. 1973. Les axones à LH-RH de l'émence médiane. Mise en évidence chez le Canard par une technique immunocytochimique. *C. R. Acad. Sci., ser. D (Paris)*, 277: 2765-2768.
- CALDER, W. A., AND J. R. KING. 1974. Thermal and caloric relations of birds. Pp. 260-413, in *Avian biology* (D. S. Farner and J. R. King, eds.), Academic Press, New York and London, 4:xxi+1-504 pp.
- CALLARD, G. V., S. W. C. CHAN, AND I. P. CALLARD. 1975. Negative feedback control of the lizard adrenal gland by corticosterone and aldosterone. *Gen. Comp. Endocrinol.*, 25: 387-390.
- CALLARD, I. P., AND I. CHESTER JONES. 1971. The effect of hypothalamic lesions and hypophysectomy on adrenal weight in *Sceloporus cyanogenys*. *Gen. Comp. Endocrinol.*, 17:194-202.
- CALLARD, I. P., AND J. P. DOOLITTLE. 1973. The influence of intrahypothalamic implants of progesterone on ovarian growth and function in the ovoviparous iguanid lizard, *Sceloporus cyanogenys*. *Comp. Biochem. Physiol.*, 44A:625-629.
- CALLARD, I. P., AND E. WILLARD, III. 1969. Effects of intrahypothalamic betamethazone implants on adrenal function in male *Sceloporus cyanogenys*. *Gen. Comp. Endocrinol.*, 13:460-467.
- CALLARD, I. P., C. G. BAYNE, AND W. F. MCCONNELL. 1972. Hormones and reproduction in the female lizard *Sceloporus cyanogenys*. *Gen. Comp. Endocrinol.*, 18:175-194.
- CASEY, J. M., J. J. REEVES, P. C. HARRISON, AND R. P. PETERSON. 1971. Dual bio-radio-immuno detection of LH-RH like activity in hypothalamic tissue of *Coturnix* quail. *Poultry Sci.*, 50:1562-1563.
- COMPHER, M. K., AND J. DENT. 1973. Effects of hypothalamic extirpations on thyroidal and intermedial activity in the red spotted newt. *Gen. Comp. Endocrinol.*, 20:29-40.
- CRIM, L. W., R. E. PETER, AND R. BILLARD. 1976. Stimulation of gonadotropin secretion by intraventricular injection of hypothalamic extracts in the goldfish, *Carassius auratus*. *Gen. Comp. Endocrinol.*, 30:77-82.

- CROIX, D., J. C. HENDRICK, J. BALTHAZART, AND P. FRANCHIMONT. 1974. Dosage radio-immunologique de l'hormone folliculo-stimulante (FSH) hypophysaire de Canard à l'aide d'un système de mammifères. C. R. Soc. Biol., 168:136-140.
- DANIEL, J. Y., AND I. ASSENMACHER. 1969. Dosage de la corticostérone et de l'aldostérone dans la surrénale du Canard, après hypophysectomie ou autogreffe hypophysaire. C. R. Acad. Sci., Ser. D (Paris), 269:1308-1311.
- DANIEL, P. M., AND M. M. L. PRICHARD. 1975. Studies of the hypothalamus and the pituitary gland. Acta Endocrinol., suppl., 201:1-216.
- DAUGHERTY, D. R., AND I. P. CALLARD. 1972. Plasma corticosterone in the male iguanid lizard *Sceloporus cyanogenys* under various physiological conditions. Gen. Comp. Endocrinol., 19:69-79.
- DAVIES, D. T., AND B. K. FOLLETT. 1975a. The neuroendocrine control of gonadotropin release in the Japanese quail. I. The role of the tuberal hypothalamus. Proc. Roy. Soc. London, ser. B, 191:285-301.
- . 1975b. The neuroendocrine control of gonadotrophin release in the Japanese quail. II. The role of the anterior hypothalamus. Proc. Roy. Soc. London, ser. B., 191: 303-315.
- . 1975c. Electrical stimulation of the hypothalamus and luteinizing hormone secretion in Japanese quail. J. Endocrinol., 67:431-438.
- DEERY, D. J. 1974. Determination by radioimmunoassay of the luteinizing hormone-releasing hormone (LHRH) content of the hypothalamus of the rat and some lower vertebrates. Gen. Comp. Endocrinol., 24:280-285.
- . 1975. Effect of catecholamines and synthetic mammalian hypothalamic hormones on the adenylyl cyclase activity of the pituitary of the teleost, *Carassius auratus*. Gen. Comp. Endocrinol., 25:295-299.
- DEERY, D. J., AND A. C. JONES. 1975. Effects of hypothalamic extracts, neurotransmitters and synthetic hypothalamic releasing hormones on adenylyl cyclase activity in the lobes of the pituitary of the dogfish (*Scyliorhinus canicula* L.). J. Endocrinol., 64:49-57.
- DE ROOIJ, J. A. M., AND O. R. HOMMES. 1974. The tubero-infundibular region in man: structure-monoamines-karyometrics. Pp. 79-96, in Integrative hypothalamic activity (D. F. Swaab and J. P. Schade, eds.), Elsevier Sci. Pub., Amsterdam and New York, xi+516 pp.
- DIERICKX, K. 1964. The nerve fibers controlling the gonadotropic activity of the hypophysis of *Rana temporaria*. Z. Zellforsch. Mikrosk. Anat., 63:938-949.
- . 1965. The origin of the aldehyde-Fuchsin-negative nerve fibers of the median of the hypophysis: a gonadotrophic centre. Z. Zellforsch. Mikrosk. Anat., 66:504-518.
- . 1966. Experimental identification of a hypothalamic gonadotropic centre. Z. Zellforsch. Mikrosk. Anat., 74:53-79.
- . 1967a. The gonadotropic centre of the tuber cinereum hypothalami and ovulation. Z. Zellforsch. Mikrosk. Anat., 77:188-203.
- . 1967b. The function of the hypophysis without preoptic neurosecretory control. Z. Zellforsch. Mikrosk. Anat., 78:114-130.
- DIERICKX, K., AND N. GOOSSENS. 1970. The residual adrenocorticotrophic activity of the hypophysis after its neural disconnection. Z. Zellforsch. Mikrosk. Anat., 106:371-375.
- DIERICKX, K., A. DRYTS, M. P. VANDENBERGHE, AND N. GOOSSENS. 1972. Identification of adeno-hypophysiotropic neurohormone producing neurosecretory cells in *Rana temporaria*. I. Ultrastructural evidence for the presence of neurosecretory cells in the tuber cinereum. Z. Zellforsch. Mikrosk. Anat., 134:459-504.
- DIERICKX, K., N. GOOSSENS, AND M. P. VANDENBERGHE. 1973a. Identification of adeno-hypophysiotropic neurohormone producing neurosecretory cells in *Rana temporaria*. III. The tubero-hypophysial monoaminergic fibers and the role of the tubero-hypophysial neurosecretory system. Z. Zellforsch. Mikrosk. Anat., 143:93-106.
- DIERICKX, K., M. P. VANDENBERGHE, AND N. GOOSSENS. 1973b. Identification of adeno-hypophysiotropic neurohormone producing neurosecretory cells in *Rana temporaria*. II.

- Identification, in the median eminence, of the terminal arborizations of axons of the tubero-hypophysial neurosecretory system. *Z. Zellforsch. Mikrosk. Anat.*, 142:479-513.
- DODD, J. M. 1972a. Ovarian control in cyclostomes and elasmobranchs. *Amer. Zool.*, 12: 325-339.
- . 1972b. The endocrine regulation of gametogenesis and gonad maturation in fishes. *Gen. Comp. Endocrinol.*, suppl. 3:675-687.
- . 1975. The hormones of sex and reproduction and their effects in fish and lower chordates: twenty years on. *Amer. Zool.*, 15(suppl. 1):137-171.
- DODD, J. M., P. J. EVENNETT, AND C. K. GODDARD. 1960. Reproductive endocrinology in cyclostomes and elasmobranchs. *Symp. Zool. Soc. London*, 1:77-103.
- DODD, J. M., B. K. FOLLETT, AND P. J. SHARP. 1971. Hypothalamic control of pituitary function in submammalian vertebrates. *Adv. Comp. Physiol. Biochem.*, 4:113-223.
- DUBOIS, M. P., J. BARRY, AND J. LEONARDELLI. 1974. Mise en évidence par immunofluorescence et repartition de la somatostatine (SRIF) dans l'eminence mediane des vertebres (mammifères, oiseaux, amphibiens, poissons). *C. R. Acad. Sci., ser D (Paris)*, 279:1899-1902.
- EGGE, A. S., AND R. B. CHIASSON. 1963. Endocrine effects of diencephalic lesions in the white leghorn hen. *Gen. Comp. Endocrinol.*, 3:346-361.
- EGGE, A. S., W. J. RADKE, AND R. B. CHIASSON. 1975. The effect of hypothalamic lesions on thyroid-stimulating hormone concentration in the chicken pituitary. *Poultry Sci.*, 54:1628-1630.
- ENSOR, D. M., AND J. N. BALL. 1972. Prolactin and osmoregulation in fishes. *Fed. Proc.*, 31:1615-1623.
- EPPLE, A., AND M. H. STETSON. 1976. Einige endokrinologische Besonderheiten der Vogel. *J. Ornithol.*, 117:257-278.
- EPSTEIN, A. N., H. R. KISSILEFF, AND E. STELLAR. 1973. The neuropsychology of thirst: new findings and advances in concepts. Wiley, New York, xv+354 pp.
- ERICKSON, J. E. 1975. Hypothalamic gonadotropin releasing hormone and the photoperiodic control of the testis in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. Unpublished Ph.D. dissertation, Univ. Washington, 247 pp.
- ETKIN, W., AND A. G. GONA. 1968. Failure of mammalian thyrotropin releasing factor preparation to elicit metamorphic responses in tadpoles. *Endocrinology*, 82: 1067-1068.
- ETKIN, W., A. DERBY, AND A. G. GONA. 1969. Prolactin-like antithyroid action of pituitary grafts in tadpoles. *Gen. Comp. Endocrinol.*, suppl. 2:253-259.
- EVENNETT, P. J., AND J. M. DODD. 1963. Endocrinology of reproduction in the river lamprey. *Nature*, 197:715-716.
- FLAKMER, S., AND A. J. MATTY. 1966. Blood sugar regulation in the hagfish, *Myxine glutinosa*. *Gen. Comp. Endocrinol.*, 6:334-346.
- FERNHOLM, B. 1972. Neurohypophysial-adenohypophysial relations in hagfish (*Mixinoidea, Cyclostomata*). *Gen. Comp. Endocrinol.*, suppl. 3:1-10.
- FIRTH, A. J., AND L. VOLLRATH. 1973. Determination of the distribution of luteinizing hormone-like activity within the dogfish pituitary gland by means of the *Xenopus* oocyte meiosis assay. *J. Endocrinol.*, 58:347-348.
- FLOSS, H. G., J. M. CASSADY, AND J. E. ROBBERS. 1973. Influence of ergot alkaloids on pituitary prolactin and prolactin-dependent processes. *J. Pharmaceutical Sci.*, 62:699-715.
- FOLLETT, B. K. 1970. Gonadotropin-releasing activity in the quail hypothalamus. *Gen. Comp. Endocrinol.*, 15:165-179.
- . 1973. The neuroendocrine regulation of gonadotropin secretion in avian reproduction. Pp. 209-243, in *Breed-ing biology of birds* (D. S. Farner, ed.), Nat. Acad. Sci., Washington, D.C., xi+515 pp.
- . 1976. Plasma follicle-stimulating hormone during photoperiodically induced sexual maturation in male Japanese quail. *J. Endocrinol.*, 69:117-126.

- FOLLETT, B. K., C. G. SCANES, AND F. J. CUNNINGHAM. 1972. A radioimmunoassay for avian luteinizing hormone. *J. Endocrinol.*, 52:359-378.
- FRANKEL, A. I., J. W. GRABER, AND A. V. NALBANDOV. 1967a. The effect of hypothalamic lesions on adrenal function in intact and adenohipophysectomized cockerels. *Gen. Comp. Endocrinol.*, 8:387-396.
- . 1967b. Adrenal function in cockerels. *Endocrinology*, 80:1013-1019.
- FURR, B. J. A., G. I. ONVORO, R. C. BONNEY, AND F. J. CUNNINGHAM. 1973. The effect of synthetic hypothalamic releasing factors on plasma levels of luteinizing hormone in the cockerel. *J. Endocrinol.*, 59:495-502.
- GOGAN, F. 1968. Sensibilité hypothalamique à la testostérone chez le Canard. *Gen. Comp. Endocrinol.*, 11:316-327.
- GOOS, H. J. TH., P. J. M. LIGTENBERG, AND P. G. W. J. OORDT. 1976. Immunofluorescence studies on gonadotropin releasing hormone (GRH) in the fore-brain and the neurohypophysis of the green frog, *Rana esculenta* L. *Cell. Tissue Res.*, 168:325-333.
- GORBMAN, A. 1965. Vascular relations between neurohypophysis and adenohipophysis of cyclostomes and the problem of evolution of hypothalamic neuroendocrine control. *Arch. Anat. Microsc. Morphol. Exp.*, 54:163-194.
- . 1973. Comments on "Neuroendocrinology of teleosts." *Amer. Zool.*, 13:757-758.
- GORBMAN, A., AND M. HYDER. 1973. Failure of mammalian TRH to stimulate thyroid function in the lungfish. *Gen. Comp. Endocrinol.*, 20:588-589.
- GORBMAN, A., AND K. TSUNEKI. 1975. A technique for hypophysectomy of the Pacific hagfish: first observations. *Gen. Comp. Endocrinol.*, 26:420-422.
- GORBMAN, A., H. KOBAYASHI, AND H. UEMURA. 1963. The vascularization of the hypophysial structures of the hagfish. *Gen. Comp. Endocrinol.*, 3:505-514.
- GOUDJI, D. 1970. Prolactine et relations photosexuelles chez les oiseaux. Pp. 233-257, in *La photoregulation de la reproduction chez les oiseaux et les mammifères* (J. Benoit and I. Assenmacher, eds.), C.N.R.S., Paris, xi+397 pp.
- GOUDJI, D., AND A. TIXIER-VIDAL. 1966. Mise en évidence d'un contrôle hypothalamique stimulant de la prolactine hypophysaire chez le canard. *C. R. Acad. Sci., ser. D (Paris)*, 263:162-165.
- GRABER, J. W., A. I. FRANKEL, AND A. V. NALBANDOV. 1967. Hypothalamic center influencing the release of LH in the cockerel. *Gen. Comp. Endocrinol.*, 9:187-192.
- GRAU, E. G., AND M. H. STETSON. 1977. Pituitary autotransplants in *Fundulus heteroclitus*: effect on thyroid function. *Gen. Comp. Endocrinol.*, 32:427-431.
- HARDISTY, M. W. 1972. Quantitative and experimental studies on the interrenal tissues of the upstream migrant stage of the river lamprey, *Lampetra fluviatilis* L. *Gen. Comp. Endocrinol.*, 18:501-504.
- HARRISON, P. C., J. M. CASEY, R. L. ADAIR, AND J. J. REEVES. 1974. Fluctuation of hypothalamic luteinizing hormone releasing hormone and pituitary gonadotropins in laying and non-laying hens. *Poultry Sci.*, 53:554-559.
- HENDERSON, N. E. 1976. Thyroxine concentrations in plasma of normal and hypophysectomized hagfish, *Myxine glutinosa* (Cyclostomata). *Canadian J. Zool.*, 54:180-184.
- HENDERSON, N. E., AND F. L. LORSCHIEDER. 1975. Thyroxine and proteinbound iodine concentrations in plasma of the Pacific hagfish, *Eptatretus stouti* (Cyclostomata). *Comp. Biochem. Physiol.*, 51A:723-726.
- HIGGINS, K. M., AND J. N. BALL. 1970. Investigations on the hypothalamic control of thyroid-stimulating hormone secretion in the teleost *Poecilia latipinna*. *J. Endocrinol.*, 48:xxix.
- HOLMES, R. L., AND J. N. BALL. 1974. The pituitary gland: a comparative account. Cambridge Univer. Press, London and New York, x+397 pp.
- HOLMES, W. N., M. Y. CHAN, J. S. PORADLEY, AND I. M. STAINER. 1970. Salt regulation in aquatic birds with special reference to the control of pituitary and adrenal function. *Mem. Soc. Endocrinol.*, 18:87-110.
- JACKSON, G. L. 1971a. Avian luteinizing hormone-releasing factor. *Endocrinology*, 89:1454-1459.

- . 1971b. Comparison of rat and chicken luteinizing hormone-releasing factors. *Endocrinology*, 89:1460-1463.
- . 1972. Partial purification and characterization of chicken and rat follicle stimulating hormone-releasing factors. *Endocrinology*, 91:1090-1094.
- JACKSON, G. L., AND A. V. NALBANDOV. 1969. Luteinizing hormone releasing activity in the chicken hypothalamus. *Endocrinology*, 84:1262-1265.
- JACKSON, I. M. D., AND S. REICHLIN. 1974. Thyrotropin-releasing hormone (TRH): distribution in hypothalamic and extra-hypothalamic brain tissues of mammalian and sub-mammalian chordates. *Endocrinology*, 95:854-862.
- JACKSON, I. M. D., R. SAPERSTEIN, AND S. REICHLIN. 1977. Thyrotropin releasing hormone (TRH) in pineal and hypothalamus of the frog: effect of season and illumination. *Endocrinology*, 100:97-100.
- JACKSON, R. G., AND M. SAGE. 1973. Regional distribution of thyroid stimulating hormone activity in the pituitary gland of the Atlantic stingray, *Dasyatis sabina*. *Fishery Bull., Fish Wildlife Serv., U.S. Dept. Interior*, 71:93-97.
- JASINSKI, A. 1969. Vascularization of the hypophyseal region in lower vertebrates (cyclostomes and fishes). *Gen. Comp. Endocrinol.*, 2:510-521.
- JASINSKI, A., AND A. GORBMAN. 1966. Hypothalamo-hypophyseal vascular and neurosecretory links in ratfish, *Hydrolagus collei* (Lay and Bennett). *Gen. Comp. Endocrinol.*, 6: 476-490.
- JØRGENSEN, C. B. 1968. Central nervous control of adenohypophyseal functions. Pp. 469-541, in *Perspectives in endocrinology* (E. J. W. Barrington and C. B. Jørgensen, eds.), Academic Press, New York and London, xvi+583 pp.
- . 1970. Hypothalamic control of hypophyseal function in anurans. Pp. 649-661, in *The hypothalamus* (L. Martini, M. Motta, and F. Fraschini, eds.), Academic Press, New York and London, xi+705 pp.
- . 1974. Integrative functions of the brain. Pp. 1-51, in *Physiology of the Amphibia* (B. Lofts, ed.), Academic Press, New York and London, 2:xi+1-592 pp.
- KANEMATSU, S., AND S. I. MIKAMI. 1970. Effects of hypothalamic lesions on protein-bound ¹³¹Iodine and thyroidal ¹³¹I uptake in the chicken. *Gen. Comp. Endocrinol.*, 14:25-34.
- KAUL, S., AND L. VOLLRATH. 1974a. The goldfish pituitary: II. Innervation. *Cell Tissue Res.*, 154:231-249.
- . 1974b. The goldfish pituitary: I. Cytology. *Cell Tissue Res.*, 154:211-230.
- KNIGHT, P. J., AND A. CHADWICK. 1975. The effect of *in vivo* administration of chicken hypothalamic extract on the crop sac and the pituitary gland of the pigeon. *Gen. Comp. Endocrinol.*, 27:488-494.
- KNOWLES, G. 1965. Evidence for dual control, by neurosecretion, of hormone synthesis and hormone release in the pituitary of the dogfish, *Scyliorhinus stellaris*. *Phil. Trans. Roy. Soc. London, ser. B*, 249:435-456.
- KNOWLES, F., AND L. VOLLRATH. 1965. A dual neurosecretory innervation of the pars distalis of the pituitary of the eel. *Nature*, 208:1345.
- KOBAYASHI, H., AND M. WADA. 1973. Neuroendocrinology in birds. Pp. 287-347, in *Avian biology* (D. S. Farner, J. King, and K. C. Parkes, eds.), Academic Press, New York and London, 3:xix+573 pp.
- KOBAYASHI, H., T. MATSUI, AND S. ISHII. 1970. Functional electron microscopy of the hypothalamic median eminence. *Int. Rev. Cytol.*, 29:282-381.
- KRAGT, C. L., AND J. MEITES. 1965. Stimulation of pigeon pituitary prolactin release by pigeon hypothalamic extract *in vitro*. *Endocrinology*, 76:1169-1176.
- KRAMER, K. L., M. P. SCHREIBMAN, AND P. K. T. PANG. 1973. The effect of 2-Br- α -ergocryptine-methane sulfonate on freshwater survival and pituitary cytology in the teleost, *Fundulus heteroclitus*. *Amer. Zool.*, 13:1279.
- KUHN, E. R., AND H. ENGELN. 1976. Seasonal variation in prolactin and TSH releasing activity in the hypothalamus of *Rana temporaria*. *Gen. Comp. Endocrinol.*, 28: 277-282.

- LAM, T. J., S. PANDEY, AND W. S. HOAR. 1975. Induction of ovulation in goldfish by synthetic luteinizing hormone-releasing hormone (LH-RH). *Canadian J. Zool.*, 53: 1189-1192.
- LAM, T. J., S. PANDEY, Y. NAGAHAMA, AND W. S. HOAR. 1976. Effect of synthetic luteinizing hormone-releasing hormone (LH-RH) on ovulation and pituitary cytology of the goldfish, *Carassius auratus*. *Canadian J. Zool.*, 54:816-824.
- LAMMERS, H. J., AND A. H. LOHMAN. 1974. Structure and fiber connections of the hypothalamus in mammals. Pp. 61-78, in *Integrative hypothalamic activity* (D. F. Swaab and J. P. Schade, eds.), Elsevier Scientific Publishing Co., Amsterdam, Oxford, and New York, xi+705 pp.
- LARSEN, L. O. 1965. Effects of hypophysectomy in a cyclostome, *Lampetra fluviatilis* (L.) Gray. *Gen. Comp. Endocrinol.*, 5:16-30.
- . 1969. Hypophysial functions in river lampreys. *Gen. Comp. Endocrinol.*, suppl. 2:522-527.
- LARSEN, L. O., AND P. ROSENKILDE. 1971. Iodine metabolism in normal, hypophysectomized and thyrotropin-treated river lampreys, *Lampetra fluviatilis* (Gray) L. (Cyclostomata). *Gen. Comp. Endocrinol.*, 17:94-104.
- LAUB, J. M., G. V. CALLARD, AND I. P. CALLARD. 1975. The role of adrenal steroids in the negative feedback control of the amphibian adrenal gland. *Gen. Comp. Endocrinol.*, 25:425-431.
- LISK, R. D. 1967. Neural control of gonad size by hormone feedback in the desert iguana *Dipsosaurus dorsalis dorsalis*. *Gen. Comp. Endocrinol.*, 8:258-266.
- MATTY, A. J., K. TSUNEKI, W. W. DICKHOFF, AND A. GORBMAN. 1976. Thyroid and gonadal function in hypophysectomized hagfish, *Eptatretus stouti*. *Gen. Comp. Endocrinol.*, 30:500-516.
- McFARLAND, L. Z., M. K. YOUSEF, AND W. O. WILSON. 1966. The influence of ambient temperature and hypothalamic lesions on the disappearance rates of thyroxine ¹³¹I in the Japanese quail. *Life Sci.*, 5:309-315.
- McKEOWN, B. A. 1972a. Effect of 2-Br- α -ergocryptine on fresh water survival in the teleosts, *Xiphophorus hellerii* and *Poecilia latipinna*. *Experientia*, 28:675-676.
- . 1972b. Prolactin and growth hormone concentrations in the plasma of the toad *Bufo bufo* (L.) following ectopic transplantation of the pars distalis. *Gen. Comp. Endocrinol.*, 19:167-174.
- McNEILL, T. H., G. P. KOZLOWSKI, J. H. ABEL, AND E. A. ZIMMERMAN. 1976. Neurosecretory pathways in the mallard duck (*Anas platyrhynchos*) brain: localization by aldehyde fuchsin and immunoperoxidase techniques for neurophysin (NP) and gonadotrophin releasing hormone (Gn-Rh). *Endocrinology*, 99:1323-1332.
- MEURLING, P. 1960. Presence of a portal system in elasmobranchs. *Nature*, 187:336-337.
- . 1967. The vascularization of the pituitary in elasmobranchs. *Sarsia*, 28:1-104.
- MOTTA, M., P. G. CROSIGNANI, AND L. MARTINI, EDS. 1975. *Hypothalamic hormones*. Academic Press, London, New York and San Francisco, 352 pp.
- MULLER, E. E., S. SAWANO, AND A. V. SCHALLY. 1967. Growth hormone-releasing activity in the hypothalamus of animals of different species. *Gen. Comp. Endocrinol.*, 9:349-352.
- MYERS, R. D. 1969. Temperature regulation: neurochemical systems in the hypothalamus. Pp. 506-523, in *The hypothalamus* (W. Haymaker, E. Anderson, and W. J. H. Nauta, eds.), Thomas, Springfield, Illinois, xiv+805 pp.
- NAGAHAMA, Y., R. S. NISHIOKA, AND H. A. BERN. 1974. Structure and function of the transplanted pituitary in the seawater goby, *Gillichthys mirabilis*. I. The rostral pars distalis. *Gen. Comp. Endocrinol.*, 22:21-34.
- NAGAHAMA, Y., R. S. NISHIOKA, H. A. BERN, AND R. L. GUNTHER. 1975. Control of prolactin secretion in teleosts, with special reference to *Gillichthys mirabilis* and *Tilapia mossambica*. *Gen. Comp. Endocrinol.*, 25:166-168.
- NAUTA, W. J., AND W. HAYMAKER. 1969. Hypothalamic nuclei and fiber connections. Pp. 136-209, in *The hypothalamus* (W. Haymaker, E. Anderson, and W. J. Nauta, eds.) Thomas, Springfield, Illinois, xiv+805 pp.

- NICOLL, C. S. 1965. Neural regulation of adenohipophysial prolactin secretion in tetrapods: indications from *in vitro* studies. *J. Exp. Zool.*, 158:203-210.
- NOVIN, D., W. WYRWICKA, AND G. A. BRAY. 1976. Hunger: basic mechanisms and clinical implications. Raven Press, New York, xv+494 pp.
- OKSCHE, A., AND D. S. FARNER. 1974. Neurohistological studies of the hypothalamo-hipophysial system of *Zonotrichia leucophrys gambelii*. With special attention to its role in the control of reproduction. *Adv. Anat. Embryol. Cell Biol.*, 48:1-136.
- OLIVEREAU, M. 1975. Dopamine, prolactin control and osmoregulation in eels. *Gen. Comp. Endocrinol.*, 26:550-561.
- OLIVEREAU, M., AND A. LEMOINE. 1973a. Endocrinologie comparée. Action de la l-dopa sur la sécrétion de prolactine chez l'anguille. *C. R. Acad. Sci., ser. D (Paris)*, 276:1325-1327.
- . 1973b. Endocrinologie comparée. Effet de la 2-Br- α -ergocryptine (CB 154) sur la sécrétion de prolactine chez l'anguille. *C. R. Acad. Sci., ser. D (Paris)*, 276:1883-1886.
- OPEL, H., AND P. D. LEPORE. 1972. *In vivo* studies of luteinizing hormone-releasing factor in the chicken hypothalamus. *Poultry Sci.*, 51:1004-1014.
- PÉCZELY, P. 1972a. Corticotropic and ADH system in the hypothalamus of the pigeon. *Acta Physiol. Acad. Sci. Hungaricae*, 41:37-47.
- . 1972b. Effect of ether stress on CRF-ACTH system of the domestic pigeon. *Acta Biol. Acad. Sci. Hungaricae*, 23:23-29.
- PÉCZELY, P., AND G. ZBORAY. 1967. CRF and ACTH activity in the median eminence of the pigeon. *Acta Physiol. Acad. Sci. Hungaricae*, 32:229-239.
- PÉCZELY, P., J. D. BAYLÉ, J. BOISSIN, AND I. ASSENMACHER. 1970. Activités corticotrope et CRF dans l'émience médiane, et activité corticotrope de greffes hypophysaires chez le pigeon. *C. R. Acad. Sci., ser. D (Paris)*, 270:3264-3267.
- PETER, R. E. 1970. Hypothalamic control of thyroid gland activity in the goldfish, *Carassius auratus*. *Gen. Comp. Endocrinol.*, 14:334-356.
- . 1972. Feedback effects on thyroxin in goldfish, *Carassius auratus*, with an autotransplanted pituitary. *Neuroendocrinology*, 17:273-281.
- . 1973. Neuroendocrinology of teleosts. *Amer. Zool.*, 13:743-755.
- PETER, R. E., AND B. A. MCKEOWN. 1974. Effects of hypothalamic and thalamic lesions on prolactin secretion in goldfish (*Carassius auratus*). *Gen. Comp. Endocrinol.*, 23:438-452.
- . 1975. Hypothalamic control of prolactin and thyrotropin secretion in teleosts, with special reference to recent studies on the goldfish. *Gen. Comp. Endocrinol.*, 25:153-165.
- PICKERING, A. D. 1972. Effects of hypophysectomy on the activity of the endostyle and thyroid gland in the larval and adult river lamprey, *Lampetra fluviatilis* L. *Gen. Comp. Endocrinol.*, 18:335-343.
- . 1976. Iodide uptake by the isolated thyroid gland of the river lamprey, *Lampetra fluviatilis* (L.). *Gen. Comp. Endocrinol.*, 28:358-364.
- PLATT, J. E. 1976. The effects of ergocornine on tail height, spontaneous and T4-induced metamorphosis and thyroidal uptake of radioiodide in neotenic *Ambystoma tigrinum*. *Gen. Comp. Endocrinol.*, 28:71-81.
- RAISMAN, G. 1970. Some aspects of the neural connections of the hypothalamus. Pp. 1-15, in *The hypothalamus* (L. Martini, M. Motta, and F. Fraschini, eds.), Academic Press, New York and London, xi+705 pp.
- RAISMAN, G., AND P. M. FIELD. 1971. Anatomical considerations relevant to the interpretation of neuroendocrine experiments. Pp. 45-66, in *Frontiers in neuroendocrinology* (L. Martini and W. F. Ganong, eds.), Oxford Univ. Press, London, xii+419 pp.
- RAVONA, H., N. SNAPIR, AND M. PEREK. 1973a. The effect on the gonadal axis in cockerels of electrolytic lesions in various regions of the basal hypothalamus. *Gen. Comp. Endocrinol.*, 20:112-124.

- . 1973b. Histological changes of the pituitary gland in cockerels bearing basal hypothalamic lesions and demonstration of its anti-HCG immunofluorescence reacting cells. *Gen. Comp. Endocrinol.*, 20:490-497.
- REDGATE, E. S. 1974. Neural control of pituitary adrenal activity in *Cyprinus carpio*. *Gen. Comp. Endocrinol.*, 22:35-41.
- REEVES, J. J., P. C. HARRISON, AND J. M. CASEX. 1973. Ovarian development and ovulation in hens treated with synthetic (Porcine) luteinizing hormone releasing hormone follicle stimulating hormone releasing hormone (LH-RH/FSH-RH). *Poultry Sci.*, 52:1883-1886.
- REICHLIN, S., R. SAPERSTEIN, I. M. D. JACKSON, A. E. BOYD, III, AND Y. PATEL. 1976. Hypothalamic hormones. *Ann. Rev. Physiol.*, 38:389-424.
- RESKO, J. A., H. W. NORTON, AND A. V. NALBANDOV. 1964. Endocrine control of the adrenal in chickens. *Endocrinology*, 75:192-200.
- REVIERS, M. DE, AND M. P. DUBOIS. 1974. Binding of synthetic LHRF antibodies in the median eminence of the cockerel. *Horm. Metabol. Res.*, 6:94.
- ROSENBERG, L. L., H. ASTIER, G. LA ROCHE, J. D. BAYLÉ, A. TIXIER-VIDAL, AND I. ASSENMACHER. 1967. The thyroid function of the drake after hypophysectomy or hypothalamic pituitary disconnection. *Neuroendocrinology*, 2:113-125.
- ROSENKILDE, P. 1972. Hypothalamic control of thyroid function in Amphibia. *Gen. Comp. Endocrinol.*, suppl. 3:32-40.
- SAGE, M. 1968. Responses to steroids of *Xiphophorus* ACTH cells in organ culture. *Z. Zellforsch. Mikrosk. Anat.*, 92:34-42.
- SAGE, M., AND R. J. PURBOTT. 1969. The control of teleost ACTH cells. *Z. vgl. Physiol.*, 63:85-90.
- SAINT GIRON, H. 1970. The pituitary gland. Pp. 135-199, in *Biology of the Reptilia* (C. Gans and T. S. Parsons, eds.), Academic Press, New York and London, 3:xiv +1-385 pp.
- SALEM, M. H. M., H. W. NORTON, AND A. V. NALBANDOV. 1970a. A study of ACTH and CRF in chickens. *Gen. Comp. Endocrinol.*, 14:281-289.
- . 1970b. The role of vasotocin and of CRF in ACTH release in the chicken. *Gen. Comp. Endocrinol.*, 14:281-289.
- SATO, T., AND J. C. GEORGE. 1973a. Evidence for the existence of a corticotropin-releasing factor in the pigeon hypothalamus. *Canadian J. Physiol. Pharmacol.*, 51:737-742.
- . 1973b. Diurnal rhythm of corticotropin-releasing factor activity in the pigeon hypothalamus. *Canadian J. Physiol. Pharmacol.*, 51:743-747.
- SCANES, C. G., S. DOBSON, B. K. FOLLETT, AND J. M. DODD. 1972. Gonadotrophic activity in the pituitary gland of the dogfish (*Scyliorhinus canicula*). *J. Endocrinol.*, 54:343-344.
- SHARP, P. J., AND B. K. FOLLETT. 1969a. The effect of hypothalamic lesions on gonadotrophin release in Japanese quail (*Coturnix coturnix japonica*). *Neuroendocrinology*, 5:205-218.
- . 1969b. The blood supply to the pituitary and basal hypothalamus in the Japanese quail (*Coturnix coturnix japonica*). *J. Anat.*, 104:227-232.
- SHARP, P. J., E. HAASE, AND H. M. FRASER. 1975. Immunofluorescent localization of sites binding anti-synthetic LHRH serum in the median eminence of the Greenfinch (*Chloris chloris* L.). *Cell Tissue Res.*, 162:83-91.
- SMITH, P. M., AND B. K. FOLLETT. 1972. Luteinizing hormone releasing factor in the quail hypothalamus. *J. Endocrinol.*, 53:131-138.
- STAINER, I. M., AND W. N. HOLMES. 1969. Some evidence for the presence of a corticotropin-releasing factor (CRF) in the duck (*Anas platyrhynchos*). *Gen. Comp. Endocrinol.*, 12:350-359.
- STETSON, M. H. 1969. The role of the median eminence in control of photoperiodically induced testicular growth in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Z. Zellforsch. Mikrosk. Anat.*, 93:369-394.
- . 1972a. Hypothalamic regulation of testicular function in Japanese quail. *Z. Zellforsch. Mikrosk. Anat.*, 130:389-410.

- . 1972b. Hypothalamic regulation of gonadotropin release in female Japanese quail. *Z. Zellforsch. Mikrosk. Anat.*, 130:411-428.
- . 1972c. Feedback regulation of testicular function in Japanese quail: testosterone implants in the hypothalamus and adenohipophysis. *Gen. Comp. Endocrinol.*, 19:37-47.
- . 1972d. Feedback regulation by oestradiol of ovarian function in Japanese quail. *J. Reprod. Fert.*, 31:205-213.
- . 1973. Recovery of gonadal function following hypothalamic lesions in Japanese quail. *Gen. Comp. Endocrinol.*, 20:69-75.
- STEVENSON, J. A. F. 1969. Neural control of food and water intake. Pp. 524-621, in *The hypothalamus* (W. Haymaker, E. Anderson, and W. J. H. Nauta, eds.), Thomas, Springfield, Illinois, xiv+805 pp.
- TANAKA, K., AND M. KAMIYOSHI. 1976. Induction of ovulation in hens by an analogue of LH-RH. *J. Reprod. Fert.*, 46:243-244.
- TANAKA, K., M. KAMIYOSHI, AND M. TAGAMI. 1969. *In vitro* demonstration of luteinizing hormone releasing activity in the hypothalamus of the hen. *Poultry Sci.*, 48:1985-1987.
- TANAKA, K., M. KAMIYOSHI, AND M. SAKAIDA. 1974a. Effects of progesterone on the hypothalamic gonadotropin-releasing activity and on the pituitary gonadotropic activity in hens and cocks. *Poultry Sci.*, 52:1772-1776.
- . 1974b. Changes in gonadotropin-releasing activity of the hypothalamus during an ovulatory cycle of the hen. *Poultry Sci.*, 53:1555-1560.
- TAUROG, A., C. OLIVER, R. L. ESKAY, J. C. PORTER, AND J. M. MCKENZIE. 1974. The role of TRH in the neoteny of the Mexican axolotl (*Ambystoma mexicanum*). *Gen. Comp. Endocrinol.*, 24:267-279.
- THORNTON, V. F., AND I. I. GESCHWIND. 1974. Hypothalamic control of gonadotropin release in Amphibia: evidence from studies of gonadotropin release *in vitro* and *in vivo*. *Gen. Comp. Endocrinol.*, 23:294-301.
- TIXIER-VIDAL, A., AND D. GOURDJ. 1972. Cellular aspects of the control of prolactin secretion in birds. *Gen. Comp. Endocrinol.*, suppl. 3:51-64.
- TSUNEKI, K., AND B. FERNHOLM. 1975. Effect of thyrotropin-releasing hormone on the thyroid of a teleost, *Chasmichthys dolichognathus*, and a hagfish, *Eptatretus burgeri*. *Acta Zool.*, 56:61-65.
- VALE, W., P. BRAZEAU, C. RIVIER, M. BROWN, B. BOSS, J. RIVIER, R. BURGUS, N. LING, AND R. GUILLEMIN. 1975. Somatostatin. *Rec. Progr. Hormone Res.*, 31:365-392.
- VANDESANDE, F., AND M. ASPESLAGH. 1974. Failure of thyrotropin releasing hormone to increase ¹²⁵I uptake by the thyroid in *Rana temporaria*. *Gen. Comp. Endocrinol.*, 23:355-356.
- VIJAYAKUMAR, S., C. B. JØRGENSEN, AND K. KJÆR. 1971. Regulation of ovarian cycle in the toad, *Bufo bufo bufo* (L.): effects of autografting pars distalis of the hypophysis, of extirpating gonadotropic hypothalamic region, and of partial ovariectomy. *Gen. Comp. Endocrinol.*, 17:432-443.
- VITUMS, A., S. I. MIKAMI, A. OKSCHE, AND D. S. FARNER. 1964. Vascularization of the hypothalamo-hypophyseal complex in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Z. Zellforsch. Mikrosk. Anat.*, 64:541-569.
- WADA, M. 1972. Effect of hypothalamic implantation of testosterone on photostimulated testicular growth in Japanese quail (*Coturnix coturnix japonica*). *Z. Zellforsch. Mikrosk. Anat.*, 124:507-519.
- . 1974a. Effect of hypothalamic implantation of puromycin on photostimulated testicular growth in the Japanese quail (*Coturnix coturnix japonica*). *Gen. Comp. Endocrinol.*, 22:54-61.
- . 1974b. Blockade of photoperiodically induced testicular growth by hypothalamic deafferentation in Japanese quail (*Coturnix coturnix japonica*). *Gen. Comp. Endocrinol.*, 24:113-120.
- . 1975. Cell types in the adenohipophysis of the Japanese quail and effects of injection of luteinizing hormone-releasing hormone. *Cell. Tissue Res.*, 159:167-178.

- WHITTO, G. C. 1970. Comparative physiology of thermoregulation: invertebrates and nonmammalian vertebrates. Academic Press, New York and London, 1:ix+1-333 pp.
- . 1971. Comparative physiology of thermoregulation: mammals. Academic Press, New York and London, 2:xi+1-410 pp.
- . 1973. Comparative physiology of thermoregulation: special aspects of thermoregulation. Academic Press, New York and London, 3:xii+1-278 pp.
- WILSON, F. E. 1967. The tubero-infundibular neuron system. A component of the photoperiodic control of the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. Z. Zellforsch. Mikrosk. Anat., 81:1-24.
- WILSON, S. C., AND P. J. SHARP. 1975. Effects of progesterone and synthetic luteinizing hormone during sexual maturation in the hen (*Gallus domesticus*). J. Endocrinol., 67:359-369.
- ZAMBRANO, D. 1970. The nucleus lateralis tuberis system of the gobiid fish *Gillichthys mirabilis*. II. Innervation of the pituitary. Z. Zellforsch. Mikrosk. Anat., 110:496-516.
- ZAMBRANO, D., R. S. NISHIOKA, AND H. A. BERN. 1972. The innervation of the pituitary gland of teleost fishes: its origin, nature, and significance. Pp. 50-66, in Brain-endocrine integration. Median eminence: structure and function (K. Knigge, D. F. Scott, and A. Weindl, eds.), Karger, Basel, xii+368 pp.
- ZAMBRANO, D., W. C. CLARKE, E. F. HAWKINS, M. SAGE, AND H. A. BERN. 1973/74. Influence of 6-hydroxydopamine on hypothalamic control of prolactin and ACTH secretion in the teleost fish *Tilapia mossambica*. Neuroendocrinology, 13:284-298.
- ZIMMERMAN, E. A. 1976. Localization of hypothalamic hormones by immunocytochemical techniques. Pp. 25-62, in Frontiers in neuroendocrinology (L. Martini and W. F. Ganong, eds.), Raven Press, New York, xii+419 pp.

EVOLUTIONARY ASPECTS OF ESTROGEN-SENSITIVE STRUCTURES IN THE VERTEBRATE FOREBRAIN

WALTER E. STUMPF, MADHABANANDA SAR, YE SOON KIM, DONALD A. KEEFER,
AND MARIE CHRISTINE MARTINEZ-VARGAS

The introduction of the dry-mount and thaw-mount autoradiographic techniques established the topographical pattern of distribution of estrogen target sites in the brain (Stumpf, 1968, 1970). Accordingly, the concept of the phylogenetically old periventricular brain, as the site of accumulation of estrogen concentrating neurons, was developed (Stumpf, 1970). The structures involved agree, in part, with the "limbic system," including in the forebrain parts of the septum, preoptic nuclei, anterior and central hypothalamic regions, anterior thalamus, epithalamus, amygdala, claustrum, parts of the hippocampus, allocortex and proisocortex; in addition, involved are regions of the midbrain, pons, medulla and spinal cord (Stumpf *et al.*, 1975). Therefore, several questions arose, such as, how does the anatomical distribution of estrogen target cells compare with the concepts of a "sex center" or "sex centers," the "hypophysiotrophic area" and the "limbic system." Phylogenetic and ontogenetic studies were initiated in order to help answer such questions.

MATERIALS AND METHODS

At least three animals each of different vertebrate species and sex were injected subcutaneously with ^3H estradiol-17 β , specific activity 40 to 100 C/mM, 0.5 μg per 100 g. body weight. The species included squirrel monkey (*Saimiri sciureus*), tree shrew (*Tupaia glis*), Sprague Dawley rat (*Rattus norvegicus*), Swiss albino mouse (*Mus musculus*), ring dove (*Streptopelia risoria*), lizard (*Anolis carolinensis*) and goldfish (*Carassius auratus*). Mammalian species were gonadectomized 48 hours prior to the experiment, whereas nonmammalian species were left intact. The experiments were performed at different seasons so that different levels of endogenous hormones and receptors must be considered. Mammals and doves were decapitated at 30 minutes or one hour and cold-blooded animals at four or six hours after the injection. These time intervals approximate the highest differential uptake of radioactivity between nuclear and nonnuclear concentration. The brains were removed, placed on tissue holders, and frozen and mounted simultaneously in liquified propane at -180°C ; mounted tissues were stored in liquid nitrogen. Frozen sections were cut at 4 μm in a Wide-Range Cryostat and dry or thaw-mounted on photographic emulsion-precoated (Kodak NTB3) slides. After different lengths of photographic exposure at -15°C in desiccator boxes, slides were photographically processed and stained. Details of the autoradiographic procedure have been described by Stumpf and Sar

(1975a). Competition studies were performed in all of the species, in order to characterize the chemical nature and specificity of the radioactivity that was concentrated in cell nuclei. In the competition experiments, 10 to 30 minutes prior to the administration of ^3H estradiol-17 β , a 500 or 1000 times higher dose of unlabeled estradiol-17 β was injected intravenously or subcutaneously. The autoradiograms obtained showed that the nuclear uptake of radioactivity was absent or greatly diminished.

RESULTS AND DISCUSSION

Only the forebrain is considered here because the data available for the lower brain stem are limited. In all of the animals studied, concentration and retention of radioactivity were observed in the nuclei of certain neurons (referred to as labeled cells or estrogen target cells) but not in others. There is controversy among some investigators about the detailed distribution of estrogen target cells. Although most investigators agree with our observations, Pfaff claims that estrogen is concentrated in "neurons and glial cells throughout the brain. No evidence was seen for exclusive uptake by, or absence of, uptake from any particular type of nerve cells or glial cell" (Pfaff, 1968:1355). Pfaff also states that "the ratio of the number of reduced grains over cell bodies in limbic and hypothalamic structures to that in non-limbic structures was 1.75 in male brains and 1.73 in female brains" (Pfaff, 1968:1355). In a later publication, Pfaff and Keiner (1973:155) arrived at the conclusion that "the limbic-hypothalamic distribution found in the present experiment agrees with conclusions based on our earlier work (Pfaff, 1968), in which a combined fixation procedure with osmium and formalin was used for brain tissues," a statement made in spite of the fact that a topographical distribution of estrogen target cells was obtained that resembles the one published by Stumpf (1968, 1970). It should be noted that the technique used by Pfaff and his coworkers since 1972 is in essence the thaw-mount autoradiographic technique of Stumpf and Roth (1966) and Stumpf (1971).

Figs. 1-7 show representative cross sections through the preoptic (top section) and central hypothalamic (bottom section) regions of the squirrel monkey, tree shrew, rat, mouse, dove, lizard, and goldfish. Homologous areas with estrogen target neurons may be recognized. In general, estrogen target neurons are most conspicuously present in the vicinity of ventricles, especially their ventral extensions. Characteristic accumulations of estrogen target neurons are seen in several forebrain regions that may be designated as central hypothalamic, preoptic-septal, thalamic, amygdaloid, hippocampal, and cortical region. The presence of estrogen labeled cells in the so-called circumventricular organs or ventricular recess organs is considered important because circumventricular organs are considered nodal points of the periventricular brain.

Detailed anatomical descriptions for the localization of ^3H estradiol are published elsewhere for the squirrel monkey and tree shrew (Keefer and

Stumpf, 1975), the rat (Stumpf *et al.*, 1975), the mouse (Stumpf and Sar, 1975b) and the ring dove (Martinez-Vargas *et al.*, 1975), and are to be published for the lizard (Keefer, Martinez-Vargas, and Stumpf, unpublished data) and the goldfish (Kim, Stumpf, and Sar, unpublished data). Since this meeting was held in 1977, estrogen distribution has been published for the lizard (J. Exp. Zool., 205:141-147, 1978), goldfish (J. Comp. Neurol., 182:611-620, 1978), and platyfish (Brain Res., 170:43-59, 1979).

Central hypothalamic region.—The central hypothalamus is a conspicuous site of accumulation of strongly labeled estrogen target neurons in all species studied. In the rodents and primate examined this involves the n. infundibularis sive arcuatus, the n. periventricularis, the n. premammillaris ventralis and the n. ventromedialis pars ventrolateralis as well as scattered labeled neurons in the n. dorsomedialis and the lateral and dorsal hypothalamic area. The central hypothalamus of birds and lizards is less differentiated into distinct nuclei. However, in the rat and mouse, transitions between the n. arcuatus, n. ventromedialis, and the n. premammillaris ventralis have been noted in our earlier studies. The goldfish shows a labeled portion of the n. lateralis tuberis, including its pars anterior, posterior and inferior, that seems to be homologous to the labeled central hypothalamic complex in higher vertebrates.

Preoptic-septal region.—All animals studied, from teleost to primate, showed an accumulation of estrogen target neurons in the vicinity of the optic recess of the third ventricle. The nuclear concentration of ^3H estradiol is high, when compared with other regions, and includes in rodents and primates such specific areas as the n. preopticus medialis and lateralis, the n. preopticus periventricularis, and the n. preopticus pars suprachiasmatica. In the bird, reptile, and teleost fish, estrogen labeled cells are accumulated in the homologous n. preopticus parvocellularis, that includes the n. preopticus periventricularis.

Neurons of the magnocellular n. paraventricularis and n. supraopticus in primates and rodents concentrate ^3H estradiol moderately. Similarly, the homologous n. preopticus pars magnocellularis in the nonmammalian vertebrates, where it could be identified, shows weak labeling or strong labeling of a subpopulation of cells.

All species exhibit a group of labeled cells dorsal to the n. preopticus, which is partially separated from it by the anterior commissure. These cells in mammals comprise the bed nucleus of the stria terminalis, the n. triangularis septi, and a ventral part of the n. septi lateralis. Nonmammalian species also show labeled supracommissural cells. In these species, these cells are difficult to group into separate nuclei because there are obvious transitional types of cells among the supracommissural "nuclei" and even between the supracommissural septal and infracommissural preoptic nuclei. Thus, it appears that preoptic and septal estrogen target neurons originally formed one group that subsequently became separated by the anterior commissure and the columns of the fornix.

FIGS. 1-7.—Cross-sections through the preoptic-septal (left) and central-hypothalamic (right) planes of brains from animals of different vertebrate classes, showing schematically sites of accumulation of estrogen target neurons in squirrel monkey (Figs. 1a, 1b), tree shrew (Figs. 2a, 2b), rat (Figs. 3a, 3b), mouse (Figs. 4a, 4b), ring dove (Figs. 5a, 5b), lizard (Figs. 6a, 6b), and goldfish (Figs. 7a, 7b). Prepared after autoradiograms following injection of ^3H estradiol. Size and density of dots on left half represent intensity and frequency of neuronal nuclear labeling with radioactivity. Designation of structures on right half: nuclear groups, lowercase letters; neural pathways, capital letters. Ventricles, black. Abbreviations: a, nucleus accumbens; aa, area amygdala; aaa, area amygdala anterior; aba, n. basalis accessorius amygdalae; abal, n. basalis accessorius lateralis amygdalae; abam, n. basalis accessorius medialis amygdalae; abl, n. amygdaloideus basalis pars lateralis; abm, n. amygdaloideus basalis pars medialis; ac, n. amygdaloideus centralis; aco, n. amygdaloideus corticalis; AL, ansa lenticularis; al, n. lateralis amygdalae; ala, n. amygdaloideus lateralis pars anterior; alp, n. amygdaloideus lateralis pars posterior; am, n. amygdaloideus medialis; ar, n. arcuatus; c, caudatum; CA, commissura anterior; CAI, capsula interna; CER, cerebellum; cl, claustrum; CO, chiasma opticum; CP, commissura posterior; cp, n. caudatus putamen; CPF, cortex piriformis; CS, corpus striatum; dli, n. diffusio lobi inferioris; ep, n. entopeduncularis; F, columna fornicis; FMP, fasciculus medialis prosencephali; FMT, fasciculus mammillothalamicus; gl, n. glomerulosus; gp, globus pallidus; ndm, n. dorsomedialis hypothalami; HI, hippocampus; hl, n. habenularis lateralis; hm, n. habenularis medialis; hpm, n. hypothalamicus posterior medialis; hpv, n. periventricularis hypothalami; hpvp, n. periventricularis posterior hypothalami; hvm, n. ventromedialis hypothalami; ltp, n. lateralis tuberis pars posterior; OM, tractus occipitomesencephalicus; OSC, organum subcommissurale; P, putamen; pa, n. paraventricularis; pgl, n. preglomerulosus pars lateralis; po, n. preopticus; pol, n. preopticus lateralis; pom, n. preopticus medialis; popv, n. preopticus peri-

ventricularis; **posc**, n. preopticus pars suprachiasmatica; **pvr**, n. periventricularis rotundocellularis; **pvs**, n. periventricularis stellatocellularis; **QF**, tractus quintofrontalis; **re**, n. reuniens; **rh**, n. rhomboideus; **rl**, n. recessus lateralis; **s**, n. septi; **sc**, n. suprachiasmaticus; **sf**, n. septalis fimbrialis; **sl**, n. septi lateralis; **SM**, stria medullaris thalami; **so**, n. supraopticus; **spc**, n. superficialis parvocellularis; **SR**, sulcus rhinalis; **st**, n. interstitialis striae terminalis; **ta**, n. anterior tubercis; **TCC**, truncus corporis callosi; **TD**, tractus diagonalis; **tdm**, n. dorsomedialis thalami; **TEO**, tectum opticum; **TI**, tractus infundibularis; **TL**, torus longitudinalis; **tml**, n. medialis thalami pars lateralis; **tmm**, n. medialis thalami pars medialis; **TMT**, tractus mammillothalamicus; **TO**, tractus opticus; **TOL**, tractus olfactorius lateralis; **ts**, n. triangularis septi; **TSM**, tractus septomesencephalicus; **tv**, n. ventralis thalami; **tvd**, n. ventralis thalami pars dorsomedialis; **tvm**, n. ventralis medialis thalami pars magnocellularis; **vcgl**, n. ventralis corporis geniculati lateralis; **zi**, zona incerta. Continued on following page.

FIGS. 1-7.—Continued.

Thalamic regions.—In contrast to the above mentioned regions of estrogen localization, thalamic target areas are less well defined; their functions are little understood and not well implicated in the control of gonadotropin secretion and sex behavior. Small groups of labeled neurons or scattered labeled cells were observed in dorsal and ventral segments of the thalamus in the specimens we examined. In rodents, this includes ventrally, the zona incerta, dorsally, the n. parataenialis, the n. interanterodorsalis, and the periventricular nuclei, and caudally, certain structures of the epithalamus, the n. habenularis lateralis, the lamina intercalaris, the substantia grisea centralis, and the n. parafascicularis. Labeled cell groups in juxtaventricular thalamic regions seen in the non-mammalian species are probably homologous to those defined in rodents; however, a detailed relationship remains to be established.

Amygdaloid region.—Concentrations of estrogen target neurons exist at the level of and caudal to the n. preopticus in a region that is rostro-lateral and ventral to the ventral tip of the lateral ventricle and close to the ventral surface of the hemisphere in all vertebrate species studied except in the teleosts *Carassius auratus* and *Xiphophorus maculatus* (Kim, Stumpf and Sar, unpublished data). In nonmammalians this cell group, associated with the *ventro-medial* outgrowth of the lateral ventricle, does not seem to be differentiated into nuclei, although in the ring dove an incipient differentiation is

suggested. In mammalian species, this estrogen target cell group is associated with the *ventro-lateral* outgrowth of the lateral ventricle and is differentiated into different subgroups or nuclei. The separation and identification of the different amygdaloid nuclei, labeled or unlabeled, is not always distinct and probably is the reason for varying descriptions and nomenclature of this region.

Regarding the location of the estrogen labeled cells in this region, homology is suggested between the "nucleus taeniae" in reptiles and birds and the labeled amygdaloid nuclei in mammals. Therefore, in nonmammalian species, the term *area amygdala* is employed here.

Hippocampal region.—Weakly and scattered labeled neurons have been found in hippocampal regions in rodents, the ring dove, and tree shrew, but no such cells have been observed in the squirrel monkey, lizard, and goldfish. Inasmuch as the labeling is weak or sparse and long autoradiographic exposure times are required, it is possible that estrogen target cells are present in all species but simply have not been demonstrated thus far in the latter three taxa. From the results of the autoradiographic studies with other steroid hormones, it is apparent that the hippocampus contains preferential target sites for glucocorticosteroids (Stumpf and Sar, 1975c) and androgen (Sar and Stumpf, 1975). Weak estrogen localization has been reported in rats and mice, mainly in areas CA2 and CA3 and in the subiculum.

Cortex.—Estrogen concentration is observed in certain cortical regions of the mouse, and, to a lesser degree, the rat. Labeled cortical areas in the mouse correspond to portions of the allocortex, the periallocortex, and proisocortex. No indications for cortical labeling, other than amygdala and hippocampus, have been obtained thus far from other species, including non-mammalian vertebrates.

Ventricular recess organs and ependyma.—Attention has been focused on circumventricular organs in rodents. Estrogen target cells have been identified in all of these structures, except the ependyma of the subcommissural organ.

With the exception of the weak labeling of the ependyma of the recessus mammillare in rodents, no ependymal nuclear uptake is seen in the third and lateral ventricles. Ependymal nuclear labeling with ^3H estradiol and ^3H dihydrotestosterone, however, is distinct in certain regions of the lower brain stem and spinal cord. Functional significance has been attributed to the localization of steroid hormone target cells within ventricular recess organs (including the optic recess organ, the subfornical organ, the pineal organ and lamina intercalaris, the infundibular recess, the collicular recess organ, and the area postrema) and the accumulations of steroid hormone target neurons in the vicinity of these structures. Thus, it appears that the ventricular recess organs with their individual surrounding steroid hormone target areas constitute nodal points within the phylogenetically old periventricular brain.

CONCLUSIONS

Within the forebrain of female and male representatives from mammalian and nonmammalian species, groups of estrogen target neurons have been identified. During evolution, these neuronal groups have shown a tendency to grow and differentiate into subgroups. In teleosts, accumulations of estrogen target neurons are present in central hypothalamic, preoptic-septal and dorsal and ventral thalamic regions; hippocampal and amygdaloid estrogen target cells were not found. More qualitative and quantitative data will be required in order to define further evolutionary trends and to identify homologies and progression indices of the steroid hormone target areas. The present data suggest that steroid hormones may be useful as markers in order to establish homologies.

A close topographical relationship is apparent between the different brain target regions for ^3H estradiol among the nonmammalian animals studied. For instance, the preoptic-septal-amygdaloid estrogen target neurons are located so closely together that it may be considered a single entity. This relationship remains visible and can be traced phylogenetically, even when neural fiber tracts separate these regions and "differentiate" them into "nuclei." Our data suggest a close functional relationship among preoptic, septal, and amygdaloid steroid hormone target regions. In addition, there is a continuum of estrogen target neurons in the periventricular stratum of the third ventricle. The accumulation of steroid hormone target neurons in the vicinity of the ventricular system was noted earlier in the rat and is a feature present in all of the species we examined. In teleosts, the supracommissural "septal" accumulation appears related to the interhemispheric ventricle; lateral ventricles are still absent. In reptiles, birds, rodents, and primates (probably also amphibians), the supracommissural "septal" group lies rostral and lateral to the rostral wall of the third ventricle and connects to the ventromedial extension of the lateral ventricles. This *ventromedial* extension of the lateral ventricle is caudally associated with the labeled nucleus taeniae, designated here as area amygdala (Figs. 5b, 6b) in reptiles, birds, and possibly amphibians. In mammals, amygdaloid estrogen target regions are associated with the *ventrolateral* extension of the lateral ventricle (visible in Fig. 1b). Probably the growth of the hemispheres and the lateral extension of the lateral ventricle play a role here.

This paper is concerned only with topographic aspects, but there may well be an association between topography and function. This, however, remains to be elucidated. The estrogen target areas in the forebrain of vertebrates probably are involved in the induction of gonadotropin secretion, reproductive and instinctive behavior, and the regulation of autonomic functions.

ACKNOWLEDGMENT

This work was supported by U.S. PHS grant NS 09914.

LITERATURE CITED

- KEEFER, D. A., AND W. E. STUMPF. 1975. Estrogen localization in the brain. Pp. 153-165, in *Anatomical neuroendocrinology* (W. E. Stumpf and L. D. Grant, eds.), Karger, Basel, vi+472 pp.
- MARTINEZ-VARGAS, M. C., W. E. STUMPF, AND M. SAR. 1975. Estrogen localization in the dove brain. Phylogenetic considerations and implications for nomenclature. Pp. 166-175, in *Anatomical neuroendocrinology* (W. E. Stumpf and L. D. Grant, eds.), Karger, Basel, vi+472 pp.
- PFAFF, D. 1968. Autoradiographic localization of radioactivity in rat brain after injection of tritiated sex hormones. *Science*, 161:1355-1356.
- PFAFF, D., AND M. KEINER. 1973. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *J. Comp. Neurol.*, 151:121-158.
- SAR, M., AND W. E. STUMPF. 1975. Distribution of androgen-concentrating neurons in rat brain. Pp. 120-133, in *Anatomical neuroendocrinology* (W. E. Stumpf and L. D. Grant, eds.), Karger, Basel, vi+472 pp.
- STUMPF, W. E. 1968. Estradiol concentrating neurons: topography in the hypothalamus by dry-mount autoradiography. *Science*, 162:1001-1003.
- . 1970. Estrogen-neurons and estrogen-neuron systems in the periventricular brain. *Amer. J. Anat.*, 129:207-218.
- . 1971. Autoradiographic techniques for the localization of hormones and drugs at the cellular and subcellular level. *Acta Endocrinol.*, 153(suppl.):205-222.
- STUMPF, W. E., AND L. J. ROTH. 1966. High resolution autoradiography with dry mounted, freeze-dried frozen specimens. Comparative study of six methods using two diffusible compounds ^3H -estradiol and ^3H -mesobilirubinogen. *J. Histochem. Cytochem.*, 14:274-287.
- STUMPF, W. E., AND M. SAR. 1975a. Autoradiographic techniques for localizing steroid hormones. Pp. 135-156, in *Methods in enzymology* (B. W. O'Malley and J. G. Hardman, eds.), Academic Press, New York, xviii+575 pp.
- . 1975b. Hormone-architecture of the mouse brain with c1rH-estradiol. Pp. 82-103, in *Anatomical neuroendocrinology* (W. E. Stumpf and L. D. Grant, eds.), Karger, Basel, vi+472 pp.
- . 1975c. Anatomical distribution of corticosterone-concentrating neurons in rat brain. Pp. 254-261, in *Anatomical neuroendocrinology* (W. E. Stumpf and L. D. Grant, eds.), Karger, Basel, vi+472 pp.
- STUMPF, W. E., M. SAR, AND D. A. KEEFER. 1975. Atlas of estrogen target cells in rat brain. Pp. 104-119, in *Anatomical neuroendocrinology* (W. E. Stumpf and L. D. Grant, eds.), Karger, Basel, vi+472 pp.

EVOLUTION OF NEUROHYPOPHYSIAL PEPTIDE FUNCTIONS

P. J. BENTLEY

The physiological role of the vertebrate neurohypophysis was reviewed recently by Bentley (1974) and Maetz and Lahlou (1974). Few advances have been made since in this area. At present, there are approximately six peptide hormones for which no function has been described. The principal reason for this problem is lack of methodology sufficiently sensitive, specific, and convenient to detect and measure the small quantities of such peptides that may occur in the blood. The development of radioimmunoassay techniques may serve as the solution for tracing microcomplements in vertebrate systems and already have been applied successfully by Rosenbloom and Fisher (1974) and Sawyer and Pang (1975).

Much is known about the physiological roles of the neurophysial hormones in mammals, and this information has provided some useful clues about their functions in nonmammals. It is however, also possible that this approach has resulted in some strictures and solidification of our thinking about the problem. Unlike many of the hormones that are being discussed at this symposium, those from the neurohypophysis do not seem to be essential for life. For example, in man the absence of the antidiuretic hormone (ADH) results in the condition known as diabetes insipidus, when up to about 20 liters of urine may be formed in a day. Provided that the water is replaceable, there is a normal life expectancy, even though the frequent necessity to empty the urinary bladder is uncomfortable, inconvenient, and limits periods of sleep. The other neurohypophysial hormone in mammals is oxytocin, which may promote labor by contracting the uterus; parturition, however, still occurs in its absence. The release of milk from the mammary glands in response to suckling is a reflex, and the final arc in the sequence is the release of oxytocin, which contracts the myoepithelial cells surrounding the secretory alveoli. Although oxytocin release appears essential for normal milk yield in many mammalian species, it has not been found to be essential in all species. Thus, the neurohypophysial hormones appear to improve the quality but not necessarily the quantity of our lives.

The most obvious way to look for roles of the neurohypophysial hormones in nonmammals is to consider the effects that they may have when injected. Some of these effects are outlined as follows. Pharmacological activity in the posterior pituitary gland was identified first by Oliver and Schäfer in 1895 when they showed that pituitary gland extract could increase the blood pressure of dogs and produce vasoconstriction in frogs. In 1912, Paton and Watson demonstrated that such an extract could also *decrease* blood pressure in decapitated ducks. The ability of these peptide hormones to constrict and dilate blood vessels is widespread in the tetrapods

(Woolley, 1959), and vascular constriction has been demonstrated in fish as primitive as cyclostomes (Somlyo and Somlyo, 1968). Although the discovery of a vasopressor response was important, for it showed that the posterior lobe contained biologically active materials, the response does not appear to mean that vasopressin has a physiological role in cardiovascular regulation in mammals. It may however, afford an important clue to the possible actions of these peptides in lower vertebrates.

Henry Dale in 1906 discovered that posterior pituitary extracts could contract the uterus of a pregnant cat. Four years later, in 1910, Isaac Ott and John Scott performed what has been called an 'unlikely experiment.' They injected a posterior pituitary extract into the ear vein of a nursing goat and demonstrated the galactobolic action of the extract.

The posterior pituitary gland was at first thought to contain a diuretic principle, but this subsequently was found to be an artifact of the experimental procedures involving large doses of the extract. In 1913, von den Velden showed that pituitary extracts had an antidiuretic effect in human patients who were unanesthetized and suffering from diabetes insipidus. It is interesting that such hormones have been shown since to have a diuretic effect in some fishes. It is known now that the effects of mammalian posterior pituitary extracts are due to two hormones present in the neurohypophysis: vasopressin (or ADH) and oxytocin. There are a whole variety of other assorted effects of the posterior pituitary hormones. For instance, they contract intestinal smooth muscle. Vasopressin also has a hyperglycemic action and may raise or lower free fatty acid levels in the plasma. Furthermore, the posterior pituitary hormones are known to influence Na metabolism by increasing or decreasing Na excretion in the urine, promoting Na uptake across amphibian skin and urinary bladder, and facilitating exchanges of Na across the gills of some fish. These effects may be due to hemodynamic changes or a more direct action on the permeability of epithelia. A rather intriguing capability of neurohypophysial peptides, which is seen either *in vitro* or *in vivo*, is their ability to stimulate release of some adenohypophysial hormones (Doepfner, 1968). Indeed at one time, it appeared possible that vasopressin might be the still elusive corticotrophin-releasing factor (CRF).

NEUROHYPOPHYSIAL PEPTIDES

Structure.—Neurohypophysial hormones are octapeptides with a molecular weight of about 1000 (see review by Acher, 1974). They consist of a ring of 5 amino acids, stabilized by a disulfide bridge provided by cystine, and a side chain of three amino acids. The hormones display structural polymorphism and at present nine different ones have been identified in nature. They display a distinct and orderly phyletic distribution. There are usually two such variants in the posterior pituitary of a single animal. In mammals, these are [8-arginine]-vasopressin (or ADH) and oxytocin, but in some pigs

[8-lysine]-vasopressin and oxytocin are present. Vasopressin and oxytocin differ by two amino acid substitutions, at positions 3 and 8, and these differences confer the relatively specific effects—an antidiuretic effect to ADH and uterotonic and galactobolic effects to oxytocin. Vasopressin is a uniquely mammalian hormone. The distribution of oxytocin in lower vertebrates is not clear but it appears to be rather sporadic in its occurrence. All non-mammalian vertebrate classes possess a neurohypophysial peptide that is a hybrid between mammalian arginine-vasopressin and oxytocin; it has the ring of oxytocin and side chain of arginine-vasopressin and is thus called *vasotocin*. The spectrum of its pharmacological activities, when it is injected into mammals, is intermediate between those of oxytocin and vasopressin. Vasotocin possesses substantial vasopressor and antidiuretic as well as oxytocic and galactobolic activities. It would not be a very efficient hormone in mammals because it would lack the specificity to control the different physiological responses. Vasotocin, however, has some unique properties when injected into nonmammals.

The other neurohypophysial peptides make a more sporadic appearance; *isotocin* is present in most bony fishes but is replaced by *mesotocin* in the lungfishes. Of special note is the presence of the latter peptide in the neurohypophysis of amphibians, reptiles, and birds. The persistence of vasotocin throughout the vertebrates is quite remarkable and points to its conservative nature as a hormone. Considering the propensity of the other peptides to change from one phyletic group to another, raises the question of why this should be so. All that can be suggested at this point is that the vasotocin molecule possesses a somewhat ideal structure with respect to its three-dimensional conformation, stability, and specificity. Such properties could facilitate its interaction with appropriate receptors and generally promote its function as a hormone. Mainly on the basis of its broad phyletic distribution starting in the cyclostomes (where it appears to be the sole neurohypophysial peptide), it is often suggested that vasotocin is the primeval ancestral neurohypophysial hormone, the others having arisen from it as a result of a series of mutations.

Activity.—Each neurohypophysial hormone has a distinct spectrum of activities when tested on recognized standard pharmacological preparations. These tests, with a single exception (the chicken blood depressor assay), use mammals and so do not provide many clues about the relative potencies of the hormones in the species in which they occur. With the exception of the chicken blood depressor assay (Table 1) vasotocin does, however, have a far greater potency when tested in nonmammalian species. This is seen clearly in its effects in amphibians. For instance, when compared for their abilities to increase water movement across the toad urinary bladder, vasotocin is 200-times as active as the other amphibian neurohypophysial peptide, mesotocin, or mammalian oxytocin. Vasotocin is about 10-times more active in effecting antidiuresis in chickens than mammalian ADH but is only half as active as the latter in the rat.

TABLE 1.—Relative activities, measured in units/ 10^{-6} mole, of some neurohypophysial peptides when tested on preparations from homologous species.

Specimen	Vasotocin	Mesotocin	8-Arg. vasopressin	Oxytocin
Chicken antidiuresis ¹ *	3,500		400	weak and diphasic
Chicken oviduct ¹ *	2,600		240	29
Fowl blood depressor	300	500	60	450
Turtle oviduct ¹	880			
Toad bladder ²				
water	90,000	450	120	450
natriferic	45,000	450		450
Bullfrog bladder				
water ¹	47,000		≈30	450
Frog skin				
natriferic ³	1,700		44	450
Bullfrog antidiuresis ⁴	225,000			450

¹Recalculated from data in Munsick *et al.*, 1960²Bentley, 1969a³Morel and Jard, 1968⁴Recalculated from data in U'ringa and Sawyer, 1960

*In terms IU of oxytocin except for chicken antidiuresis (IU vasopressin) and chicken and turtle oviduct (IU/0.5 mg. whole pituitary I.U.S. standard)

MORPHOLOGY OF THE NEUROHYPOPHYSIS

There is an enlargement of the neurohypophysis in tetrapods to form the neural lobe, which is absent in the fishes though the beginning of it can be seen in the lungfishes. When comparing the quantities of stored vasotocin in the glands (Table 2), it can be seen that there is a remarkable increase that is first apparent in the Amphibia. Tadpoles and neotenus amphibians, like the mudpuppy *Necturus maculosus*, are exceptions. Some years ago, Wingstrand (1959) speculated that the appearance of the neural lobe in tetrapods may be associated with an increased requirement for the hormone for regulating the water metabolism in terrestrial animals. The formation of large cleidoic eggs and the development of viviparity conceivably could result also in the need for an ecobolic or uterotonic hormone.

The functions of the neurohypophysial hormones can be divided into two categories: direct effects on the permeability of epithelial membranes, which are related to the hormones' ability to activate adenylyl cyclase and promote the formation of cyclic AMP; and effects on the contractility of smooth muscle, such as that occurring in the vasculature and reproductive tract. These latter effects appear to be universally present but are not dependent on the activation of adenylyl cyclase. In mammals, oxytocin has a unique related function in contracting the myoepithelial cells of the mammary glands.

FUNCTION IN TETRAPODS

Osmoregulation.—Evidence for the physiological roles of the neurohypophysial peptides in nonmammals is usually rather weak because the

TABLE 2.—Quantities of vasotocin stored in the neurohypophyses of species from the major phyletic groups of vertebrates.

Species	Vasotocin 10 ⁻⁹ M/kg. body wt.	Source
Lamprey (cyclostomes)	0.34	Follett, 1963
Eel (Teleostei)	0.33	"
Trout "	0.71	"
African lungfish (choanichthyes)	0.10	"
Frog (Amphibia, Anura)	6.2	"
Tadpole " "	0.5	Bentley and Greenwald, 1970
Toad " "	16.2	Follett, 1963
Newt (Amphibia, Urodela)	12.7	"
Salamander " "	6.2	Bentley, 1969a
Mudpuppy " "	0.7	"
Grass snake (Reptilia)	23.6	Follett, 1963
Herring gull (Aves)	9.5	"

accepted criteria of physiological deficits due to glandular extirpation and their correction by synthetic replacement usually have not been satisfied. All available evidence, however, indicates that vasotocin, like vasopressin in mammals, acts as an antidiuretic hormone in birds, reptiles, and amphibians. The precise mechanism whereby this response operates may differ somewhat from mammals for although vasotocin appears to increase water reabsorption across the renal tubule, as in mammals, it also can inhibit urine flow by reducing the renal glomerular filtration rate. Reduction in the filtration rate appears to require higher concentrations of vasotocin than the tubular response. As there are few measurements of the precise concentrations of vasotocin in the plasma of such species, it is uncertain whether or not both responses constitute part of a dual physiological control.

In some amphibians such as the urodeles and the aquatic toad *Xenopus laevis*, the glomerular effect may be predominant (see Bentley and Heller, 1964; Henderson *et al.*, 1972; Galli-Gallardo, *et al.*, 1977) and may even be the sole renal action of vasotocin. This possibility needs more extensive investigation. Animals that cannot form a hyperosmotic urine, like reptiles and amphibians, would benefit from an ability to limit glomerular filtration for it would afford them a way of controlling urine volume in extreme situations when water supplies are limited. The kidney of such animals can do little to control the total osmotic concentration of the body fluids under dry conditions, and if glomerular filtration continued unabated, the animals would be continually forming urine with little or no restoration of the plasma osmolality. This would deplete the volume of body fluids and the prevention of this effect may be a primary consideration in the animal's survival.

It is interesting that the effect of neurohypophysial peptides on epithelial permeability is not confined to the renal tubule in nonmammalian species. In the Amphibia, these peptides can increase the permeability of the skin and the urinary bladder to water. Indeed it has been estimated that in the

toad *Bufo marinus* reabsorption of water from the urinary bladder accounts for about 50 per cent of the apparent antidiuretic response (Bentley and Ferguson, 1967), and in the fire salamander *Salamandra maculosa* increasing bladder permeability may be the sole mechanism by which vasotocin can reduce urinary water loss (Bentley and Heller, 1965).

The water-related responses of amphibians to vasotocin are not quantitatively uniform in all species. Those anurans that live in arid areas where water is expected to be scarce and only available sporadically, tend to produce a stronger reaction to the injection of vasotocin. Aquatic anurans, like *Xenopus laevis* and urodeles such as *Necturus maculosus* and *Siren lacertina*, only exhibit small responses to vasotocin (Heller and Bentley, 1965; Bentley, 1973). These differences appear to reflect a weak antidiuretic effect and the lack of an action on the skin and the urinary bladder in aquatic anurans. We have, however, shown recently that the skin of *Xenopus* and *Necturus* is responsive to vasotocin (Bentley and Yorio, 1977, and unpublished observations), but the response can only be detected by the use of tritiated water and it appears to be physiologically insignificant. The ventral skin of certain species of tree frogs (Hylidae) show *in vitro* responses that are relatively enormous; vasotocin can stimulate an osmotic water movement of about 250 to 300 $\mu\text{l}/\text{H}_2\text{O cm}^{-2}$ (Yorio and Bentley, 1977). This value is approximately 10 times as great as that seen in frogs like *Rana pipiens* and *R. esculenta*. The amphibians thus have utilized the same hormone, vasotocin, to elicit responses from renal and nonrenal tissues in order to conserve water and increase water uptake.

Neurohypophyseal peptides also increase the rate of active sodium transport across the skin and urinary bladder of many but not all amphibians. Like the water response, this is mediated by adenyl cyclase and the formation of cyclic AMP. The response (the natriuretic response) of sodium is of a rather transient nature and may be of little physiological significance.

Reproduction.—The oviducts of vertebrates other than mammals contract in response to neurohypophyseal peptides and vasotocin appears to be especially active (see Heller, 1972). These effects can be seen *in vivo* with injections of the peptides; injections promote egg laying in chickens and parturition in several species of ovoviparous reptiles and amphibians. The oviduct of the chicken and several reptiles and amphibians are also highly responsive to the presence of such peptides under *in vitro* conditions. Changes in sensitivity have been related to the particular period of the breeding cycle (see Heller, 1972) in a manner consistent with a physiological function. Neurohypophysectomized chickens can still lay eggs (Opel, 1965), although there appears to be a release of vasotocin into the circulation during egg laying (Sturkie and Lin, 1966). These observations are similar to those seen in relation to the role of oxytocin in mammalian parturition (Fitzpatrick, 1966). It thus seems likely that the neurohypophyseal peptides may exert an ecobolic or oxytocic effect in nonmammals, but the evidence is equivocal.

FUNCTION IN FISHES

We experience particular difficulties in assessing the physiological role of the neurohypophysial hormones in fish. These vertebrates are especially interesting because of their relative phyletic proximity to the tetrapods; vasotocin and its related peptides are present in the neurohypophyses of all the major vertebrate classes. So far, we are not certain that these peptides are actually released into the circulation of fishes (see Bentley, 1971), but if they are they would appear to be present at much lower concentrations than are those found in tetrapods.

Responses to the injection of neurohypophysial peptides vary depending on the particular species of fish.

1. Blood pressure may be increased and this could be associated with regional changes in the blood supply to such organs as the gills and the kidneys (see Maetz and Lahlou, 1974).

2. Urine flow is increased in the goldfish, *Carassius auratus*, and the eel, *Anguilla anguilla*, but this response is not seen in the flounder, *Platichthys*. It is interesting that in the lungfishes, which are the presumed phyletic forebears of the tetrapods, vasotocin also has a diuretic effect. This response is seen in all three extant genera *Protopterus*, *Lepidosiren*, and *Neoceratodus* (Sawyer, 1970; Sawyer and Pang, 1972; Sawyer *et al.*, 1976). Diuretic effects of vasotocin are due to an increased glomerular filtration rate and do not involve changes in renal tubular absorption of water. In some teleosts, it has been suggested that there is a recruitment of functioning glomeruli (glomerular intermittency), but in the lungfishes vasotocin changes the hemodynamic situation in individual glomeruli (Sawyer, 1972a).

Henderson and Wales (1974) confirmed that a diuretic response to vasotocin injections (>1 ng/kg) occurs in European freshwater eels, *Anguilla anguilla*. They, however, found the response to be paradoxical for when lower dosages were used (<0.1 ng/kg) antidiuresis resulted. The effects on urine flow were due to changes in the glomerular filtration rate; tubular water reabsorption was not involved. Measurement of the TMG (glucose renal tubular transport maximum, a function of the number of functioning glomeruli) indicated that vasotocin changes the number of active glomeruli (glomerular intermittency). It was suggested that vasotocin may have two effects: if it constricted the afferent preglomerular arterioles, antidiuresis would result (as in tetrapods), but at higher doses an action on the efferent postglomerular arteriole may predominate, which would produce a diuresis. In the latter instance, however, the blood pressure also increased and this effect could mediate the response. We must await an extension of these observations to other species of teleosts in order to assess their phyletic and physiological significance.

3. Changes in Na metabolism may occur in response to neurohypophysial peptides and this is usually associated with the renal diuretic actions when natriuresis occurs. Changes in Na transfer across the gills also have been observed (see Maetz and Lahlou, 1974). In goldfish, the uptake of Na from

the external solution is increased whereas in freshwater flounder, transferred to sea water, there is an increased branchial loss of Na. Rankin and Maetz (1971) have shown that vasotocin and isotocin, *in vitro*, at low concentrations (10^{-3} and 10^{-14} M respectively) can bring about regional changes in branchial blood flow that could account for altered Na transfer. It is possible that, in contrast to tetrapods, neurohypophysial peptides do not directly influence Na transport across epithelial membranes of fishes. The possible physiological significance of the action of the neurohypophysial peptides on sodium metabolism in fishes is unknown but it could constitute a nonspecific side-effect of the vascular actions of the peptide.

4. Injected neuropophysial hormones have been shown to induce spawning behavior in *Fundulus heteroclitus* (Wilhelmi *et al.*, 1955) and the delivery of the young in some ovoviviparous teleosts (see Heller, 1972). Heller noted that these hormones could promote contraction of the smooth muscle of the oviduct *in vitro* and this response is seen also in elasmobranchs. In the latter, it is interesting that elasmobranch neurohypophysial extracts are more active than vasotocin.

5. The neurohypophysial hormones have not yet been demonstrated in the systemic circulation of fishes (seen Bentley, 1971), though it is possible that the methods available at present are not sufficiently sensitive to detect them. In 1959, Wingstrand suggested that the neurohypophysial peptides could have a role in controlling adenohypophysial function by acting as local transmitters, in which case they need not reach the general circulation in significant quantities. This possibility merits further investigation in the fishes.

FUNCTION AND THE RELEASE OF NEUROHYPOPHYSIAL PEPTIDES

The different stimuli that influence the release of neurohypophysial peptides in mammals (from Ginsburg, 1968) include: increased plasma osmotic pressure; suckling (milk-ejection reflex); stretching of uterus, cervix, or vagina (parturition, copulation); hemorrhage (decreased blood volume); emotion and stress (CNS effect). Increases in the osmotic concentration and decreases in the volume of the plasma appear to be the main physiological stimuli for the release of the antidiuretic hormone. These stimuli are consistent with the ability of ADH to activate homeostatic mechanisms concerned with the maintenance and restoration of the concentrations and volume of the body fluids. Similar mechanisms have been shown to influence the release of vasotocin in amphibians (Bentley, 1969b; Sawyer and Pang, 1975), but there appear to be no direct measurements in other tetrapods. Such a release mechanism would appear inappropriate in the fishes if the principal osmotic action of vasotocin is really to produce diuresis. Sawyer (1972b) has suggested that this diuretic response could prevent hyperhydration in some lungfishes especially when they are exposed suddenly to water during their awakening from estivation. It is also conceivable that such a diuretic hormone could be physiologically useful in freshwater tele-

TABLE 3.—*Summary of probable and possible physiological effects of neurohypophyseal hormones in nonmammalian vertebrates.*

Vertebrate	Response
Birds	1. Antidiuresis: increased tubular reabsorption of water and (at higher doses) decreased GFR. 2. Ecboic; contracts the oviduct and promotes egg laying.
Reptiles	1. Antidiuresis; tubular and glomerular action 2. Contracts the oviduct but possible significance in egg-laying and parturition not explored.
Amphibians	1. Antidiuretic; tubular and glomerular 2. Increased reabsorption of urine from urinary bladder. 3. Increased absorption of water across skin. 4. Contracts oviduct.
Lungfishes	1. Diuresis; increased GFR (no tubular action).
Teleosts	1. Diuresis (some species); increased GFR 2. Antidiuresis (very small dose in eels); decreased GFR. 3. Induces spawning and parturition; contracts oviduct.
Elasmobranchs	1. Contracts oviduct; no effects on water metabolism have been demonstrated.
Cyclostomes	1. ? No effects on water metabolism.

osts. For an appropriate hormone secretion to occur in these animals, however, one must postulate a basic change in its release mechanisms. Sawyer (1972b) suggested that this may have occurred so that the release of the hormone takes place in response to an expansion of the body fluids, rather than a contraction or increased concentration as seen in tetrapods. If the hormones and their functions can evolve then it is not unreasonable to expect that the mechanisms for their release may also change. Experimental confirmation is, however, needed.

CONCLUSION AND SUMMARY

The neurohypophysis is present in all vertebrates from cyclostomes to mammals and contains a variety of octapeptides that have a precise phyletic distribution. There are nine such peptides (usually two are present in a single species) and at least some appear to fulfill an endocrine function. This role (see Table 3) is seen clearly in mammals where vasopressin (or ADH) limits urine secretion and oxytocin has uterotonic and galactabolic effects. Vasotocin is present in the neurohypophysis of all the other groups of vertebrates and (except in cyclostomes) is accompanied by one or more similar peptides, the functions of which we know almost nothing. Vasotocin appears to act as an antidiuretic hormone in birds, reptiles, and amphibians where it can exert its effect both by constricting the afferent preglomerular arterioles and increasing water reabsorption across the renal tubular epithelium. In amphibians, the latter membrane response is displayed also by the skin and urinary bladder. This effect is due to activation of adenylyl cyclase and it may be confined to tetrapods.

In some fishes, including lungfishes, injected vasotocin has a diuretic effect and this response, as in nonmammalian tetrapods, reflects an increase

in the glomerular filtration rate. In the freshwater eel, vasotocin also has an antidiuretic action at very low, but not high, doses that is mediated by a reduced glomerular filtration rate. The condition in the freshwater eel is due apparently to a tetrapod-like response to vasotocin by the preglomerular vessels. Vasotocin can influence the tone of vascular muscle in all groups of vertebrates and its effect on the glomerular vessels may be due to this type of effect. Its action on vascular smooth muscle may thus reflect a primeval endocrine role, but it is still unknown if these responses in fishes are of a physiological nature.

The neurohypophysial peptides also have a phylogenetically persistent ability to contract the smooth muscle of the uterus and oviduct. In mammals, this effect can assist parturition and it possibly may be important in oviparous and ovoviviparous species lower down the phyletic scale.

Neurohypophysial peptides, when injected, can influence a variety of other processes in the body such as blood pressure, blood glucose and salt metabolism. It does not seem likely that these effects are physiological ones.

In the future, it is hoped that sensitive, convenient, and specific assay methods will become available that will allow identification and measurement of the concentrations of the neurohypophysial peptides present in the circulation of lower vertebrates. It is only in this way that we will really be able to assess the possible endocrine roles of not only vasotocin but also the six other similar peptides that are found in the neurohypophysis of various nonmammalian vertebrates.

ACKNOWLEDGMENT

The recent work cited by the author was supported by National Science Foundation Grant No. BMS 75-07684.

LITERATURE CITED

- ACHER, R. 1974. Chemistry of the neurohypophysial hormones: an example of molecular evolution. Pp. 103-117, in *Handbook of physiology, Section 7, Endocrinology* (R. O. Greep and E. B. Astwood, section eds.), The pituitary gland and its neuroendocrine control, Pt. 1 (E. Knobil and W. H. Sawyer, volume eds.), Amer. Physiol. Soc., Washington, D.C., 4:vii+1-576 pp.
- BENTLEY, P. J. 1969a. Neurohypophysial function in Amphibia: hormone activity in the plasma. *J. Endocrinol.*, 43:359-369.
- . 1969b. Neurohypophysial hormones in Amphibia: a comparison of their actions and storage. *Gen. Comp. Endocrinol.*, 13:39-44.
- . 1971. Failure to detect levels of vasotocin similar to those in Amphibia in the plasma of the eel, *Anguilla rostrata*. *J. Endocrinol.*, 49:183-184.
- . 1973. Osmoregulation in the aquatic urodeles *Amphiuma means* (the Congo eel) and *Siren lacertina* (the mud eel). Effects of vasotocin. *Gen. Comp. Endocrinol.*, 20:386-391.
- . 1974. Actions of neurohypophysial peptides in amphibians, reptiles and birds. Pp. 545-563, in *Handbook of Physiology, Section 7, Endocrinology* (R. O. Greep and E. B. Astwood, section eds.), The pituitary gland and its neuroendocrine control, Pt. 1 (E. Knobil and W. H. Sawyer, volume eds.), Amer. Physiol. Soc., Washington, D.C., 4:vii+1-576 pp.

- BENTLEY, P. J., AND D. R. FERGUSON. 1967. The role of the toad urinary bladder in the amphibian "water balance effect" of neurohypophysial hormones. *J. Endocrinol.*, 37:349-350.
- BENTLEY, P. J., AND L. GREENWALD. 1970. Neurohypophysial function in bullfrog (*Rana catesbeiana*) tadpoles. *Gen. Comp. Endocrinol.*, 14:412-415.
- BENTLEY, P. J., AND H. HELLER. 1964. The actions of neurohypophysial hormones on the water and sodium metabolism of urodele amphibians. *J. Physiol. (London)*, 171: 434-453.
- . 1965. The water-retaining action of vasotocin on the fire-salamander (*Salamandra maculosa*): the role of the urinary bladder. *J. Physiol. (London)*, 181:124-129.
- BENTLEY, P. J., AND T. YORIO. 1977. The permeability of the skin of a neotenous urodele amphibian, the mudpuppy *Necturus maculosus*. *J. Physiol. (London)*, 265:537-547.
- DALE, H. H. 1906. On some physiological actions of ergot. *J. Physiol. (London)*, 34:163-206.
- DOEPFNER, W. 1968. The influence of neurohypophysial polypeptides and adenohypophysial function. Pp. 625-654, in *Neurohypophysial hormones and similar polypeptides* (B. Berde, ed.). Springer-Verlag, Berlin, Heidelberg, New York, xix+967 pp.
- FITZPATRICK, R. J. 1966. The posterior pituitary gland and the female reproductive tract. Pp. 453-504, in *The pituitary gland: Pars intermedia and neurohypophysis* (G. W. Harris and B. T. Donovan, series eds.). Univ. California Press, Berkeley and Los Angeles, 3:xiv+1-678 pp.
- FOLLETT, B. K. 1963. Mole ratios of the neurohypophysial hormones in the vertebrate neural lobe. *Nature*, 198:693-694.
- GALLI-GALLARDO, S. M., P. K. T. PANG, AND W. H. SAWYER. 1977. Arginine-vasotocin actions on arterially perfused kidneys of bullfrogs, mudpuppies and South American lungfish. *Fed. Proc.*, 36:593.
- GINSBURG, M. 1968. Production, release, transportation and elimination of the neurohypophysial hormones. Pp. 286-371, in *Neurohypophysial hormones and similar polypeptides* (B. Berde, ed.). Springer-Verlag, Berlin, Heidelberg, New York, xix+967 pp.
- HELLER, H. 1972. The effect of neurohypophysial hormones on the female reproductive tract of lower vertebrates. *Gen. Comp. Endocrinol.*, suppl. 3:703-714.
- HELLER, H., AND P. J. BENTLEY. 1965. Phylogenetic distribution of the effects of neurohypophysial hormones on water and salt metabolism. *Gen. Comp. Endocrinol.*, 5:96-108.
- HENDERSON, I. W., AND N. A. M. WALES. 1974. Renal diuresis and antidiuresis after injections of arginine vasotocin in the freshwater eel (*Anguilla anguilla* L.). *J. Endocrinol.*, 61:487-500.
- HENDERSON, I. W., B. R. EDWARDS, H. O. GARLAND, AND I. CHESTER JONES. 1972. Renal function in two toads, *Xenopus laevis* and *Bufo marinus*. *Gen. Comp. Endocrinol.*, suppl. 3:350-359.
- MAETZ, J., AND B. LAHLOU. 1974. Actions of neurohypophysial hormones in fishes. Pp. 521-544, in *Handbook of physiology, Section 7, Endocrinology* (R. O. Greep and E. B. Astwood, section eds.), *The pituitary gland and its neuroendocrine control*, Pt. 1 (E. Knobil and W. H. Sawyer, volume eds.), Amer. Physiol. Soc., Washington, D.C., 4:vii+1-576 pp.
- MOREL, F., AND S. JARD. 1968. Actions and functions of the neurohypophysial hormones and related peptides in lower vertebrates. Pp. 655-716, in *Neurohypophysial hormones and similar peptides* (B. Berde, ed.). Springer-Verlag, Berlin, Heidelberg, New York, xix+967 pp.
- MUNSICK, R. A., SAWYER, W. H., AND H. B. VAN DYKE. 1960. Avian neurohypophysial hormones: pharmacological properties and tentative identification. *Endocrinology*, 66:860-871.
- OLIVER, G., AND E. A. SCHÄFER. 1895. On the physiological action of extracts of pituitary body and certain other glandular organs. *J. Physiol. (London)*, 18:277-279.

- OPEL, H. 1965. Oviposition in chickens after removal of the posterior lobe of the pituitary by an improved method. *Endocrinology*, 76:673-677.
- OTT, I., AND J. C. SCOTT. 1910. The action of infundibulum upon the mammary secretion. *Proc. Soc. Exp. Biol. Med.*, 8:48-49.
- PATON, D. N., AND A. WATSON. 1912. The actions of pituitrin, adrenalin and barium on the circulation of the bird. *J. Physiol. (London)*, 44:413-424.
- RANKIN, J. C., AND J. MAETZ. 1971. A perfused teleostean gill preparation: vascular actions of neurohypophysial hormones and catecholamines. *J. Endocrinol.*, 51:621-635.
- ROSENBLOOM, A. A., AND D. A. FISHER. 1974. Radioimmunoassay of arginine-vasotocin. *Endocrinology*, 95:1726-1732.
- SAWYER, W. H. 1970. Vasopressor, diuretic and natriuretic responses by lungfish to arginine vasotocin. *Amer. J. Physiol.*, 218:1789-1794.
- . 1972a. Neurohypophysial hormones and water and sodium excretion in the African lungfish. *Gen. Comp. Endocrinol.*, suppl. 3:345-349.
- . 1972b. Lungfishes and amphibians: endocrine adaptation and the transition from aquatic to terrestrial life. *Fed. Proc.*, 31:1609-1614.
- SAWYER, W. H., AND P. K. T. PANG. 1972. Neuropophysial hormones and water and sodium balances in the South American lungfish (*Lepidosiren paradoxa*). *Proc. IVth Int. Congr. Endocrinol. Int. Congr. Ser.*, 256:137.
- . 1975. Endocrine adaptation to osmotic requirements of the environment: endocrine factors in osmoregulation by lungfishes and amphibians. *Gen. Comp. Endocrinol.*, 25:224-229.
- SAWYER, W. H., J. R. BLAIR-WEST, P. A. SIMPSON, AND M. K. SWYER. 1976. Renal responses of Australian lungfish to vasotocin, angiotensin II and NaCl infusion. *Amer. J. Physiol.*, 231:593-602.
- SOMLYO, A. V., AND A. P. SOMLYO. 1968. Vasotocin-magnesium interaction in vascular smooth muscle of the hagfish (*Eptatretus stoutii*). *Comp. Biochem. Physiol.*, 24: 267-270.
- STURKIE, P. D., AND Y. C. LIN. 1966. Release of vasotocin and oviposition. *J. Endocrinol.*, 35:325-326.
- URANGA, J., AND W. H. SAWYER. 1960. Renal responses of the bullfrog to oxytocin, arginine vasotocin and frog neurohypophysial extract. *Amer. J. Physiol.*, 198:1287-1290.
- VELDEN, R. VON DEN. 1913. Die Nierenwirkung von Hypophysenextrakten beim Menschen. *Berlin Klin. Wochschr.*, 50:2083-2086.
- WILHELM, A. E., G. E. PICKFORD, AND W. H. SAWYER. 1955. Initiation of the spawning reflex response in *Fundulus* by the administration of fish and mammalian neurohypophysial preparations and synthetic oxytocin. *Endocrinology*, 57:243-252.
- WINGSTRAND, K. G. 1959. Attempts at a comparison between the neurohypophysial region in fishes and tetrapods, with particular reference to amphibians. Pp. 393-403, in *Comparative endocrinology* (A. Gorbman, ed.), Wiley and Sons, New York, xx +746 pp.
- WOOLLEY, P. 1959. The effect of posterior lobe pituitary extracts on blood pressure in several vertebrate classes. *J. Exp. Biol.*, 36:453-458.
- YORIO, T., AND P. J. BENTLEY. 1977. Asymmetrical permeability of the integument of tree frogs (Hylidae). *J. Exp. Biol.*, 67:197-204.

ADENOHYPOPHYSIS: STRUCTURE AND FUNCTION

MARTIN P. SCHREIBMAN

The pituitary gland provides very special material for a study of the evolution of structure and function of endocrine systems in vertebrates. The diversity and complexity of this gland would be difficult to evaluate within the limited confines of this presentation were it not for several reviews that deal specifically with adenohipophysial evolution (Harris and Donovan, 1966; Wingstrand, 1966a; Holmes and Ball, 1974; Fontaine and Olivereau, 1975).

Our discussion of the evolution of an organized pituitary gland begins with the cyclostomes. It is true that the literature abounds with attempts to homologize Hatschek's pit of amphioxus or the subneural gland of tunicates with the vertebrate pituitary. There are even those who would suggest that the forerunner of the hypophysis may be found in the nemertean worms (Willmer, 1970). These structures, which may have had a sensory function or whose mucous secretions served as feeding mechanisms, originate as invaginations from the roof of the oral cavity. The fact that Rathke's pouch, the anlage for the vertebrate adenohipophysis, has a similar origin and that at least some pituitary cells may have been derived from a mucoid cell-type, makes Hatschek's pit and the subneural gland attractive for consideration as pituitary gland progenitors.

This paper is in two parts. An orthodox presentation of pituitary structure and function in vertebrates will be followed by a less conventional presentation of ideas and observations that is intended to stimulate thought and discussion.

Class AGNATHA

Agnathans, the most primitive living vertebrates, are represented by two orders: Petromyzoniformes (lamprey eels) and Myxiniiformes (hagfish). The hagfish pituitary gland appears to be the most primitive of the two. Its most striking feature is an adenohipophysis that is separated from the neurohipophysis and not differentiated into zones except, perhaps, in the posterior region of older and larger animals (Holmes and Ball, 1974). Generally, islets of cells are embedded in a poorly vascularized connective tissue (Fernholm, 1972; Olsson, 1969). Some cells form follicles and contain PAS-positive (periodic acid-Schiff-positive) colloid.

Cell types are difficult to characterize but there may be several if they are classified by their affinities for standard pituitary stains. They have no clear cut pattern of distribution in the adenohipophysis and their function is obscure. Recent studies have shown the presence of a thyrotropic substance in the adenohipophysis of the hagfish, *Eptatretus stouti* (Dickhoff and Gorb-

man, 1977), but attempts to demonstrate a gonadotropic factor have not been successful (Matty *et al.*, 1976). It may be that a single class of cells secretes more than one hormone, all of similar chemical structure. This will be discussed later.

The question of the structure and function of a hypophysial-portal system is not resolved. Structurally, it is markedly reduced but may show species variation. There is no developed portal system in the Eastern Pacific hagfish, *Eptatretus (Polistotrema) stouti*, (Gorbman, 1965). In *Eptatretus burgeri*, the Western Pacific hagfish, some 20 portal vessels have been recorded and the suggestion is presented that the ventral floor of the neurohypophysis is a primitive median eminence structurally similar to that of tetrapods (Kobayashi and Uemera, 1972). However, there are still many questions to be resolved, including a determination of the direction of blood flow and whether this structure exercises control over the adenohypophysis. It is interesting to note that *E. stouti* and the Atlantic hagfish, *Myxine glutinosa*, do not migrate from a consistently cold and dark environment and do not display seasonal cycles (Gorbman *et al.*, 1963). These organisms have few (if any) blood vessels. *E. burgeri*, on the other hand, has a seasonal gonadal cycle, moves into shallow water (Kobayashi *et al.*, 1972), and may have a better developed "median eminence" with greater potential for hypothalamic control of pituitary functions, although this has not been demonstrated yet. This may very well be a case of a structure appearing before its function has developed. The entire question requires additional study.

The lamprey adenohypophysis has a more orthodox organization. It is compact and divided into three zones. Only the pars intermedia is in contact with the neurohypophysis, but there are no direct nervous or vascular connections between the two (Tsuneki and Gorbman, 1975a). The pars distalis is separated from the infundibulum by connective tissue and its cells display a regional distribution, at least according to their stain affinity, ultrastructure, and purported function (see Ball and Baker, 1969; Larsen and Rothwell, 1972; Holmes and Ball, 1974). The rostral pars distalis (RPD) contains either chromophobes or PAS-positive and aldehyde fuchsin-positive basophils. The caudal pars distalis (CPD) contains both acidophils and fewer basophils. The function of the pars distalis cells is not clear despite attempts to relate them to metamorphosis, spawning, hypophysectomy, the action of inhibiting agents, and immunochemical analysis. Reports for the pars intermedia vary but generally one cell type is present that is carminophilic, either PAS-positive or negative and lead hematoxylin-positive. These cells undergo cytological change with varying illumination, migration, and spawning and presumably secrete MSH (van Oordt, 1968; Ball and Baker, 1969; Larsen and Rothwell, 1972).

There is little evidence to suggest that the hypothalamus exerts any influence on the pars distalis. Larsen (1969) showed that even with an ectopic adenohypophysis, complete gonadal maturation may take place in male lampreys. This is supported by ultrastructural studies, which fail to demon-

strate the presence of nervous or vascular connections between the anterior neurohypophysis and the pars distalis even though this region in lampreys is similar to the median eminence of higher vertebrates (Tsuneki and Gorbman, 1975b). Tsuneki and Gorbman (1975b) have suggested that neurosecretions diffuse across the thin connective tissue separation into the adenohypophysis. This could represent the most primitive of hypothalamus-pituitary associations.

Adult lampreys, unlike hagfish, generally lead an active predatory life. Changes in the amount of neurosecretory material in their neural lobe may reflect accommodations to varying environments brought about by their migrations between rivers and the sea (Ball and Baker, 1969; Holmes and Ball, 1974), variations in daylight (Öztan and Gorbman, 1960), and a variety of other seasonally-related phenomena (Sterba, 1969).

Class CHONDRICHTHYES

The living cartilaginous fish diverged early and pursued an evolution independent of all other fish groups. Recent palaeontological studies have indicated that the two extant subclasses—the sharks, skates, and rays comprising the Elasmobranchii and the rat-fishes or rabbit fishes and chimaeroids of the Holocephali—are independent derivations of placoderm ancestors (Moy-Thomas and Miles, 1971). Although most of our knowledge of adenohypophysial structure is derived from studies of the elasmobranchs, the phylogenetic concepts are supported by the existence of many similarities accompanied by significant differences when compared to the holocephalian gland.

In elasmobranchs, the adenohypophysis is divided into four areas. There is an elongated pars distalis (the "dorsal lobe") with its characteristic RPD and CPD, a ventral lobe attached to the CPD, and a large pars intermedia that is heavily penetrated by neurohypophysial tissue to form a typical neurointermediate lobe.

Follicles and spaces are characteristic of the chondrichthyan pituitary and range from a highly developed system of vesicles and tubules that communicate with a hollow pars distalis in sharks and dogfish, to a situation that is found in skates and rays where the hypophysial cavity is small or lost entirely. The ventral lobe is also hollow and often vesicular (Ball and Baker, 1969). All of the cavities and spaces, including a persistent hypophysial cleft, are apparently remnants of Rathke's pouch. (Elasmobranchii are the only fishes to have a hollow Rathke's pouch during development.) The cavity frequently contains a PAS, aldehyde fuchsin, and alcian blue-positive colloid. The concept that this represents stored hormones is no longer tenable (Holmes and Ball, 1974). It is likely that the secretions emanate from non-endocrine, chromophobic cells that line the walls of the cavity. Their structure and the chemistry of their secretion suggest that they are similar to the mucous-secreting epithelial cells of the buccal cavity (Mellinger, 1969; Alluchon-Gérard, 1971). They have been homologized with the stellate cells

that are found in the adenohypophysis of most vertebrates (Vila-Porcile, 1972).

Although certain tropic hormone functions have been defined clearly and associated with specific areas of the gland, others remain elusive. Attempts to link hormones to specific cells have spawned contradictions. Adrenocorticotrophic hormone (ACTH) and prolactin presumably are produced in the RPD (Fontaine and Olivereau, 1975). There is one cell type present that stains with PAS and orange G but not with aldehyde fuchsin or alcian blue. This cell, therefore, is unlike the corticotropin and prolactin-producing cells of teleosts. Earlier reports that ACTH may be found in the pars intermedia as well as in the RPD (deRoos and deRoos, 1967) have been confirmed by the use of immunofluorescence. There is, however, no cross reactivity with antialpha or antibeta MSH antibodies or with porcine anti-ACTH (Mellinger and Dubois, 1973). The CPD cells are acidophils that have a varied response to PAS and an unknown function. Identification of the hormones that originate in the ventral lobe continues to be a source of confusion. The cells of this lobe are large and stain with aldehyde fuchsin, PAS, and alcian blue. According to Ball and Baker (1969) and Dodd *et al.* (1960), they may represent thyrotrops and gonadotrops. Scanes *et al.* (1972) reported that gonadotropic activity, as judged by ^{32}P uptake in the chick gonad, occurs only in the ventral lobe. Immunofluorescence confirms luteinizing hormone-like (LH) activity in this region (Mellinger, 1972). In addition, removal of the ventral lobe from the dogfish results in the degeneration and phagocytosis of a specific stage of spermatogenesis (Dobson and Dodd, 1977a, 1977b, 1977c; Dodd *et al.*, 1960; Mellinger, 1965).

In holocephalians, the entire pars distalis is hollow and not clearly divisible into rostral and caudal regions. A ventral lobe, characteristic of the elasmobranchs, is not present. In adult holocephalians, there is a large compact follicular structure called the Rachendachhypophyse (the pharyngeal lobe). It lies outside the cranium in the roof of the buccal cavity and has an independent blood supply (Jasinski and Gorbman, 1966). In young *Hydrolagus*, the pharyngeal lobe is vesicular and composed of a single layer of follicular epithelium (Sathyanesan and Das, 1975). According to Ball and Baker (1969), the Rachendachhypophyse contains two kinds of basophils; both are PAS-positive but only one is aldehyde fuchsin-positive. It is not likely that the Rachendachhypophyse and the ventral lobe are homologues, because their embryogenesis differs (Honma, 1969). Rather, a "homology" has been suggested with the buccal part of the *Latimeria* pituitary gland (Holmes and Ball, 1974; see page 16). The cell types of the *Hydrolagus* pituitary have recently been described (Sathyanesan and Das, 1975) but their function(s) remains unknown.

A portal system and a median eminence are present in elasmobranchiomorphs. In elasmobranchs, but not in holocephalians, the median eminence is divisible into an anterior and posterior region that corresponds to the RPD and CPD. This is reminiscent of the median eminence seen in birds,

which also is divided into an anterior and posterior portion and which serves in a "point to point" delivery of vessels to the adenohypophysis. There is no direct innervation of the pars distalis (in contrast to teleosts) so that the portal system is the only route for hypothalamic control. In elasmobranchs, too, there is a portal supply to the neurointermediate lobe.

Class OSTEICHTHYES

Subclass ACTINOPTERYGII

Another group of fishes was evolving towards the dominant fishes alive today, the teleosts, at the same time that the sharks, skates, and rays were moving away from their ancestral stock. This group, the Actinopterygii (fish with bony skeleton and paired ray fins), includes the infraclasses Telostei, Chondrostei, and Holostei—the latter two taxa are often referred to as the ganoid fishes. An understanding of the structure and function of the pituitary gland in extant ganoids may help us to better evaluate the evolution of the teleostean gland.

Infraclass CHONDROSTEI

The Chondrostei contain two orders, Polypteriformes (for example, *Polypterus* and *Calamoichthys*) and Acipenseriformes (paddlefish and sturgeons). The pituitary gland of both *Polypterus* and *Calamoichthys* are apparently similar in structure. Their most remarkable feature is the presence of a duct or canal that connects a cavity in the ventral region of the RPD to the roof of the mouth (the buccohypophysial or orohypophysial duct). In section, they sometimes appear as follicles within the RPD. The canal presumably arises from a solid Rathke's pouch. The gland subdivisions are easily discernible and resemble those seen in teleosts. The only cell that has been identified with any certainty by structure, stain, and the use of immunofluorescence has been the one producing prolactin. Fluorescent-labeled antibodies to ovine prolactin are bound by cells scattered throughout the RPD of *Polypterus* and *Calamoichthys* (Aler, 1971). The duct cells do not stain with the labeled antibodies and are, therefore, not homologous with the prolactin cells that surround the follicles of the Salmonidae and the Clupeidae. The duct cells are mucous-producing (Lagios, 1968) and nonendocrine.

Calamoichthys and *Polypterus* possess typical median eminences and portal systems and in this respect are different from the phylogenetically more recent teleosts (Lagios, 1968). There is no innervation of the pars distalis cells.

In the order Acipenseriformes, the most notable anatomic feature is a large central hypophysial cavity with many tubular extensions. Unlike the palaeoniscoids, however, there is no hypophysial duct. There are also many "follicles" that contain PAS-positive colloid and are surrounded by acidophils, basophils, and chromophobes. These acidophils may be the source of

the prolactin detected by bioassay (Sage and Bern, 1972). All these observations have led to the speculation that these vesicles are homologous to the RPD follicles in holosteans and some "primitive" teleosts (Holmes and Ball, 1974).

A thin-walled neural lobe penetrates into the deepest regions of a large pars intermedia. There is no direct innervation of the pars distalis (Sathyanesan and Chavin, 1967), but there is a well-developed median eminence and portal system (Ball and Baker, 1969; Hayashida and Lagios, 1969).

Infraclass HOLOSTEI

The Holostei are the closest living primitive relatives of the teleosts. Neither genus of the two living holosteans, *Amia* (bowfin) and *Lepisosteus* (garpiki), has a hypophysial cavity or duct in the adult form. The gland is attached along most of its length to the infundibular floor and a vascular connective tissue is found in between. There is a large saccus vasculosus (Sathyanesan and Chavin, 1967).

The RPD contains closed follicles that are lined with the acidophils that may be the source of prolactin (Sage and Bern, 1972). These cells are in close association with an erythrosin, aniline blue, and lead hematoxylin positive cell that may be the corticotrop. A so-called "basophil" (it stains with periodic acid-Schiff, aldehyde fuchsin, alcian blue, and lead hematoxylin) also takes erythrosin and could be (although no experimental evidence is available) the gonadotrop (Holmes and Ball, 1974). In the CPD, there is an acidophil that differs from those of the RPD, stains with orange G, and is purported to be a somatotrop. Two types of basophils, one of which is a TSH cell and the other unknown, are present also in the CPD. In the pars intermedia, a prominent lead hematoxylin-positive cell and a less numerous PAS-positive cell are present.

In contrast to teleosts, the pars distalis is not penetrated by large strands of neurohypophysial tissue. Lagios (1970) has shown, however, that there are small numbers of fibers with neurosecretory material (alcian blue-positive) that extend into and abut against the cell cords of the CPD. Innervation of the pars intermedia of *Amia* is similar to that of other ganoids and primitive teleosts in that type A fibers contact the basement membrane of the intervacular space that separates the neural processes from endocrine cells (Holmes and Ball, 1974). Occasionally type A fibers appear to synapse with pars intermedia cells (Lagios, 1970). This type of novel contact is rare in other ganoids but common in teleosts. Holosteans also maintain a median eminence that is somewhat better developed than their phylogenetic predecessors, the acipenseroids.

Thus, it appears from an examination of the pituitary in *Amia* that innervation of the adenohypophysial cells, a characteristic that typifies teleosts, may have preceded the change in the median eminence-portal system that we see in the bony fishes.

Infraclass TELEOSTEI

Reviews on the structure and function of the teleostean pituitary gland have appeared with a regular periodicity in recent years (Ball and Baker, 1969; Sage and Bern, 1971; Schreibman *et al.*, 1973). Teleosts represent the most diverse group of vertebrates with more than 20,000 extant species recorded. Inasmuch as they occupy every conceivable habitat, it would not be surprising to find this variability reflected in a number of different patterns of pituitary organization and function. However, this is generally not the case, for within all this diversity are common anatomical, histological, and cytological denominators. The most notable of these is that the adeno-hypophysis is clearly divided into three regions. The clarity of this separation is essentially due to a restriction of specific cell types to distinct regions of the gland. In teleosts, there is also a structural intimacy of neuro and adeno-hypophysis. Adeno-hypophysial cells are innervated directly and there is a modified portal system and median eminence but no pars tuberalis.

Variations in the structure of the pituitary gland appear in the axis formed between the pituitary gland and the brain and, thus, in the orientation of the various adeno-hypophysial regions. Specific cell types may not be found in the same region of the gland in all fishes. This is especially true for the thyrotrops. The arrangement of cells may vary too. For example, follicles that are common in the Salmonidae, Clupeidae and Anguillidae generally are not found in other fish. A persistent orohypophysial duct may be seen in adult *Chanos chanos* (Olsson, 1974) and in some Clupeidae (Misra and Sathyanesan, 1958). Different hypothalamic control mechanisms also have been noted among the teleosts (see Holmes and Ball, 1974, for discussion).

The neurohypophysis generally can be divided into two parts, an anterior region with aldehyde fuchsin-negative type B fibers (and a modicum of pituicites) in contact with the pars distalis, and a posterior region composed mainly of aldehyde fuchsin-positive type A fibers (but with many pituicites) in direct association with the pars intermedia. It has been suggested from functional, anatomical, and embryological evidence that these two regions correspond to the median eminence and the neural lobe, respectively (Ball and Baker, 1969; Perks, 1969; Vollrath, 1972).

Subclass SARCOPTERYGII

This subclass, appears as a separate stock from the chondrichthyans and actinopterygians. It consists of the orders Dipnoi and Crossopterygii. Inasmuch as tetrapods presumably evolved from a rhipidistian stock, the single Coelacanth genus, *Latimeria*, and the half dozen species of lungfish have a very special interest in our consideration of the evolution of pituitary gland structure and function.

Order DIPNOI

The adenohipophysis of lungfish is more similar in structure to amphibians than it is to other fishes (Holmes and Ball, 1974). Among the amphibian characteristics of the lungfish pituitary are a well-formed neural lobe, a prominent median eminence, the absence of a saccus vasculosus, and a less obvious regional distribution of cell types (Kerr and van Oordt, 1966). Nevertheless certain fish-like characteristics persist. Most notable is the direct innervation of the pars distalis cells (Zambrano and Iturriza, 1973), the interdigitation of the neurohypophysis and pars intermedia, a prominent hypophysial cleft between the pars distalis and the pars intermedia, an absence of a pars tuberalis, and the presence of follicles especially in *Neoceratodus*.

Lack of experimental evidence precludes assigning specific functions to distinct cells. Nevertheless, investigators do extrapolate from the data in amphibians and justify this scientifically unsound action by citing the similarities of stain response, distribution of cells, and ontogenetic appearance.

The size of the pars intermedia, the arrangement of its cells, and its structural association with the neural lobe processes varies among the lungfish. In all forms it is separated from the pars distalis by a hypophysial cleft.

The neurohypophysis clearly is divisible into a median eminence, infundibular stalk, and neural lobe (Perks, 1969). The preoptic nucleus (PON) is the major and perhaps the only hypothalamic nucleus and stains with the usual neurosecretory material methods. The neurons are peptidergic and terminate around the primary portal vessels, the plexus intermedius, the neural lobe, and in the pars intermedia. In *Lepidosiren*, Zambrano and Iturriza (1973) described many small aminergic neurons whose perikarya lie just above the ependyma of the median eminence, and whose axons end in the pars distalis, pars intermedia, and in the neural lobe close to the pars intermedia. These neurons resemble the NLT (nucleus lateralis tuberis) of teleosts and the infundibular and arcuate nucleus of tetrapods. We have a system here of type A fibers terminating on capillaries anteriorly but on an avascular connective tissue posteriorly.

There is still much to be learned about the lungfish pituitary. We need to know what variations there are in hypophysial structure, aside from relative number of follicles, size of intermedia and innervation of cells, that would account for differences in the physiological activities of the three living genera of Dipnoi. This would be especially interesting in regard to the differences in ability of lungfish to aestivate (Thomson, 1969).

As we have seen, there is a portal system in all fish groups as well as in the tetrapods. Direct innervation developed (apparently independently) in both actinopterygians and crossopterygians. In teleosts, the neural link has essentially become more dominant to the portal link. The detail of the sequence of events and their evolutionary significance requires further study.

Order CROSSOPTERYGII

Latimeria chalumnae is the only living representative of the Suborder Coelacanthini. From its phylogenetic position one would expect to find a gland that heralds the structures of the tetrapods while retaining the characteristics of the preceding and more primitive fish groups, that is, a true intermediate stage.

Recently Lagios (1972) and van Kemenade and Kremers (1975) described single specimens collected in 1970 and 1972, respectively. The orthodox features of the coelacanth pituitary include a neurointermediate lobe (typically fish-like) ventral to the brain, direct contact between pars distalis and pars intermedia, follicles and tubules containing glycoprotein colloid seen in many fish, and a well-developed hypothalamo-hypophysial portal system (Lagios, 1972). Other "fish-like" features include a small saccus vasculosus, penetration of neurohypophysial tissue into the pars distalis, and regional differentiation of the gland based on cell type.

Among the exceptional features is a greatly extended pars distalis that lies, in part, in a dorsal depression of the neurointermediate lobe. Especially unique is a long cylinder of connective tissue that extends from the pars distalis and within which are vascularized masses of adenohypophysial cells, the so-called "rostral islets" or "pars buccalis." These structures are comparable with the Rachendachhypophyse of holocephalians rather than the ventral lobe of elasmobranchs at least insofar as anatomical connection is concerned, although cell types of the islets are similar to those in the ventral lobe.

Thus, the pituitary gland of *Latimeria* shows many specializations and peculiarities. It possesses many features of the teleost and elasmobranchiomorph gland rather than those of dipnoans and amphibians. As Holmes and Ball (1974) indicate, *Latimeria* is probably not going to provide much information on how the tetrapod pituitary evolved from the primitive fish glands. It certainly appears that the dipnoan gland, even though phylogenetically more isolated, is a much better intermediate form between fishes and amphibians.

Class AMPHIBIA

Most will agree that modern amphibians should not be considered ancestral to reptiles, birds, or mammals, and yet the amphibian gland is very much like the hypophysis of the other tetrapods. This is taken as strong evidence that the basic structure of the tetrapod pituitary developed very early in evolution from its crossopterygian predecessors (Holmes and Ball, 1974).

The adenohypophysis is generally composed of a compact pars distalis that is differentiated into cephalic (rostral) and caudal regions. In anurans, it lies ventral and posterior to the neural lobe. The pars distalis is flattened and often wider than long. Dorsally it continues with the pars intermedia

without an intervening cleft and anteriorly it connects to the median eminence by strands of connective tissue through which the portal vessels pass. The pars tuberalis is represented by a pair of epithelial plaques attached on either side of the tuber cinereum anterior to the median eminence.

In urodeles, the pars distalis occupies the same position but is more elongated. The median eminence is almost always less well formed than in the anurans. Perhaps the fact that the pituitary gland is more "fish-like" in its structure may be related to the fact that they are highly specialized for aquatic life (Holmes and Ball, 1974). Our knowledge of pituitary structure and function does not shed any light on the question of whether anurans and urodeles evolved independently or as a unit.

Pituitary structure is least known for the order Apoda (includes the blind worm-like burrowers from tropical regions). The gland is considerably more flattened. Although the median eminence is relatively well developed, it is not known how it functions.

There is a wealth of information, not all of it conclusive, that identifies five functional cell types in the pars distalis and one MSH cell (PAS and lead hematoxylin-positive in most) in the pars intermedia. Classification by new techniques, especially immunocytochemistry, provides an important and useful complement to structural and physiological studies (Doerr-Schott, 1976a; Hansen and Hansen, 1976).

The neurohypophysis is differentiated into a neural lobe and a median eminence, which varies greatly in its complexity. The neural lobe is generally largest in the more terrestrial amphibians (Dodd *et al.*, 1971). In anurans, the median eminence can be as elaborate a structure as one finds in mammals (Holmes and Ball, 1974). The pars intermedia receives blood from the neural lobe, the median eminence, and from the general circulation. The pars distalis, however, receives blood only from the median eminence (Rodriguez and Piezzi, 1967).

Class REPTILIA

The morphology of the reptilian hypophysis is well known from the works of Wingstrand (1966a, 1966b) and Saint-Girons (1963, 1967, 1970). In general, it is felt that the gland strongly resembles both the amphibian and avian structures—a reassuring observation considering the phylogenetic position of reptiles.

In *Sphenodon*, turtles, and crocodiles there is a prominent pars intermedia, pars tuberalis, and a pars distalis that has clearly defined cephalic (rostral) and caudal lobes. The pars tuberalis is reduced or absent in lizards and never develops in snakes. Portal vessels pass from the median eminence to the pars distalis in connective tissue but without the company of pars tuberalis tissue (a situation reminiscent of that which exists in fishes). In some adult lizards, the pars tuberalis, which forms plaques on either side of the median eminence, invades the hypothalamus and median eminence when the basement membrane and pia separating the tuberalis disappear (Holmes

and Ball, 1974). In *Typhlops* and *Leptotyphlops* (blind and burrowing snakes) and in the burrowing lizards, there is no pars intermedia and no intermedia cells are found in the pars distalis (Saint-Girons, 1963, 1967). This is unlike the situation in birds and some mammals that, although they lack a discrete pars intermedia, have intermedia cells in their pars distalis. In most snakes, however, the pars intermedia is massive.

As is typical in tetrapods, the pars distalis is made up of branching cords of cells that are highly vascularized by intervening sinusoids. Occasionally follicular structures may form. Recent immunocytochemical studies by Doerr-Schott (1976b) have demonstrated the similarity of distribution of hypophysial cell types in reptiles and amphibians. That is, ACTH and prolactin-producing cells are found in the rostral zone, STH-producing cells are found in the caudal zone, and gonadotrops and thyrotrops are found in both lobes (see Doerr-Schott, 1976a, 1976b, for discussion; Schreibman and Holtzman, 1975).

Class AVES

The hypophysis of birds has been described by Wingstrand (1951). Several features distinguish their gland from that of other vertebrates. In many birds, the oral part of Rathke's pouch gives rise to an epithelial stalk that persists as a connection between the pars distalis and the buccal epithelium. The separation of the adenohypophysis and the neurohypophysis makes it less of a compact unit as, for example, is seen in mammals.

The pars tuberalis is located between the median eminence and the pars distalis although in some species (for example, *Diomedea melanophris*) it may lie within the median eminence. In those birds where the tuberalis is not closely applied to the neural downgrowth, an isolated porto-tuberal tract may form (Benoit and Assenmacher, 1953) that serves to bridge neural and glandular regions. There is no organized pars intermedia although intermedia cells (lead hematoxylin-positive) can be found in the pars distalis.

The median eminence differs from mammals in that the primary capillaries of the portal system are more superficial and form a somewhat less complex arrangement in birds. The median eminence can be differentiated into the so-called "point-to-point" portal system, which comprises a rostral portion that supplies blood to the cephalic lobe and a caudal area that delivers blood to the caudal pars distalis (Vitums *et al.*, 1964; Sharp and Follett, 1969). This arrangement of the vessels may have considerable importance in light of the distribution of cell types into the different lobes for it may provide a mechanism for the separation and deliverance of specific stimuli from the central nervous system to the adenohypophysis. The differentiation of the median eminence into two regions is reminiscent of the situation in reptiles and elasmobranchs.

Class MAMMALIA

Despite the fact that there is a basic similarity in the structure of the mammalian pituitary gland, departures from the pattern can be found in various genera (see reviews by Hanström, 1966, and Holmes and Ball, 1974). Generally, these differences are in the degree of development of the parts of the neurohypophysis, the axis of the gland in relation to the brain, the presence of an intraglandular cleft, and the extent of development of the three parts of the adenohypophysis and their relationship to the neurohypophysis.

One variation is the failure of the pars intermedia to form in cetaceans (whales and dolphins), presumably as a result of a connective tissue barrier that separates the neuro and adenohypophysis. Although a pars tuberalis is a characteristic of the mammalian gland, in some animals such as the sloth (Wislocki, 1938) and the pangolin (Herlant, 1958) it may be absent. In more primitive forms (for example, members of the order Monotremata, the duck-billed platypus and spiny anteaters) a typical porto-tuberal tract is present similar to that of many birds and reptiles.

Most adenohypophysial cells occur in cords or small groups and in close proximity to blood vessels. The regional distribution of cells seen so frequently in nonmammalian vertebrates may sometimes occur in mammals too. For example, a differential distribution of basophils has been noted in the pars distalis of rats (Purves and Griesbach, 1951). In other mammals, like the tree shrew and the potto (Holmes and Ball, 1974), typical follicular structures containing PAS-positive colloid are clearly evident. It appears, therefore, that pituitary cells arranged in follicles is a ubiquitous feature in vertebrates. Perhaps it is an inherent property of adenohypophysial cells to be able to reverse polarity and secrete their product into a central depository.

Generally, it is accepted that direct innervation of the adenohypophysis does not play an important role among the mammals if, indeed, it plays any at all. However, innervation of delivering and draining blood vessels may be an important control mechanism. The dominant regulatory mechanism of the adenohypophysis in mammals is via the vascular system. This feature shows phylogenetic constancy for a portal system is found in mammals, birds, reptiles, amphibians, and in some fishes.

DISCUSSION

The remainder of this chapter will deal with a number of more or less related topics that may have significance in the history of the hypophysis or that signal new areas of exploration to be pursued in our attempt to understand better the evolution of endocrine structure and function. This discussion will begin by posing a series of questions.

Extra-pituitary Pituitary Hormones

Is it conceivable that hypophysial hormones may originate from sources other than the pituitary gland; that is, what are the possibilities for extra-

pituitary pituitary hormones? This notion, I am certain, must have occurred to each of us at one time or another—perhaps when we observed what appeared to be a persistent pituitary-mediated action or ever-present circulating levels of a tropic hormone even in the absence of a pituitary gland. A prime example was the unexpected survival of hypophysectomized fish, noted by Schreibman and Kallman (1969), which were expected to die in freshwater in the absence of prolactin. Extra-pituitary secretion was again suggested when normal levels of prolactin were detected in the plasma of hypophysectomized teleosts (Schreibman *et al.*, 1976; Nicoll *et al.*, 1977).

The possibility of hormones emanating from other than an *in situ* gland is a possibility for there are several instances among the vertebrates of functional ectopic pituitary tissue. For example, in humans there is a pharyngeal hypophysis (craniopharyngeal) that is an elongated body in the roof of the nasopharynx. It is encapsulated, vascularized, and exhibits staining properties similar to the sellar hypophysial cells (McGrath, 1971). ACTH, STH, TSH, and prolactin have been reported in this structure (Hachmeister, 1967; McGrath, 1968, 1971), and the ultrastructure of one of its four cells resembles a pituitary somatotrop (González *et al.*, 1977). The pharyngeal hypophysis also undergoes sexual dimorphic, age-related volume changes (Boyd, 1956; McGrath, 1968, 1971).

The Rachendachhypophyse of elasmobranchs and the scattered pituitary islets in *Latimeria* are other examples of pituitary tissue that no longer maintain their connection with the diencephalon. By contrast, adenohypophysial cells may occur within the brain. By way of illustration, the pars tuberalis, during its normal development, is incorporated into the median eminence and hypothalamus of birds and reptiles. The pituitary gland may also extend to the pharynx. Epithelial stalks persist as remnants of Rathke's pouch in birds, reptiles, and in a number of fishes and may retain a potential for redifferentiation.

It may be that unorganized ectopic tissue with the ability to secrete pituitary hormones goes undetected when sought by dissection or histological means because the cellular conformation has been altered. The chromophobic appearance of several hormones (such as, prolactin) secreting tumors may be related to their intense secretory or release activity (Furth, 1955; Holmes, 1964; Lundin and Schelin, 1964).

Related more directly to hormone production are the reports that ACTH may originate from the diencephalon as well as from the hypophysis. Krieger *et al.* (1977) demonstrated immunoreactive and bioreactive ACTH-like activities in the brain of normal and hypophysectomized rats. Similar findings have been made in hogs (Guillemin *et al.*, 1962) and dogs (Schally *et al.*, 1962). The question arises as to the biological significance of this extra-hypophysial ACTH since plasma ACTH is undetected and adrenal weight and function is impaired in hypophysectomized rats. It has been suggested that ACTH in the brain may have an extra-endocrine role as in learning, memory, and motivation (deWied, 1974, 1977). Recently a substance was

extracted from the rat amygdaloid nucleus that was identical to pituitary growth hormone as judged by immunology, chromatography, and bioassay analysis (Pacold *et al.*, 1978). These authors also reported that amygdaloid extracts had stimulatory effects on the adrenal, gonad, and thyroid of the rat. They speculate that these products serve as neurotransmitter substances that affect neurosecretory influences on the pituitary (Pacold *et al.*, 1978). That neurons of the central nervous system may synthesize pituitary hormones lends support to those who postulate a neuroectodermal origin for endocrine secretory cells (Takor and Pearse, 1975).

The placenta is another nonpituitary structure that produces several pituitary-like hormones (Josimovich and Venning, 1975). It has been suggested, too, that human amnionic epithelial cells synthesize prolactin (Healy *et al.*, 1977). In addition, it has been observed that a thyroid carcinoma produced several polypeptide substances with no less than ACTH-like, CRF-like, and prolactin-like activities (Birkenhäger *et al.*, 1976). This also serves to demonstrate that cells may change their secretory product under certain physiological conditions (a topic to be discussed below).

Immunohistochemical and RIA methods have demonstrated hypothalamic (hypophysiotropic) hormones in other portions of the central nervous system as well as in non-neural tissues (Jackson and Reichlin, 1974; Brownstein *et al.*, 1974; Hökfelt *et al.*, 1975; Yasuda and Greer, 1976; Currie *et al.*, 1977; Jackson and Reichlin, 1977). Even if it should develop that there is mimicry of molecule action or that the methods of measurement are subject to criticism, the observation that these various hypophysiotropic substances have similar biological activity leads to interesting speculation concerning the origin of hormone producing structures and is pertinent to our consideration of the evolution of pituitary control. The extra-hypothalamic occurrence of releasing factors enjoys widespread phylogenetic distribution and this suggests a basic role for them in all vertebrates. Convincing evidence is being amassed to support the view that these substances may function in the regulation of neuronal activity and the elicitation of specific behavioral effects (Jackson, 1977).

Cell Lineage and Multiplicity of Roles for Pituitary Cells

All cells are endowed with identical genetic information. Although specialized cells use specific parts of the chromosome to perform their functions, we must recognize the distinct possibility that a cell, either under normal or pathological situations, may retain the ability to draw on different portions of the information that is stored in the genome. In this regard, another persistent and basic question is, can certain cells transform into others and what is the relationship of the number of different types of cells to the number of hormones that are produced?

The concept of cell lineage, origin of adenohypophysial cells, and multiplicity of cell functions is especially thought provoking if one considers the *Myxine* pituitary gland. Fernholm (1972) identified PAS-positive cells that

could not be differentiated based on their ultrastructure. They were not considered endocrine cells because they were associated with colloid-containing cysts, their structure was unlike thyrotrops and gonadotrops of other vertebrates, and the existence of gonadotropins is questionable in the hagfish pituitary. He suggested, rather, that they are mucous-secreting cells (structurally they resemble them) that have become part of the adenohypophysis but have not transformed as yet into endocrine cells. This has been proposed by others as well and is supported by the observation that the adenohypophysis develops from an epithelium that is characterized by a large population of mucous-elaborating cells. Even in the more advanced teleost *Xiphophorus maculatus*, mucous-containing cysts may develop within the adenohypophysis (Schreibman, 1966). These cysts are lined with typical goblet cells characteristic of the buccal epithelium. From an evolutionary viewpoint, these observations point out that cell organization could precede cell function. However, the reverse sequence, function first followed by organization, may be possible also.

The other cell types of the hagfish pituitary are no less exciting as models for speculation of evolutionary lineage. Fernholm (1972) identified two cell types with the use of the electron microscope that secrete protein hormones. Based on morphological observations, he speculated that cell "type I" produces a hormone that is similar to ACTH and MSH and "type II," a substance similar to prolactin and somatotropin (STH). The idea was favored that there is a common parent molecule with the action of both hormones rather than two separate products manufactured by a single cell. The similarity of chemical structure and biological action of ACTH/MSH and prolactin/STH makes this an attractive hypothesis. This explanation could be extended to gonadotrops and thyrotrops based on the demonstration that mammalian LH, FSH, and TSH share a common chemical subunit (Pierce, 1971; Papkoff, 1972; Fontaine and Burzawa-Gerard, 1977). It would appear, therefore, that the hagfish pituitary could represent a stage in evolution that precedes the differentiation of ACTH from MSH and prolactin from STH as well as the formation of separate cells for their production.

The task of determining lineage for the chemical structure of pituitary hormones, as well as for the cells that make them, is replete with problems. This is illustrated by several reports of corticotropin activity in the pars intermedia, a region of the gland generally associated with MSH (a hormone whose similarity to ACTH already has been discussed). Kraicer *et al.* (1973) found ACTH in the pars intermedia of the rat that is biologically different from, but immunologically similar to, the ACTH of the pars distalis. Stoeckel *et al.* (1973) discovered cells in the pars intermedia of rats that are similar in their ultrastructure to the corticotrops of the pars distalis. The cells of the pars intermedia reacted strongly to anti-beta 1-24 ACTH antibodies but displayed no reaction to anti-alpha and anti-beta MSH antibodies.

ACTH has been reported also in the pars intermedia of teleosts. Haider and Sathyanesan (1975) examined the effect of formalin stress in *Clarias*

batrachus using autoradiographic methods. Cells of both the pars distalis and pars intermedia showed an increase in cytological and synthetic activity concomitant with an increase in the uptake of ^3H -thymidine by interrenal cells. This would suggest that cells of both regions produce corticotropin.

The similarity of the chemical content of cells in the pars distalis and the pars intermedia is illustrated further in a number of vertebrates by their uniform response to lead hematoxylin staining (Olivereau, 1970). In teleosts, lead hematoxylin-positive cells occur in both the rostral pars distalis and pars intermedia. It is supposed that in the pars distalis they produce ACTH and in the pars intermedia they synthesize MSH. The infrequent occurrence of isolated cells in the caudal pars distalis that stain with lead hematoxylin and have immunocytochemical similarities (Follenius and Dubois, 1976; Schreibman, Holtzman and Stricoff, unpublished data) leads us to hypothesize that the cells of the pars intermedia could originate from the RPD by migrating posteriorly during development. This is being investigated.

The above observations, which deal with the distribution of ACTH activity in the adenohipophysis as well as the similarity of chemistry and stainability of ACTH and MSH, leads to the following speculations. It is quite probable that one cell in the pars distalis synthesizes ACTH. In the pars intermedia, ACTH could be produced as a separate molecule in the MSH cell along with MSH or else a single parent molecule could be synthesized that has both ACTH and MSH activity. The suggestion that ACTH in the pars intermedia of mammals could serve as a precursor for two hormonal substances [MSH, which shares a common sequence of amino acids 1-13, and a corticotropin-like intermediate lobe peptide (CLIP) which carries the 18-39 amino acid sequence of corticotropin (see Fontaine and Olivereau, 1975)] lends support to the notion that a single parent molecule resides in a single cell type and can give rise to two different active adenohipophysial principles.

Multiple Forms of Pituitary Hormones?

What is the evolutionary significance of the observations that a single pituitary hormone may exist in several different forms? Attempts to answer this question by studying cell lines and tracing hormones to specific adenohipophysial cells are complicated inordinately by continued observations that a peptide hormone may occur in a variety of sizes (Yalow, 1974). These hormones are indistinguishable by immunochemical methods. For example, circulating immunoreactive prolactin exists in "little" (75-90 per cent of total circulating levels), "big" (8-20 per cent), and recently "big big" (0-10 per cent) forms (Suh and Frantz, 1974; Farkouh *et al.*, 1977). Luteinizing hormone also occurs in several forms in human serum (Graesslin *et al.*, 1976). The large, small, and "mini" forms are, except for the size heterogeneity, qualitatively indistinguishable as determined by RIA, radioligand, receptor assay, and bioassay methods.

Reports indicate that hormones also assume multiple forms *within* their cells of origin (Burgers, 1963; Nicoll, 1972; Weatherhead and Whur, 1972). Bogdanove *et al.* (1975) have made the thought-provoking suggestion that a change in the hormonal environment might influence an endocrine gland to alter its form of hormone. They present evidence that a rat gonad can dictate both the amount and kind of tropic hormone that is produced. These statements may receive support from observed alterations in cytological profiles that reflect changes in synthetic or release activity of endocrine cells with varying physiological states (see discussion by Schreibman *et al.*, 1973). Knowles (1965) differentiated peripheral and central cells in the pars intermedia of *Scyliorhinus stellaris* on the basis of location and morphology. He suggested that although they both produce MSH they have different methods of hormone storage or release and that there could be "fast" and "slow" cells depending on their proximity to blood vessels. A similar zonation of prolactin cells according to size and extent of stored granules has been described by Holtzman and Schreibman (1975) in the RPD of *Xiphophorus*.

That prolactin cells may change their pattern of synthesis and perhaps their product under different physiological states may be indicated by the marked shift in granule size that occurs when freshwater swordtails are placed in dilute sea water (Holtzman and Schreibman, 1972). This dramatic change in granule dimensions, along with a modification in the structure of the synthetic apparatus of the cell, has been noted by others (see discussion in Schreibman *et al.*, 1973).

These observations would suggest alterations in cell type and the nature of the products produced (modifications that could be considered of evolutionary significance and scope) may occur in a relatively short time and may be of transitory nature. These questions deserve continued attention. The more sophisticated methods of research currently being employed will not necessarily increase our understanding of endocrine mechanisms. It appears that our problems and questions are merely compounded by technological advances.

There are many topics that have been excluded from this presentation. For example, what is the sequence in evolutionary time for the development of, and the interaction between, definitive pituitary hormones, prostaglandins, cyclic nucleotides, and other neural and nonneural regulatory substances? Did these latter substances operate before *bona fide* pituitary agents assumed their present day role and did they have even greater importance than they do today? This is a rapidly advancing area of comparative endocrine investigation and hopefully in the near future we may be provided with answers to these questions (see Honn and Chavin, this symposium; Labrie *et al.*, 1976).

Genetics and Endocrine Evolution

We have already briefly discussed how a cell could conceivably change its functional potential by drawing information from different portions of its chromosomes. But what is the role of genetics on the larger scale of the evolution of endocrine gland interaction? Our knowledge of the genetic regulation of the endocrine system is rather limited. Generally, evolutionary trends are studied by examining a particular structure or physiological parameter in as many diverse forms as are available. In recent years, however, it has become apparent that endocrine structure and function is under specific regulation of the genome and that marked variations can be achieved even within a species. Identifiable gene loci dictate the precise time of sexual maturation and ultimate adult size by regulating gonadotrop development and, thus, gametogenesis and steroidogenesis (Kallman *et al.*, 1973; Kallman and Schreibman, 1973; Schreibman and Kallman, 1977). The profound influence on patterns of reproduction and body dimensions that is dictated by genetic information, could explain the evolution of new species or the modification of existing ones. For example, in poeciliid fishes, the variation in total length and habitus resulting from differences in the time of sexual maturation between brothers of the same brood may be of such magnitude that they could easily be mistaken for two separate species.

Endocrine Activity for Nonendocrine Cells

We also have much to learn about the significance of a group of cells that are generally considered nonendocrine types. Although we long have been aware of their presence, our understanding of what they do is being reevaluated. Tanycytes, stellate cells, and pituicytes are intimately involved in pituitary function and are ubiquitous among the vertebrates. It is clear that they play an important, albeit undefined, role in the storage, modification, and transport of pituitary hormones (see Holmes and Ball, 1974, for discussion). For example, tanycytes apparently are modified ependymal cells and were first described in elasmobranchs (Horstmann, 1954). Their potential importance is clearly indicated by their anatomical location between the ventricular system and portal and pituitary elements and from their cytological changes associated with certain endocrine processes (for example, reproductive cycles; Akmayev and Fidelina, 1976). These cells provide yet another important link between the central nervous system, cerebrospinal fluid, and adenohypophysial cells. Stellate cells may serve as an additional mechanism for the release of pituitary hormones by translocating (and modifying?) hormones from their site of synthesis to the vascular system. Whether this is an advanced or more primitive mechanism is subject to speculation. Clarification of the role of this class of cells and how they fit into the overall picture of pituitary function and of signal transmission between central nervous system and endocrine system should provide exciting evolutionary food for thought.

SUMMARY

Comparing the architecture of the adenohypophysis and relating it to its function, even within a class of vertebrates, is not a simple task and is complicated, not made easier, by the newer tools of investigation. On a phylogenetic basis, the evolution of the pituitary gland cannot as yet be dealt with comprehensively. We have gained much knowledge about the structure of the gland, but there are more than 60,000 species of vertebrates and most of them have not been examined. The inevitable structural variability to be expected when all of these forms are studied will doubtlessly complicate our understanding of the morphological events involved in the evolution of the pituitary. A consideration of the evolution of hypophyseal function is even more difficult to deal with than is an analysis of its morphology. The diversity of functions served by similar hormones, and the difficulty of using a single physiological parameter that could be examined in each vertebrate class, hampers analysis. Indeed, even posing meaningful questions becomes a task.

It is important that we exercise caution against making sweeping generalizations based on our limited information. It is true that we have begun to delineate some main trends in pituitary gland evolution, but our evolutionary tree must remain young and flexible in order to accommodate the inevitable, and essential, influx of new information. Our discussion of extra-pituitary pituitary hormones, whether manufactured by derivatives of Rathke's pouch or by specific neurons of the central nervous system, and of hypophysiotropic factors that are synthesized at some distance from the hypothalamus suggests that we may have to modify our focus and explore other avenues of investigation in order to continue our analysis of the evolution of pituitary gland structure, function, and control mechanisms.

LITERATURE CITED

- AKMAYEV, I. G., AND O. V. FIDELINA. 1976. Morphological aspects of the hypothalamic-hypophyseal system. VI. The tanocytes: their relation to the sexual differentiation of the hypothalamus. An enzyme-histochemical study. *Cell Tiss. Res.*, 173: 407-416.
- ALER, G. M. 1971. Prolactin-producing cells in *Clupea harengus membras*, *Polypterus palmas* and *Calamoichthys calabaricus* studied by immuno-histochemical methods. *Acta Zool. (Stockholm)*, 52:275-286.
- ALLUCHON-GÉRARD, M. J. 1971. Types cellulaires et étapes de la différenciation de l'adenohypophyse chez l'embryon de roussette (*Scyllium canicula*, *Chondrichthys*). Étude au microscope électronique. *Z. Zellforsch. Mikrosk. Anat.*, 120:525-545.
- BALL, J. N., AND B. I. BAKER. 1969. The pituitary gland: anatomy and histophysiology. Pp. 1-110, in *Fish physiology* (W. S. Hoar and D. J. Randall, eds.), Academic Press, New York, 2:xiv+1-446 pp.
- BENOIT, J., AND I. ASSENMACHER. 1953. Rapport entre la stimulation sexuelle prehypophysaire et la neurosécrétion chez l'oiseau. *Arch. Anat. Microsc. Morph. Exp.*, 42:334-386.
- BIRKENHÄGER, J. C., G. V. UPTON, H. J. SELDENRATH, D. T. KRIEGER, AND A. H. TASHJIAN, JR. 1976. Medullary thyroid carcinoma: ectopic production of peptides with ACTH-like, corticotropin releasing factor-like and prolactin-stimulating activities. *Acta Endocrinol.*, 83:280-292.

- BOGDANOVE, E. M., J. M. NOLIN, AND G. T. CAMPBELL. 1975. Qualitative and quantitative gonad-pituitary feedback. *Rec. Prog. Horm. Res.*, 31:567-626.
- BOYD, J. D. 1956. Observations on the human pharyngeal hypophysis. *J. Endocrinol.*, 14:66-77.
- BROWNSTEIN, M. J., M. PALKOVITS, J. M. SAAVEDRA, R. M. BASSIRI, AND R. D. UTIGER. 1974. Thyrotropin-releasing hormone in specific nuclei of rat brain. *Science*, 185:267-269.
- BURGERS, A. C. J. 1963. Melanophore-stimulating hormones in vertebrates. *Ann. New York Acad. Sci.*, 100:669-677.
- CURRIE, B. L., J. WARBERG, K. FOLKERS, C. Y. BOWERS, E. PAULSEN, J. MULDER, AND F. PAULSEN. 1977. Presence of peptide hormones that control gonadotropin secretion in extra-hypothalamic areas of the brain. *Biochem. Biophys. Res. Commun.*, 79:1207-1211.
- DEROOS, R., AND C. C. DEROOS. 1967. Presence of corticotropin activity in the pituitary gland of chondrichthyan fish. *Gen. Comp. Endocrinol.*, 9:267-275.
- DEWIED, D. 1974. Pituitary-adrenal system hormones and behavior. Pp. 653-666, in *The neurosciences third study program* (F. O. Schmitt and F. G. Worden, eds.), Massachusetts Institute of Technology Press, Cambridge, Mass., xxvii+1107 pp.
- . 1977. ACTH effects on learning processes. Pp. 51-56, in *Endocrinology. Proc. Vth Int. Congr. Endocrinol.* (V. James, ed.), Int. Congr. Ser. 402, Excerpta Medica, Amsterdam, 1:xiv+1-603 pp.
- DICKHOFF, W. W., AND A. GORBMAN. 1977. *In vitro* thyrotropic effect of the pituitary of the Pacific hagfish, *Eptatretus stouti*. *Gen. Comp. Endocrinol.*, 31:75-79.
- DOBSON, S., AND J. M. DODD. 1977a. Endocrine control of the testes in the dogfish *Scyliorhinus canicula* L. I. Effects of partial hypophysectomy on gravimetric, hormonal and biochemical aspects of testes function. *Gen. Comp. Endocrinol.*, 32:41-52.
- . 1977b. Endocrine control of the testes in the dogfish *Scyliorhinus canicula* L. II. Histological and ultrastructural changes in the testes after partial hypophysectomy (ventral lobectomy). *Gen. Comp. Endocrinol.*, 32:53-71.
- . 1977c. The roles of temperature and photoperiod in the response of the testes of the dogfish, *Scyliorhinus canicula* L. to partial hypophysectomy (ventral lobectomy). *Gen. Comp. Endocrinol.*, 32:114-115.
- DODD, J. M., P. J. EVENNETT, AND C. K. GODDARD. 1960. Reproductive endocrinology in cyclostomes and elasmobranchs. *Symp. Zool. Soc. London*, 1:77-103.
- DODD, J. M., B. K. FOLLETT, AND P. J. SHARP. 1971. Hypothalamic control of pituitary function in submammalian vertebrates. Pp. 113-223, in *Advances in comparative physiology and biochemistry* (O. Lowenstein, ed.), Academic Press, New York and London, 4:xii+1-410 pp.
- DOERR-SCHOTT, J. 1976a. Immunohistochemical detection, by light and electron microscopy, of pituitary hormones in cold-blooded vertebrates. I. Fishes and amphibians. *Gen. Comp. Endocrinol.*, 28:487-512.
- . 1976b. Immunohistochemical detection, by light and electron microscopy, of pituitary hormones in cold-blooded vertebrates. II. Reptiles. *Gen. Comp. Endocrinol.*, 28:513-529.
- FARKOUH, N. H., M. G. PACKER, AND A. G. FRANTZ. 1977. Variations in size of circulating human prolactin. *Endocrinology*, 100(suppl.):124.
- FERNHOLM, G. 1972. The ultrastructure of the adenohypophysis of *Myxine glutinosa*. *Z. Zellforsch. Mikrosk. Anat.*, 132:451-472.
- FOLLENIUS, E., AND M. P. DUBOIS. 1976. Étude immunocytoologique des cellules corticotropes de plusieurs espèces de poissons téléostéens. *Gasterosteus aculeatus* L., *Carassius auratus* L., *Lebistes reticulatus* P., *Salmo irideus* Gibbs and *Perca fluviatilis* L. *Gen. Comp. Endocrinol.*, 28:339-349.
- FONTAINE, M., AND M. OLIVEREAU. 1975. Some aspects of the organization and evolution of the vertebrate pituitary. *Amer. Zool.*, 15(suppl.):61-79.
- FONTAINE, Y., AND E. BURZAWA-GERARD. 1977. Esquisse de l'évolution des hormones gonadotropes et thyreotropes des vertébrés. *Gen. Comp. Endocrinol.*, 32:341-347.

- FURTH, J. 1955. Experimental pituitary tumours. *Rec. Prog. Horm. Res.*, 11:221-249.
- GONZÁLEZ, C. B., G. F. VALDES, AND D. R. CIOCCA. 1977. Ultrastructure of the human pharyngeal hypophysis. *Acta Anat.*, 97:224-230.
- GORBMAN, A. 1965. Vascular relations between the neurohypophysis and adenohypophysis of cyclostomes and the problem of evolution of hypothalamic endocrine control. *Arch. Anat. Microsc. Morph. Exp.*, 54:163-194.
- GORBMAN, A., H. KOBAYASHI, AND H. UEMURA. 1963. The vascularization of the hypophysial structures of the hagfish. *Gen. Comp. Endocrinol.*, 3:505-514.
- GRAESSLIN, D., F. A. LEIDENBERGER, V. LICHTENBERG, D. GLISMANN, N. HESS, P. J. CZYGAN, AND G. BETTENDORF. 1976. Existence of big and little forms of luteinizing hormone in human serum. *Acta Endocrinol.*, 83:466-482.
- GUILLEMIN, R., A. V. SCHALLY, H. S. LIPSCOMB, R. N. ANDERSON, AND J. M. LONG. 1962. On the presence in hog hypothalamus of β -corticotropin releasing factor, α - and β -melanocyte stimulating hormones, adrenocorticotropin, lysine vasopressin and oxytocin. *Endocrinology*, 70:471-477.
- HACHMEISTER, W. 1967. Die Stellung der rachendachhypophyse im system hypothalamus-adenohypophyse. *Endokrinologie*, 51:145-163.
- HAIDER, S., AND A. G. SATHYANESAN. 1975. On the similarity between the corticotrophs and pars intermedia cells in a teleost fish (*Clarias batrachus*). *Experientia*, 31:423-424.
- HANSEN, B. L., AND G. N. HANSEN. 1976. Immunohistochemical localization and identification of the prolactin and growth hormone producing cells in the pituitary gland of the toad *Bufo bufo bufo* (L.). *Gen. Comp. Endocrinol.*, 28:52-58.
- HANSTRÖM, B. 1966. Gross anatomy of the hypophysis of mammals. Pp. 1-57, in *The pituitary gland: anterior pituitary* (G. W. Harris and B. T. Donovan, series eds.), Butterworths, London, 1:xiv+1-587 pp.
- HARRIS, G. W., AND B. T. DONOVAN. (eds.). 1966. *The pituitary gland: anterior pituitary*. University of California Press, Berkeley and Los Angeles, 1:xiv+1-587 pp.
- HAYASHIDA, T., AND M. D. LAGIOS. 1969. Fish growth hormone: a biological immunohistochemical and ultrastructural study of sturgeon and paddlefish pituitaries. *Gen. Comp. Endocrinol.*, 13:403-411.
- HEALEY, D. L., H. K. MULLER, AND H. G. BURGER. 1977. Immunofluorescence shows localization of prolactin to human amnion. *Nature*, 265:642-643.
- HERLANT, M. 1958. L'Hypophyse et le système hypothalamo-hypophysaire du pangolin (*Manis tricuspis* et *Manis tetradactyla*). *Arch. Anat. Microsc. Morph. Exp.*, 47:1-23.
- HÖKFELT, T., S. EFENDIC, C. HELLERSTRÖM, O. JOHANSSON, R. LUFT, AND A. ARIMURA. 1975. Cellular localization of somatostatin endocrine-like cells and neurons of the rat with special reference to the A1-cells of the pancreatic islets and to the hypothalamus. *Acta Endocrinol.*, 200(suppl.):5-41.
- HOLMES, R. L. 1964. Experimental, histochemical and ultrastructural contributions to our understanding of mammalian pituitary function. *Int. Rev. Gen. Exp. Zool.*, 1:187-240.
- HOLMES, R. L., AND J. N. BALL. 1974. *The pituitary gland: a comparative account*. Cambridge Univ. Press, Cambridge and New York, x+397 pp.
- HOLTZMAN, S., AND M. P. SCHREIBMAN. 1972. Morphological changes in the "prolactin" cell of the freshwater teleost, *Xiphophorus hellerii*, in salt water. *J. Exp. Zool.*, 180:187-196.
- . 1975. The effect of altering the ambient salinity of the freshwater teleost *Xiphophorus maculatus* on the histophysiology of its prolactin cells. I. Progressive changes in one-third sea water. *Gen. Comp. Endocrinol.*, 25:447-455.
- HONMA, Y. 1969. Some evolutionary aspects of the morphology and role of the adenohypophysis in fishes. *Gunma Symp. Endocrinol.*, 6:19-36.
- HORSTMANN, E. 1954. Die Faserglia des Selachiergehirns. *Z. Zellforsch. Mikrosk. Anat.*, 39:588-617.

- JACKSON, I. M. D. 1977. Extrahypothalamic distribution of TRH, LRH and somatostatin, and their function. Pp. 62-66, in *Endocrinology. Proc. Vth Int. Congr. Endocrinol.* (V. James, ed.), Int. Congr. Ser. 402, Excerpta Medica, Amsterdam, 1:xiv+1-603 pp.
- JACKSON, I. M. D., AND S. REICHLIN. 1974. Thyrotropin releasing hormone (TRH): distribution in the brain, blood and urine of the rat. *Life Sci.*, 14:2259-2266.
- . 1977. Thyrotropin-releasing hormone: abundance in the skin of the frog *Rana pipiens*. *Science*, 198:414-415.
- JASINSKI, A., AND A. GORBMAN. 1966. Hypothalamo-hypophysial vascular and neurosecretory links in the ratfish, *Hydrolagus colliei* (Lay and Bennett). *Gen. Comp. Endocrinol.*, 6:476-490.
- JOSIMOVICH, J. B., AND E. H. VENNING. 1975. Hormonal physiology of the placenta: polypeptide and steroid hormones. Pp. 78-98, in *Gynecologic endocrinology* (J. J. Gold, ed.), Harper and Row, Baltimore, Maryland, xxxii+690 pp.
- KALLMAN, K. D., AND M. P. SCHREIBMAN. 1973. A sex-linked gene controlling gonadotrop differentiation and its significance in determining the age of sexual maturation and size of the platyfish, *Xiphophorus maculatus*. *Gen. Comp. Endocrinol.*, 21:287-304.
- KALLMAN, K. D., M. P. SCHREIBMAN, AND V. BORKOSKI. 1973. Genetic control of gonadotrop differentiation in the platyfish, *Xiphophorus maculatus* (Poeciliidae). *Science*, 181:678-680.
- KERR, T., AND P. G. W. J. VAN OORDT. 1966. The pituitary of the African lungfish *Protopterus* sp. *Gen. Comp. Endocrinol.*, 7:549-558.
- KNOWLES, F. 1965. Evidence for a dual control, by neurosecretion, of hormone synthesis and hormone release in the pituitary of the dogfish, *Scylliorhinus stellaris*. *Phil. Trans. Roy. Soc., ser. B*, 249:435-456.
- KOBAYASHI, H., AND H. UEMURA. 1972. The neurohypophysis of the hagfish, *Eptatretus burgeri* (Girard). *Gen. Comp. Endocrinol.*, suppl. 3:114-124.
- KOBAYASHI, H. T., T. ICHIKAWA, H. SUZUKI, AND M. SEKIMOTO. 1972. Seasonal migration of the hagfish, *Eptatretus burgeri*. *Japan J. Ichthyol.*, 19:191-194.
- KRAICER, J., J. L. GOSBEE, AND S. A. BENCOSME. 1973. Pars intermedia and pars distalis: two sites of ACTH production in the rat hypophysis. *Neuroendocrinology*, 11:156-176.
- KRIEGER, D. T., A. LIOTTA, AND M. J. BROWNSTEIN. 1977. Presence of corticotropin in brain of normal and hypophysectomized rats. *Proc. Nat. Acad. Sci.*, 74:648-652.
- LABRIE, F., G. PELLETIER, P. BORGEAT, J. DROUIN, L. FERLAND, AND A. BELANGER. 1976. Mode of action of hypothalamic regulatory hormones in the adenohypophysis. Pp. 63-93, in *Frontiers in neuroendocrinology* (L. Martini and W. F. Ganong, eds.), Raven Press, New York, 4:xii+1-419 pp.
- LAGIOS, M. D. 1968. Tetrapod-like organization of the pituitary gland of the polypteriform fishes, *Calamoichthys calabaricus* and *Polypterus palmas*. *Gen. Comp. Endocrinol.*, 11:300-315.
- . 1970. The median eminence of the bowfin, *Amia calva*. L. *Gen. Comp. Endocrinol.*, 15:453-463.
- . 1972. Evidence for a hypothalamo-hypophysial portal vascular system in the coelacanth *Latimeria chalumnae* Smith. *Gen. Comp. Endocrinol.*, 18:73-82.
- LARSEN, L. O. 1969. Hypophyseal functions in river lampreys. *Gen. Comp. Endocrinol.*, suppl. 2:522-527.
- LARSEN, L. O., AND B. ROTHWELL. 1972. Adenohypophysis. Pp. 1-67, in *The biology of lampreys* (M. W. Hardisty and I. E. Potter, eds.), Academic Press, New York and London, 2:xiv+1-466 pp.
- LUNDIN, P. M., AND U. SCHELIN. 1964. Light and electron microscopic studies on thyrotrophic pituitary adenomas in the mouse. *Lab. Invest.*, 13:62-68.
- MATTY, A. J., K. TSUNEMI, W. W. DICKHOFF, AND A. GORBMAN. 1976. Thyroid and gonadal function in hypophysectomized hagfish, *Eptatretus stouti*. *Gen. Comp. Endocrinol.*, 30:500-516.

- McGRATH, P. 1968. Prolactin activity and human growth hormone in pharyngeal hypophysis from embalmed cadavers. *J. Endocrinol.*, 42:205-212.
- . 1971. The volume of the human pharyngeal hypophysis in relation to age and sex. *J. Anat.*, 110:275-282.
- MELLINGER, J. C. A. 1965. Stades de la spermatogenèse chez *Scyliorhinus caniculus* (L.): description données histochimiques, variation normales et expérimentales. *Z. Zellforsch. Mikrosk. Anat.*, 67:653-673.
- . 1969. Développement post-embryonnaire de l'adénohypophyse de la torpille (*Torpedo marmorata*, Chondichthyens); évolution du système des cavités et manifestations du dimorphisme sexuel. *Ann. Univ. A.R.E.R.S.*, 7:33-48.
- . 1972. Types cellulaires et fonction de l'adénohypophyse de la Torpille (*Torpedo marmorata*). *Gen. Comp. Endocrinol.*, 18:608.
- MELLINGER, J., AND M. P. DUBOIS. 1973. Confirmation par l'immunofluorescence, de la fonction corticotrope du lobe rostral et de la fonction gonadotrope du lobe ventral de l'hypophyse d'un Poisson cartilagineux, la Torpille marbrée (*Torpedo marmorata*). *C. R. Acad. Sci., ser. D, (Paris)*, 276:1879-1881.
- MISRA, A. B., AND A. G. SATHYANESAN. 1958. On the persistence of the orohypophysial duct in some clupeoid fishes. *Proc. 15th Int. Zool. Congr., London*, 5:27.
- MOY-THOMAS, J. A., AND R. S. MILES. 1971. *Palaeozoic Fishes*. 2nd ed. W. B. Saunders Co., Philadelphia and Toronto, xi+259 pp.
- NICOLL, C. S. 1972. Some observations and speculations on the mechanism of "depletion", "repletion", and release of adenohypophyseal hormones. *Gen. Comp. Endocrinol.*, suppl. 3:86-96.
- NICOLL, C. S., J. BUNTIN, G. K. CLEMONS, M. P. SCHREIBMAN, AND S. RUSSELL. 1977. Comparative aspects of prolactin physiology: radioimmunoassay of the hormone in non-mammalian vertebrates. Pp. 293-297, in *Endocrinology. Proc. Vth Int. Cong. Endocrinol. (V. James, ed.), Int. Congr. Ser 403, Excerpta Medica, Amsterdam*, 2:xiv+1-616 pp.
- OLIVEREAU, M. 1970. Coloration de L'Hypophyse avec l'hématoxyline au plomb (H. Pb): Données nouvelles chez les téléostéens et comparaison avec les résultats obtenus chez d'autres vertébrés. *Acta Zool.*, 51:229-247.
- OLSSON, R. 1969. General review of the endocrinology of the Protochordata and Myxinoidea. *Gen. Comp. Endocrinol.*, suppl. 2:485-499.
- . 1974. Fine structure of the pituitary eta cells of larval *Chanos chanos* (Teleostei). *Gen. Comp. Endocrinol.*, 22:364.
- ÖZTAN, N., AND A. GORBMAN. 1960. The hypophysis and hypothalamo-hypophyseal neurosecretory system of larval lampreys and their response to light. *J. Morphol.*, 103:243-261.
- PACOLD, S. T., L. KIRSTEINS, S. HOJVAT, A. M. LAWRENCE, AND T. C. HAGEN. 1978. Biologically active pituitary hormones in the rat brain amygdaloid nucleus. *Science*, 199: 804-806.
- PAPKOFF, H. 1972. Subunits interrelationships among the pituitary glycoprotein hormones. *Gen. Comp. Endocrinol.*, suppl. 3:609-616.
- PERKS, A. M. 1969. The neurohypophysis. Pp. 111-205, in *Fish physiology* (W. S. Hoar and D. J. Randall, eds.), Academic Press, New York and London, 2:xiv+ 1-446 pp.
- PIERCE, J. G. 1971. The subunits of pituitary thyrotropins. Their relationship to other glycoprotein hormones. *Endocrinology*, 89:1331-1344.
- PURVES, H. D., AND W. E. GRIESBACH. 1951. The site of the thyrotrophin and gonadotrophin production in the rat pituitary studied by the McManus-Hotchkiss staining for glycoprotein. *Endocrinology*, 49:244-264.
- RODRIGUEZ, E. M., AND R. S. PIEZZI. 1967. Vascularization of the hypophysial region of the normal and adenohypophysectomized toad. *Z. Zellforsch. Mikrosk. Anat.*, 83:207-218.
- SAGE, M., AND H. A. BERN. 1971. Cytophysiology of the teleost pituitary. *Int. Rev. Cytol.*, 31:339-376.

- . 1972. Assay of prolactin in vertebrate pituitaries by its dispersion of xanthophore pigment in the teleost *Gillichthys mirabilis*. J. Exp. Zool., 180:169-174.
- SAINT-GIRONS, H. 1963. Histologie comparée de l'adénohypophyse chez les reptiles. Colloq. Int. Cent. Nat. Res. Sci., 128:275-285.
- . 1967. Morphologie comparée de l'hypophyse chez les squamata. Ann. Sci. Nat. (Zool.), ser. 12 (Paris), 9:229-308.
- . 1970. The pituitary gland. Pp. 135-199, in Biology of the Reptilia (C. Gans and T. S. Parsons, eds.), Academic Press, New York and London, 3:xiv+1- 385 pp.
- SATHYANESAN, A. G., AND W. CHAVIN. 1967. Hypothalamo-hypophyseal neurosecretory system in the primitive actinopterygian fishes (Holostei and Chondrostei). Acta Anat., 68:284-299.
- SATHYANESAN, A. G., AND R. C. DAS. 1975. Pituitary cytology of the chimaeroid fish *Hydrolagus collieri* (Lay and Bennett). Z. Mikrosk. Anat. Forsch., 89:715-726.
- SCANES, C. G., S. DOBSON, B. K. FOLLETT, AND J. M. DODD. 1972. Gonadotrophic activity in the pituitary gland of the dogfish (*Scyliorhinus canicula*). J. Endocrinol., 54:343-344.
- SCHALLY, A. V., M. S. LIPSCOMB, I. H. LONG, W. E. DEAR, AND R. GUILLEMIN. 1962. Chromatography and hormonal activities of dog hypothalamus. Endocrinology, 70:478-480.
- SCHREIBMAN, M. P. 1966. Hypophysial cysts in a teleost fish. J. Exp. Zool., 162:57-68.
- SCHREIBMAN, M. P., AND S. HOLTZMAN. 1975. The histophysiology of the prolactin cell in non-mammalian vertebrates. Amer. Zool., 15:867-880.
- SCHREIBMAN, M. P., AND K. D. KALLMAN. 1969. The effect of hypophysectomy on fresh-water survival in teleosts of the order Atheriniformes. Gen. Comp. Endocrinol., 13:27-38.
- . 1977. The genetic control of the pituitary-gonadal axis in the platyfish, *Xiphophorus maculatus*. J. Exp. Zool., 200:277-294.
- SCHREIBMAN, M. P., J. F. LEATHERLAND, AND B. A. McKEOWN. 1973. Functional morphology of the teleost pituitary gland. Amer. Zool., 13:719-742.
- SCHREIBMAN, M. P., J. D. BUNTING, AND C. S. NICOLL. 1976. Radioimmunoassay (RIA) of teleost prolactin (PRL). Proc. Vth. Int. Congr. Endocrinol., Hamburg, Germany, 1:255.
- SHARP, P. J., AND B. K. FOLLETT. 1969. The blood supply of the pituitary and the basal hypothalamus in the Japanese quail (*Coturnix coturnix japonica*). J. Anat., 104:227-232.
- STERBA, G. 1969. Endocrinology of the lampreys. Gen. Comp. Endocrinol., suppl. 2:500-509.
- STOECKEL, M. E., J. DOERR-SCHOTT, A. PORTE, H. D. DELLMAN, AND M. P. DUBOIS. 1973. Immunohistochemical demonstration of corticotrophic cells concentrated in the rostral zone of the pars intermedia of the mouse hypophysis. Experientia, 29:1289-1290.
- SUH, H. K., AND A. G. FRANTZ. 1974. Size heterogeneity of human prolactin in plasma and pituitary extracts. J. Clin. Endocrinol. Metab., 39:928-935.
- TAKOR, T., AND A. G. E. PEARSE. 1975. Neuroectodermal origin of avian hypothalamo-hypophysial complex: the role of the ventral neural ridge. J. Embryol. Exp. Morphol., 34:311-325.
- THOMSON, K. S. 1969. The biology of the lobe-finned fishes. Biol. Rev., 44:91-154.
- TSUNEKI, K., AND A. GORBMAN. 1975a. Ultrastructure of pars nervosa and pars intermedia of the lamprey, *Lampetra tridentata*. Cell Tiss. Res., 157:165-184.
- . 1975b. Ultrastructure of the anterior neurohypophysis and the pars distalis of the lamprey, *Lampetra tridentata*. Gen. Comp. Endocrinol., 25:487-508.
- VAN KEMENADE, J. A. M., AND D. W. KREMERS. 1975. The pituitary gland of the coelacanth fish *Latimeria chalumnae* Smith: general structure and adeno-hypophysial cell types. Cell Tiss. Res., 163:291-311.
- VAN OORDT, P. G. W. J. 1968. The analysis and identification of the hormone producing cells of the adeno-hypophysis. Pp. 405-467, in Perspectives in endocrinology (E. J. W. Barrington and C. B. Jørgensen, eds.), Academic Press, New York and London, xvi+583 pp.
- VILA-PORCILE, E. 1972. Le réseau des cellules folliculo-stellaires et les follicles de l'adénohypophyse du rat (pars distalis). Z. Zellforsch. Mikrosk. Anat., 129:328-369.

- VITUMS, A., S. MIKAMI, A. OKSCHE, AND D. S. FARNER. 1964. Vascularization of the hypothalamo-hypophysial complex in the white-crowned sparrow, *Zonotrichia leucophrys gambellii*. *Z. Zellforsch. Mikrosk. Anat.*, 64:541-569.
- VOLLRATH, L. 1972. Morphological correlates of releasing factors in the pituitary of the eel, *Anguilla anguilla*. Pp. 154-163, in *Brain-endocrine interaction. Median eminence: structure and function* (K. M. Knigge, D. E. Scott, and A. Weindl, eds.), Karger, Basel, xii+368 pp.
- WEATHERHEAD, B., AND P. WHUR. 1972. Quantification of ultrastructural changes in the melanocyte-stimulating hormone cells of the pars intermedia of the pituitary of *Xenopus laevis*, produced by change of background colour. *J. Endocrinol.*, 53: 303-310.
- WILLMER, E. N. 1970. *Cytology and evolution*. 2nd ed. Academic Press, New York and London, x+649 pp.
- WINGSTRAND, K. G. 1951. The structure and development of the avian pituitary. C. W. K. Gleerup, Lund, Sweden, xi+316 pp.
- . 1966a. Comparative anatomy and evolution of the hypophysis. Pp. 58-126, in *The pituitary gland: anterior pituitary*. (G. W. Harris and B. T. Donovan, series eds.), Univ. California Press, Berkeley, California, 1:xiv+1-586 pp.
- . 1966b. Microscopic anatomy, nerve supply and blood supply of the pars intermedia. Pp. 1-27, in *The pituitary gland: pars intermedia and neurohypophysis* (G. W. Harris and B. T. Donovan, series eds.), Butterworths, London, III:xiv+678 pp.
- WISLOCKI, G. B. 1938. The topography of the hypophysis in the Xenarthra. *Anat. Rec.*, 70:451-471.
- YALOW, R. S. 1974. Heterogeneity of peptide hormones. *Rec. Prog. Horm. Res.*, 30:597-633.
- YASUDA, M., AND M. A. GREER. 1976. Distribution of corticotropin releasing factor(s) activity in neural and extraneural tissues of the rat. *Endocrinology*, 99:944-948.
- ZAMBRANO, D., AND F. C. ITURRIZA. 1973. Hypothalamo-hypophysial relationships in the South American lungfish, *Lepidosiren paradoxa*. *Gen. Comp. Endocrinol.*, 20:256-273.

MECHANISM OF ACTION OF SOME POLYPEPTIDE HORMONES IN AN EVOLUTIONARY SERIES OF VERTEBRATES

KENNETH V. HONN AND WALTER CHAVIN

The mechanism of hormone action, in a broad sense, may encompass a large variety of cell responses. Among these are transcription, translation, various aspects of biosynthesis, and the like, as well as hormone-receptor interaction with ensuing reactions. As hormones impinge on cells and subsequently evoke specific responses, this aspect of hormone mechanistics is of fundamental interest in evolutionary studies. The hormone-cell interaction is dependent on the nature of the hormone and that of the receptor. Sutherland and collaborators beginning their work in the 1950s, ultimately suggested that cyclic 3', 5'-adenosine monophosphate (cAMP) was the unitary second messenger in the mechanism of action of a variety of polypeptide hormones and established criteria for defining the second messenger concept (Robison, 1971). In order to establish the role of a second messenger it is necessary that cAMP production in a given target cell be specific for a hormone effector. In addition, a significantly increased cAMP level should temporally precede the event (biochemical or physiological) used as the criterion of hormone action. Inhibitors of phosphodiesterase (methylxanthines, theophylline) should enhance cellular cAMP levels and thereby the hormonal effects in an experimental situation. The hormonal effects may be mimicked by exogenous cAMP or derivatives, as far as cell permeability permits. These four basic considerations, applied to a large variety of cells and organisms, have established firmly the role of cAMP in cell function. Furthermore, the work of a number of investigators has demonstrated the presence of another cellular cyclic nucleotide, cyclic 3', 5'-guanosine monophosphate (cGMP), which also is involved in hormone mediated processes (Goldberg *et al.*, 1973a, 1973b; Goldberg and Haddox, 1977). However, in many, but not all, described cases, cAMP and cGMP appear to act in opposition to each other (Goldberg *et al.*, 1973a, 1973b, 1974). The findings in a wide variety of systems suggests that the two cyclic nucleotides interacting in an unknown manner and, perhaps, with Ca^{+2} ions as well (Berridge, 1975; Rasmussen and Goodman, 1977) form a complex that regulates cellular processes. The state of knowledge at present is not such to permit precise definition of the roles of these and additional factors controlling cellular activity, but it is clear that the cyclic nucleotides are fundamental in hormonal regulation of cellular processes.

Our interests, therefore, deal with the evaluation of the initial polypeptide hormone effects upon endocrine and nonendocrine target cells and the means by which such may be modulated. The cellular responses occur early in hormone-cell reaction time and may be most effectively studied as cyclic

nucleotide alterations occurring pursuant to polypeptide hormone-membrane receptor interaction. Modulation of such cyclic nucleotide responses by prostaglandins occurs in mammals (Kuehl, 1974). Consideration of this sophisticated modulatory mechanism may also provide insight into changes occurring with evolutionary progress. Furthermore, the ensuant productivity of the target cell is of importance as the alterations of cyclic nucleotide levels alone do not reveal their biological significance. Therefore, in considering the evolutionary aspects of hormone mechanisms of action, several basic questions may be posed: When in the course of vertebrate evolution was the hormonally controlled cyclic nucleotide mechanism established? May the cyclic nucleotide mechanism be generalized for a variety of polypeptide hormones in lower vertebrates, as has been demonstrated in mammals? May the cyclic nucleotide mechanism of lower vertebrates be modulated or was such sophistication acquired during the course of evolution of the higher vertebrates? May the study of the mechanism of hormone action in lower vertebrates help elucidate the apparent complex array of events that occur in mammals?

PROCEDURES

The findings described in the present report are based upon *in vitro* studies of target tissues. In all but one of the animal studies, the organisms were maintained under constant conditions of light, temperature, and diet for the given species. Only in the study dealing with shark adrenals were feral animals (*Prionace glauca*, *Isurus oxyrinchus*) utilized immediately upon capture. The human studies involved normal adrenal glands taken at surgery (8-10 A.M.) from female patients who had received no previous anti-mitotic or radiation therapy. The animals were sacrificed rapidly by decapitation to minimize stress and at a given time of day to obviate possible differences produced by diel alterations. The appropriate tissues were immediately and rapidly removed, placed in cold (0-4° C), buffered physiological saline appropriate to the species (including 200 mg. glucose/dl. and 0.5% bovine serum albumin, fraction V), trimmed, and diced. The dice were incubated at the environmental temperature of the poikilothermic species or at the appropriate body temperature of the homeothermic species. After preincubation (45 minutes) at the test temperature, the dice were each incubated in 1 milliliter of the above appropriate buffer solution in a Dubnoff metabolic incubator (Honn and Chavin, 1975a, 1976a, 1976b) for given time intervals with the test hormone. The dice were individually quenched and stored in liquid nitrogen. The incubation medium was immediately frozen (-25° C). Cyclic nucleotide and steroid assays were performed by RIA (Honn and Chavin, 1975a, 1976a, 1976b, 1977a) as well as thyroxine levels (Hollander and Shenkman, 1974). The Na⁺-K⁺-ATPase and Mg²⁺-ATPase assays were performed by modification of existent procedures (Bonting *et al.*, 1963; Bonting, 1966). Pituitaries were removed from the heads of the decapitated organisms and immediately frozen in liquid nitrogen until used. The

pituitary material was weighed and homogenized in cold (0-4°C) buffer appropriate to the species, immediately prior to use. Tissue protein was determined by the automated biuret procedure (Honn and Chavin, 1975b) and the findings indicated per milligram of tissue protein. Data were analyzed by Student's *t* test and analysis of variance.

ADRENOCORTICOTROPIN

One of the early cell types studied in regard to the hormonal (ACTH) mechanism of action via cAMP is the adrenocortical cell (Haynes and Berthet, 1957; Grahame-Smith *et al.*, 1967). ACTH has been shown to stimulate an increase in cAMP levels with subsequent steroid release in adrenocortical cells of a number of mammalian species (Halkerston, 1975). Plasma membrane fractions of adrenocortical cells containing ACTH receptors (Lefkowitz *et al.*, 1970; Finn *et al.*, 1972) respond to ACTH with increased cAMP levels. Theophylline and methylxanthines increase cAMP levels by inhibition of phosphodiesterase. Exogenous cAMP or dibutyryl cAMP increase steroid release. Furthermore, a substance that is not a hormone, cholera toxin, increases cAMP levels and thereby affects subsequent steroid release (Donta, 1974; Haksar *et al.*, 1974). Considerable support, therefore, is present for the concept that cAMP is a critical factor in the action of ACTH upon the adrenal cortex. However, additional factors appear to play a role in this fundamental response and will be discussed below.

The ACTH-adrenocortical cell cAMP system has been studied extensively in several mammalian species, but, surprisingly, few reports deal with humans (Pearlmutter *et al.*, 1974; Honn and Chavin, 1976b, 1977b; Kolanowski and Crabbe, 1976). In view of our long term interest in the adrenal cortex, we examined the mechanism of ACTH action in the human adrenal as well as the adrenals of a number of vertebrate classes forming an evolutionary series. The nonmammalian vertebrate species were selected on the basis of the presence of discrete adrenal tissue, which could readily be dissected free of adherent non-adrenal tissue, and the availability of sufficient numbers of individuals. In all species used, responses were studied on a temporal basis.

In the human adrenal dice, response to ACTH is rapid at the higher doses studied (Honn and Chavin, 1977b). At 10-100 mIU, ACTH stimulates a rapid (1-2 min.) increase in cAMP synthesis. The lowest doses of ACTH stimulate cAMP levels at the 4 min. intervals. However, cortisol production by human adrenocortical tissue occurs rapidly (2 min.) and significantly in response to all ACTH doses (Honn and Chavin, 1977b). From the available temporal data, therefore, it cannot be stated unequivocally that significantly increased cAMP levels precede cortisol output in the human adrenal. Kolanowski and Crabbe (1976) examined the temporal relationship between cAMP accumulation and steroid output by human adrenals employing dispersed cells from a Cushingoid adrenal. These investigators reported that increased cAMP levels temporally preceded cortisol production at an ACTH dose of 1

$\mu\text{g/ml}$. At the highest ACTH dose ($0.67 \mu\text{g/ml}$.) used in our studies, cAMP production also preceded cortisol output. Therefore, at moderately high ACTH doses the classic cAMP-steroid relationship may be expected. Examining the relationship between cAMP levels and cortisol release (Honn and Chavin, 1977b), a small increase in cAMP levels above that of the control group, evokes a large steroid release. With increasing ACTH doses, the cAMP levels continue to increase but steroid release achieves a plateau level. Thus, in the human adrenal, a lack of correlation exists between the amount of cAMP generated and the amount of steroid released. ACTH stimulation of cAMP beyond levels necessary for maximal steroidogenesis also has been reported in other mammalian systems (Beall and Sayers, 1972; Sayers *et al.*, 1974); however, the function of these high levels is unknown. It has been postulated that such could be a mechanism for regulating total steroid output (Honn and Chavin, 1977b). In human adrenal dice, continued elevation of cAMP levels is not necessary for continued steroid output. In fact, when cAMP levels remain elevated for prolonged periods ($10\text{--}100 \text{ mIU ACTH}$), cortisol output achieves a plateau. It would appear that prolonged cAMP elevation may have an inhibitory effect on steroid output. The same phenomenon has been observed in isolated perfused rat adrenocortical cells (Hudson and McMartin, 1975). In this rat system, corticosterone production achieves a plateau despite continuous stimulation (20 min.) with exogenous cAMP. In contrast, pulse stimulation with exogenous cAMP followed by washout of this cyclic nucleotide, does not result in a plateau of corticosterone production. In this case, steroid production continues to increase and the production curve is steeper than during the initial cAMP stimulation (Hudson and McMartin, 1975). Therefore, the phenomenon of ACTH stimulation of cAMP levels beyond that necessary to achieve maximal steroidogenic levels may have physiological significance in the control of total steroid output.

It was stated earlier, that a criterion used to establish cAMP as a second messenger is that a phosphodiesterase inhibitor (that is, theophylline) should enhance cellular cAMP levels and potentiate hormone action. In the mammalian literature, a discrepancy exists with respect to theophylline action upon the adrenal (Halkerston, 1975). In the human adrenal, theophylline increases cAMP levels (Fig. 1) and, subsequently, increases cortisol output (Fig. 2). However, aldosterone output by the human adrenal is depressed by theophylline (Table 1). Pretreatment with ACTH followed by ACTH plus theophylline results in cortisol levels significantly lower than those evoked by ACTH alone (Fig. 3). Pretreatment with theophylline followed by transfer to theophylline plus ACTH results in cortisol levels that do not differ from basal levels (Fig. 3). Similarly, theophylline depresses ACTH stimulated aldosterone output by the human adrenal (Table 1). These unexpected effects of theophylline may be due to its effect upon adrenal protein synthesis (Halkerston *et al.*, 1966). However, recent evidence (Horrobin *et al.*, 1977) demonstrates that the methylxanthines, in particular

FIG. 1 (Left).—*In vitro* temporal (1-32 min.) cAMP responses of normal human adrenocortical tissue to phosphodiesterase inhibitor, theophylline (10 mM), compared to nontreated (KRBGA vehicle) controls.

FIG. 2 (Right).—*In vitro* temporal (1-32 min.) cortisol responses of normal human adrenocortical tissue to theophylline (10 mM) compared to basal cortisol output in KRBGA vehicle.

theophylline, are potent prostaglandin antagonists. These results are intriguing considering the demonstrated role of prostaglandins in both cortisol and aldosterone output by the human adrenal (Honn and Chavin, 1976a, 1976b, 1977a, 1977c, 1977d, 1978a) as will be discussed below. These results also indicate that cAMP may not be the sole factor involved in ACTH stimulated steroidogenesis.

It has been known for some time that high levels of exogenous cGMP stimulate rat adrenal steroidogenesis (Glinsmann *et al.*, 1969). Subsequently, ACTH has been demonstrated to stimulate cGMP levels in reptilian (Honn and Chavin, 1974, 1975a), rat (Kitabchi *et al.*, 1974; Perchellet *et al.*, 1978), and bovine (Rubin *et al.*, 1977) adrenocortical tissue. Therefore, the relative amounts of cyclic AMP and cyclic GMP rather than the absolute levels of cAMP, may be the key factor in the regulation of steroid biosynthesis and release (Honn and Chavin, 1977e; Rubin *et al.*, 1977). In the human adrenal, ACTH (100 mIU) significantly increases cGMP levels at 1 minute followed by a precipitous decline during the 2-8 min. interval (Table 2). A second cGMP peak occurred later (16-32 min.) temporally (Table 2). This second cGMP peak correlates temporally with observations in the reptilian (Honn and Chavin, 1975a) and bovine (Rubin *et al.*, 1977) adrenals.

From the above it is clear that cAMP may be correlated with steroid secretion in mammals, but that additional factors are involved. Whether the basic relationship between cyclic nucleotides and adrenocortical steroid secretion exists in other vertebrate classes was evaluated in a number of representative species.

The most primitive group studied, the Chondrichthyes, is represented by two elasmobranch species, the blue shark (*Prionace glauca*) and the mako shark (*Isurus oxyrinchus*). ACTH has been demonstrated to be an effective agent in the stimulation of adrenocortical function in several elasmobranch species (Honn and Chavin, 1969, 1976c; Klesch and Sage, 1973, 1975; Idler, 1973), but the underlying mechanism(s) remains unstudied.

TABLE 1.—In vitro temporal effect of theophylline (10 mM) on basal and ACTH (100 mIU/ml.) stimulated aldosterone output (ng./mg. protein; $\bar{X} \pm \text{SEM}$) by human adrenocortical dice.

Time (min.)	Control	ACTH	Theophylline	ACTH pretreatment followed by	Theophylline pretreatment followed by
				ACTH + Theophylline	ACTH + Theophylline
1	1.7 $\pm$ 0.60	2.8 $\pm$ 0.45	1.2 $\pm$ 0.20	1.9 $\pm$ 0.37	1.2 $\pm$ 0.21
2	2.4 $\pm$ 0.25	3.6 $\pm$ 0.25	1.7 $\pm$ 0.42	3.2 $\pm$ 0.25	1.5 $\pm$ 0.10
4	4.2 $\pm$ 0.50	5.0 $\pm$ 0.50	2.0 $\pm$ 0.28	3.4 $\pm$ 0.33	2.5 $\pm$ 1.0
8	4.6 $\pm$ 0.78	5.8 $\pm$ 0.30	3.2 $\pm$ 0.30	4.1 $\pm$ 0.59	2.9 $\pm$ 0.60
16	4.8 $\pm$ 0.47	6.3 $\pm$ 0.37	3.4 $\pm$ 0.50	4.5 $\pm$ 0.36	3.3 $\pm$ 0.36
32	5.3 $\pm$ 0.28	8.3 $\pm$ 1.0	3.7 $\pm$ 0.33	5.0 $\pm$ 1.0	4.0 $\pm$ 0.50

The adrenocortical tissue of the blue shark responded to the two doses of mammalian ACTH with a significantly increased cAMP level at 5 min. (Fig. 4). The cAMP levels remained elevated in respect to the control incubates for 45 min. This type of response cannot be generalized as characteristic of shark adrenocortical tissue for that of the mako shark reacted somewhat differently. In the mako shark adrenocortical tissue, ACTH also significantly elevated cAMP levels, but these declined after the 5 min. peak (Fig. 5) and returned to control levels at 30 min. This type of response may be the result of high phosphodiesterase levels in the mako shark adrenal as theophylline, a phosphodiesterase inhibitor, was only moderately effective in elevation of cAMP levels (Fig. 5).

Correlation of steroid secretion with cAMP levels in sharks would appear to be difficult for two reasons. First, antibodies to 1 α -OH-corticosterone, the major adrenal corticoid in these forms (Idler and Truscott, 1966, 1967), were not available. (Since completion of this work Kinne (1977) has developed a radioimmunoassay for 1 α -hydroxy-corticosterone). Secondly, the total volume per incubate is 1 ml., a quantity too small to use for other analytical approaches. However, another major corticoid produced by the two shark species studied is corticosterone (Truscott and Idler, 1968) so that evaluation of steroid secretion in these species is feasible. In the blue shark, a slow and gradual accumulation of corticosterone is present in the control groups (Fig. 6). Corticosterone secretion is significantly stimulated by ACTH at 15 min. (Fig. 6) following the initial rise in cAMP (Fig. 4). Interestingly, the adrenocortical response to the lower ACTH dose (1 mIU/ml.) is more rapid although the total steroid output at 60 min. is the same at both ACTH doses. The mako shark adrenocortical tissue also shows significant elevation of corticoid output at 15 min. (Fig. 7), following the cAMP peak at 5 (Fig. 5). The subsequent corticoid release appears slower but the steroid levels are almost twice that produced by the blue shark tissue in response to the same ACTH dose. It is noteworthy that the ACTH stimulated cAMP levels of the mako shark adrenocortical tissue are only about two-thirds that of the blue shark material. The cAMP levels of the mako shark tissue rapidly decrease rather than remaining elevated as in the blue shark tissue. Thus, as in the human adrenal, continued elevation of cAMP levels in shark adrenocortical tissue may blunt steroid output.

FIG. 3.—Effects of pretreatment (4 min.) in theophylline (10 mM) or ACTH (100 mIU/ml.) followed by transfer to ACTH + theophylline subsequent cortisol production by human adrenocortical tissue. Results are compared to basal cortisol production and to the response to 100 mIU ACTH/ml. Symbols are closed circle, KRBGA control; open circle, ACTH; square, ACTH + theophylline following ACTH preincubation; triangle, theophylline + ACTH following theophylline preincubation.

As discussed above, cGMP may modulate ACTH increased cAMP levels in the mammalian adrenal. Kitabchi *et al.* (1974) reported that low ACTH doses increased rat adrenal cGMP levels with little or no cAMP accumulation. In evaluation of the blue shark adrenocortical cGMP response, it was found that ACTH significantly elevates cGMP levels above that of the control groups (Table 3). The larger cGMP response to ACTH is present with the lower dose used. Similarly, an increase in cGMP levels also is evoked by ACTH in the mako shark adrenocortical tissue (Table 4). Therefore, as elevation of both cAMP and cGMP levels precede significant steroid output, it is possible that cAMP is not the sole effector of ACTH action, but that a combination of the two nucleotides is necessary.

Considering a more advanced group, the Amphibia, the frog, *Rana berlandieri forreri*, was studied. The adrenal tissue shows a rapid increase in the cAMP levels with a peak at 4 min. in response to homologous pituitary homogenate or ACTH (Fig. 8). The cAMP level then declines rapidly and is at the control level at 16 min. The cAMP peak precedes the increase in corticosterone output which is significantly greater than the control levels at 8 min. Similarly, increased cAMP levels precede significant aldosterone secretion in frog adrenals in response to ACTH or frog pituitary homogenate (Fig. 9). Exposure of this adrenal material to theophylline results in elevation of cAMP levels prior to the dramatic elevations of corticosterone and aldosterone output (Fig. 10). It would appear that in contrast to the shark adrenocortical tissue reaction to theophylline, the phosphodiesterase levels in this amphibian may not be as great, so that the cyclic nucleotide and steroid responses are quite high. In addition, theophylline does not depress frog

TABLE 2.—In vitro temporal effect of ACTH (100 mIU), prostaglandin E_1 (10 $\mu\text{g}/\text{ml.}$) and prostaglandin E_2 (10 $\mu\text{g}/\text{ml.}$) on cGMP levels (per cent control) in normal human adrenocortical tissue.

Time (min.)	ACTH	PGE ₁	PGE ₂
1	250±27	108±15	1242±200
2	12± 6	296±20	274± 23
4	27± 3	250±22	250± 15
8	25±12	125± 7	163± 3
16	652±38	115±30	170± 29
32	653±45	100± 6	122± 19

adrenal basal aldosterone output as observed in the human adrenal. Unfortunately, we have been unable to evaluate the cGMP levels in the frog so that the role of this cyclic nucleotide is not known. However, it is clear that the basic ACTH-cAMP relationship evolutionarily first seen in the shark is also present in this amphibian species.

The first dramatic alteration of the mechanistic concept described to this point occurs in a reptile, *Caiman sclerops* (Honn and Chavin, 1973, 1975a, 1977e). Normal adrenals of this crocodile when quickly excised postdecapitation show cGMP levels to be significantly higher than cAMP levels. When these adrenals are kept in cold buffer prior to initiation of an incubation series (zero-time), cAMP levels remain unchanged but cGMP levels decrease. Incubation in buffer alone at 30° C, further decreases cGMP levels and elevates cAMP levels. These reactions suggest that cGMP is under positive hormone control *in vivo* to an even greater degree than that observed in shark adrenocortical tissue. Conversely, cAMP levels appear inhibited in the normal *in vivo* crocodilian adrenal. Removal of the adrenals from the animal, which simulates the hypophysectomized state, results in a release from inhibition and expression of the potential adenylate cyclase activity. These results are opposite to that observed in the hypophysectomized rat adrenal wherein cAMP levels decrease concomitant with increased cGMP levels (Whitley *et al.*, 1975).

In the absence of ACTH, control adrenal cAMP levels increase significantly during the course of incubation to a maximum response at 8 min. (Honn and Chavin, 1977c). ACTH depresses *Caiman* adrenal cAMP levels in a dose dependent manner, below control cAMP levels. Crocodile pituitary homogenate also depresses adrenal cAMP levels. These depressed levels are not significantly different from the cAMP depression evoked by 100 mIU or 1000 mIU porcine ACTH. Therefore, the effect of mammalian ACTH upon the crocodilian adrenal cannot be attributed to the use of a heterologous ACTH as similar results were obtained with homologous pituitary homogenate containing immunoreactive ACTH (Honn and Chavin, 1975c). This crocodilian cAMP response to ACTH is unique among the vertebrates studied to date, including the human. In contrast to cAMP, control cGMP levels remain essentially unchanged during the incubation period (Honn and

FIG. 4 (Upper left).—*In vitro* temporal (2-60 min.) cAMP response of blue shark (*Prionace glauca*) adrenocortical tissue to given doses of porcine ACTH (150 mIU/mg.; Schwarz/Mann). Blue sharks were caught by a hand line off Catalina Island, California, in January. Water temperature 15°C. Three tissue replicates were used per datum point.

FIG. 5 (Upper right).—*In vitro* temporal (2-60 min.) cAMP response of mako shark (*Isurus oxyrinchus*) adrenocortical tissue to porcine ACTH (150 IU/mg.; Schwarz/Mann), 100 mIU/ml., and theophylline (10 mM). Mako sharks were caught by a hand line off Catalina Island, California, in January. Three tissue replicates were used per datum point.

FIG. 6 (Lower left).—*In vitro* temporal (2-60 min.) corticosterone output by blue shark (*Prionace glauca*) adrenocortical tissue in response to porcine ACTH (1, 100 mIU/ml.).

FIG. 7 (Lower right).—*In vitro* temporal (2-60 min.) corticosterone output by mako shark (*Isurus oxyrinchus*) adrenocortical tissue in response to porcine ACTH (100 mIU/ml.) or theophylline (10 mM).

Chavin, 1975a). Both crocodile pituitary homogenate and ACTH significantly increase crocodile adrenal cGMP levels above that of the control groups although the responses differ temporally (Honn and Chavin, 1975a, 1977e). *Caiman* pituitary homogenate produces an early cGMP peak (8 min.) followed by a decline to control levels at 32 min. The cGMP response to mammalian ACTH is remarkably similar to that observed in the human adrenal, that is, an early increase above controls followed by a depression and subsequent secondary peak at 32 min. The temporal nature of this secondary cGMP peak also coincides with observations in the bovine adrenals (Rubin *et al.*, 1977). Theophylline increases *Caiman* adrenal cAMP and cGMP levels although the responses are quantitatively and temporally distinct (Honn and Chavin, 1975a). Corticosterone output is increased slightly

TABLE 3.—In vitro temporal cGMP responses of *Prionace glauca* adrenals to porcine ACTH. Units are pM/mg. protein, $\bar{X} \pm \text{SEM}$.

Parameters	Time (min.)						
	2	5	10	15	30	45	60
Control	undetect.	undetect.	0.019 $\pm$ 0.005	0.038 $\pm$ 0.007	1.88 $\pm$ 0.05	0.88 $\pm$ 0.34	undetect.
100 mIU ACTH/ml.	0.41 $\pm$ 0.004	0.32 $\pm$ 0.10	undetect.	undetect.	undetect.	undetect.	undetect.
1 mIU ACTH/ml.	1.54 $\pm$ 0.59	0.60 $\pm$ 0.20	undetect.	undetect.	undetect.	undetect.	undetect.

with theophylline (Honn and Chavin, 1977e), however, as in the human adrenal, theophylline depresses basal aldosterone output by the *Caiman* adrenal (unpublished observation).

Porcine ACTH does not alter *Caiman* adrenal corticosterone output during the first 16 min. of incubation, however, steroid output increases in a dose related fashion at 32 min. Gist and Kaplan (1976) found that mammalian ACTH first increases *Caiman* plasma corticosterone levels *in vivo* 30 minutes post injection providing excellent correlation of *in vitro* and *in vivo* results. *Caiman* pituitary homogenate also produces a dose related increase in corticosterone output at the 16 min. interval. The relative changes (depression) in cAMP levels do not provide an explanation for ACTH or pituitary homogenate increased corticosterone output. In addition, although cGMP levels are increased prior to (pituitary homogenate) or coexistent (ACTH) with the first burst in steroid output there is no significant correlation between cGMP and steroid output.

In view of the fact that both cAMP and cGMP levels may be important in some groups, as seen in human, bovine (Rubin *et al.*, 1977), and shark adrenocortical tissue, the cAMP/cGMP ratio (A/G ratio) in the crocodile adrenal was explored. Interestingly, ACTH produced a dose dependent decrease in A/G ratio with a simultaneous dose dependent increase in corticosterone output (Honn and Chavin, 1977e). At 32 min., a significant and high degree

TABLE 4.—In vitro temporal cGMP responses of *Isurus oxyrinchus* adrenals to porcine ACTH and theophylline. Units are pM/mg. protein, $\bar{X} \pm \text{SEM}$.

Parameters	Time (min.)						
	2	5	10	15	30	45	60
Control	undetect.	undetect.	0.04 $\pm$ 0.01	0.08 $\pm$ 0.02	0.02 $\pm$ 0.01	0.07 $\pm$ 0.01	0.250 $\pm$ 0.100
100 mIU ACTH/ml.	0.67 $\pm$ 0.13	undetect.	undetect.	undetect.	undetect.	undetect.	undetect.
10 mM Theophylline	undetect.	undetect.	0.69 $\pm$ 0.08	0.03 $\pm$ 0.01	undetect.	undetect.	undetect.

FIG. 8 (Upper left).—*In vitro* temporal (1-16 min.) cAMP and corticosterone response of frog (*Rana berlandieri forreri*) adrenocortical tissue to porcine ACTH (134.8 IU/mg.; Schwarz/Mann), 10 mIU/ml., and a homologous pituitary homogenate (0.5 mg./ml.; wet weight/v) expressed as per cent control. Frogs of mixed sex were obtained from a commercial supplier in August and housed in large open tanks with running water (25°C) for two weeks prior to use. A minimum of four adrenals were utilized per datum point.

FIG. 10 (Lower left).—*In vitro* temporal (1-16 min.) cAMP, corticosterone, and aldosterone response of frog (*Rana berlandieri forreri*) adrenocortical tissue to theophylline (10 mM) expressed as per cent control. A minimum of four adrenals were utilized per datum point.

FIG. 9 (Upper right).—*In vitro* temporal (1-16 min.) cAMP and aldosterone response of frog (*Rana berlandieri forreri*) adrenocortical tissue to porcine ACTH (134.8 IU/mg.; Schwarz/Mann), 10 mIU/ml., and a homologous pituitary homogenate (0.5 mg./ml.; wet weight/v) expressed as per cent control. A minimum of four adrenals were utilized per datum point.

of correlation ($r+0.911$) exists between the A/G ratio and steroid output. The relationship between this ratio and the quantity of steroid secreted is inverse (that is, lowest ratio with highest corticosterone output). Temporal examination of this ratio in terms of corticosterone secretion reveals a gradual decrease in the A/G ratio to 32 min. followed by a gradual increase in the

A/G ratio. Steroid output is not correlated with the A/G ratio prior to 32 min.; after the 32 min. interval the correlation decreases with time.

Although a highly significant correlation between the A/G ratio and steroid secretion is present at the 32 min. interval, other factors also appear to be involved in secretion. For example, the A/G ratio (4.33; 16 min.) in response to 1000 mIU ACTH is almost identical to the ratio (4.34; 32 min.) in response to 100 mIU ACTH, however, no significant steroid output above control levels occur in the former instance whereas significantly increased steroid output is observed in the latter (Honn and Chavin, 1977e). If the ratios are physiologically significant, it is possible that an additional factor(s), a modulator, may be necessary before significant steroid output occurs. The nature of some of the modulators that may affect such adrenal function will be discussed below.

The cyclic nucleotide response of the crocodilian adrenal to ACTH differs from that of the other vertebrates studied. This difference appears to be inherent in the reptilian adrenocortical cell as both mammalian ACTH and crocodile pituitary homogenate depress cAMP. In order to ensure that the reptilian ACTH molecule present in the pituitary homogenate is not responsible for the fundamental difference observed in the mechanism of ACTH action, rat adrenals were used as controls. Rat adrenal incubates show significantly increased cAMP levels above control levels in response to this homogenate. Rat adrenal corticosterone secretion is also increased above control levels by the crocodile pituitary homogenate. In fact, no significant difference is present in the rat adrenal responses to ACTH or crocodile pituitary homogenate (Honn and Chavin, 1977e). These normal responses of the rat adrenal demonstrate that the *Caiman* adrenal reactions are unique and that the correlation of a number of factors may be necessary for adrenal productivity in terms of steroid. It may very well be the case, that our present state of knowledge regarding the mechanisms of hormone action is overly simplified.

Although the effects of ACTH upon the avian adrenal cortex has been reviewed recently (Holmes and Phillips, 1976) the underlying mechanism(s) has not been investigated. Birds appear to be derived from the same stem reptiles that gave rise to the Crocodilia. It was of considerable interest, therefore, to determine the controlling mechanism of corticoid secretion in an avian species. The white leghorn chicken was used in these studies. Surprisingly, ACTH (10 mIU) significantly increases cAMP levels above that of the control groups at 1 min., with a peak occurring at 2. (Fig. 11). This increase precedes the first observed increase in corticosterone secretion (Fig. 11). Chicken pituitary homogenate also produces a rapid increase in cAMP levels and subsequent corticosterone output (Fig. 12). The pituitary homogenate evokes a more prolonged cAMP response, but the steroid output is temporally similar to that evoked by ACTH, although of lesser magnitude. In addition, theophylline produced a transient (2-16 min.) increase in chicken adrenal cAMP levels and a slight increase in corticosterone output

FIG. 11 (Left).—*In vitro* temporal (1-32 min.) chicken (*Galus domesticus*) adrenal cAMP levels and corticosterone output in response to porcine ACTH (134.8 IU/mg.; Schwarz/Mann). Thirteen-day-old white leghorn chickens were used, and minimum of four adrenals were used per datum point.

FIG. 12 (Right).—*In vitro* temporal (1-32 min.) chicken (*Galus domesticus*) adrenal cAMP levels and corticosterone output in response to chicken pituitary homogenate (5 mg. wet weight/ml. KRBGA). Pituitaries from 106 chickens were utilized. A minimum of four adrenals were used per datum point.

at 32 min. The reactions would appear to be similar to those in the mammals studied.

Evaluation of the chicken adrenal cGMP response to ACTH reveals an inverse log-dose relationship with lower ACTH doses producing the higher cGMP levels (Table 5). However, the temporal cGMP response pattern to all ACTH doses is similar to that observed in the human adrenal. Strong similarities to the response of shark, reptilian, rat (Kitabchi *et al.*, 1974), and bovine (Rubin *et al.*, 1977) adrenals also exist. For example, an early (1-5 min.) cGMP elevation is observed in the shark, *Caiman*, chicken, rat (Kitabchi *et al.*, 1974; Perchellet *et al.*, 1978), and human adrenals, followed by return to baseline levels or depression below same. A temporally later (16-32 min.) peak also is observed in the *Caiman*, chicken, bovine (Rubin *et al.*, 1977), and human adrenals. In addition, chicken pituitary homogenate also elevates chicken adrenal cGMP levels at the 2 (6.0 ± 0.1 pM/mg. protein) and 16 min. (0.78 ± 0.05 pM/mg. protein) intervals.

Comparison of the A/G ratio found in the chicken adrenal at 16 min. with the total corticosterone output (32 min.) in response to different ACTH doses reveals an interesting inverse relationship (Fig. 13). There is no significant difference between the steroid response to either 1 or 10 mIU ACTH, however, a lower corticosterone response is observed with the highest (100 mIU) ACTH dose (Fig. 13). A slight decrease in corticosterone production by rat adrenals in response to high ACTH doses also is reported (Beall and

TABLE 5.—In vitro temporal effect of ACTH on cGMP levels in the chicken adrenal. Units are pM/mg. protein, $\bar{X} \pm \text{SEM}$.

Time (min.)	Control	ACTH 100 mIU	ACTH 10 mIU	ACTH 1 mIU
1	0.16 $\pm$ 0.03	0.39 $\pm$ 0.04	0.41 $\pm$ 0.01	0.7 $\pm$ 0.02
2	0.16 $\pm$ 0.02	0.86 $\pm$ 0.02	0.39 $\pm$ 0.07	0.7 $\pm$ 0.1
4	0.5 $\pm$ 0.07	0.34 $\pm$ 0.02	0.67 $\pm$ 0.01	0.58 $\pm$ 0.04
8	0.3 $\pm$ 0.06	0.46 $\pm$ 0.1	0.68 $\pm$ 0.08	0.63 $\pm$ 0.07
16	0.37 $\pm$ 0.06	1.11 $\pm$ 0.06	1.12 $\pm$ 0.02	1.43 $\pm$ 0.1
32	0.24 $\pm$ 0.04	0.79 $\pm$ 0.02	0.53 $\pm$ 0.2	0.55 $\pm$ 0.07

Sayers, 1972). Interestingly, the A/G ratio in the chicken adrenal decreases with decreasing ACTH dose (Fig. 13). In addition, steroid output is correlated with A/G ratio such that the highest output occurs at the lowest ratio (Fig. 13).

This type of relationship is similar to that present in the reptile. Therefore, two mechanisms producing the same adrenocortical secretory effect appear to be present in vertebrates. The ratio of the cyclic nucleotides may be important in at least two lower vertebrate classes, and recent evidence obtained from bovine adrenocortical cells also suggests that the ratio of cAMP/cGMP is important in the control of steroid output (Rubin *et al.*, 1977). It is, perhaps, too early to be more precise, for detailed studies in all vertebrate classes involving larger number of species and evaluation of the total cyclic nucleotide effects evoked by ACTH with subsequent alterations in steroid secretion remain to be undertaken. However, it is clear from the present findings that the concept of a unitary second messenger requires extension, for control or modulation of adrenocortical function is more complex than originally conceived. This is illustrated again in the chicken adrenal response in which the basal A/G ratio of the control group (Fig. 13) is lower than that of the 100 mIU ACTH stimulated group, but the basal steroid output of the control group is lower. Clearly, additional factors appear to be involved.

Consideration of such possible factors suggests that prostaglandins may play a role in this regard. Considerable evidence supports the concept that prostaglandins may stimulate cAMP and subsequent steroidogenesis and release in human (Fichman *et al.*, 1972; Fichman and Horton, 1973; Honn and Chavin, 1976a, 1976b, 1977a, 1977c, 1977d; Golub *et al.*, 1976), bovine (Saurta and Kaplan, 1972; Rubin *et al.*, 1977), cat (Laychock and Rubin, 1975; Warner and Rubin, 1975; Laychock *et al.*, 1977a), and rat (Flack and Ramwell, 1972; Gallant and Brownie, 1973; Spat and Jozan, 1975; Spat *et al.*, 1977a, 1977b, 1977c) adrenals. Initially, such cAMP mediated control of steroid output was believed to occur via pituitary ACTH release (Peng *et al.*, 1970), but direct prostaglandin effects have been demonstrated. The presence of prostaglandin receptors in human and bovine adrenal cell plasma membranes provide direct support to the effects of exogenous prostaglandins in adrenal physiology (Dazord *et al.*, 1974; Saez *et al.*, 1975). In addition, ACTH induces E and F type prostaglandin biosynthesis (Laychock and

FIG. 13.—Comparison of cAMP/cGMP ratio (16 min.) to corticosterone output (32 min.) by chicken (*Galus domesticus*) adrenals in response to porcine ACTH 1-100 mIU/ml.) or KRBGA.

Rubin, 1975) via increased phospholipase A activity in adrenal cell plasma membranes (Laychock *et al.*, 1977b). The majority of work deals with prostaglandins of the E and F series. The A and B series prostaglandins cannot be overlooked for they have potent adrenocortical effects (Fichman *et al.*, 1972, Fichman and Horton, 1973; Spat and Jozan, 1975; Honn and Chavin, 1977a). Despite the available information, it is still unclear whether prostaglandins function in addition to or as an integral part of ACTH-adrenocortical cell interaction. In this regard, prostaglandins may modulate the ACTH-cyclic nucleotide mechanism at the transducer level by affecting the appropriate cyclase or membrane lipids. In addition, effects may be expressed as alterations in cAMP or possibly cGMP, as will be discussed below. As the most readily available material was the human adrenal, our initial studies of this problem were undertaken in this form.

Indomethacin is an inhibitor of prostaglandin biosynthesis (Gallant and Brownie, 1973) and 7-oxa-13 prostynoic acid is a competitive inhibitor of prostaglandin binding to its receptor (Kuehl, 1974). These agents, therefore, are used to determine the role of prostaglandins in the adrenal responses to ACTH. Pretreatment of human adrenal dice with ACTH for 4 min. then exposing the dice to these agents in the presence of ACTH, increases the cAMP response above that obtained with ACTH at the same dose level (Honn and Chavin, 1976b). Conversely, pretreatment (4 min.) with either

inhibitor followed by exposure to the inhibitor in the presence of ACTH, depresses the cAMP response. This set of experiments suggests that the ACTH-adrenocortical cell interaction may be modulated in a bidirectional fashion in that prostaglandins may initially potentiate the ACTH effect but subsequently depress that effect or vice versa. This may occur via a negative feedback by a single type of prostaglandin or an antagonistic interaction between prostaglandins of the E and F series or possibly the A and B series. Considering this possibility, it is known that ACTH induces biosynthesis of both E and F series prostaglandins in addition to a third component tentatively considered a member of the A or B series (Laychock and Rubin, 1975). In the human adrenal, PGE_1 and PGE_2 increase cAMP levels above that of the controls in a dose related manner despite the absence of ACTH (Honn and Chavin, 1976b, 1978a). The F series prostaglandins $\text{PGF}_{1\alpha}$ and $\text{PGF}_{2\alpha}$, however, depress cAMP levels (Honn and Chavin, 1978a). Steroid output by the human adrenal in response to prostaglandins follows the nucleotide alterations such that the E series prostaglandins increase cortisol output in a dose related fashion while the F series prostaglandins depress cortisol output (Honn and Chavin, 1976b, 1978a). Similarly, prostaglandins E_1 and E_2 increase human adrenal aldosterone output while prostaglandins $\text{F}_{1\alpha}$ and $\text{F}_{2\alpha}$ depress aldosterone output (Honn and Chavin, 1976a, 1977d). The A and B series prostaglandins increase or decrease cAMP levels depending upon the dose used (Honn and Chavin, 1977a). Changes in human adrenal steroid output in response to A and B series prostaglandins are dependent upon the dose used. High doses of PGA_1 and PGA_2 increase cortisol output but low doses depress same. Aldosterone output is exactly opposite, high doses of PGA_1 and PGA_2 depressed mineralocorticoids while the lowest dose increases aldosterone output. PGB_1 and PGB_2 behaved similarly to the E type prostaglandins (Honn and Chavin, 1977a).

The discussion of the complexity of prostaglandin-cyclic nucleotide interactions has been limited to changes in cAMP. However, as we have seen above, ACTH may also effect cGMP levels in a variety of vertebrates, including man. Kuehl (1974) suggests that the observed antagonism between cAMP and cGMP observed in some systems may be related to antagonism between various prostaglandins. In this regard, Rubin *et al.* (1977) have recently demonstrated a positive effect of PGE_2 upon bovine adrenocortical cell cGMP levels. In contrast to the effects of ACTH upon bovine adrenocortical cGMP (Rubin *et al.*, 1977), PGE_2 effects a rapid (5 min.) increase in bovine adrenocortical cGMP levels, which return to baseline by the 30 min. interval. Both PGE_1 and PGE_2 effect a rapid increase in human adrenocortical cGMP levels within 1-2 min. (Table 2) returning to baseline levels thereafter. In the case of PGE_1 , this cGMP increase coexists with increased cAMP levels and precedes PGE_2 increased cAMP (Honn and Chavin, 1978a). Rubin *et al.* (1977) suggest that as the PGE_2 induced cGMP peak preceded the maximal effect of ACTH on cGMP levels, prostaglandins may be the intermediary through which ACTH modulates cGMP formation. Clearly, such a

FIG. 14 (Left).—*In vitro* effect of prostaglandins A₁, A₂, B₁, B₂, E₁, E₂, F_{1α}, F_{2α}, (100 μg/ml.) on corticosterone output relative to controls (C) and porcine ACTH (100 mIU/ml.), by frog (*Rana berlandieri forreri*) adrenals.

FIG. 15 (Right).—*In vitro* effect of prostaglandins A₁, A₂, B₁, B₂, E₁, E₂, F_{1α}, F_{2α}, (100 μg/ml.) on aldosterone output relative to controls (C) and porcine ACTH (100 mIU/ml.), by frog (*Rana berlandieri forreri*) adrenals.

hypothesis has merit as one of the early effects of ACTH is to induce prostaglandin biosynthesis (Laychock and Rubin, 1975; Laychock *et al.*, 1977a). However, in the human adrenal, ACTH produces an early (1 min.) increase in cGMP levels. Interestingly, cGMP has been demonstrated to increase E type prostaglandin biosynthesis in rat graafian follicles (Zor *et al.*, 1977). Therefore, not only may prostaglandins affect cyclic nucleotide levels but the reciprocal is also possible, suggesting an elaboration upon the hypothesis of Rubin *et al.* (1977). Thus, the degree of cyclic nucleotide (cAMP, cGMP) change in the adrenal is modulated by prostaglandins and the steroid output reflects such modulation. Furthermore, the prostaglandins may stimulate or inhibit the cyclic nucleotide changes so that the interaction of both stimulatory and inhibitory prostaglandin effects may be responsible for the eventual total steroid yield in a given situation. This complex ACTH, prostaglandin stimulation-prostaglandin inhibition, cyclic nucleotide mechanism controlling adrenocortical function in mammals, including man, raised the question of the evolutionary origin of such a sophisticated mechanism.

Examining the effects of a number of prostaglandins at one dose level (100 μg/ml) upon the frog adrenal, steroid release was stimulated, unaffected or inhibited depending upon the prostaglandin tested. Corticosterone output by the frog adrenal is increased above controls by all prostaglandins tested except PGB₁ and PGF_{1α} which were ineffective in this regard (Fig. 14). The most effective stimulant of corticosterone release, PGB₂, is about 4-fold more potent than porcine ACTH. In the human adrenal, the E series prostaglandins are most effective. In regard to frog adrenal aldosterone secretion, PGB₂

TABLE 6.—In vitro effects of E and F series prostaglandins on chicken adrenal cAMP and cGMP levels in addition to corticosterone and aldosterone output expressed as a per cent of their relative controls. Mean values $\pm$ one standard error of the mean are given.

Parameter	Prostaglandin (100 μ g/ml)			
	E ₁	E ₂	F _{1α}	F _{2α}
cAMP	364 $\pm$ 41	320 $\pm$ 66	245 $\pm$ 66	248 $\pm$ 30
cGMP	160 $\pm$ 10	167 $\pm$ 22	250 $\pm$ 40	263 $\pm$ 30
Corticosterone	140 $\pm$ 3	237 $\pm$ 22	85 $\pm$ 23	48 $\pm$ 17
Aldosterone	150 $\pm$ 2	200 $\pm$ 20	143 $\pm$ 11	132 $\pm$ 11

also is the most effective prostaglandin, being 5-6 fold more effective than porcine ACTH (Fig. 15). PGA₁, PGE₂ and PGF_{1 α} are ineffective with respect to eliciting a mineralocorticoid effect in the frog adrenal whereas PGB₁ inhibits aldosterone release (Fig. 15). The antagonism between E and F series prostaglandins, as observed in the human adrenal, appears absent in this lower vertebrate. However, these findings require extension as only a single prostaglandin dose was tested. Nevertheless, a differential effect of the A and B series prostaglandins upon frog adrenal aldosterone output is observed. Differential effects of A and B series prostaglandins have been observed in the human adrenal (Honn and Chavin, 1977a). The prostaglandin modulation of cAMP or cGMP levels in the frog adrenal has not been investigated.

The action of prostaglandins upon adrenocortical function was considered further in the white leghorn chicken (Table 6). At a dose of 100 μ g/ml. both E and F series prostaglandins increase chicken adrenal cAMP and cGMP levels. At the dose studied, no apparent antagonism with F series prostaglandins occurs at the level of cyclic nucleotide biosynthesis. Nevertheless, PGE₁ and PGE₂ increase chicken adrenal corticosterone output and PGF_{1 α} and PGF_{2 α} depressed same (Table 6). Both the E and F series prostaglandins increase chicken adrenal aldosterone output (Table 6). From these initial less complex findings it appears that further studies of prostaglandin modulation of the mechanism of hormone action in lower vertebrates may be fruitful and provide insight into the reactions of the higher vertebrates.

Clearly the mechanism of ACTH upon the adrenocortical cell via the cyclic nucleotides is a mechanism common to all the vertebrate classes examined to date. In addition, the action of prostaglandins may be superimposed upon this cyclic nucleotide mechanism from the Amphibia to man. However, generalization of polypeptide hormone action requires first a number of tests using hormones other than ACTH. To this end, a more primitive vertebrate group, the elasmobranchs, was used. It is reasonable to assume that mechanisms revealed in elasmobranchs could be operative in more advanced poikilotherms and, perhaps, mammals as well.

THYROTROPIN

In mammals, considerable information exists regarding the mechanism of TSH action in the thyroid. This mechanism postulates that TSH binds to a specific receptor on the outer side of the thyroid cell plasma membrane (Schell-Frederick and Dumont, 1970; Dumont, 1971, Lissitzky *et al.*, 1973, 1975; Macchia and Meldolesi, 1974; Winand and Kohn, 1975). After TSH is bound to the plasma membrane receptor, membrane bound adenylate cyclase is activated with a resultant increase in cAMP level (Gilman and Rall, 1968; Schell-Frederick and Dumont, 1970; Dumont, 1971; Wolff and Hope-Cook, 1973; Pochet *et al.*, 1974). In the presence of TSH, peak cAMP levels are attained within 3-6 min. and slowly decline thereafter (Gilman and Rall, 1968; Dumont, 1971). Other polypeptide hormones (ACTH, LH, FSH, GH, HCG, prolactin) do not stimulate cAMP production (Gilman and Rall, 1968) in the mammalian thyroid. However, NaF (Zor *et al.*, 1969; Wolff and Hope-Cook, 1973; Kariya *et al.*, 1974), epinephrine (Gilman and Rall, 1968; Schell-Frederick and Dumont, 1970; Dumont, 1971), and prostaglandins E_1 and E_2 (Zor *et al.*, 1969; Burke *et al.*, 1973; Haye *et al.*, 1973; Sato *et al.*, 1974) are effective in this regard in a variety of thyroid systems studied. The resultant generated cAMP increases specific protein kinase activity (Spaulding and Burrow, 1975) and subsequent phosphorylation of intracellular proteins.

A number of experimental designs have been employed to demonstrate that the TSH effects on the thyroid are cAMP mediated. Among these are reproduction of the effects with exogenous cyclic AMP and acyl derivatives of cAMP and use of phosphodiesterase inhibitors such as the methylxanthines (Dumont, 1971). It is generally concluded that a large number of TSH effects on the thyroid (such as iodide trapping and organification, glucose oxidation, colloid droplet formation, iodide and thyroxine release, ornithine decarboxylase activation and RNA and protein synthesis) are cAMP mediated (Schell-Frederick and Dumont, 1970; Dumont, 1971; Zusman and Burrow, 1975). In the mammalian thyroid, cGMP levels do not appear to be affected by TSH or prostaglandin E_1 (Yamashita and Field, 1972), suggesting that thyroid cAMP and cGMP levels may be regulated by separate hormonal mechanisms. Indeed, acetylcholine increases thyroidal cGMP levels (Yamashita and Field, 1972; Fallon *et al.*, 1974) and alters the intracellular cGMP distribution (Fallon *et al.*, 1974). In addition to affecting thyroid cAMP levels, TSH has been demonstrated to increase thyroid prostaglandin biosynthesis (Haye *et al.*, 1973; Burke *et al.*, 1973), which in turn may modulate adenylate cyclase activity (Zor *et al.*, 1969; Sato *et al.*, 1974; Wolff and Hope-Cook, 1973; Pochet *et al.*, 1974).

Using thyroids of the warm water nurse shark, *Ginglymostoma cirratum*, and the cold water horn shark, *Heterodontus francisci*, the action of thyrotropin was evaluated. Both species of shark were acclimatized (6 months) in a closed marine facility (Honn and Chavin, 1975c) prior to use in this study.

Bovine TSH produces a significant rapid and sustained elevation of cAMP in the nurse shark thyroid *in vitro* (Fig. 16). Nurse shark pituitary homogenate also elevates nurse shark thyroid cAMP levels above the control groups, but the increase is temporally delayed to the 20 min. interval (Fig. 16). Similar results were obtained with the horn shark thyroid (Honn and Chavin, 1976d). Both hormone preparations, bTSH and homologous pituitary homogenates, produce a significant increase in thyroxine secretion compared to that of the control groups at the 10 min. and 20 min. interval by the nurse shark (Fig. 17) and horn shark (Fig. 18) thyroids, respectively.

Considering the interaction of cAMP and cGMP in adrenocortical cell physiology, the effects of bTSH and pituitary homogenate upon cGMP were examined in these thyroids. As in the mammalian thyroid, bTSH does not alter thyroidal cGMP in the nurse shark (Fig. 19) and the horn shark (Fig. 20). However, nurse shark pituitary homogenate significantly increases nurse shark thyroid cGMP levels at 10 min. with the peak response occurring at 40 min. (Fig. 19). The horn shark pituitary homogenate also increases horn shark thyroid cGMP levels with the peak occurring at 40 min. (Fig. 20). It is generally accepted that mammalian TSH does not stimulate thyroid cGMP accumulation in the mammalian thyroid (Yamashita and Field, 1972), although a detailed temporal study has not been reported to date. As mammalian TSH does not produce stimulation of either mammalian or shark thyroid cGMP, it appears reasonable to conclude that shark TSH is not the cGMP effective agent present in the pituitary homogenates. Nevertheless, such a possibility can only be excluded when purified shark TSH becomes available. As shark pituitary homogenate contains a number of hormonal agents, other alternatives are possible. Acetylcholine is a known effector of thyroid cGMP in mammals (Yamashita and Field, 1972). However, as precautions to prevent cholinesterase activity in the shark pituitary homogenate were not taken, it is doubtful whether this effect (increased cGMP) can be attributed to endogenous acetylcholine. A more plausible suggestion is that the cGMP effector is a polypeptide hormone. Prolactin has been found to stimulate several aspects of thyroid physiology in fishes (Bern and Nicoll, 1968), amphibians (Peyrot *et al.*, 1971), reptiles (Chiu *et al.*, 1975), and birds (Wada *et al.*, 1975). These effects of prolactin include

FIG. 16 (Upper left).—*In vitro* temporal (5-60 min.) cAMP response of nurse shark (*Ginglymostoma cirratum*) thyroid tissue to bovine TSH (NIH, lot B-7) 10 mIU/ml. ESGA (Honn and Chavin, 1976c) and nurse shark pituitary homogenate (10 μ l/ml. ESGA). Sexually immature nurse sharks were acclimatized (25°C) for 6 months in a closed marine facility designed (Honn and Chavin, 1975c) to permit maximum stabilization of environmental factors. A minimum of four replicates were used per datum point.

FIG. 17 (Upper right).—*In vitro* temporal (5-60 min.) thyroxine release from nurse shark (*Ginglymostoma cirratum*) thyroid dice in response to 10 mIU bovine TSH or homologous pituitary homogenate 10 μ l/ml. ESGA. A minimum of four replicates were used per datum point.

FIG. 18 (Lower left).—*In vitro* temporal (5-60 min.) thyroxine release from horn shark (*Heterodontus francisci*) thyroid dice in response to 10 mIU bovine TSH or homologous pituitary

homogenate (10 $\mu\text{l}/\text{ml}$. ESGA). The immunoreactive TSH content of the pituitary homogenate was, 0.63 $\mu\text{IU}/\text{ml}$. A minimum of four replicates were used per datum point.

FIG. 19 (Lower right).—*In vitro* temporal (5-60 min.) cGMP response of nurse shark (*Ginglymostoma cirratum*) thyroid dice to 10 mIU bovine TSH (NIH, lot B-7) or homologous pituitary homogenate (10 $\mu\text{l}/\text{ml}$. ESGA). A minimum of four replicates were used per datum point.

FIG. 20.—*In vitro* temporal (5-60 min.) cGMP response of horn shark (*Heterodontus francisci*) thyroid dice to 10 mIU bovine TSH (NIH, lot B-7) or homologous pituitary homogenate (10 μ l/ml. ESGA). A minimum of four replicates were used per datum point.

epithelial cell hypertrophy, iodine uptake, and T_4 release. Interestingly, cGMP in the mammalian thyroid stimulates protein synthesis (Yamashita and Field, 1972; deNayer, 1973), growth (Pisarev *et al.*, 1971), and iodination of thyroglobulin (Fallon *et al.*, 1974). Prolactin has been demonstrated to have no effect on mammalian thyroid cAMP levels (Gilman and Rall, 1968) but its effects on cGMP stimulation have not been investigated. In addition, thyrotropin releasing hormone (TRH) has been demonstrated to stimulate pituitary prolactin release (Porteus and Malven, 1974; Sievertsson *et al.*, 1975), thus indicating that a more than casual relationship may exist between TSH and prolactin secretion by the pituitary. Therefore, the interesting possibility of bidirectional (TSH, prolactin) control or modulation of thyroid activity in vertebrates emerges, although the mechanism(s) for such control/modulation is unknown. Fallon *et al.* (1974) have provided evidence

that thyroid guanylate cyclase may be bound to the plasma membrane, suggesting that a prolactin receptor-guanylate cyclase mechanism may be operative in the vertebrate thyroid. Whether prolactin is present in shark pituitary homogenates is difficult to determine in light of the fact that shark prolactin has not been isolated and the amino acid sequence determined. Nevertheless, these results are intriguing and await further clarification.

PROLACTIN

Prolactin, believed to be one of the most primitive polypeptide hormones, produces effects on a number of tissues in a variety of vertebrates. The role of prolactin in control of hydromineral metabolism in vertebrates has been the subject of a number of excellent reviews (Ball, 1969; Lam, 1972; Ensor and Ball, 1972; Utida *et al.*, 1972; Utida and Hirano, 1973; Bradley, 1974; Massry, 1975; Bern, 1975; Katz and Lindheimer, 1977). The literature reveals no common pathway of prolactin action but, rather, a variety of osmoregulatory mechanisms that are influenced by prolactin, to a greater or lesser extent. These osmoregulatory mechanisms may have varying degrees of importance in different species, which adds to the complexity of the prolactin literature. Investigation of the mechanism of prolactin action, therefore, may provide information useful not only from the evolutionary point of view, but also may aid in the clarification of the mechanism of prolactin action in mammals and provide a unifying concept. As prolactin has osmoregulatory effects in a number of vertebrate classes, this fundamental aspect of its action was explored using both cyclic nucleotide level alterations and the subsequent changes in activity of enzymes affecting osmoregulation. The role of cyclic nucleotides in the mechanisms of action of many hormones is well documented (Robison *et al.*, 1968). As far as the permeability and transport properties of epithelial cells are concerned, little is known about the role of cyclic nucleotides. However, several hormones (such as oxytocin and vasopressin) stimulate sodium transport in isolated frog skin and also activate adenylate cyclase (Morel and Jard, 1971). Furthermore, a cAMP activated ATPase fraction with a high affinity for Na^+ ions have been observed in frog skin and bladder epithelial cells (Gachelin and Bastide, 1968). Considering the above, prolactin has been demonstrated to initiate both cAMP and cGMP accumulation in mammary glands of lactating rats (Rillema, 1976). Therefore, the possibility that the mechanism of action of prolactin involves cyclic nucleotide alterations appears worthwhile.

Prolactin produces an immediate decrease in nurse shark rectal gland cAMP levels compared to the control groups with maximum depression observed at 40 min. (Fig. 21). Prolactin, at doses of 1 and 10 mIU/ml., also decreases nurse shark kidney cAMP levels. In conjunction with the prolactin depressed cAMP levels, the Na^+ - K^+ -ATPase activity of the rectal gland also is depressed to barely detectable levels (Fig. 22). Kidney Na^+ - K^+ -ATPase activity is depressed as well with the higher prolactin dose producing the greater

FIG. 21 (Upper left).—*In vitro* temporal (5-120 min.) cAMP response of nurse shark (*Ginglymostoma cirratum*) rectal gland tissue dice to 10 mIU ovine prolactin/ml. ESGA (NIH, Lot S-7). A minimum of four replicates were used per datum point.

FIG. 22 (Upper right).—*In vitro* temporal (5-120 min.) effect of 10 mIU ovine prolactin on Na⁺-K⁺-ATPase activity in nurse shark (*Ginglymostoma cirratum*) rectal gland tissue dice. A minimum of four replicates were used per datum point.

FIG. 23 (Lower left).—*In vitro* temporal (5-120 min.) effect of two doses (1 and 10 mIU/ml.) of ovine prolactin (NIH, lot S-7) on Na⁺-K⁺-ATPase activity in tissue dice of nurse shark (*Ginglymostoma cirratum*) kidney. A minimum of four replicates were used per datum point.

FIG. 24 (Lower right).—*In vitro* temporal (5-120 min.) cGMP response of nurse shark (*Ginglymostoma cirratum*) rectal gland tissue dice to 10 mIU ovine prolactin/ml. A minimum of four replicates were used per datum point.

inhibition of enzymic activity (Fig. 23). It appears that Na⁺-K⁺-ATPase activity is extremely sensitive to depression of cAMP levels.

In contrast, prolactin rapidly and significantly elevates nurse shark rectal gland cGMP levels above the control levels (Fig. 24). Subsequent to this cGMP elevation, rectal gland Mg²⁺-ATPase activity is increased (Fig. 25), demonstrating the correspondence between this nucleotide and the enzyme. However, in the kidney, prolactin depresses cGMP levels relative to the control levels, and the Mg²⁺-ATPase activity is decreased below the control levels (Fig. 26).

Inasmuch as prolactin has different effects upon cyclic nucleotide levels in the rectal gland and kidney, the third major osmoregulatory organ, the gills,

FIG. 25 (Upper left).—*In vitro* temporal (5-120 min.) effect of 10 mIU ovine prolactin/ml. on Mg^{2+} -ATPase activity in nurse shark (*Ginglymostoma cirratum*) rectal gland dice. A minimum of four replicates were used per datum point.

FIG. 26 (Upper right).—*In vitro* temporal (5-120 min.) effect of two dose (1 and 10 mIU/ml.) of ovine prolactin of Mg^{2+} -ATPase activity in nurse shark (*Ginglymostoma cirratum*) kidney tissue dice. A minimum of four replicates were used per datum point.

FIG. 27 (Lower left).—*In vitro* temporal (5-120 min.) cAMP response of nurse shark (*Ginglymostoma cirratum*) gill tissue dice to two doses (1 and 10 mIU/ml.) of ovine prolactin (NIH, lot S-7). A minimum of four replicates were used per datum point.

FIG. 28 (Lower right).—*In vitro* temporal (5-120 min.) effect of two doses (1 and 10 mIU/ml.) of ovine prolactin on Na^+ - K^+ -ATPase activity in nurse shark (*Ginglymostoma cirratum*) gill tissue dice. A minimum of four replicates were used per datum point.

was examined. Nurse shark gills respond to the higher prolactin dose with significantly increased cAMP levels compared to the control group at 10 min. (Fig. 27). The lower prolactin dose depresses cAMP levels from 10 to 20 min. compared to the control levels (Fig. 27). If the mechanism of hormone action in the gill is similar to that of the rectal gland, the higher prolactin dose should stimulate Na^+ - K^+ -ATPase activity and the lower dose should depress enzymatic activity. However, both prolactin doses stimulate gill Na^+ - K^+ -ATPase activity (Fig. 28) with the higher dose producing the greater response. The gill Na^+ - K^+ -ATPase response to the lower prolactin dose was an early stimulation (5-20 min.) and subsequent depression (120 min.). This suggests that the mechanism of prolactin action occurring in

FIG. 29 (Left).—*In vitro* temporal (5-120 min.) cGMP response of nurse shark (*Ginglymostoma cirratum*) gill tissue dice to two doses (1 and 10 mIU/ml.) of ovine prolactin. A minimum of four replicates were used per datum point.

FIG. 30 (Right).—*In vitro* temporal (5-120 min.) effect of two doses (1 and 10 mIU/ml.) of ovine prolactin on Mg²⁺-ATPase activity in nurse shark (*Ginglymostoma cirratum*) gill tissue dice. A minimum of four replicates were used per datum point.

the rectal gland and kidney cannot be extended to the gill or that Na⁺-K⁺-ATPase activity may be increased by more than one effector post prolactin stimulation.

Clearly, the remaining factor to be considered in the mechanism of prolactin action is cGMP. Examination of the alterations in nurse shark gill cGMP levels after prolactin stimulation reveal that both doses of the hormone increase this cyclic nucleotide (Fig. 29). The cGMP response is rapid, showing a more than 10-fold increase at the earliest time interval (5 min.) at the low prolactin dose. It is possible that these high cGMP levels elevated by the low prolactin dose may mimic the cAMP effect upon Na⁺-K⁺-ATPase activity producing the unexpected increase in this enzyme activity in the presence of depressed cAMP levels. Both doses of prolactin increase gill Mg²⁺-ATPase activity in a dose related manner (Fig. 30) in accord with increased cGMP levels.

The relationships between the cyclic nucleotide levels and the ATPase levels in response to prolactin are summarized in Table 7. It is clear that Na⁺-K⁺-ATPase activity is increased following an increase in cAMP levels. For example, in the nurse shark gill, Na⁺-K⁺-ATPase activity is increased with 10 mIU prolactin, concomitant with increased cAMP levels. However, when cGMP levels are extremely highly elevated, as in the gill response to 1 mIU prolactin, Na⁺-K⁺-ATPase activity may increase in the face of depressed cAMP levels. In general, depression of cAMP levels results in depressed Na⁺-K⁺-ATPase activity, as present in the kidney and rectal gland. Mg²⁺-ATPase activity is generally regulated by cGMP, independent of cAMP alterations. Depressed cAMP levels do not affect Mg²⁺-ATPase activity unless cGMP levels are unaltered (Table 7: kidney). Thus, the positive modulatory effects of prolactin upon elasmobranch Na⁺-K⁺-ATPase activity may occur via two positive modulators, cAMP and cGMP, whereas Mg²⁺-ATPase activity is

TABLE 7.—Summary of prolactin effects in gill, kidney, and rectal gland tissue of the normal (25°C) acclimated nurse shark. Symbols are: +, increase; —, decrease; +—, increase and decrease; 0, no change.

Parameter	Prolactin 1 mIU/ml.	Prolactin 10 mIU/ml.
Gill		
cAMP	—	+
cGMP	+	+
Na ⁺ -K ⁺ -ATPase	+—	+
Mg ²⁺ -ATPase	+	+
Kidney		
cAMP	—	—
cGMP	0	0
Na ⁺ -K ⁺ -ATPase	—	—
Mg ²⁺ -ATPase	—	—
Rectal gland		
cAMP		—
cGMP		+
Na ⁺ -K ⁺ -ATPase		—
Mg ²⁺ -ATPase		+

positively modulated by a single effector, cGMP. Negative modulation of Na⁺-K⁺-ATPase activity by prolactin is accomplished only by depression of cAMP levels, but negative modulation of Mg²⁺-ATPase by cAMP requires the permissive cooperation of cGMP. Clearly, the two nucleotides controlled by prolactin play a major role in osmoregulation and this action appears correlated with Na⁺-K⁺-ATPase and Mg²⁺-ATPase activities.

CONCLUSIONS

From the foregoing discussion it is obvious that the mechanism of hormone action, as originally proposed by Sutherland and coworkers (Robison *et al.*, 1968), has increased in sophistication and complexity. In the adrenal, the mechanism of action of ACTH cannot be totally explained via increased intracellular cAMP levels. It is clear that the action of ACTH involves a complex sequence of coordinated events involving, in addition to cAMP, cGMP and prostaglandins. Therefore, ACTH action is expressed as the activation or generation of a number of different intracellular effectors. It is reasonable to presume that these effectors may interact at sites other than the plasma membrane (Honn and Chavin, 1978b). For example, studying the distribution of exogenous prostaglandins in the adrenal, Penney *et al.*, (1973a, 1973b) reported that ³H-PGE₁ localizes in rat adrenocortical mitochondria. In addition, mitochondrial receptors for prostaglandins have been reported (Rao and Mitra, 1977). The fact that prostaglandins specifically interact with adrenal cytochrome-P-450 (Tan *et al.*, 1973), a necessary component of adrenal hydroxylation reactions in mammals (Jefcoate *et al.*, 1976) including man (deAlvare *et al.*, 1977), as well as a number of lower vertebrates (Kimura *et al.*, 1976) offers yet another locus for prostaglandin modu-

lation of adrenal function. The complexity of hormone action may be extended to the thyroid. The effects of TSH upon thyroidal prostaglandin levels have been reported (Haye *et al.*, 1973; Burke *et al.*, 1973). In the shark, there appears to exist a pituitary factor that increases shark cGMP. This factor may be prolactin, which increases shark gill and rectal gland cGMP levels. Finally, although prostaglandin modulation of prolactin effects upon $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ and $\text{Mg}^{2+} - \text{ATPase}$ activities have not been studied, such may prove to be a fruitful area of research because $\text{PGF}_{2\alpha}$ has been demonstrated to stimulate $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity in mouse fibroblasts (Lever *et al.*, 1976).

Considering the evolutionary aspects of hormone mechanisms of action, several basic questions were posed. In answer to those questions it seems apparent that cyclic nucleotide involvement in the mechanism of hormone action was established early in evolution and appears functional in the lowest vertebrate form studied, the elasmobranchs. In addition, this cyclic nucleotide mechanism is generalized for at least three polypeptide hormones, ACTH, TSH, and prolactin, as early in evolution as the elasmobranch. Clearly, modulation of the unitary cAMP second messenger concept is already established in the Amphibia and probably exists in earlier evolutionary vertebrate species. As the basic cyclic nucleotide mechanism appears essentially unchanged from shark to man, with the exception of the crocodilia, it is probably in the modulation of this mechanism where comparative endocrinologists can expect to find the evolutionary diversity. Therefore the study of the mechanism of hormone action in lower vertebrates may prove to be an invaluable aid in the elucidation of the complex array of events that occur in the mammals and culminate in man.

ACKNOWLEDGMENTS

This work was supported, in part, by funds from the Detroit General Hospital Research Corporation and Department of Radiology, School of Medicine, Wayne State University. The expert technical assistance of Dr. Joel Abramowitz, Mr. David Bailey, Mr. Greg Busacker, and Ms. Nancy Capoferi is gratefully acknowledged.

LITERATURE CITED

- BALL, J. N. 1969. Prolactin and osmoregulation in teleost fishes: a review. *Gen. Comp. Endocrinol.*, suppl. 2:10-25.
- BEALL, R. J., AND G. SAYERS. 1972. Isolated adrenal cells: steroidogenesis and cyclic AMP accumulation in response to ACTH. *Arch. Biochem. Biophys.*, 148:70-76.
- BERN, H. A. 1975. Prolactin and osmoregulation. *Amer. Zool.*, 15:937-949.
- BERN, H. A., AND C. S. NICOLL. 1968. The comparative endocrinology of prolactin. *Rec. Progr. Horm. Res.*, 24:681-719.
- BERRIDGE, M. J. 1975. The interaction of cyclic nucleotides and calcium in the control of cellular activity. Pp. 2-97, in *Advances in cyclic nucleotide research* (P. Greengard and G. A. Robinson, eds.), Raven Press, New York, 6:vii+1-350 pp.
- BONTING, S. L. 1966. Studies on sodium-potassium-activated adenosine triphosphatase-XV. The rectal gland of the elasmobranch. *Comp. Biochem. Physiol.*, 17:953-966.

- BONTING, S. L., L. L. CARAVAGGIO, AND N. M. HAKINS. 1963. Studies on sodium-potassium-activated adenosine triphosphatase. *Arch. Biochem. Biophys.*, 101:47-55.
- BRADLEY, S. E. 1974. Hormones and the kidney. *Kidney Int.*, 6:261-376.
- BURKE, G., L. CHANG, AND M. SZABO. 1973. Thyrotropin and cyclic nucleotide effects on prostaglandin levels in isolated thyroid cells. *Science*, 180:872-875.
- CHIU, K. W., C. C. WONG, F. H. LEI, AND V. TAM. 1975. The nature of thyroidal secretions in reptiles. *Gen. Comp. Endocrinol.*, 25:74-82.
- DAZORD, A., A. M. MORERA, J. BERTRAND, AND J. M. SAEZ. 1974. Prostaglandin receptors in human and ovine adrenal glands: binding and stimulation of adenylyl cyclase in subcellular preparations. *Endocrinology*, 95:352-359.
- DEALVARE, L. R., T. KIMURA, A. SINGHAKOWINTA, K. V. HONN, AND W. CHAVIN. 1977. Mitochondrial redox components of human adrenocortical steroid hydroxylases under physiological conditions and in focal hyperplasia of the zona fasciculata. *Acta Endocrinol.*, 84:780-788.
- DENAYER, P. 1973. Effect of cyclic 3', 5' GMP on *in vitro* protein synthesis. *Biochimie*, 55:1507-1509.
- DONTA, S. T. 1974. Comparison of the effects of cholera enterotoxin and ACTH on adrenal cells in tissue culture. *Amer. J. Physiol.*, 227:109-113.
- DUMONT, J. E. 1971. The action of thyrotropin on thyroid metabolism. *Vitamin Horm.*, 29:287-412.
- ENSOR, D. M., AND J. N. BALL. 1972. Prolactin and osmoregulation in fishes. *Fed. Proc.*, 31:1615-1623.
- FALLON, E. F., R. AGRAWAL, E. FURTH, A. L. STEINER, AND R. COWDEN. 1974. Cyclic guanosine and adenosine 3', 5'-monophosphate in canine thyroid: localization by immunofluorescence. *Science*, 184:1089-1091.
- FICHMAN, M., AND R. HORTON. 1973. Significance of the effects of prostaglandins on renal and adrenal function in man. *Prostaglandins*, 3:629-646.
- FICHMAN, M., G. LITTENBERG, J. WOO, AND R. HORTON. 1972. The effect of prostaglandin (PGA₁) on adrenal function in man. *Adv. Biosci.*, 9:313-320.
- FINN, F. M., C. C. WIDNESS, AND K. HOFMANN. 1972. Localization of an adrenocorticotrophic hormone receptor on bovine adrenal cortical membranes. *J. Biol. Chem.*, 247:5695-5702.
- FLACK, J. D., AND P. W. RAMWELL. 1972. A comparison of the effects of ACTH, cyclic AMP, dibutyryl cAMP and PGE₂ on corticosteroidogenesis *in vitro*. *Endocrinology*, 90:371-377.
- GACHELIN, G., AND F. BASTIDE. 1968. Mise en évidence de deux ATPase activées le sodium et le potassium dans les cellules épithéliales de la vessie de grenouille. *C. R. Acad. Sci., ser D (Paris)*, 267:906-908.
- GALLANT, S., AND A. C. BROWNIE. 1973. The *in vivo* effect of indomethacin and prostaglandin E₂ on ACTH and DBcAMP-induced steroidogenesis in hypophysectomized rats. *Biochem. Biophys. Res. Commun.*, 55:831-836.
- GILMAN, A. G., AND T. W. RALL. 1968. Factors influencing adenosine 3', 5'-monophosphate accumulation in bovine thyroid slices. *J. Biol. Chem.*, 243:5867-5871.
- GIST, D. H., AND M. C. KAPLAN. 1976. Effects of stress and ACTH on plasma corticosterone levels in the caiman *Caiman crocodilus*. *Gen. Comp. Endocrinol.*, 28:413-419.
- GLINSMANN, N. H., E. P. HERN, L. G. LINARRELLI, AND R. V. FARESE. 1969. Similarities between effects of adenosine 3', 5'-monophosphate on liver and adrenal metabolism. *Endocrinology*, 85:711-719.
- GOLDBERG, N. D., AND M. K. HADDOX. 1977. Cyclic GMP metabolism and involvement in biological regulation. *Ann. Rev. Biochem.*, 46:823-896.
- GOLDBERG, N. D., M. K. HADDOX, D. K. HARTLE, AND J. W. HADDEN. 1973a. The biological role of cyclic 3', 5'-guanosine monophosphate. *Proc. Fifth, Int. Congr. Pharmacol.*, 5:146-169.

- GOLDBERG, N. D., P. F. O'DEA, AND M. K. HADDOX. 1973b. Cyclic GMP. Pp. 155-223, in *Advances in cyclic nucleotide research* (P. Greengard and G. A. Robinson, eds.), Raven Press, New York, 3:x+1-406 pp.
- GOLDBERG, N. D., M. K. HADDOX, R. ESTENSEN, J. G. WHITE, C. LOPEZ, AND J. W. HADDEN. 1974. Evidence of a dualism between cyclic GMP and cyclic AMP in the regulation of cell proliferation and other cellular processes. Pp. 247-262, in *Cyclic AMP, cell growth and the immune response* (W. Braum, L. M. Lichtenstein, and C. W. Parker, eds.), Springer-Verlag, New York, xv+416 pp.
- GOLUB, M. S., P. F. SPECKART, P. K. ZIA, AND R. HORTON. 1976. The effect of prostaglandin A_1 on renin and aldosterone in man. *Cir. Res.*, 39:574-579.
- GRAHAME-SMITH, D. G., R. W. BUTCHER, R. L. NEY, AND E. W. SUTHERLAND. 1967. Adenosine 3', 5'-monophosphate as the intracellular mediator of the action of adrenocorticotrophic hormone on the adrenal cortex. *J. Biol. Chem.*, 242:5535-5541.
- HALKERSTON, I. D. K. 1975. Cyclic AMP and adrenocortical function. in *Advances in cyclic nucleotide research* (P. Greengard and G. A. Robinson, eds), Raven Press, New York, 6:vii+1-350 pp.
- HALKERSTON, I. D. K., M. FEINSTEIN, AND O. HECHTER. 1966. An anomalous effect of theophylline on ACTH and adenosine 3', 5'-monophosphate stimulation. *Proc. Soc. Exp. Biol. Med.*, 122:896-900.
- HAKSAR, A., D. V. MAUDSLEY, AND F. G. PERON. 1974. Neuraminidase treatment of adrenal cells increases their response to cholera enterotoxin. *Nature*, 251:514-515.
- HAYE, B., S. CHAMPION, AND C. JACQUEMIN. 1973. Control by TSH of a phospholipase A_2 activity, a limiting factor in the biosynthesis of prostaglandins in the thyroid. *FEBS Letters*, 30:253-260.
- HAYNES, R. C., AND L. BERTHET. 1957. Studies on the mechanism of action of the adrenocorticotrophic hormone. *J. Biol. Chem.*, 225:115-124.
- HOLLANDER, C. S., AND L. SHENKMAN. 1974. Thyroxine and triiodothyronine. Pp. 215-230, in *Methods of hormone radioimmunoassay* (B. M. Jaffe and J. R. Behrman, eds.), Academic Press, New York, xxi+520 pp.
- HOLMES, W. N., AND J. G. PHILLIPS. 1976. The adrenal cortex of birds. Pp. 293-420, in *General comparative and clinical endocrinology of the adrenal cortex* (I. Chester Jones and I. W. Henderson, eds.), Academic Press, New York, 1:1-416 pp.
- HONN, K. V., AND W. CHAVIN. 1969. Some *in vitro* hormonal requirements for maintenance of adrenocortical dehydrogenases and diaphorases in the nurse shark, *Ginglymostoma cirratum* (Bonnaterre). *Amer. Zool.*, 9:1082.
- . 1973. *In vitro* inhibition of adrenal cyclic AMP by mammalian ACTH in the crocodilian *Caiman sclerops*. *Amer. Zool.*, 13:1281.
- . 1974. Control of cyclic nucleotide levels in the reptilian adrenal. *Endocrinology*, 94 (suppl.):A-161.
- . 1975a. ACTH control of adrenocortical cGMP. *Gen. Comp. Endocrinol.*, 26:374-381.
- . 1975b. An improved automated biuret method for the determination of microgram protein concentrations. *Anal. Biochem.*, 68:230-235.
- . 1975c. Prototype design for a closed marine system employing quarternary water processing. *Marine Biol.*, 31:293-298.
- . 1976a. Role of prostaglandins in aldosterone production by the human adrenal. *Biochem. Biophys. Res. Commun.*, 72:1319-1326.
- . 1976b. Prostaglandin modulation of the mechanism of ACTH action in the human adrenal. *Biochem. Biophys. Res. Commun.*, 73:164-170.
- . 1976c. *In vitro* trophic action of ACTH and insulin upon adrenocortical enzymes of the squaliform *Ginglymostoma cirratum* (Bonnaterre). *Gen. Comp. Endocrinol.*, 29:360-368.
- . 1976d. *In vitro* cAMP response of shark thyroid to TSH. *Amer. Zool.*, 16:243.
- . 1977a. Effects of A and B series prostaglandins on cAMP, cortisol and aldosterone production by the human adrenal. *Biochem. Biophys. Res. Commun.*, 76:977-982.

- . 1977c. Temporal effects of ACTH, indomethacin and 7-oxa-13 prostynoic acid upon glucocorticoid production by the human adrenal. *Experientia*, 33:545-546.
- . 1977d. Antagonistic action of E and F series prostaglandins upon mineralocorticoid production by the human adrenal. *Experientia*, 33:398-400.
- . 1977e. *In vitro* ACTH stimulation of corticosterone output in relation to cyclic nucleotide alterations in the crocodilian (*Caiman sclerops*) adrenal. *Gen. Comp. Endocrinol.*, 32:330-340.
- . 1978a. Temporal effect of prostaglandins E and F on human adrenal cAMP and cortisol output. *Life Sciences*, 22:543-552.
- . 1978b. Effect of prostaglandins upon cortisol production by bovine adrenocortical mitochondria. *Fed. Proc.*, 37:812.
- HORROBIN, D. F., M. J. MANKU, D. J. FRANKS, AND P. HAMET. 1977. Methyl xanthine phosphodiesterase inhibitors behave as prostaglandin antagonists in a perfused rat mesenteric artery preparation. *Prostaglandins*, 13:33-40.
- HUDSON, A. M., AND C. McMARTIN. 1975. An investigation of the involvement of adenosine 3', 5'-cyclic monophosphate in steroidogenesis by using isolated adrenal cell column perfusion. *Biochem. J.*, 148:539-544.
- IDLER, D. R. 1973. Comments on "Structure and function of the adrenal gland of fishes" by David Gordon Butler. *Amer. Zool.*, 13:881-884.
- IDLER, D. R., AND B. TRUSCOTT. 1966. 1 α -hydroxycorticosterone from cartilaginous fish: a new adrenal steroid in blood. *J. Fish. Res. Bd. Canada*, 23:615-619.
- . 1967. 1 α -hydroxycorticosterone: synthesis *in vitro* and properties of an interrenal steroid in the blood of cartilaginous fish (Genus *Raja*). *Steroids*, 9:457-477.
- JEFCOATE, C. R., W. H. ORME-JOHNSON, AND H. BEINERT. 1976. Cytochrome P-450 of bovine adrenal mitochondria. *J. Biol. Chem.*, 251:3706-3715.
- KARIYA, T., M. KOTANI, AND J. B. FIELD. 1974. Effects of sodium fluoride and other metabolic inhibitors on basal and TSH-stimulated cyclic AMP and thyroid metabolism. *Metabolism*, 23:967-973.
- KATZ, A. I., AND M. D. LINDHEIMER. 1977. Actions of hormones on the kidney. *Ann. Rev. Physiol.*, 39:97-134.
- KIMURA, T., H. P. WANG, L. R. DEALVARE, K. V. HONN, AND W. CHAVIN. 1976. Cytochrome P-450 and adrenodoxin in adrenal mitochondrial fraction of several lower vertebrates. *Comp. Biochem. Physiol.*, 53A:273-276.
- KINNE, D. E. 1977. Measurement of 1 α -hydroxycorticosterone and other corticosteroids in elasmobranch plasma by radioimmunoassay. *Gen. Comp. Endocrinol.*, 33:344-351.
- KITABCHI, A. E., A. H. NATHANS, P. JAMES, F. BOWYER, D. B. WILSON, AND L. C. KITCHELL. 1974. Cyclic 3', 5' (GMP (cGMP) as possible mediator of steroidogenic action of ACTH at physiologic concentrations. *Endocrinology*, 94(suppl.):1-160.
- KLESCH, W. L., AND M. SAGE. 1973. The control of the interrenal by the pituitary in the elasmobranch, *Dasyatis sabina*. *Comp. Biochem. Physiol.*, 45A:961-967.
- . 1975. The stimulation of corticosteroidogenesis in the interrenal of the elasmobranch *Dasyatis sabina* by mammalian ACTH. *Comp. Biochem. Physiol.*, 52A:145-146.
- KOLANOWSKI, J., AND J. CRABBE. 1976. Characteristics of the response of human adrenocortical cells to ACTH. *Mol. Cell. Endocrinol.*, 5:255-267.
- KUEHL, Jr., F. A. 1974. Prostaglandins, cyclic nucleotides and cell function. *Prostaglandins*, 5:325-340.
- LAM, T. J. 1972. Prolactin and hydromineral regulation in fishes. *Gen. Comp. Endocrinol.*, suppl. 3:328-338.
- LAYCHOCK, S. G., AND R. P. RUBIN. 1975. ACTH-induced prostaglandin biosynthesis from ³H-arachidonic acid by adrenocortical cells. *Prostaglandins*, 10:529-540.
- LAYCHOCK, S. G., W. WARNER, AND R. P. RUBIN. 1977a. Further studies on the mechanisms controlling prostaglandin biosynthesis in the cat adrenal cortex: the role of calcium and cyclic AMP. *Endocrinology*, 100:74-81.

- LAYCOCK, S. G., R. C. FRANSON, W. B. WEGICKI, AND R. P. RUBIN. 1977b. Identification and partial characterization of phospholipases in isolated adrenocortical cells. *Biochem. J.*, 164:753-756.
- LEFKOWITZ, R. J., J. ROTH, W. PRICER, AND I. PASTAN. 1970. ACTH receptors in the adrenal: specific binding of ACTH-¹²⁵I and its relation to adenyl cyclase. *Proc. Nat. Acad. Sci.*, 65:745-752.
- LEVER, J. E., D. CLINGAN, AND L. JIMINEZ DE ASUA. 1976. Prostaglandin F₂ and insulin stimulate phosphate uptake and (Na⁺, K⁺) ATPase activity in resting mouse fibroblast cultures. *Biochem. Biophys. Res. Commun.*, 71:136-143.
- LISSITZKY, S., G. HENNEN, AND P. JAQUET. 1973. Thyroid-stimulating hormone binding to cultured thyroid cells. *FEBS Letters*, 29:20-24.
- LISSITZKY, S., G. FAYET, AND B. VERRIER. 1975. Thyrotropin-receptor interaction and cyclic AMP-mediated effects in thyroid cells. Pp. 133-152, in *Advances in cyclic nucleotide research* (G. I. Drummond, P. Greengard, and G. A. Robinson, eds.), Raven Press, New York, 5:xiii+1-872 pp.
- MACCHI, V., AND M. F. MELDOLESE. 1974. Cell membrane of thyroid and its altered responsiveness to the hormone TSH. *Adv. Cytopharmacol.*, 2:33-37.
- MASSRY, S. G. 1975. Symposium on kidney and hormones. *Nephron*, 15:165-408.
- MOREL, F., AND S. JARD. 1971. Cyclic AMP and sodium transport in frog skin. *Ann. New York Acad. Sci.*, 185:351-362.
- PEARLMUTTER, A. F., R. FRANCE-SAENZ, E. RAPINE, AND M. SAFFRAN. 1974. Human adrenal tissue in vitro: steroidogenic properties. *J. Clin. Endocrinol. Metab.*, 39:150-153.
- PENG, T. C., K. M. SIX, AND P. L. MUNSON. 1970. Effects of prostaglandin E₁ on the hypothalamo-hypophyseal-adrenocortical axis in rats. *Endocrinology*, 86:202-206.
- PENNEY, D. P., J. OLSON, G. V. MARINETTI, S. VAALA, AND K. AVERILL. 1973a. Localization of tritiated prostaglandin E₁ in rat adrenal cortices. *Z. Zellforsch. Mikrosk. Anat.*, 146:308-317.
- PENNEY, D. P., J. OLSON, AND K. AVERILL. 1973b. Fine structural studies of rat adrenal cortices following prostaglandin administration. *Z. Zellforsch. Mikrosk. Anat.*, 146:297-307.
- PERCHELLET, J. P., G. SHANKER, AND R. K. SHARMA. 1978. Regulatory role of guanosine 3', 5'-monophosphate in adrenocorticotropin hormone-induced steroidogenesis. *Science*, 199:311-312.
- PEYROT, A., C. VELLANO, G. E. ANDREOLETTI, G. PONS, AND M. BICIOTTI. 1971. On the activating effect of prolactin on thyroid metabolism in the crested newt. Comparison between the effect of prolactin and methylthiouracil. *Gen. Comp. Endocrinol.*, 16:524-534.
- PISAREV, M. A., L. J. DEGROOT, J. F. WILBER, AND N. ALTSCHULES. 1971. Action of cyclic guanosine monophosphate on thyroid weight and protein. *Endocrinology*, 88:1074-1076.
- POCHET, R., J. M. BOEYNAEMS, AND J. E. DUMONT. 1974. Stimulation by thyrotropin of horse thyroid plasma membranes adenylate cyclase: evidence of cooperativity. *Biochem. Biophys. Res. Commun.*, 58:446-453.
- PORTEUS, S. E., AND P. V. MALVEN. 1974. Stimulation of prolactin release *in vivo* by thyrotropin-releasing hormone (TRH) through direct action on the ectopic rat pituitary. *Endocrinology*, 94:1699-1703.
- RAO, C. V., AND S. MITRA. 1977. Subcellular distribution of prostaglandin and gonadotropin receptors in bovine corpora lutea. *Biochem. Biophys. Res. Commun.*, 76:636-643.
- RASMUSSEN, H., AND D. B. P. GOODMAN. 1977. Relationships between calcium and cyclic nucleotides in cell activation. *Physiol. Rev.*, 57:421-509.
- RILLEMA, J. A. 1976. Cyclic nucleotides, adenylate cyclase, and cyclic AMP phosphodiesterase in mammary glands from pregnant and lactating mice. *Proc. Soc. Exp. Biol. Med.*, 151:748-751.
- . 1977b. *In vitro* temporal cAMP and cortisol response to ACTH by the normal human adrenal gland. *Acta Endocrinol.*, 86:363-368.

- ROBISON, G. A. 1971. Cyclic AMP and hormone action. Pp. 17-47, in *Cyclic AMP* (G. A. Robison, R.W. Butcher, and E.W. Sutherland, eds.), Academic Press, New York, ix+531 pp.
- ROBISON, G. A., R. W. BUTCHER, AND E. W. SUTHERLAND. 1968. Cyclic AMP. *Ann. Rev. Biochem.*, 37:149-174.
- RUBIN, R. P., S. G. LAYCHOCK, AND D. W. END. 1977. On the role of cyclic AMP and cyclic GMP in steroid production by bovine cortical cells. *Biochem. Biophys. Acta.*, 496:329-338.
- SAEZ, J. M., A. DAZORD, AND D. GALLET. 1975. ACTH and prostaglandin receptors in human adrenocortical tumors. *J. Clin. Invest.*, 56:536-547.
- SAURTA, T., AND N. M. KAPLAN. 1972. Adrenocortical steroidogenesis: the effects of prostaglandins. *J. Clin. Invest.*, 51:2246-2251.
- SATO, S., T. YAMADA, R. FURIHATA, AND M. MAKIUCHI. 1974. Effect of guanyl nucleotides on the stimulation of adenylyl cyclase activity in human thyroid plasma membranes by thyroid-stimulating hormone and prostaglandin E₂. *Biochem. Biophys. Acta*, 332: 166-174.
- SAYERS, G., S. SELLIG, S. KUMAR, G. KARLAGANIS, R. SCHWYZER, AND M. FUJINO. 1974. Isolated adrenal cortex cells: ACTH4-23 (NH₂), ACTH5-24, ACTH6-24 and ACTH7-23 (NH₂); cyclic AMP and corticosterone production. *Proc. Soc. Exp. Biol. Med.*, 145: 176-181.
- SHELL-FREDERICK, E., AND J. E. DUMONT. 1970. Mechanism of action of thyrotropin. Pp. 415-463, in *Biochemical action of hormones* (G. Litwack, ed.), Academic Press, New York, 1:xvi+1-415 pp.
- SIEVERTSSON, H., S. CASTENSSON, K. ANDERSSON, AND S. BJORKMAN. 1975. Thyrotropin and prolactin inhibitory studies by compounds related to the thyrotropin releasing hormone. *Biochem. Biophys. Res. Commun.*, 66:1401-1407.
- SPAT, A., AND S. JOZAN. 1975. Effect of prostaglandin E₂ and A₂ on steroid synthesis by the rat adrenal gland. *J. Endocrinol.*, 65:55-63.
- SPAT, A., S. JOZAN, K. GAAL, AND T. MOZES. 1977a. Effect of indomethacin on the adrenal response to frusemide in the rat. *J. Endocrinol.*, 73:401-402.
- SPAT, A., F. A. ANTONI, T. BALLA, E. BONTA, AND P. SIKLOS. 1977b. A study on the interaction of ACTH and prostaglandin F_{2α}. *J. Steroid Biochem.*, 8:247-248.
- SPAT, A., P. SIKLOS, F. A. ANTONI, K. NAGY, AND K. SZIRANYI. 1977c. Effect of prostaglandin synthetase inhibitors on basal and ACTH-stimulated steroid synthesis by separated adrenocortical zones. *J. Steroid Biochem.*, 8:293-298.
- SPAULDING, S. W., AND G. N. BURROW. 1975. Effect of PGE₁ and TSH on cAMP dependent protein kinase activity in the thyroid. *Endocrinology*, 96:1018-1021.
- TAN, L., H. M. WANG, AND J. G. LEHOUX. 1973. Binding of prostaglandins and cytochrome P-450. *Prostaglandins*, 4:9-17.
- TRUSCOTT, B., AND D. R. IDLER. 1968. The widespread occurrence of a corticosteroid 1-hydroxylase in the interrenals of elasmobranchii. *J. Endocrinol.*, 40:515-526.
- UTIDA, S., AND T. HIRANO. 1973. Effects of changes in environmental salinity on salt and water movement in the intestine and gills of the eel. Pp. 240-269, in *Responses of fish to environmental changes* (W. Chavin, ed.), Charles C. Thomas, Springfield, x+459 pp.
- UTIDA, S., T. HIRANO, H. OIDE, M. ANDO, D. W. JOHNSON, AND H. A. BERN. 1972. Hormonal control of the intestine and urinary bladder in teleost osmoregulation. *Gen. Comp. Endocrinol.*, suppl. 3:317-327.
- WADA, M., Y. ARIMATSU, AND H. KOHAYASHI. 1975. Effect of prolactin on thyroid function in Japanese quail (*Coturnix coturnix japonica*). *Gen. Comp. Endocrinol.*, 27:28-33.
- WARNER, W., AND R. P. RUBIN. 1975. Evidence for a possible prostaglandin link in ACTH-induced steroidogenesis. *Prostaglandins*, 9:83-95.
- WHITLEY, T. H., N. W. STOWE, S. H. ONG, R. L. NEY, AND A. L. STEINER. 1975. Control and localization of rat adrenal cyclic guanosine 3', 5'-monophosphate. *J. Clin. Invest.*, 56:146-154.

- WINAND, R. J., AND L. D. KOHN. 1975. Thyrotropin effects in thyroid cells in culture. *J. Biol. Chem.*, 250:6534-6540.
- WOLFF, J., AND G. HOPE-COOK. 1973. Activation of thyroid membrane adenylate cyclase by purine nucleotides. *J. Biol. Chem.*, 248:350-355.
- YAMASHITA, K., AND J. B. FIELD. 1972. Elevation of cyclic guanosine 3', 5'-monophosphate levels in dog thyroid slices caused by acetylcholine and sodium fluoride. *J. Biol. Chem.*, 247:7062-7066.
- ZOR, U., T. KANEKO, I. P. LOWE, G. BLOOM, AND J. B. FIELD. 1969. Effect of thyroid stimulating hormone and prostaglandins on thyroid adenylyl cyclase activation and cyclic adenosine 3', 5'-monophosphate. *J. Biol. Chem.*, 244:5189-5195.
- ZOR, U., B. STRULOVICI, AND H. R. LINDNER. 1977. Stimulation by cyclic GMP of prostaglandin E production in isolated graffian follicles. *Biochem. Biophys. Res. Commun.*, 76:1086-1091.
- ZUSMAN, D. R., AND G. N. BURROW. 1975. Thyroid-stimulating hormone regulation of ornithine decarboxylase activity in the thyroid. *Endocrinology*, 97:1089-1095.

PHYLOGENETIC TRENDS IN HORMONAL CONTROL OF GONADAL STEROIDOGENESIS

VALENTINE LANCE AND IAN P. CALLARD

The successful adaptive radiation of the recent vertebrates has depended ultimately on the successful adaptation of their reproductive cycles to the environments in which they evolved. Because almost all habitable environments show some degree of fluctuation, successful adaptation to these environments has necessitated, in most instances, some control of the reproductive cycle to produce offspring at the time of year most advantageous to their survival. Such control of the vertebrate reproductive cycle requires that there be a degree of independence from the external environment and that the organism be able to sense changes in the environment and modify its physiology accordingly. This has been accomplished by the evolutionary modification of the neuroendocrine system.

It is likely that the ancestral group from which the vertebrates arose already possessed a pituitary gland (Barrington, 1968), but there is no way of knowing whether or not this ancestral pituitary had any control over the reproductive processes as there are no protochordates with a recognizable pituitary. Although there is some evidence of a relationship between the neural complex and the gonads of ascidians (Georges, 1976), attempts to trace the antecedents of the vertebrate pituitary to this specialized structure in tunicates (Berrill, 1955) or the wheel organ and Hatschek's pit of *Amphioxus* (Barrington, 1964) have been singularly unsuccessful (see reviews by Dodd, 1955; Charniaux-Cotton and Kleinholz, 1964; Bern and Nandi, 1964; Barrington, 1968; Olsson, 1969; Dodd, 1975). Likewise, the theory of Willmer (1974, 1975) also is difficult to demonstrate. He has proposed, reviving Hubrecht's long vilified theory (1887), that the nemertine worms are the true ancestors of the vertebrates and that the specialized secretory cells found in the buccal epithelium of these worms are the forerunners of the hormone secreting cells of the adenohypophysis. Although provocative, such theories are impossible to prove, making it unlikely that we ever will be able to trace the evolutionary origins of the vertebrate pituitary gland (see Gorbman, 1941; Dodd, 1975).

If the origins of the vertebrate gonadotropins are obscure, there is ample evidence that the steroid hormones secreted by the vertebrate gonads are very ancient molecules, and that steroid synthesizing enzymes occur in the gonadal tissues of many invertebrate groups (see Sandor *et al.*, 1975, for references). However, a recent study on the metabolism of progesterone- ^{14}C by the gonads of breeding tunicates (*Styela plicata*) failed to demonstrate any evidence of androgen or estrogen production by this species. The authors were forced to conclude "that the metabolic profiles produced from progesterone

by the gonads of *Styela plicata* have fewer vertebrate features than those of several molluscs" (Colombo *et al.*, 1977). Although in no way conclusive, these results only serve to emphasize the distance, both biochemically and phylogenetically, of these protochordates from the living vertebrates (see for example, Russell and Subak-Sharpe, 1977), and the difficulty of drawing any evolutionary inferences from such studies. Any review on the evolution of the pituitary control of gonadal function must, therefore, ignore the protochordates and examine the evidence for phylogenetic trends in the living vertebrates available for study.

It is generally assumed that the highly complex endocrine control of reproduction in mammals evolved in many stages over many millions of years from the perhaps less complex system of the egg-laying vertebrates. It is also one of the assumptions of comparative endocrinology that the extant vertebrates still retain some of the characteristics of the ancestral groups from which they evolved and that by studying the living fishes, amphibians, reptiles, and birds we may gain some insight into how the endocrine system has evolved. The dangers inherent in such an approach have been pointed out by numerous investigators (Barrington, 1968), for it is obvious that the extant classes of vertebrates have all evolved along independent lines for many millions of years and are uniquely adapted to their present day habitat (see also Jarvik, 1968). However, despite these drawbacks, some limited phylogenetic extrapolations are possible from the study of living vertebrates (for example, Turner and Bagnara, 1976; Bentley, 1976).

Although the cyclostomes represent a separate line of evolution and are not the direct ancestors of the recent vertebrates (Jarvik, 1968; Stensio, 1968; Hubbs and Potter, 1971) the structural characteristics of the hagfish and lampreys are truly primitive and there is no doubt that they are derived from a stock that is ancestral to the vertebrates. It is possible that in addition to ancient morphological characteristics they also retain some ancient endocrinological mechanisms intact.

Class AGNATHA

The two existing groups of cyclostomes, the orders Myxinoidei and Petromyzontia, differ from each other in so many respects that many authorities consider that they are diphyletic in origin (Stensio, 1968; Hubbs and Potter, 1971). The considerable list of differences between the two groups also includes marked differences in their modes of reproduction; it is unwarranted, therefore, to extend any finding from hagfish to lampreys and vice versa (Falkmer *et al.*, 1974).

Order MYXINOIDEI

Almost nothing is known of the life history of the myxinoids. In 1864 and 1865, the Academy of Science of Copenhagen offered a prize for the elucidation of the problem of *Myxine* reproduction (Walvig, 1963). The prize was never awarded, and it is unlikely that the accumulated knowledge on all of

the myxinoid species to date could warrant a presentation today. In fact, the discovery of a third embryo of *Myxine* was in itself worthy of a detailed study (Fernholm, 1969).

Hagfishes lack secondary sex characters and often show incomplete sex differentiation of the gonads. The gonads of both sexes are unpaired and there are no sex ducts, the ova and spermatozoa being released into the body cavity and passed to the exterior, presumably by muscular contractions of the body wall (Walvig, 1963).

The testis consists of follicles or cysts containing synchronously developing germ cells, each follicle generally at a different stage of development. Spermiogenesis is achieved by the rupture of the thin connective tissue wall of the follicle and the release of the spermatozoa into the body cavity. Interstitial cells have not been identified (Walvig, 1963; Matty *et al.*, 1976).

The ovary contains large yolked follicles in various stages of development, which at maturity are released into the body cavity and thus to the exterior. The eggs are covered by a tough, horny shell with anchor filaments at each pole, apparently secreted by the follicular granulosa cells (Patzner, 1975). This phenomenon appears to be unique to the myxinoids inasmuch as no other vertebrate is known to produce a shell with the ovary.

Most species of hagfish live in muddy sediments at very great depths and, as far as is known, breed year round. The one known exception is the Pacific hagfish, *Eptatretus burgeri*, which lives in the shallow waters off Japan from October to the beginning of June and then migrates into deep water to breed (Kobayashi *et al.*, 1972). The gonads of this species show a seasonal cycle of activity.

The question of whether the gonads of these primitive vertebrates secrete steroids is still open to question. Fernholm (1972) was unable to demonstrate any histochemical or ultrastructural evidence for steroid secreting cells in the ovaries of *Myxine glutinosa*, though the thecal cells did contain large lipid droplets of unknown function; also, he was unable to detect any progesterone in the plasma, by use of a relatively insensitive (1 ng/ml.) protein binding assay. In this same species, however, we have been able to show the presence of aromatizing enzymes in minces of ovarian tissue incubated in the presence of androstenedione-³H. Estrone and estradiol-17 β have been tentatively identified (Table 1), but a fraction copolar with authentic testosterone in three separate chromatographic systems failed to recrystallize to constant specific activity. Hirose *et al.* (1975) incubated ovarian tissue from *Eptatretus burgeri* with radioactive pregnenolone, progesterone, androstenedione, and testosterone. Although neither testosterone nor estradiol were positively identified (Table 2), there were several unidentified polar fractions that might have been estrogen metabolites. The tissue did not metabolize 17-alpha hydroxy progesterone (17OH-progesterone) to any significant degree. From the results of these two experiments we can tentatively conclude that myxinoid ovarian tissue possesses the enzymatic equipment to produce both androgens and estrogens. What is also of interest is that the *Eptatretus* ovary

TABLE 1.—*Estrogen biosynthesis in vitro by hagfish Myxine glutinosa ovary from androstenedione-³H. Three aliquots (487, 494, and 308 mg.) of minced ovarian tissue from Myxine glutinosa were incubated in 10 ml. of cyclostome Ringer solution for six hours at 18°C with 5 μ Ci of androstenedione-³H in each flask as substrate. Following extraction, steroids were purified on thin layer chromatography and recrystallized to constant specific activity, (Callard and Fedele, unpublished data).*

Steroid identified	Specific activity in crystals (cpm./mg.)			Per cent conversion of substrate
	4th	5th	6th	
Estrone	65	65	66	0.089
	96	93	90	0.130
	117	117	108	0.150
Estradiol-17 β	458	421	445	0.600
	490	519	540	0.680
	419	390	415	0.460

also is capable of synthesizing 11-deoxycortisol and pregnanolone (3 α -hydroxy-5 β pregnan-20-one) from radioactive precursors. These two compounds also have been isolated from a number of other vertebrates, and their significance will be discussed later.

Evidence for pituitary control of gonadal function in the Myxinoidei is lacking. Acetone-dried extracts of *Myxine* pituitary had no effect on spermatogenesis when injected into frogs, but the same preparation was reported to have stimulated uterine activity in house mice (Strahan, 1959). Although the *Myxine* pituitary contains PAS-positive cells, which were tentatively indentified as gonadotropes (Fernholm and Olsson, 1965), these cells were unaffected by gonadectomy or steroid injection and showed no morphological changes correlated with the functional stage of the gonads (Fernholm and Olsson, 1969). Matty *et al.* (1976) successfully hypophysectomized a large number of *Eptatretus stouti* and were able to maintain them in the laboratory for periods up to seven months after the operation. The animals did not feed but appeared healthy, and all females (both experimental and control) continued to ovulate. The authors also measured testosterone and estradiol-17 β in the plasma by radioimmunoassay and found similar levels in both sexes. The level of testosterone in females showed a negative correlation with body size and, although the mean level in hypophysectomized (male plus female) animals was lower than intact (19.5 ± 5.5 pg/ml., range ND-44 versus 29.0 ± 5.2 pg/ml., range, 15-64), the difference was not significant. The majority of animals of both sexes had undetectable estradiol levels, but seven individuals had levels in the range of 100-1016 pg/ml. Hypophysectomy or multiple subcutaneous implants of fresh pituitary had no detectable effect on spermatogenesis, steroid levels, or ovulation. There was a suggestion of some testicular disorganization in a number of the hypophysectomized hagfish, but, in most cases, spermatogenesis appeared to be proceeding normally. These results would suggest that there is little or no pituitary control of gonadal function in this species and that gametogenesis, steroidogenesis, and ovulation are largely autonomous. However, there is no

TABLE 2.—In vitro steroid metabolism by hagfish, *Eptatretus burgeri*, ovary (from Hirose et al., 1975).

Substrate	Metabolite identified	Per cent conversion
Pregnenolone	Progesterone	2.6
	17-OH Progesterone	2.0
	Androstenedione	1.7
	11-Deoxycortisol	3.4
Progesterone	Pregnanolone	35.5
17-OH Progesterone		
Androstenedione	Epiandrosterone	11.8
	5 α -Androstenedione	8.7
Testosterone	Androstenedione	15.2

information on sexual maturation in this species, which may be under pituitary control inasmuch as the PAS-positive cells (putative gonadotropes) that are present in the pituitary of adults do not occur in the pituitary of immature specimens (Fernholm and Olsson, 1969).

Eptatretus stouti, as does *Myxine glutinosa*, breeds year round in very deep water and probably receives little environmental stimulus to influence breeding habits, and may, therefore, require little or no gonadotropic regulation. It has been suggested by Tsuneki *et al.* (1974), however, that the seasonally breeding *E. burgeri* probably possesses functional gonadotropes in its pituitary, and that environmental stimuli might reach the adenohypophysis via neurosecretory neurons in the ventral wall of the neurohypophysis. Further investigation of this species is of the utmost importance. If this myxinoid can be shown to possess pituitary gonadotropin(s) it might help to determine whether the deep-water forms are degenerate. If they have lost pituitary regulation of reproduction, a functional pituitary gonad axis indeed could be a primitive vertebrate feature.

Order PETROMYZONTIA

Unlike the hagfish, the lampreys are active predators and undergo a complicated life cycle that includes a prematuration metamorphosis under pituitary (Larsen and Rothwell, 1972) and pineal (Eddy, 1969) control, a migration into salt water to feed as adults (except for a few fresh water species), and a return to fresh water to breed and die. During the migration to the breeding grounds, the lampreys stop feeding, the gut atrophies, tissue mobilization occurs and well-defined secondary sexual characters develop. Like the hagfish, the lampreys lack sex ducts and release their gametes into the body cavity, but the structure of the ovary and testis shows many differences. Hagfish produce around 18 to 20 large-yolked eggs and breed for several seasons, whereas the lampreys produce thousands of small eggs that are ovulated at one time, shortly before they die.

The spermatogonia are arranged in lobules or cysts and possess well-developed interstitial or interlobular cells that exhibit all the histochemical

(Chieffi and Botte, 1962; Hardisty and Barnes, 1968) and ultrastructural (Barnes and Hardisty, 1972) characteristics of steroid-secreting cells. The ovaries also give a positive reaction for Δ^5 3β -hydroxysteroid dehydrogenase (3β -HSD), and ultrastructural studies indicate that the thecal cells might be steroidogenic (Busson-Mabillot, 1967). Estradiol, estrone, and progesterone have been identified in tissue extracts of *Petromyzon* ovary (Botticelli *et al.*, 1963), and testosterone has been identified in testis extracts of *Lampetra* (Parkinson, unpublished, *vide* Hardisty, 1972), but as yet there are no reports of circulating steroid levels.

A number of workers have performed hypophysectomy, gonadectomy, and pituitary implant experiments in lampreys (see reviews by Dodd, 1972a, 1975; Larsen, 1969, 1973; Larsen and Rothwell, 1972; Hardisty, 1972) and, in general, the results are in agreement. Hypophysectomy prevents the appearance of the secondary sexual characteristics, ovulation, spermiation, and atrophy of the gut, but has only a delaying effect on spermatogenesis and does not cause atresia of the yolked follicles in the ovary. Gonadectomy prevents the appearance of the secondary sexual characteristics and the atrophy of the gut, both of which can be induced by the injection of the appropriate steroid (Pickering, 1976a, 1976b). Moreover, the gonadal steroids have also been implicated in the loss of osmoregulatory ability in the sexually mature adults (Pickering and Dockray, 1972). Injections of estradiol cause liver hypertrophy and an increase in protein-bound calcium in the plasma (Pickering, 1976b), suggesting that vitellogenesis is an estrogen-mediated phenomenon, probably under the control of pituitary gonadotropins, as it is in all other vertebrates.

Pituitary implants or injections of mammalian gonadotropins are able to stimulate the appearance of secondary sexual characteristics and induce ovulation in hypophysectomized or sexually immature animals (Evennett and Dodd, 1963), suggesting that the steroid secretion is stimulated by gonadotropins and that the pituitary is able to secrete gonadotropins without neural input. In all of these experiments, however, the timing of hypophysectomy or gonadectomy is critical. If the operations are performed during the latter half of the sexual maturation, they often have no effect (Larsen, 1973). This would suggest that the pituitary hormones are necessary for initiating sexual maturation and steroidogenesis but once underway these processes require little gonadotropic regulation.

There have been two reports (Eddy, 1971; Joss, 1973) on the function of the pineal in the lamprey, and both have demonstrated that extirpation of the pineal prior to sexual maturation retards growth of the gonads and appearance of the secondary sexual characters.

In summary, it can be concluded that 1) the gonads of lampreys produce sex steroids; 2) sex steroids are necessary for the development of the secondary sexual characters and the production of vitellogenic protein by the liver—furthermore, they are involved in some way in the atrophy of the gut and loss of osmoregulatory ability in the sexually mature animals; and 3)

the synthesis and secretion of the sex steroids are controlled by pituitary gonadotropins and also can be stimulated by large doses of mammalian gonadotropins. Unusual, or perhaps primitive, features of gonadal function in lampreys are: 1) hypophysectomy does not result in gonadal regression or atresia but does prevent further rapid growth; 2) spermatogenesis and possibly ovarian follicular growth (Larsen, 1973) are able to proceed in the absence of the pituitary, but at a slower rate; 3) there is no evidence for hypothalamic control of pituitary function (Gorbman, 1965; Gorbman *et al.*, 1963); and 4) the pineal appears to exert a positive gonadotropic action.

Class CHONDRICHTHYES

The limited amount of information on the endocrinology of reproduction in elasmobranch fishes has been reviewed in great detail by Chieffi (1967) and by Dodd (1955, 1972a, 1972b, 1975). Nothing is known of the endocrinology of Holocephali, and all of our information comes from work on a few common species of Rajiformes (skates and rays) and Squaliformes (dogfish and sharks).

The gonads of both sexes are invested by a hemopoietic tissue, known as the epigonal organ, not known to occur in other vertebrates. The testes of elasmobranchs are unusual in that the Sertoli cells and germ cells are enclosed in concentric zones of ampullae rather than tubules, with all of the germ cells in each ampulla at the same stage of development (Dodd *et al.*, 1960; Holstein, 1969). Various histochemical studies have implicated both the interstitial cells (Della Corte *et al.*, 1961) and the Sertoli cell (Collenot and Ozon, 1965; Simpson and Wardle, 1967) as sources of steroid hormones, and testosterone, androstenedione, progesterone and estradiol-17 β have been identified in extracts of testicular tissue from *Scyliorhinus stellaris* (Chieffi and Lupo, 1961). Simpson *et al.* (1964) identified testosterone, DOC and a number of metabolites from *Squalus acanthias* testis incubated with radioactive progesterone, but were unable to detect DOC in *Scyliorhinus* testis incubates.

It has also been established that the secondary sexual characters of dogfish are dependent on androgens from the testis (Dodd *et al.*, 1960; Dodd, 1975). Androgens were tentatively identified in the plasma of male *Scyliorhinus* by Simpson *et al.*, 1969a) using gas liquid chromatography, and testosterone has been definitively identified in plasma of *Raja radiata* and *R. ocellata* (Idler and Truscott, 1966; Fletcher *et al.*, 1969; Darrow and Fletcher, 1972). Levels of testosterone of between 20 and 200 ng/ml. were estimated. Using radioimmunoassay (RIA), Dobson (quoted by Dodd, 1975) measured testosterone in the plasma of *Scyliorhinus canicula* throughout the year and estimated levels from a mean low of 2 ng/ml. in February to a mean high of 6 ng/ml. in August.

The ovaries of elasmobranchs are superficially similar to those of birds in that they contain large-yolked follicles in various stages of development. There are, however, considerable variations within the class as might be

expected considering the range of reproductive patterns encountered. Lance and Callard (1969) pointed out that from the limited data available it appeared that the microscopic structure of the follicle wall of the ovary might show differences between the *Rajiformes* and the *Squaliformes*: in the former the granulosa being composed of two cell types; in the latter, a single layer of columnar epithelium. There have been no additional histological studies to bear out this observation. There have been a number of studies on the histochemistry of elasmobranch ovaries (see reviews by Lofts and Bern, 1972; Dodd, 1972a; Guraya, 1976), and, in all species examined positive reactions for the enzyme 3-beta-hydroxysteroid dehydrogenase (3β -HSD) have been obtained in the follicular granulosa. However, interspecific differences have been observed in other ovarian sites of activity. In *Torpedo marmorata*, atretic follicles show activity, and postovulatory follicles (corpora lutea) do not (Lupo *et al.*, 1965); whereas in *Squalus acanthias* and *Scyliorhinus canicula*, the corpora lutea show intense activity, and the atretic follicles are consistently negative (Lance and Callard, 1969; Lupo *et al.*, 1965).

Tissue extracts of elasmobranch ovaries and *in vitro* experiments with radioactive precursors have clearly established that the elasmobranch ovary is capable of synthesizing progesterone, testosterone, androstenedione and estradiol-17 β (Wotiz *et al.*, 1958, 1960; Simpson *et al.*, 1963; Chieffi and Lupo, 1963; Callard and Leathem, 1965; Simpson *et al.*, 1968). Lupo *et al.* (1967) tentatively identified progesterone, testosterone, estrone, and estriol in the plasma of female *Torpedo marmorata*. The levels that they estimated for testosterone (15.6-35 ng/ml.) are remarkably close to those reported by Fletcher *et al.* (1969), who used far more rigorous identification procedures for female *Raja radiata* (11-62 ng/ml.). There has been only one additional report of steroid levels in the plasma of female elasmobranchs. Dobson (1975, quoted by Dodd, 1975) measured plasma testosterone in the plasma of *Scyliorhinus canicula* throughout the year. Lowest testosterone levels (approximately 1 ng/ml.) occurred in late summer when most of the ovarian follicles were atretic and highest levels (approximately 6 ng/ml.) occurred during the vitellogenic and ovulation phase. It is surprising that testosterone levels were identical in males and females inasmuch as male secondary sexual characters (clasper growth) can be induced in immature males by testosterone implants (Dodd *et al.*, 1960).

The elasmobranchs offer a unique model for the study of pituitary control of reproduction because of the unusual morphology of the adenohypophysis (see Ball and Baker, 1969). Dodd *et al.* (1960) showed that after removal of the anatomically discrete ventral lobe in *Scyliorhinus canicula*, testes would regress in males, and yolked follicles would undergo atresia and ovulation would cease in females. Removal of the rostral or median lobe alone had no detectable effect. Mellinger (1972) reported identical results on removal of the ventral lobe from female *Torpedo marmorata*. Additional indirect evidence of this segregation of the gonadotropes from the rest of the pituitary comes from a study in which antibodies to sheep LH were used to locate the

gonadotropes in the pituitary of the ray, *Torpedo marmorata*, by immunofluorescence (Mellinger and Dubois, 1973). A specific reaction, observed only in the ventral lobe, could be blocked by excess unlabelled ovine LH. Scanes *et al.* (1972a) examined extracts from the different lobes of the pituitary of *Scyliorhinus canicula* by using an antibody raised against chicken LH and found that only extracts from the ventral lobe showed significant cross reaction and that parallel dose response curves could be generated with increased concentrations.

Bioassay studies of ^{32}P uptake by day-old chick testes have been performed and similar results have been obtained, only the ventral lobe showing significant activity (Scanes *et al.*, 1972b). However, using a more sensitive bioassay, Firth and Vollrath (1973) were able to demonstrate gonadotropic activity in both the ventral lobe and the median lobe, although levels reported for the ventral lobe were considerably lower than those reported by Scanes *et al.* This assay, which uses *Xenopus* oocyte meiosis as an end point, is relatively specific for mammalian LH, whereas the ^{32}P uptake assay measures both FSH and LH activity. Firth and Vollrath suggested that Scanes *et al.* were able to measure such high activity in the ventral lobe and none in the median lobe because they were measuring total gonadotropin, whereas in their assay they were able to detect the small amount of LH-like material present in the median lobe. A further indication that there might be more than one gonadotropin in elasmobranch pituitary was suggested by Knowles *et al.* (1975) in a detailed ultrastructural study on the adenohypophysis of *Scyliorhinus canicula*. The authors tentatively identified six cell types based on granule size. Their cell type IV, found only in the ventral lobe contained particles from 100 to 700 nanometers in diameter, and another cell type (V), found in the median lobe contained secretory granules 500 nanometers in diameter, both of which could be gonadotropes.

Despite their unusual structural features and the unique gonadotropin containing pituitary ventral lobe, which lacks either vascular or neural connection with the hypothalamus, the elasmobranchs appear to follow the general vertebrate pattern in the hormones produced by the gonads and their roles in reproduction. It is obvious, however, that additional studies are required to characterize the gonadotropin(s) of sharks and rays, preferably using bioassays in which homologous gonadal tissue is used. In addition, more work is needed to determine the nature of the circulating steroids, especially with regard to the ten-fold concentration differences reported for Rajiformes and Squaliformes.

Class OSTEICHTHYES

Subclass ACTINOPTERYGII

It has been suggested that the teleosts represent the culmination of a highly successful adaptive radiation of a group that is in a direct line of the main vertebrate stem, and that "the fleshy-finned vertebrates" such as man and his tetrapod relatives are a specialized offshoot that diverged from the

main vertebrate stock more than 400 million years ago (Nelson, 1969). Whether we accept this partisan view, it is obvious that the teleosts are of great economic importance and have thus received far more attention than other nonmammalian vertebrates. Consequently, a voluminous literature has accumulated that not only emphasizes their interest to comparative endocrinologists but also the enormous variety encountered in the modes of reproduction among the 20,000 or so species (Breder and Rosen, 1966). Of the many thousands of species known, however, the endocrinology of reproduction of only a dozen or so has been studied in any detail, and even among these there are substantial biochemical differences in the putative sex hormones. Not only are the differences among the teleosts confusing to comparative endocrinologists, but the long separation from the rest of the vertebrates and spectacular adaptive radiation of the modern teleosts has, in many cases, obscured phylogenetic relationships. If we are to draw any broad evolutionary inferences on the hormonal control of reproduction it is necessary that we ignore the many unique features of teleost reproduction and look for features that are common to all teleosts and vertebrates alike. The pituitary gonad axis appears to be such a feature; and despite the bewildering variation in reproductive patterns found within the teleosts, there is good evidence that in all of them, gonadal function is dependent on the pituitary gland (Pickford and Atz, 1957). There also are data implicating specific areas in the hypothalamus (nucleus lateralis tuberis and nucleus preopticus) as centers that exert control over gonadotropin secretion by the anterior pituitary (Peter, 1973). Abundant evidence also exists for steroid secretion by the gonads of fish (see extensive bibliography compiled by Bern and Chieffi, 1968, and reviews by Gottfried, 1964; Nandi, 1967; Dodd, 1972b; Ozon, 1972a; Reinboth, 1972; Colombo *et al.*, 1972) and that these steroids are important in stimulating the appearance of the secondary sexual characters (Hoar, 1969), vitellogenesis (Chester Jones *et al.*, 1972), and mating behavior (Liley, 1969).

The structure of the teleost testis shows more variation than that of other vertebrates (Lofts, 1968; Lofts and Bern, 1972; Hoar, 1969; Dodd, 1972b; DeVlaming, 1974). In some species it consists of thin-walled lobules that contain germ cells and Sertoli cells, but no interstitial cells. The Leydig cell homologue, the lobule boundary cell (Marshall and Lofts, 1956), may develop from connective tissuelike cells that surround the spermatogenetic cyst. Other species may have well-developed Leydig cells in the connective tissue matrix between lobules of germinal elements, and yet other species that exhibit variations on both of the basic types, including some that apparently possess both lobule boundary cells and Leydig cells (Yaron, 1966). Fishes in common with other anamniote vertebrates lack a permanent germinal epithelium.

There is histochemical and ultrastructural evidence for steroid secretion by the lobule boundary cells (Yaron, 1966; O'Halloran and Idler, 1970; Upadhyay and Guraya, 1971; Nicholls and Graham, 1972), the interstitial cells

(Lupo di Prisco and Chieffi, 1963; Stanley *et al.*, 1965; Bara, 1969; Follenius, 1968; Horstmann and Brucker, 1972), and the Sertoli cells (Wiebe, 1969; Billard, 1970). All of the known mammalian gonadal steroids have been identified in teleost testicular tissue extracts or by *in vitro* incubations with radioactive steroid precursors (see reviews by Gottfried, 1964; Nandi, 1967; Chester Jones *et al.*, 1974; Colombo *et al.*, 1972).

Although testosterone has been identified in the plasma of *Serranus cabrilla* (D'Istria *et al.*, 1973) and *Pleuronectes platessa* (Wingfield and Grimm, 1977), and increased concentrations appear to correlate with the onset of spawning, it now appears that in some species the major testicular and circulating androgens are the 11-oxygenated metabolites of testosterone. Idler *et al.* (1960) first isolated 11-ketotestosterone from the peripheral plasma of the sockeye salmon, *Oncorhynchus nerka*, and subsequent studies have demonstrated its presence in the plasma of *Salmo salar* (Idler *et al.*, 1964) and *Pseudopleuronectes americanus* (Campbell *et al.*, 1976). In a number of ambisexual species, however, 11-hydroxytestosterone appears to be the major circulating androgen (Idler *et al.*, 1976). A number of *in vitro* studies have identified 11-ketotestosterone and 11 β -hydroxytestosterone as the major metabolites of radioactive precursors in testicular tissue of *Salmo salar* (Idler and MacNab, 1967; Idler *et al.*, 1968), *Salmo gairdnerii* (Arai and Tamaoki, 1967), *Microstomus kitt* (Simpson *et al.*, 1969b) and *Poecilia latipinna* (Van den Hurk and Lambert, 1977). It has been shown that 11-ketotestosterone has 10 times the potency of testosterone in promoting the appearance of male secondary sexual characters in the female medaka, *Oryzias latipes* (Arai, 1967; Hishida and Kawamoto, 1970).

The concentration of 11-ketotestosterone increases in the peripheral plasma of salmon at the approach of sexual maturity (Schmidt and Idler, 1962; Idler *et al.*, 1971), and in *Pleuronectes americanus* just prior to spawning (Campbell *et al.*, 1976). In the latter species, however, elevated plasma levels (170 ng/ml.) did not always correlate with testis weight, inasmuch as some apparently sexually mature individuals had levels of only 20 to 40 ng/ml. The authors suggested that 11-ketotestosterone might play a role in the final stages of spermatogenesis.

The ovary of teleosts is a hollow saccular organ within which are branched connective tissue septae or ovigerous lamellae that contain the germinal epithelium and the developing oocytes (see Hoar, 1969; Lambert, 1970, for details and a bibliography). Developing follicles exhibit the typical structure of egg-laying vertebrates in having a single layer of granulosa epithelium separated from the thecal layers by a basement membrane. Histochemical studies have shown that the granulosa and/or theca of the preovulatory follicle are the principle sites of steroid production (Bara, 1965; Lambert, 1970; Livni, 1971; Yaron, 1971).

Progesterone, testosterone, and estradiol have been identified in tissue extracts (Gottfried, 1964; Nandi, 1967; Barr, 1968; Katz *et al.*, 1971; Ozon, 1972a, 1972b) by *in vitro* studies using radioactively labelled precursors

(Lambert *et al.*, 1971; Reinboth, 1972, 1975) and in the plasma (Eleftheriou *et al.*, 1966; Wingfield and Grimm, 1976, 1977; Campbell *et al.*, 1976). However, an unusual feature of many of these *in vitro* studies is that testosterone, 11-ketotestosterone, and DOC have been identified as the major products from radioactive precursors in ovarian tissue (Eckstein and Katz, 1971; Colombo *et al.*, 1972, 1973; Lambert and Pot, 1975). The same three steroids also have been identified in the peripheral plasma of female *Tilapia* (Katz and Eckstein, 1974), *Pleuronectes* (Wingfield and Grimm, 1976) and *Pseudopleuronectes* (Campbell *et al.*, 1976). However, plasma testosterone levels in prespawning female flounder were even higher than those in males of the same species (Campbell *et al.*, 1976). They suggested that the testosterone in vitellogenic teleosts could be serving as a precursor for estrogen synthesis. Wingfield and Grimm (1977) came to the same conclusion as the seasonal pattern of plasma testosterone in the female plaice lags behind but follows that of the plasma estradiol (Fig. 1). The latter authors, however, found that although plasma testosterone was significantly elevated in the prespawning female plaice, the levels attained were less than 50 per cent of those found in the spawning males. Whereas Campbell *et al.* (1976) found that plasma levels of 11-ketotestosterone in female flounders were insignificant throughout the annual reproductive cycle, Katz and Eckstein (1974) found a 2.6-fold increase in the plasma levels in spawning versus nonbreeding female *Tilapia*.

Colombo *et al.* (1972, 1973) suggested that the ability of teleost ovarian tissues to synthesize DOC *in vitro* might be related to the ability of this steroid to induce ovulation. Sundararaj and Goswami (1969) showed that either LH or DOC could induce ovulation in hypophysectomized catfish, *Heteropneustes fossilis*. They observed a significant lag period between the time taken to induce ovulation with LH when compared to DOC, which suggested to them that the LH might have been acting on the fish interrenal tissue to induce the secretion of a corticosteroid that then acted on the ovary to trigger ovulation. Colombo *et al.* (1972, 1973) suggested, rather, that the ovary itself could be secreting the ovulatory surge of DOC in response to the gonadotropin. Katz and Eckstein (1974) also are of the opinion that ovarian DOC might be the trigger for ovulation in *Tilapia* inasmuch as they found a 38-fold increase in plasma DOC levels in spawning versus nonbreeding fish. However, Ungar *et al.* (1977) could find no evidence of DOC formation by *Heteropneustes* ovarian tissue when incubated *in vitro* with radioactive prenenolone, whereas head kidney tissue from the same animals was shown to synthesize DOC and a number of other corticosteroids from the same precursor. Prior *in vivo* treatment of the catfish with either ACTH or salmon gonadotropin stimulated the interrenal and ovary to convert more precursor *in vitro*, but there was no evidence that the gonadotropin stimulated DOC or corticoid production by the interrenal tissues.

The role of the gonad-interrenal relationship in teleosts remains uncertain. In the few species examined there are elevated plasma corticosteroid

FIG. 1.—Seasonal changes in plasma steroids in the male and female plaice, *Pleuronectes platessa*. Redrawn from Wingfield and Grimm (1977).

levels prior to spawning (Idler *et al.*, 1959; Schmidt and Idler, 1962; Campbell *et al.*, 1976; Wingfield and Grimm, 1977), which could be due to an effect of gonadotropin on the interrenal tissue as suggested by Goswami *et al.* (1974), to increased ovarian secretion of corticosteroids in response to gonadotropins as suggested by Colombo *et al.* (1973), or to increased secretion of ACTH due the stress of breeding. Another possibility is that the interrenal tissue is converting steroid precursors secreted by the gonad into corticosteroids and that these corticosteroids in turn induce ovulation. This heightened interrenal activity associated with breeding is not peculiar to the teleosts. Corticosteroids can induce ovulation in Amphibia, and amphibian interrenal tissue shows heightened activity during the breeding season (Lofts, 1974) as does that of reptiles (Callard and Callard, 1977) and birds (Lofts and Bern, 1972). Injections of DOC can induce ovulation in the domestic chicken (Etches and Cunningham, 1976a). On the other hand, this heightened corticosteroid activity could be merely a seasonal phenomenon not associated with breeding, inasmuch as Wingfield and Grimm (1977) observed a rise in cortisol without any concomitant rise in sex steroids in the plasma of immature plaice at the same time the mature fish were breeding.

Although there is as yet no information on the effects of gonadotropins *in vivo* on gonadal steroidogenesis and circulating steroid levels in teleosts,

there is abundant evidence that teleost pituitary extracts are able to stimulate all the indices of steroidogenesis when injected into hypophysectomized fish (Pickford and Atz, 1957; Hoar, 1969; DeVlaming, 1974). The nature of piscine gonadotropins, however, remains in dispute, and it is in this area that some of the most exciting advances have been made during the past few years. Otsuka (1956) separated by ammonium precipitation two fractions of salmon pituitary that he termed "LH" and "FSH", both of which were able to induce ovulation in hypophysectomized newts. Donaldson and his coworkers, however, were able to find only a single fraction with gonadotropic activity after partial purification of salmon pituitary extract on Sephadex G100 (Yamazaki and Donaldson, 1968; Donaldson *et al.*, 1972). This gonadotropin has since been shown to maintain spermatogenesis and induce spermiation in hypophysectomized males and maintain vitellogenesis and induce ovulation in hypophysectomized females in a number of teleost species (Donaldson, 1973). The amino acid composition of salmon GTH has now been determined (Table 3). However, there is now some doubt regarding the homogeneity of this salmon gonadotropin as the SG-100 fraction of Donaldson *et al.* (1972) separated into three distinct fractions following rigorous purification procedures. Of these, one was without any biological activity, and the other two had only 25 per cent of the original SG-100 activity as assayed by ^{32}P uptake by day-old chick testis (Pierce *et al.*, 1976). Burzawa-Gerard (1971) also has been able to demonstrate only a single gonadotropic fraction in carp pituitary extracts, which differs from the salmon GTH both in its chemical composition and biological activity when tested in non teleost species but is able to maintain gonadal function in hypophysectomized male and female fish. A single glycoprotein fraction with gonadotropic activity has since been isolated from the sturgeon, *Acipenser stellata*, which differs substantially both in biological activity (Burzawa-Gerard *et al.*, 1976) and chemical composition from the carp and salmon gonadotropins (Burzawa-Gerard *et al.*, 1975a, 1975b). The pituitaries of two additional teleost species, *Salmo gairdnerii* (Breton *et al.*, 1976) and *Sarotherodon mossambicus* (= *Tilapia mossambica*) (Farmer and Papkoff, 1977), have since been fractionated to yield a single gonadotropinlike molecule, both of which differ in chemical composition from each other and all of the other fish gonadotropins analyzed to date (see Table 3). These biochemical results provide additional support to the concept of a narrow species specificity within the teleosts as noted by Clemens and Johnson (1964) in their study on the goldfish testis hydration factor in pituitary extracts from a wide range of fish species and by Breton *et al.* (1973) when testing the ability of different fish pituitary extracts to displace radioiodinated carp-GTH from carp-GTH antisera.

Highly specific radioimmunoassays with sufficient sensitivity to measure circulating gonadotropin in carp (Breton *et al.*, 1971), salmon (Crim *et al.*, 1973), and trout (Breton *et al.*, 1976) have been developed. A peak of gonadotropin some five times above base level was found in the plasma of female

TABLE 3.—Amino acid composition of gonadotropins from salmon (*Onchorynchus tshawytscha*), trout (*Salmo gairdnerii*), carp (*Cyprinus carpio*), tilapia (*Sarotherodon mossambicus*), sturgeon (*Acipenser stellatus*), bullfrog (*Rana catesbeiana*), sea turtle (*Chelonia mydas*), turtle (*Chelydra serpentina*), alligator (*Alligator mississippiensis*), turkey (*Meleagris gallopavo*), chicken (*Gallus domesticus*), sheep (*Ovis aries*). Source of data is identified in the box heading as follows: (1) Pierce et al., 1976; (2) Breton et al., 1976; (3) Burzawa-Gerard et al., 1975; (4) Papkoff et al., 1976a; (5) Licht et al., 1976; (6) Farmer et al., 1975; (7) Godden and Scanes, 1975; (8) Ward et al., 1969; (9) Papkoff and Ekblad, 1970; (10) Papkoff et al., 1976b; (11) Farmer and Papkoff, 1977. Amino acids/100 residues (original values recalculated where necessary).

[illegible]

carp during ovulation (Breton *et al.*, 1972); seasonal changes in plasma gonadotropin in a variety of salmonid fishes were recorded by Crim *et al.* (1975), with the highest levels found in females near the time of ovulation. The levels of gonadotropin in male fish, however, remain low. These results would suggest that a large preovulatory surge in gonadotropin might be required to initiate ovulation in teleosts, as in the case in mammals.

Increased plasma gonadotropin levels following injection of carp hypothalamic extract or synthetic LRF have been reported in carp (Breton and Weil, 1973; Weil *et al.*, 1975) and brown trout (Crim and Cluett, 1974), and intraventricular injection of brain extracts or synthetic LRF have been shown to increase plasma gonadotropin in goldfish (Crim *et al.*, 1976). Although there is sound evidence for hypothalamic control of the teleost anterior pituitary (Peter, 1973), the relevance of LRF effects in teleosts is unclear inasmuch as teleost hypothalamic extracts show no cross reactivity to an LRF antisera (Deery, 1974).

The chemical nature of the fish gonadotropins suggests a close similarity to mammalian FSH and LH both in amino acid composition (Table 3) and in their subunit nature. Burzawa-Gerard (1974) was able to disassociate carp GTH into two distinct subunits, neither of which retained biological activity, whereas 60 per cent of the original biological activity was regained on reassociation. What is of even greater importance is the ability of the carp gonadotropin subunits to form hybrid gonadotropins on association with subunits from other species. Burzawa-Gerard and Fontaine (1976) formed a hybrid molecule composed of the alpha subunit of bovine LH and subunit II of carp GTH. This hybrid GTH had no biological activity when tested on its ability to stimulate adenylate cyclase in carp or rat ovarian tissue, but was more potent than the native carp GTH or the bovine α subunit in stimulating adenylate cyclase activity in frog ovarian tissue.

The ability of these fish gonadotropins to maintain gonadal function in hypophysectomized fish led Burzawa-Gerard and Fontaine (1972) to propose that teleosts possess only one gonadotropin, which combines the functions of FSH and LH. Donaldson (1973) came to the same conclusion as regards the salmon. Doerr-Schott (1976a) reached a similar conclusion based on a review of immunohistochemical studies of teleost pituitary. Fluorescent-labeled anti-ovine LH binds to teleost gonadotropes and can be inhibited by excess cold ovine LH, whereas similarly tagged anti-ovine FSH shows no reaction.

Contrary conclusions have been expressed by a number of pituitary cytologists who have shown that, in some species at least, there is morphological and histochemical evidence for two distinct gonadotropes which exhibit signs of heightened activity during different stages of the reproductive cycle (see reviews by Ball and Baker, 1969; Sage and Bern, 1971; Rai, 1972; Schreibman *et al.*, 1973; Olivereau, 1976). Similar reservations have been expressed by Reinboth (1972).

Further data on the possibility of more than one gonadotropin in fishes comes from three separate studies in which teleost pituitaries have been

separated into various fractions by electrophoresis and column chromatography. Hattingh and du Toit (1973) separated pituitary homogenates from the mudfish *Labeo umbratus* into eight fractions by means of polyacrylamide gel electrophoresis and chromatography on DEAE-cellulose and Sephadex G-50. Two of the fractions (3 and 4) showed considerable gonadotropic activity when tested by the fish spermiation assay of Yamazaki and Donaldson (1968). However, the details of the bioassay species and the magnitude of the response were not given. Idler *et al.* (1975a, 1975b, 1975c) have purified two protein fractions with gonadotropic activity from chum salmon, *Oncorhynchus nerka*, pituitary glands which exhibited distinct physicochemical behavior in the separatory systems used. Both fractions stimulated increased cAMP formation in immature trout gonads *in vitro*, a bioassay in which mammalian hormones are inactive. One contributing and, as yet, unexplained result in these experiments was that subfractions of one of the two main fractions showed sex specificity in their ability to stimulate adenylate cycle activity in the gonads of immature trout. The authors concluded that the results of their study "strongly suggests the existence of two or more distinct gonadotropins in chum salmon pituitaries."

Haider and Blüm (1977) separated goldfish pituitary extracts into several fractions using polyacrylamide gel electrophoresis and demonstrated two separate protein bands that showed differences in gonadotropic activity using the swordtail, *Xiphophorus helleri*, as the bioassay species. Fraction I was able to induce spermatogenesis and spermiogenesis and stimulate the Leydig cells, whereas the other fraction (IV) initiated and "mediated spermatogenesis," but did not affect the Leydig cells or promote spermiation in fish maintained on short photoperiod. When the fractions were tested on their ability to stimulate the uptake of ^{32}P by *Xiphophorus* testis, it was seen that fraction I increased uptake 282 per cent of control; fraction IV, 194 per cent. Another confusing observation on teleost pituitary fractions was made by Campbell and Idler (1976) in a study on vitellogenesis in the winter flounder, *Pseudopleuronectes americanus*. They found that the glycoprotein fractions isolated from frozen *Hippoglossoides* pituitaries according to the method of Idler *et al.* (1975b) failed to stimulate the incorporation of yolk into the oocytes of the flounder, whereas a nonglycoprotein fraction from the same pituitaries was stimulatory. This is in contrast to the situation in amphibians (Redshaw, 1972) and reptiles (Neaves, 1972) where mammalian gonadotropins stimulate the uptake of vitellogenin by the oocyte.

The effect of fish prolactin on the pituitary gonad axis is poorly understood, though there is a suggestion that there could be an antagonism between gonadotropin and prolactin secretion by the pituitary (Reinboth, 1972).

In summary, it would appear that reproduction in teleosts is similar to the general vertebrate pattern in its fundamental aspects but has several features that are unique to the class and a substantial degree of interspecific variation in both the structure of the gonads and the hormones they produce. Several findings that need further research are the exceptionally high

circulating testosterone levels in both males and females, the role of the unique, potent piscine androgen, 11-ketotestosterone, and the secretion of DOC by the ovary. The argument of one versus two gonadotropins is far from settled, and the possibility of even more than two, some of which may be sex specific (Idler *et al.*, 1975c) should provide fertile ground for research for a long time to come.

Class AMPHIBIA

The modern amphibia are poorly represented in the fossil record though it is generally agreed that they evolved from the rhipidistian crossopterygians (Schaeffer, 1969) and are probably more closely related to the lungfish than to any other group of living vertebrates. It has been proposed, however, that the Urodela and Anura are diphyletic in origin, each having evolved from a different family of Devonian fishes (Jarvik, 1968). Despite the large number of differences between the urodeles and the anurans and their postulated phylogenetic distance, the hormonal control of reproduction in the two groups appears to be basically similar, and, until more information is available, there is no reason to consider them separately. The relationships of the 150 known species of Apoda to living or extinct amphibians is uncertain, and nothing is known of their reproductive endocrinology other than that fertilization is internal and viviparity common.

The sensitivity of the amphibians to human chorionic gonadotropin was discovered more than 40 years ago and, subsequently, exploited in the *Xenopus* ovulation test (Bellerby, 1934; Shapiro and Zwarenstein, 1934) and the toad spermiation test (Gailli-Mainini, 1947) for pregnancy. Despite this early interest in amphibians as research animals (see reviews by Creaser and Gorbman, 1939; Smith, 1955; Van Oordt and Van Oordt, 1955; Houssay, 1954; Dodd, 1960) and the recent interest in *Xenopus* oocytes as a tool for translating heterologous mRNA (Mous *et al.*, 1976) or the process of vitellogenesis in *Xenopus* as a model for understanding the mechanism of action of estradiol (see review by Follett and Redshaw, 1974), there has been surprisingly little progress in understanding the process of reproduction in amphibians. Recent reviews on amphibian reproduction can be found in Redshaw (1972) and Lofts (1974).

The testes of anurans are paired structures consisting of a mass of convoluted seminiferous tubules containing Sertoli cells and the germinal epithelium embedded in a connective tissue matrix. As in other anamniote vertebrates, proliferation of germ cells occurs in coordinated clusters within a membranous capsule termed a germinal cyst. At any one point of the spermatogenetic cycle, all of the germinal cysts are at the same stage of development, unlike the urodele testis where different regions of the testis contains cysts at different stages of maturation. Detailed information on the structure of the anuran testis and the spermatogenic cycle can be found in Lofts (1974), Gavaud (1976), and Kalt (1976).

The testes of urodeles, however, are closer to those of teleosts in structure, with the germinal cysts enclosed within a thin-walled lobule. Typical Leydig cells are absent, but, as in teleosts, there are the Leydig cell homologues, the lobule boundary cells. These cells derive from the connective tissuelike cells lining the wall of the cysts. In some species the testis may be composed of as many as five separate lobes in a string, whereas in others the testis is a single lobe. Within each lobe (or testis) are a number of lobules containing cysts. Generally, each lobule contains cysts at an identical stage of maturation, but within each lobe there is a gradient of cyst maturation. Thus, in *Diemictylus*, where spermatogenesis occurs in a caudocephalic wave, the cephalic portion of the testis contains lobules with germ cells in the early stages of spermatogenesis and the caudal portion, lobules with evacuated cysts, remnants of degenerating Sertoli cells, and hypertrophied lobule boundary cells (see Fig. 2). For descriptions of urodele testicular cycles, the reader should consult (Humphrey, 1921, 1922, 1926; Miller and Robbins, 1954; Joly, 1971; Rouy, 1972, 1974; Lofts, 1974; Tso and Lofts, 1977a). This segregation of the germinal from the glandular regions in the urodele testis has yet to be exploited by endocrinologists, though it has been pointed out by Lazard (1976) that it should be easier to study Sertoli cell functions in axolotls than it is in mammals.

The steroidogenic function of the amphibian testis clearly has been established, and histochemical studies have identified the interstitial cells in anurans (Botte, 1964; Saidapur and Nadkarni, 1973; Pesonen and Rapola, 1962; Biswas, 1969), the lobule boundary cells in urodeles (Certain *et al.*, 1964; Della Corte *et al.*, 1962; Picheral, 1970; Vellano, 1968; Lazard, 1976; Tso and Lofts, 1977a), and the Sertoli cells in both groups (van Oordt and Brands, 1970; Lazard, 1976) as possible sites of steroid synthesis (see also reviews by Lofts and Bern, 1972; Lofts, 1972, 1974). Ultrastructural studies on the lobule boundary cells (Picheral, 1966, 1968; Tso and Lofts, 1977b) and the interstitial cells of frogs (Brokelmann, 1964; Doerr-Schott, 1964; Schulz, 1973; Aoki *et al.*, 1969; Unsicker, 1975) in general agree with the histochemical data in identifying these cell types as the principal sites of steroid synthesis in the testis. The activity of 3β -HSD in the interstitial cells of anurans, or the lobule boundary cells of urodeles, correlates with the appearance of secondary sex characters, suggesting that these cells secrete androgens. Sertoli cell 3β -HSD activity, in contrast, is correlated with spermatogonial cell division, which may take place when the indices of androgen secretion are absent. It is possible that the Sertoli cells are producing low levels of testosterone within the cysts to stimulate spermatogenesis without affecting secondary sex characters. However, the role of androgens in amphibian spermatogenesis is still controversial (Moore, 1975; Rastogi *et al.*, 1976).

Although testosterone has not been identified in extracts of testicular tissue, *in vitro* studies using radioactive precursors have shown that the tes-

FIG. 2.—Diagrammatic representation of the spermatogenic cycle of the newt (*Trituroides*) or dusky salamander (*Desmognathus*). Drawing on the left is taken from Tso and Lofts (1977a); that on the right, from Humphrey (1921). Top of the page represents the cephalic; bottom of the page, caudal direction. Regions of the testis are identified as A, spermatogonial region; B, lobules filled with maturing spermatozoa; C, evacuated lobules and developing lobule boundary cells; D, glandular region composed of hypertrophied lobule boundary cells.

tis of amphibia are capable of synthesizing testosterone and androstenedione, and that the biosynthetic pathways involved appear to be identical to those of mammalian testis (Ozon, 1967; Botte and Lupo di Prisco, 1965; Dale and Dorfmann, 1967; Rao *et al.*, 1969). In addition, it has been shown that in the testis of three anuran species, *Rana temporaria*, *Discoglossus picta*, and *Nectophrynoides occidentalis*, the major metabolite formed from testosterone is dihydrotestosterone (DHT) (Ozon *et al.*, 1964; Ozon and Stocker, 1974; Gavaud, 1975). DHT has not been identified in similar studies on urodele testes. However, it has been reported that the testes of *Triturus cristatus* convert isotopically labelled testosterone into 11-ketotestosterone and 11-hydroxytestosterone (Lupo di Prisco *et al.*, 1972). If this observation holds true for other urodele species and it can be demonstrated that urodeles but not anurans do indeed secrete these androgens, it might indicate, as is also suggested by testicular morphology, that newts and salamanders have closer affinities to the teleosts than do frogs and toads.

Testosterone has been identified in the plasma of *Necturus maculosus* (Rivarola *et al.*, 1968), *Pleurodeles waltlii* (Ozon, 1972a), *Rana esculenta* (D'Istria *et al.*, 1974; Rastogi *et al.*, 1976), *Rana pipiens* (Wada *et al.*, 1976), and *Rana catesbeiana* (Muller, 1978a). What is apparent from these limited

data is that the circulating levels of testosterone in urodeles (range 40-200 ng./ml.) are far greater than those found in the Anura (range 0.1-25 ng./ml.). We have found a similar difference in the range of values in *Bufo marinus* (0.4-9.5 ng./ml.) (Lance and Callard, unpublished data) and *Necturus maculosus* (20-150 ng./ml.) (Bolaffi and Callard, 1979).

Seasonal variations in plasma testosterone in *Rana esculenta* (D'Istria *et al.*, 1974; Rastogi *et al.*, 1976) did not appear to correlate with the annual reproductive cycle. Highest levels occurred during the winter months and were associated with the appearance of the secondary sexual characters, whereas the lowest levels occurred in the summer months when spermatogenesis was most active. The authors suggested that high levels of testosterone suppressed pituitary secretion of gonadotropin and, thereby, inhibited spermatogenesis, and that when plasma testosterone dropped at the onset of the breeding period gonadotropin secretion increased. In contrast, Gavaud (1975) showed that the ability of testicular tissue from the frog *Nectophrynoides* to metabolize testosterone to DHT varied seasonally and that the highest rate of conversion appeared to correlate with spermiation, mating, and spermatogenesis (which occurred during the wet season). The lowest levels of conversion were obtained in tissue from frogs during the dry season when primary spermatogonia were dividing.

The role of DHT, however, in male anurans is unknown. Both DHT and testosterone restore the weight of the "seminal vesicles" of castrate *Discoglossus* to those of intact controls, but the morphology of the Golgi apparatus in the secretory cells of DHT-treated frogs differed from those treated with testosterone (N'Diaya *et al.*, 1974). DHT has been identified in the plasma of *Rana pipiens* (Wada *et al.*, 1976), *Rana catesbeiana* (Muller, 1976; Callard *et al.*, 1978), and *Necturus maculosus* (Bolaffi and Callard, 1979). None of these authors has measured DHT throughout the spermatogenetic cycle. Muller (1976) found DHT levels considerably higher than those of testosterone in a small sample of frogs with spermatogenically active testes, but, in a sample taken at another time of the year, testosterone was higher than was DHT. Wada *et al.*, (1976) and Bolaffi and Callard (1977), however, found that testosterone was consistently higher than was DHT in the blood of *Rana pipiens* and *Necturus maculosus* in the limited number of samples analyzed. Implants of testosterone and DHT elicit sex behavior in *Xenopus* (Kelley and Pfaff, 1976) but are without effect in *Rana pipiens* (Palka and Gorbman, 1973).

Both structurally and biochemically, therefore, the urodeles and the anurans show considerable differences in testis structure and function. The former show the more teleostlike features in testis structure, high circulating levels of testosterone, and possibly testicular production of 11-ketotestosterone, and the latter show features closer to the amniotes in their relatively low plasma testosterone levels.

The ovaries of amphibians are hollow saclike structures containing a ridge of germinal epithelium and developing oocytes. The oocytes show the

typical features of egg-laying vertebrates in possessing a single layer of granulosa cells and a bilaminar theca. Apart from differences in the size and number of mature oocytes produced prior to ovulation, which vary from species to species, there are no structures peculiar to either urodeles or anurans. Histochemical (Botte and Cottino, 1964; Joly, 1965; Redshaw and Nicholls, 1971; Saidapur and Nadkarni, 1974) and ultrastructural studies (Kemp, 1956; Kessel and Panje, 1968; Anderson and Yatvin, 1970; Joly and Picheral, 1972; Thornton and Evenett, 1973) have identified the granulosa and possibly the theca cells of the developing oocyte, and the "corpus luteum" of the postovulatory follicle as the principle sites of steroid synthesis in the ovaries of both groups of amphibians. Generally, however, the theca does not show features of typical steroid secreting cells (see reviews by Lofts and Bern, 1972; Lofts, 1974; Guraya, 1976). Progesterone, estradiol, and estrone have been identified in ovarian tissue extracts (Gallien and Chalumeau-Le Foulgoc, 1960; Chieffi and Lupo, 1963) and by radioisotope precursor incorporation (Ozon and Breuer, 1964; Ozon *et al.*, 1964; Ozon, 1966; Callard and Leathem, 1966; Ozon and Xavier, 1968; Lupo di Prisco *et al.*, 1972; Redshaw and Nicholls, 1971). DHT was identified in incubates of *Discoglossus* ovaries (Ozon *et al.*, 1964), and the synthesis of DOC from either pregnenolone or progesterone by the ovaries of *Triturus alpestris* has been reported by Colombo and Belvedere (1975). However, the ripe ovaries of *Rana pipiens* metabolized ^3H -progesterone to mostly pregnanolone and other 5β -reduced C-21 steroids (Schatz and Morrill, 1975). Estradiol has been identified in the plasma of *Pleurodeles waltlii* and in *Rana temporaria* (Cedard and Ozon, 1962), *R. esculenta* (Polzonetti—Magni *et al.*, 1970; D'Istria *et al.*, 1974), and *Xenopus laevis* (Gallien and Chalmeau-Le Foulgoc, 1960). There is less information available on circulating steroid levels, in female amphibia than in males, but, from the few studies available, it appears that the levels of testosterone are similar to those of males. D'Istria *et al.* (1974) found that plasma testosterone in female *R. esculenta* was identical to that in the males and that levels in both showed seasonal fluctuation corresponding to the reproductive cycle. Levels of testosterone in female *Bufo marinus* ranged between 0.2 and 7.5 ng./ml., similar to those in the male (Lance and Callard, unpublished data) but seasonal variations have not been studied.

Although it is now clearly established that the gonads of amphibians secrete steroids and that the levels of these steroids circulating in the blood appear to correspond to the breeding activity, there is still no information available on what the precise relationships of the different steroids are to the spermatogenic cycle in the male and the ovulatory cycle in the female. It seems likely that the growing oocytes are secreting estradiol during the vitellogenic phase and that some hours prior to ovulation there is a surge of progesterone secretion (Redshaw, 1972). The evidence for the progesterone surge, however, is based on bioassay data only, and, inasmuch as DOC is able to stimulate ovulation *in vitro* (Schatz and Morrill, 1975), it is possible

that this preovulatory progesteronelike factor is of adrenal origin. There is evidence for a gonad-interrenal relationship in amphibians, and in most seasonally breeding species interrenal activity is heightened during the breeding period (Lofts and Bern, 1972; Van Kemenade and Van Dogen, 1968).

Although gonadal steroidogenesis clearly is regulated by hormones from the anterior pituitary, there is still some disagreement as to the nature of amphibian gonadotropins. Lofts (1961) showed that injections of mammalian FSH and LH into hypophysectomized frogs appeared to stimulate specific cell types within the testis. Spermiation was induced and Leydig cells hypertrophied following LH injections, but spermatogonia and Sertoli cells were unaffected, whereas injections of FSH stimulated spermatogonial cell division and Sertoli cells but did not affect spermiation or the Leydig cells. He proposed, therefore, that, as in mammals, testicular function in amphibians is controlled by two gonadotropins. Identical results were obtained in a urodele amphibian by Andrieux *et al.* (1973). Injections of mammalian LH into hypophysectomized male *Pleurodeles waltlii* stimulated 3β -HSD activity in the glandular tissue but did not reverse the involution of the germinal epithelium, whereas injections of FSH stimulated spermatogenesis but did not stimulate glandular (lobule boundary cell) tissue.

Results of other studies with mammalian gonadotropins, however, have generally been ambiguous as to the specificity of the amphibian response to either LH or FSH, and have not helped clarify the problem of the number of amphibian gonadotropins (see review by Barr, 1968). The frog ovulation test is relatively specific for LH (Licht and Papkoff, 1976), though FSH does have some activity in inducing meiosis in *Xenopus* oocytes (Thornton, 1971). In the frog spermiation test, however, highly purified mFSH and mLH are equipotent (Licht, 1973), and both mLH and mFSH have been used routinely to induce spawning in *Ambystoma mexicanum* (Ketterer and Forbes, 1972; Trottier and Armstrong, 1975; Humphrey, 1977). Kjaer and Jorgensen (1971) noted that both FSH and hCG could induce oocyte growth in hypophysectomized toads but that LH was without effect. The lack of an LH effect was later attributed to its shorter half-life in the circulation of the toad when compared to hCG (Roos and Jorgensen, 1974).

Evidence from pituitary cytology correlated with gonadal histology also favored the concept of two amphibian gonadotropins (see review by Holmes and Ball, 1974), though there is still some disagreement by the immunocytologists (Doerr-Schott, 1976b). Van Oordt and deKort (1969), however, argued that the cyclical changes in frog testis could be explained on the basis of a single gonadotropin with a seasonal temperature-dependent variation in tissue sensitivity. Thus, at low ambient temperatures the Leydig cells and not the germ cells respond to the single gonadotropin, and at higher temperature the spermatogonia, but not the Leydig cells respond. Furthermore, in a recent comprehensive review of the literature on amphibian pituitary, Van Oordt (1974) concluded "it seems likely the pars distalis of amphibians produces not more than one gonadotropin hormone." However,

there is now biochemical evidence that amphibians probably do possess two gonadotropins. Two fractions showing chemical similarities to mammalian LH and FSH recently have been isolated from pituitary glands of the bull frog, *Rana catesbeiana* (Licht and Papkoff, 1974a; Papkoff *et al.*, 1976a), and two fractions possessing different biological properties have been isolated from the urodele *Ambystoma tigrinum* pituitary (Licht *et al.*, 1975). The "LH" fraction from both the urodele and the anuran were able to induce ovulation in *Xenopus* or *Ambystoma* oocytes *in vitro* and were generally more potent than was mammalian LH. In contrast, the "FSH" fractions were relatively inactive in the ovulation assay but did show significant activity in their ability to maintain the testis weight of hypophysectomized *Anolis* lizards; an assay reported to be specific for mammalian FSH (Licht and Papkoff, 1974b). Two distinct biologically active fractions also have been demonstrated in extracts of *Rana esculenta* pituitary, both of which stimulated a moderate ^{32}P uptake in day-old chick testes, and induced vesicularization of immature *Xenopus* testis (Haider and Blüm, 1977). In the latter bioassay method of Simon and Reinboth (1966), one of the fractions had three times the potency of the other. Using the same purification and separation procedures, however, the authors could demonstrate only one biologically active fraction in the pituitary of the urodele *Triturus cristatus* using ^{32}P uptake by the testis of the same species as a bioassay. The purified *Rana* gonadotropins of Papkoff *et al.* (1976a) have since been tested in a number of bioassay systems, and, in each of these tests, the LH fraction has given responses that suggest that its role may be primarily steroidogenic. Progesterone secretion *in vitro* by frog, turtle, and crocodile ovarian tissue (Licht and Crews, 1976), testosterone and DHT secretion by frog (Muller, 1978b) and rabbit (Licht *et al.*, 1976a) testicular tissue *in vitro* have been shown to be stimulated by the frog LH, but generally not by the FSH. Muller (1978a, 1978b) has since shown that the *Rana* LH appears to be highly specific for steroid secretion by *Rana* testis both *in vivo* and *in vitro*. The *Rana* FSH showed no activity in these tests (Fig. 3) but did stimulate spermatogenesis in hypophysectomized frogs. The spermatogenetic response, however, appeared to be equally stimulated by both hormones, though insufficient data are available to draw any definite conclusions regarding the role of FSH. Unfortunately, none of these purified amphibian hormones has been tested on its ability to stimulate vitellogenin uptake from the plasma by the developing oocyte. Human chorionic gonadotropin has been shown to stimulate vitellogenin uptake *in vivo* by the oocytes of *Bufo bufo* (Emmersen and Kjaer, 1974), but no other gonadotropin appears to have been tested. It cannot be stated at present, therefore, if this primitive gonadotropic function is an FSH or an LH mediated response. The frog LH appears to favor the production of DHT over testosterone by the testis of *Rana* both *in vivo* and *in vitro*. Ozon and Stocker (1974) noted that prior treatment of the frog *Discoglossus* with hCG greatly enhanced the conversion of ^{14}C -testosterone to DHT by testis tissue *in vitro*. It would seem from

FIG. 3 (Left).—The effect of *Rana* LH and *Rana* FSH on the secretion of testosterone (T) and dihydrotestosterone (DHT) *in vivo* and *in vitro* by the testis of *Rana catesbeiana*. Solid and open squares represent DHT; solid and open circles, testosterone. The upper figure represents the changes in circulating androgens after a single injection of *Rana* gonadotropin. Blood samples were taken by heart puncture. The lower figure represents the changes in androgens in the incubation medium at various time intervals after the addition of *Rana* gonadotropin. (Redrawn from Muller, 1977a, 1977b).

FIG. 4 (Right).—Effect of a single injection of highly purified ovine LH (LER-1374a) or ovine FSH (LER-1881) on plasma sex steroids in male *Necturus maculosus*. Serial blood samples were taken by syringe immediately before and at two and 24 hours post injection. Duplicate aliquots of plasma were extracted with ether and assayed for total androgens and total estrogens by radioimmunoassay (Bolaffi and Callard, unpublished data).

these experiments that frog LH is similar to mammalian LH in its ability to stimulate steroidogenesis and that frog testis is similar to mammalian testis in its specificity of steroidogenic response to LH. Bolaffi and Callard (unpublished) found that male *Necturus* gave a similar response to highly purified mammalian LH *in vivo*. There was a marked increase in circulating testosterone two hours after a single 5-µg injection of purified ovine LH (LER-1374A) and a return to baseline levels after about 24 hours. Unlike the anurans, however, the testosterone levels were usually higher than the DHT

levels. Highly purified ovine FSH (LER-1881) did not increase testosterone levels over those of saline injected controls either at two or 24 hours. However, when estradiol was assayed it was seen that a massive increase occurred 24 hours after a single 2- μ g injection of FSH, whereas the LH injected animals were similar to controls (see Fig. 4). Inasmuch as estradiol was not measured in the anurans injected with FSH, we have no way of knowing at present whether this response is specific to urodeles. Although these data are preliminary, it might indicate that amphibian interstitial (or lobule boundary) cells respond to LH as do mammalian Leydig cells by secreting testosterone and DHT and that the urodele testis responds to FSH by increasing the aromatization of testosterone, as do the Sertoli cells of immature rats *in vitro* (Fritz *et al.*, 1976).

The similarity of the amphibian reproductive system to that of the mammals is further emphasized by the presence of immunoreactive of LH-RH in frog brain and hypothalamus, and by studies in which frogs (*in vivo*) have been shown to respond to synthetic LH-RH. Deery (1974) tested hypothalamic extracts from a number of nonmammalian species in a radioimmunoassay system that is specific for LH-RH. The only nonmammalian hypothalamic tissue extract to show significant cross reaction and give parallel dose response curves was that of the amphibian *Xenopus laevis*. Alpert *et al.* (1976) have since demonstrated similar cross reactivity to an LH-RH antisera with hypothalamic tissue from *Rana pipiens* and also have identified by immunohistochemistry putative LH-RH containing neurons in the frog median eminence. Goos *et al.* (1976) and Doerr-Schott and Dubois (1976) have used similar techniques to map LH-RH containing tracts in the brain of *Rana esculenta* and *Xenopus laevis*. Frog hypothalamic extracts or synthetic LH-RH have also been shown to stimulate the release of an LH-like material from the *in vitro* pituitaries of *Rana pipiens* (Thornton and Geschwind, 1974), and a number of other workers have elicited biological responses attributable to endogeneous secretion of gonadotropin by injections of synthetic LH-RH in both anurans and urodeles (Licht, 1974b; Thornton and Geschwind, 1974; Mazzi *et al.*, 1974; Vellano *et al.*, 1974).

Class REPTILIA

Although the origin of the reptiles is obscure (Carroll, 1970), the reptilian ancestry of birds and mammals is no longer doubted. All of the extant reptiles, birds, and mammals must have evolved from a single group of stem reptiles, or cotylosaurs (see Fig. 5) as it is highly unlikely that the amniote egg could have evolved in more than one group. This evolution of the amniote egg, "the most marvelous 'invention' in vertebrate history" (Romer, 1967) freed the egg-laying vertebrates from the rigid requirements of reproducing in an aqueous environment, and thus enabled them to exploit the terrestrial habitat. The resulting explosive radiation of the amniotes is testament to the success of this "invention." It would appear that this major evolutionary step was accomplished by the developing embryo (Szarski, 1968)

FIG. 5.—Phylogeny of the amniote vertebrates. The figures above the geologic eras represent the approximate number of millions of years since the beginning of each. Broken lines indicate uncertain fossil record. Based on Carroll (1969) and Bellairs (1969).

and did not involve any major structural modifications of the adult reproductive system. The gonads and reproductive systems of reptiles, therefore, are not markedly different from those of the anamniote vertebrates.

Inasmuch as the amniotes are monophyletic in origin, and the extant groups of reptiles, with the exception of the Chelononia, can be traced with a reasonable degree of accuracy to the extinct groups from which they evolved (Carroll, 1969), we should expect to find features in modern reptiles that reflect the endocrinology of the groups that gave rise to the birds and mammals. Thus, the relationship of crocodiles to birds is generally accepted (Bock, 1969), and the biochemical similarities of the yolk proteins of the two groups tend to bear this out (Clark and Zyl, 1976). We should expect to find that their endocrine systems are also closely related, and indeed, it appears that alligator LH is reasonably close to turkey and chicken LH in amino acid composition (Table 3). Reptilia, therefore, are the key group from which we would expect to gain information on the directions of early amniote evolution.

The morphology of the testis of reptiles is essentially similar to that of birds and mammals, with two unusual exceptions. The testes of the fossorial blind snakes *Leptotyphlops* and *Typhlops* are often broken up into several separate lobes, similar in gross appearance to the testes of some urodeles, but histologically each lobe contains typical seminiferous tubules and inter-

stitial cells (Fox, 1965; Werner and Drook, 1967), and the testes of lizards of the genus *Cnemidophorus* are characterized by the presence of a compact layer of Leydig cells (or circumtesticular cells) between the tunica albuginea and the seminiferous tubules (DeWolfe and Telford, 1966; Goldberg and Lowe, 1966). The ease with which this layer of Leydig cells can be separated from the seminiferous tubules and the potential of these lizards as useful research animals in the study of Leydig cell function has been commented upon by Neaves (1976).

Although the reptiles are the least studied of the nonmammalian vertebrates, it is reasonably well established that the testis secretes androgens. As in mammals and birds, the interstitial cells give a positive reaction for 3β -HSD (Arvy, 1962; Dufaure and Turchini, 1966; Callard, 1967; Botte and Delrio, 1967; Morat, 1969) and have the ultrastructural characteristics of steroid secreting cells (Dufaure, 1970) as do the circumtesticular Leydig cells of *Cnemidophorus* lizards (Currie and Taylor, 1970; Neaves, 1976; Tsui, 1976). Sertoli cells generally give a negative or weak reaction for 3β -HSD, but Lofts and Tsui (1977) have shown that the presence of this enzyme, as demonstrated by histochemical techniques, varies with the season. Testosterone has been identified in extracts of lizard testes (Lupo di Prisco *et al.*, 1968) and in the testis of *Natrix spiedon* when incubated with a radioactive pregnenolone precursor (Callard, 1967).

Plasma levels of 17-hydroxysteroids have been measured by a protein-binding assay in the lizard *Tiliqua* (Bourne and Seamark, 1975) and plasma testosterone by radioimmunoassay in the turtle *Chrysemys* (Callard *et al.*, 1976a). Whereas the peak in plasma 17-hydroxysteroids coincides with the peak of spermatogenesis in the lizard, the lowest testosterone levels are associated with the peak of spermatogenesis in the turtle. The explanation for these apparently contradictory results is in the different type of spermatogenetic cycle the two seasonally breeding species undergo.

In most species of reptiles, which mate only once a year, there is a delay of several months between the termination of one spermatogenetic cycle and the beginning of the succeeding one. At one time of the year, therefore, the seminiferous tubules contain only Sertoli cells and quiescent spermatogonia. A unique feature of many of these reptiles is a temporal separation between the functional activity of the interstitial activity and the spermatogenetic tissue (see reviews by Lofts, 1972, 1977). Thus, in *Trionyx sinensis* (Lofts and Tsui, 1977), *Chrysemys picta* (Moll, 1973; Ernst, 1971; Callard *et al.*, 1976a), and *Mauremys (Clemmys) caspica* (Lofts and Boswell, 1961), spermatogenesis takes place during the summer months and spermiogenesis in the autumn. Shortly before the animals enter hibernation, the spermatozoa are cleared from the seminiferous tubules and stored in the epididymis until the following spring when copulation occurs. During the period when mating takes place, immediately upon emergence from hibernation, the seminiferous tubules are empty of spermatazoa and spermatids, but the Leydig cells are hypertrophied and give a positive reaction for 3β -HSD (Lofts and Tsui,

1977). It is at this time that the highest plasma testosterone levels are found in *Chrysemys* (Callard *et al.*, 1976). Shortly after the mating period, the Leydig cells regress, 3β -HSD activity disappears, and plasma testosterone levels drop precipitously. About one month later, spermatogonial cell division begins, and, in *Trionyx*, 3β -HSD activity shows up in the Sertoli cells but not in the regressed Leydig cells (Lofts and Tsui, 1977). Plasma testosterone remains low to undetectable in *Chrysemys* at this time. Spermatogenetic activity peaks in late summer when testicular tubule diameter and testicular weight are maximal, but there is no change in the Leydig cells and plasma testosterone remains low. By late summer and early autumn, Sertoli cells no longer give a positive 3β -HSD response, whereas a low activity is detectable in the Leydig cells, which are beginning to recrudescence (Lofts and Tsui, 1977). This recrudescence of the Leydig cells is accompanied by an increased level of plasma testosterone and a resumption of courtship behavior without mating (Moll and Legler, 1971).

Thus, mating activity occurs when Leydig cells are most active and peripheral androgen is at a peak in early spring, whereas the period of active spermatogenesis is associated with atrophic Leydig cells and low to undetectable plasma androgen. In the lizard *Tiliqua*, however, Leydig cell hypertrophy, the plasma androgen peak, mating, and spermatogenesis are coincident (Bourne and Seamark, 1975).

If spermatogenesis is an androgen dependent process (Steinberger, 1976), this would suggest that the Sertoli cells are the source of intertubular androgens during spermatogenesis in the turtle. Lofts (1972) summarized the data on Sertoli cell function in reptiles and concluded that the evidence is highly suggestive that Sertoli cells are steroidogenic. Additional evidence for a Sertoli cell androgen secretion comes from studies in which mammalian gonadotropins have been injected into male *Chrysemys picta* and serial blood samples analyzed for testosterone (Lance *et al.*, 1977). The response, as assessed by increased plasma testosterone titers, appeared to be highly specific for mammalian FSH; the mammalian LH's tested being without effect. Furthermore, this response to FSH was minimal in turtles injected in early May when endogenous testosterone levels were high, but was maximal in midsummer when testosterone levels were low, Leydig cells atrophic, and spermatogenesis at a peak. Similar seasonal differences in turtle testicular tissue sensitivity to FSH was apparent when tested *in vitro* (Callard and Ryan, 1977). Thus, there was a far greater response to FSH both in the levels of plasma testosterone attained and in the tissue sensitivity to the hormone when Sertoli cells were most active and Leydig cells atrophic. A lack of response to injections of gonadotropins when Leydig cells are active and peripheral testosterone levels high has also been observed in a seasonally breeding mammal (Neal and Murphy, 1977). These data suggest that it is the Sertoli cells of the turtle that are responding to mammalian FSH and that the Leydig cells are unresponsive to mammalian LH or respond only to a turtle LH, or, as in the case of the seasonally breeding mammal, are already maximally stimulated and cannot respond further.

FIG. 6.—Generalized cycle of seasonally breeding male turtle. Adapted from Lofts and Boswell (1961), Lofts and Tsui (1977), Moll (1973), Callard *et al.* (1976), and Callard and Lance (unpublished data).

One could postulate, therefore, a seasonal cycle in the turtle in which an LH-like molecule activates the Leydig cells in early spring and late autumn, thereby increasing plasma testosterone levels, and that the elevated testosterone levels trigger mating behavior but suppress FSH secretion by acting in a negative feedback manner on the hypothalamus and/or pituitary. As the Leydig cells regress after mating, due to falling LH levels, and plasma testosterone levels decline, pituitary secretion of FSH increases, thereby initiating spermatogenesis and Sertoli cell androgen secretion (see Fig. 6).

The specificity of turtle testis to mammalian FSH has also been demonstrated in binding studies using highly purified, radioiodinated human FSH (Licht and Midgley, 1976a, 1976b). Isolated membrane fragments from *Chrysemys* testes specifically bind ^{125}I -hFSH, which can be displaced by excess "cold" hFSH, or *Chrysemys* pituitary homogenate, but not by ovine LH at 10,000-fold excess. These studies nicely complement the *in vivo* (Lance *et al.*, 1977) and *in vitro* (Callard and Ryan, 1977) studies in which FSH but not LH stimulated testosterone secretion (see Fig. 7). However, the cell type in the turtle that binds FSH has still not been identified, though Licht and Midgley (1977) have shown by autoradiography that ^{125}I -hFSH appears to bind to both the interstitium and tubules of snake testis. Inasmuch as the squamates respond almost equally as well to mammalian LH and FSH (see Fig. 8), and both LH and FSH are able to displace bound FSH from snake testis or ovary membrane preparations (Licht and Midgley, 1976a), the reason for the specificity of the turtle testis to mammalian FSH

FIG. 7 (Left).—Response of turtle (*Chrysemys picta*) testis to mammalian gonadotropins. Upper panel shows the response of male turtles to a single intra-arterial injection of ovine LH or FSH. Serial blood samples were taken as indicated and analyzed by radioimmunoassay for testosterone. (Redrawn from Lance and Callard, 1977). The lower panel shows the inhibitory activity of ovine FSH and LH on the binding of ¹²⁵I-human FSH to testicular homogenates from turtle testis tissue (redrawn from Licht and Midgley, 1976a).

FIG. 8 (Right).—Response of two closely related species of snakes to mammalian gonadotropins. The upper panel shows the response of female *Natrix* to a single intravenous injection of ovine LH or FSH. Blood samples were taken from the tail at two and 24 hours after injection and analyzed for total androgens by radioimmunoassay (Lance and Callard, unpublished data). The lower panel shows the inhibitory activity of ovine LH and FSH on the binding of ¹²⁵I-human FSH to ovarian tissue homogenates from the garter snake. (Redrawn from Licht and Midgley, 1976a).

is still unclear. These preliminary results from binding studies, however, should be viewed with caution inasmuch as some hormones that elicited (*in vivo*) a biological response in *Anolis* were unable to displace radioiodinated FSH from membranes prepared from snake testis or ovary (Licht and Midgley, 1976b).

The ovarian structure of crocodiles and turtles is basically similar to that of birds in that there is a connective tissue stroma containing large yolking follicles in various size classes. Histologically, the ovarian follicle of these two groups is little different from that of the hen. There is a typical single layer of granulosa cells separated from the theca by a basement membrane. From the photographs published by Gabe and Saint Girons (1964), it would

appear that the Rhynchocephalian *Sphenodon punctatus* also has this common ovarian structure, which suggests that this lizardlike creature must have separated from the stem that gave rise to the squamates long before the lizards and snakes evolved their unique follicular structure (see Fig. 5).

The granulosa layer of squamates contains cells of several different sizes, including a unique flask or pyriform cell that forms direct contact with the cytoplasm of the developing oocyte by an intercellular bridge (Neaves, 1971). This phenomenon appears to be unique to the squamates and has not been reported in any other vertebrate, though it is common in the insects (Highnam and Hill, 1969). The pyriform cell (approximately 50 nanometers in diameter) is prominent in the granulosa during the long, slow previtellogenic growth phase. At the onset of vitellogenesis, which is extremely rapid in most squamates, the pyriform cells lose their connection with the oocyte and either disintegrate or are converted into small, round, typical granulosa cells (see reviews by Loyez, 1906; Neaves, 1971; Hubert, 1973, 1976; Taddei, 1972). The function of these cells is still unclear, though it has been suggested that they contribute RNA to the developing oocyte (Taddei, 1972). Despite these unusual morphological characteristics, the ovaries of snakes and lizards seem to be basically similar to turtles in their ability to secrete steroids.

The granulosa cells of all reptiles are probably the major site of steroid synthesis during vitellogenesis. Enzyme histochemical studies have shown that the intensity of the reaction for 3β -HSD increases in the granulosa cells as the follicles enlarge (Morat, 1969; Jones *et al.*, 1974), suggesting that this may be the site of estrogen secretion. Guraya (1976), however, believes that an "interstitial gland" derived from atretic follicles is the source of ovarian steroids. In most squamates, especially ovoviviparous species, the postovulatory follicle, or corpus luteum, also gives a positive reaction (Yaron, 1972) and is probably the source of elevated progesterone levels recorded in pregnant snakes (Chan *et al.*, 1973; Highfill and Mead, 1975a; Callard and Lance, 1977a).

In vitro studies using radioactive precursors have established that the pathways of steroid biosynthesis in the ovaries of snakes, lizards, and turtles are basically similar to those in mammalian ovaries (Callard and Leatham, 1964, 1965; Callard *et al.*, 1976b; Chan and Callard, 1974; Klicka and Mahmoud, 1972, 1973; Lance and Lofts, 1977). However, in one species of snake (*Storeria deckayi*) and a lizard (*Xantusia vigilis*), it was found that DOC was a major metabolite of pregnenolone- ^{14}C in ovarian tissue incubates. In the snake, the activity of 21-hydroxylase appeared to be confined exclusively to the luteal tissue (Colombo and Yaron, 1976; Colombo *et al.*, 1974).

Whereas progesterone has been identified in the plasma of a number of squamates and has been shown to correlate with corpus luteum activity (Callard *et al.*, 1972a, 1972b; Highfill and Mead, 1975a; Veith, 1974; Callard and Lance, 1977), the changes in plasma steroids prior to ovulation have been studied in only one species of turtle, *Chrysemys picta*. This turtle ovu-

FIG. 9.—Generalized annual reproductive cycle of female snake, *Natrix*, and a female turtle, *Chrysemys picta*. Open bars represent ovarian weight. Adapted from Chan *et al.* (1973), Highfill and Mead (1976a), and Callard and Lance (1977).

lates only once a year in the northern part of its range, producing a clutch of between 4 and 8 eggs in early June. From observations made over a four-year period, we have obtained a reasonable approximation of the hormonal changes occurring during the annual ovarian cycle (see Fig. 9). However, the vitellogenic cycles of squamates and chelonians differ considerably, though the hormones controlling the process are probably similar. *Chrysemys picta* ovulates in late May or early June (in Massachusetts). From August to September, most of the larger follicles in the ovary are atretic, but prior to hibernation in the fall, there is a short period of vitellogenesis and ovarian growth—the largest follicles in the ovary reaching a size slightly less than that of the mature oocyte. On emergence from hibernation in the spring, there is a second short burst of vitellogenesis culminating in ovulation. Thus, there are two periods during the year in which vitellogenesis is occurring and each of these is associated with an increase in circulating estradiol levels (Fig. 9). Prior to, and around the point of ovulation, there is

a surge in progesterone secretion, which rapidly declines as corpus luteum function declines. This progesterone surge probably lasts less than a week as do the histologically identifiable corpora lutea. Plasma testosterone changes appear to mirror those of the estradiol, with a high level in the fall when the first vitellogenic phase of ovarian growth is occurring and a second but lesser peak during the spring.

The ovarian changes in most squamates are more dramatic. There is only a single very short period of yolk deposition just prior to ovulation in the spring, rarely lasting as long as a month. For the rest of the year, the ovarian follicles are small and translucent, the so-called hydration stage follicles (Betz, 1963). Although we have less complete data on the hormonal levels in a snake, changes in plasma vitellogenin levels, oviduct weight, and ovarian enzyme, activity during the follicular growth phase supports plasma steroid data. Thus, estrogens are the only steroids able to stimulate vitellogenesis in oviparous vertebrates (Chester Jones *et al.*, 1972), and the appearance of plasma vitellogenin in the blood can be detected very easily by measuring total plasma calcium (Urist and Schjeide, 1961; Lance, 1976). There is a single narrow peak of vitellogenin production in the snake, corresponding to the short period of follicular growth (Lance, 1976), an increasing intensity of 3β -HSD in the granulosa cells, and an increase in oviduct mass (Lance and Lofts, unpublished), all of which indicates an increase in ovarian estrogen secretion. Plasma estradiol levels in *Natrix* suggest a similar pattern. Thus, snakes with large-yolked follicles had the highest levels of estradiol; those snakes with follicles in the hydration stage, the lowest (Table 4).

Indirect evidence for a preovulatory surge of gonadotropin in squamates comes from an observation of a sharp drop in plasma cholesterol just prior to ovulation in the cobra (Lance, 1975). Follett *et al.* (1968) showed that an injection of mammalian gonadotropin caused a drop in plasma cholesterol in *Xenopus*, presumably the result of gonadotropin stimulation of oocyte uptake of lipid and vitellogenin. This would suggest that in snakes there is a preovulatory surge of gonadotropin stimulating the uptake of cholesterol by the oocyte and probably initiating ovulation.

Unlike the turtles, however, progesterone levels in squamates continue to rise after ovulation and remain high as long as the corpus luteum continues to function. This progesterone secretion by the reptilian corpus luteum does not appear to be necessary for the maintenance of pregnancy in the few viviparous species studied, though continued secretion is dependent on gonadotropins (see reviews by Callard *et al.*, 1972a, 1972b; Yaron, 1972; Highfill and Mead, 1975b). Progesterone secretion during pregnancy probably acts to inhibit further follicular growth and ovulation (Callard *et al.*, 1972b). Prolactin from mammalian species has also been shown to have antigonadal action in reptiles (Callard and Zeigler, 1970), but the role of reptilian prolactin in reproduction is unknown.

The theory that reptiles have only a single gonadotropin (Burzawa-Gerard and Fontaine, 1972; Licht, 1972, 1974a) is now no longer tenable, inasmuch

TABLE 4.—*Plasma estrogen levels in a snake, Natrix fasciata.*

Specimen number	Body weight (grams)	Ovary weight (grams)	Diameter of largest follicle (millimeters)	Plasma estrogens pg/ml.
1	473	23.4	18	206
2	607	5.5	9	204
3	762	16.8	28	65*
				20**
4	521	29.4	18	45
5	1451	156.9	28	540
6	542	23.0	18	110
7	518	74.9	20	150
8	540	3.9	5	10
9	478	3.9	7	10
10	647	63.2	20	430
11	432	8.8	12	94
12	439	42.3	25	345

*Preovulatory. Ovulation occurred the day after blood sample was taken. **Postovulatory.

as two separate molecules, similar in chemical characteristics to mammalian FSH and LH, have been identified in two species of turtle and the alligator (Table 3), though it is still argued that reptiles possess only a single gonadotropin secreting cell type in the pituitary (Doerr-Schott, 1976b). The LH molecule from the snapping turtle, *Chelydra serpentina*, can be dissociated into subunits devoid of biological activity and the subunits recombined to restore biological activity. Furthermore, the subunits can be combined with the alpha or beta subunits of ovine LH and the resulting hybrid molecules shown to possess biological activity in the *Xenopus* ovulation test (Papkoff *et al.*, 1976b). The nature of the squamate gonadotropins is still unknown, though a fraction obtained from rattlesnake (*Crotalus*) pituitary showed significant cross reaction with a chicken LH antisera (Licht *et al.*, 1974). Most vertebrate pituitary homogenates, including those of crocodilians and chelonians, show biological activity when tested in the amphibian "LH" ovulation bioassay. The pituitaries of all squamates tested to date on this bioassay are virtually devoid of activity (Licht, 1974c). However, these same squamate pituitary preparations do show biological activity in the *Anolis* bioassay (testis weight maintenance in hypophysectomized lizards), which is relatively specific for mammalian FSH (Licht and Papkoff, 1974b). It was suggested, therefore, that squamates possess only a single gonadotropin similar in biological activity to mammalian FSH (Licht, 1972, 1974b). However, the FSH-like nature of snake gonadotropin is based on a bioassay in which a squamate (*Anolis*) was the test animal, and the lack of squamate LH is based on an assay in which an amphibian was the test animal (Licht, 1974c).

The dispersed cell system of Callard and Ryan (1977) uses turtle testis tissue as the bioassay material. In all experiments, to date, it has been shown to have a narrow specificity for mammalian FSH. LH from three mammalian species is biologically inactive in this system. As can be seen

FIG. 10.—Response of enzyme dispersed turtle (*Chrysemys picta*) testis to crude pituitary homogenates and purified pituitary hormones from various species (Callard and Ryan, 1977; Rosset and Callard, unpublished).

(Fig. 10), this system can detect gonadotropic activity in microgram amounts of homogenized pituitary extracts from a wide range of species. Snake pituitary extract is consistently negative in this "FSH" bioassay. We cannot, therefore, reach any conclusion as to the FSH or LH nature of snake gonadotropins; only that they have an extremely narrow species specificity.

Although it is now clear that the Chelonia and the Crocodilia, at least, possess two gonadotropins, the biological roles of FSH and LH in the Reptilia are still far from clear. Both turtle LH and FSH stimulated progesterone production *in vitro* by ovarian follicular fragments of *Chelydra*, *Kinosternon*, *Sternotherus*, and *Gopherus* (Crews and Licht, 1975a, 1975b; Licht and Crews, 1976). The results were extremely variable, though it did appear that LH from the sea turtle was more potent than FSH from the same species in stimulating progesterone secretion by snapping turtle and caiman ovaries (Licht and Crews, 1976).

However, these reptilian FSH preparations were of unknown purity and could have contained significant amounts of LH. As in the studies on male turtles *in vivo* and *in vitro* (Callard and Ryan, 1977; Lance *et al.*, 1977) and dispersed cells from turtle ovaries (Lance and Callard, 1977), mammalian LH was ineffective in stimulating steroid secretion, whereas mammalian FSH was extremely potent. However, one of the major drawbacks of these studies on reptilian gonadotropins is that a single end point, progesterone

secretion, was measured as a biological response in a tissue composed of many different cell types. What is also apparent in studies of this nature is that the profile of steroids produced by the chelonian ovary and the tissue sensitivity of the ovary to gonadotropins varies throughout the annual reproductive cycle (Lance and Callard, 1977) and should be considered when assessing the biological activity of pituitary hormones.

In summary, the evidence suggests that the control of steroidogenesis and spermatogenesis in male reptiles may be regulated by two separate gonadotropins, an LH-like molecule controlling Leydig cell testosterone secretion and an FSH-like molecule controlling Sertoli cell function and spermatogenesis. High testosterone levels are not necessarily associated with spermatogenesis, but usually are coincident with mating activity. There is indirect evidence that the Sertoli cells secrete testosterone.

For the female reptiles, the picture is less clear. Estrogen secretion by the granulosa and/or theca of the vitellogenic follicle and progesterone secretion by the periovulatory follicle and corpus luteum are probably of universal occurrence. It also is likely that ovulation is preceded by a surge of gonadotropin secretion in all reptiles. What is not clear, however, is how the reptilian LH and FSH regulate the various stages of the cycle. The roles of prolactin and adrenal corticosteroids in regulating the ovulatory cycle and the possibility of an ovarian secretion of DOC also need further study.

The gonads of the Chelonia show an unusual specificity for mammalian FSH whereas those of the Squamata respond to both mammalian FSH and LH. In contrast, the gonadotropins of the Chelonia are biologically active in a wide variety of species, whereas those of the Squamata appear to be active only in other squamates. At present it is impossible to explain these observations in terms of an evolutionary sequence of gonadotropins and their receptors as proposed by Wallis (1975) or Haider and Blüm (1977). The data do suggest, however, that in the squamates there has been an evolutionary divergence in the structure of the pituitary hormones without the concomitant evolutionary change in the gonadal receptor proteins.

Class AVES

As far as mammalian reproduction is concerned, the birds are of little evolutionary significance inasmuch as the thecodont group from which they evolved separated from the main reptilian stem long before the therapsids appeared in the fossil record (see Fig. 5). The birds, however, have many obvious affinities with the reptiles and are, therefore, of considerable interest to comparative endocrinologists. Their economic importance and aesthetic appeal to scientists and nonscientists alike has resulted in the accumulation of vast libraries of information on many aspects of avian biology. There is, however, very little information available on the hormonal control of gonadal steroidogenesis.

Perhaps the most intensively studied of all nonmammalian vertebrates is the domestic chicken *Gallus domesticus*; hence its reproductive cycle is now

fairly well understood. Seasonally breeding birds have also been the subject of a great deal of research, especially with respect to the role of photoperiod in the control of their reproductive cycles (see reviews by Lofts, 1975; Murton, 1975; Follett and Davies, 1975; Hinde and Steel, 1975), but little is known of the hormones involved.

The testes of seasonally breeding birds undergo tremendous variations in size, often showing up to a 500 per cent increase in weight at the approach of the breeding season (Lofts and Murton, 1973). This variation in testicular weight is an extremely sensitive indicator of endogenous gonadotropin secretion; and the ease with which testis weight increases or decreases can be induced by exposing birds to different daylight regimes has been used extensively in research on avian reproductive cycles (Lofts and Murton, 1968; Farner and Lewis, 1971).

Histologically, the testes of birds are similar to those of mammals, with typical seminiferous tubules containing Sertoli cells and germ cells with Leydig cells in the interstices; but, unlike mammals, the seminiferous tubules exhibit extensive anastomoses (Lofts and Murton, 1973). Leydig cells show all the histochemical and ultrastructural features of steroid secreting cells (Woods and Domm, 1966; Connell, 1972; Scheib and Haffen, 1969; Humphreys, 1975; Lam and Farner, 1976) and histochemical evidence suggests that the Sertoli cells may be involved in steroid synthesis at the height of spermatogenetic activity (Lofts and Murton, 1973).

There is evidence for testosterone secretion by the testes from *in vitro* studies (Fevold and Eik-Nes, 1963; Nakamura and Tanabe, 1972; Chan and Lofts, 1974; Kerlan *et al.*, 1974), and circulating blood plasma levels of testosterone have been measured in a number of species (Garnier, 1971; Kerland and Jaffe, 1974; Wingfield and Farner, 1975; Lam and Farner, 1976). Concentrations of testosterone in the plasma of birds, although showing marked variation during the annual breeding cycle, are in the same range as those reported for mammals (Gustafson and Shemesh, 1976). Unlike turtles, however, elevated testosterone levels generally coincide with the peak of spermatogenesis and mating behavior. DHT has been tentatively identified in the peripheral blood of the white-crowned sparrow, *Zonotrichia leucophrys*; in general the levels are lower than those of testosterone (Wingfield and Farner, 1975; Lam and Farner, 1976). In the dove, *Streptopelia*, however, DHT plasma levels exceed those of testosterone during certain phases of the courtship cycle (Feder *et al.*, 1977).

The single left ovary of the chicken is basically similar to the paired ovaries of the turtle in gross and microscopic detail but differs from the turtle in that oogenesis is completed prior to hatching and there are fewer developing follicles. Thecal and granulosa cells of the developing follicles present all the histochemical and ultrastructural details of steroid secreting cells (Boucek and Savard, 1970; Saylor *et al.*, 1970; Dahl, 1971a, 1971b; Lofts and Murton, 1973; Guraya, 1976) and are presumably the source of circulating estrogen and progesterone. It has been claimed, however, as in mammals,

FIG. 11.—Steroid hormone levels in the peripheral plasma of the domestic chicken during an ovulatory cycle. Data taken from Haynes *et al.* (1973); Shahabi *et al.* (1975a); Shodono *et al.* (1975); Graber and Nalbandov (1976); Etches and Cunningham (1976b, 1977) and Etches (personal communication).

there is steroidogenic interstitial tissue in the bird ovary that secretes androgens (Lofts and Murton, 1973). Although there is histochemical evidence for steroidogenic cells in the stroma of the chicken ovary (Boucek and Savard, 1970), ultrastructural studies do not support a separate interstitial tissue. Dahl (1971b) suggested, rather, that these cells are actually thecal cells and prefers the term thecal gland.

There is little doubt that the growing follicles secrete estradiol during the vitellogenic phase (Kumagai and Homma, 1974; Shahabi *et al.*, 1975a; Shodono *et al.*, 1975; Graber and Nalbandov, 1976) and progesterone shortly before ovulation (Furr *et al.*, 1973a; Haynes *et al.*, 1973; Shodono *et al.*, 1975; Etches and Cunningham, 1976b). Moreover, changes in plasma testosterone appear to mirror those of estradiol during the ovulatory cycle of the chicken (Etches and Cunningham, 1977). Thus, there is a similarity between the profile of circulating steroids during the ovulatory cycle of the chicken and that of the turtle *Chrysemys picta* (see Figs. 9 and 11). In both, there are increased levels of estrogen during yolk deposition preceded by increased levels of testosterone, and a surge of progesterone shortly before ovulation. It would appear, therefore, that in terms of the reproductive cycle, the Chelonians have closer affinities to the birds than to the squamates. Although there is no information on the hormone levels in the Crocodilia, a similar avian

pattern should be expected. The similarities of the Chelonia and Crocodilia to the birds is further emphasized in their failure to evolve a viviparous mode of reproduction (Packard *et al.*, 1977).

Although an avian LH and an avian FSH have been isolated from chicken and turkey pituitaries (Scanes and Follett, 1972; Godden and Scanes, 1975; Farmer *et al.*, 1975) and radioimmunoassays for the avian LH's have been developed (Follett *et al.*, 1972; Wentworth *et al.*, 1976), the relationships of the circulating gonadotropins to the gonadal steroids is not entirely clear. In the male bird, the situation appears to be essentially like that of mammals. Injections of purified avian LH and FSH fractions were shown to stimulate the Leydig cells and seminiferous tubules respectively in immature quail (Brown *et al.*, 1975) and mammalian gonadotropins have been shown to have similar sites of action in immature or hypophysectomized birds (see review by Lofts and Murton, 1973). Similarly, avian LH stimulates Leydig cell testosterone secretion *in vitro* (Maung, 1976) and is more potent than mammalian LH or avian FSH.

Using a heterologous FSH radioimmunoassay, Follett (1976) measured circulating levels of endogenous FSH in a photostimulated quail, and his results agreed with the proposed role of FSH in initiating spermatogenesis. Highest plasma levels were found just prior to the period of maximum testicular growth and prior to the appearance of LH and testosterone. However, the lack of sensitivity of this assay has precluded its use in studies on the ovulatory cycle.

The role of the pituitary gonadotropins in the ovulatory cycle of the bird are not well understood. A peak of LH can be detected in the plasma of laying chickens four to six hours before ovulation (Wilson and Sharp, 1973; Furr *et al.*, 1973a; Lague *et al.*, 1975; Shodono *et al.*, 1975; Etches and Cunningham, 1976a; Etches and Cunningham, 1977) and a peak of plasma progesterone generally coincides with that of the LH (Mashaly *et al.*, 1976), though it is not certain if the progesterone rises before the LH and triggers its release or if the progesterone acts on the ovary to cause rupture of the ripe follicle. Injections of progesterone, testosterone or DOC can elicit an LH peak similar to that in the preovulatory bird (Wilson and Sharp, 1975, 1976a) and can induce ovulation and oviposition (Etches and Cunningham, 1976a), but prior exposure of the hypothalamus/pituitary to estradiol is necessary for progesterone to have this effect (Wilson and Sharp, 1976b).

There is no information on FSH levels during the ovulatory cycle of the bird. Partially purified avian FSH had no effect on follicle size or oviduct weight if injected into hypophysectomized chickens 14 days after the operation, but, if administered immediately after the operation, this preparation was able to maintain oviduct weight and induce ovulation of undersized eggs. All of the operated birds, however, showed some regression of ovary and oviduct (Mitchell, 1970). Injections of avian LH or mammalian LH have been shown to stimulate estrogen synthesis by the ovaries of immature chickens, however, the avian LH was far more potent than the mammalian

preparation (Burns, 1972). Shahabi *et al.* (1975b) noted increases in plasma testosterone and progesterone at 5, 30, and 90 minutes after injection of ovine LH into chickens but could not detect any change in estradiol levels. In a similar experiment in turkey hens, Camper and Burke (1977) observed variable, but significant increases in plasma progesterone and estradiol at five and 30 minutes after single injections of mammalian FSH or LH.

The steroidogenic functions of FSH and LH in birds, therefore, are not well defined. It is likely that, as in mammals, LH stimulates progesterone and testosterone secretion by the ovary, but whether FSH stimulates the conversion of androgens to estrogens as it does in mammalian ovarian tissue *in vitro* (Moon *et al.*, 1975) remains to be determined.

An antagonistic role of prolactin was demonstrated by Nalbandov (1945) and Meier and Dusseau (1968), but contested by Shani *et al.* (1973). Recently, however, Scanes *et al.* (1977) measured prolactin in the plasma of laying chickens using a radioimmunoassay specific for chicken prolactin. A sharp depression in prolactin levels was detected immediately before the preovulatory surge of LH and an increase in prolactin after the LH levels returned to baseline. The authors suggested that prolactin normally inhibits steroid secretion by the ovary but that the preovulatory suppression of prolactin might facilitate the release of ovarian steroids that in turn exert a positive feedback on LH release.

Avian pituitaries respond to synthetic LH-RH *in vivo* (Furr *et al.*, 1973b; Davies and Bicknell, 1976) and *in vitro* (Bicknell and Follett, 1975) by releasing LH in a dose-related manner. Moreover, hypothalamic extracts of chickens behave similarly to synthetic LH-RH on chromatographic columns and in a radioimmunoassay (Jeffcoate *et al.*, 1974). These data would suggest, therefore, that chickens probably possess a gonadotropic releasing hormone similar to that of mammals. However, Bicknell and Follett (1975) observed that chicken hypothalamic extract was 100 times more potent than was synthetic LH-RH in the dispersed pituitary cell system and in the LH-RH radioimmunoassay. They suggested, therefore, that chicken LH-RH might be different from that isolated from mammalian sources.

Despite their close affinities with the reptiles and lack of viviparity, birds show a similarity to the mammals in the hormonal control of the gonads. Two pituitary gonadotropins are essential for normal gonadal function in birds, and each appears to perform a role similar to its mammalian counterpart. Thus, avian or mammalian LH stimulates Leydig cell steroidogenesis in males and probably granulosa and thecal cell steroidogenesis in females; FSH stimulates spermatogenesis and Sertoli cell function in males and follicular growth and possibly estrogen secretion in females.

The fact that avian gonadotropins are consistently more potent than are those of mammals when tested in birds or reptiles (Callard and Ryan, 1977; Lance *et al.*, 1977) and show some differences in amino acid composition (Table 3) would, however, suggest that there are minor structural differences in both the gonadal receptor proteins and the pituitary gonadotropins of birds and mammals.

DISCUSSION

The oft-quoted statement "it is not the hormones which have evolved, but the uses to which they have been put" that has now become part of the "folklore of endocrinology" (Chester Jones, 1976) is perhaps true with regard to the steroids, but less well documented with regard to the pituitary gonadotropins. Thus, although it is possible to suggest reasons for the changes during evolution of the hormones (or their target tissues) controlling various physiological adaptations to the environment, it is not possible to pinpoint environmental pressures likely to lead to changes in the endocrine control of reproduction.

Steroids occur throughout the plant and animal kingdoms, and it has been proposed that their role as regulators of genomic activity is of prechordate origin (Sandor *et al.*, 1975). It would appear, therefore, that the ability of gonads to synthesize and secrete steroids did not involve any major evolutionary innovations and that very early in vertebrate evolution steroids secreted by the gonads played an essential role in the reproductive process.

In females, the storage of food reserves in the developing oocyte (vitellogenesis) as a means to ensure survival of the offspring evolved long before the appearance of the chordates and is under a sophisticated hormonal control in both invertebrates and vertebrates. Most of the mechanisms operating during oocyte maturation in insects appear analogous to those in vertebrates (Hagedorn, 1974), and it is possible that the follicular design in vertebrates could have been retained from these phylogenetically older organisms (Hisaw, 1961).

Although there is no evidence for an estrogen mediated vitellogenesis in invertebrates, in all egg-laying vertebrates, including cyclostomes, estrogen is essential for successful oocyte growth (Chester Jones *et al.*, 1972). It is now becoming clear that in all vertebrates, including mammals, the major source of this estrogen is the granulosa/theca layer of the developing follicle, and there also is fairly good evidence that the biosynthetic pathway leading to estradiol is probably similar in all vertebrate ovaries. Thus, an extreme conservatism during evolution is apparent with regard to ovarian estrogen secretion. As the vertebrate reproductive system has evolved and become more complex, however, estrogen has acquired many additional roles. In addition to its primitive role of yolk production, it has become a major trophic hormone, a regulator of hypothalamic and pituitary secretion and an important modulator of sex behavior.

The role of ovarian progesterone in nonmammalian vertebrates is less clear, though there are data to suggest that in most species it is secreted by the preovulatory follicle just before ovulation and by the corpora lutea for varying periods after ovulation. In all viviparous species studied, progesterone secretion by the corpus luteum continues through pregnancy but does not appear to be required for the maintenance of pregnancy (Yaron, 1972). It has been suggested that the role of luteal progesterone secretion in nonmammalian vertebrates is to suppress further follicular growth and ovulation during pregnancy (Callard *et al.*, 1972a).

In addition to the basic function of secreting estrogen, the ovaries of some groups of nonmammalian vertebrates secrete unusually high levels of androgen, often in excess of those secreted by the male of the same species (Campbell *et al.*, 1976). The function of these ovarian androgens is not known, though it has been suggested that they may function as estrogen precursors during vitellogenesis.

There also is *in vitro* evidence for corticosteroid production by the ovaries of poikilothermic vertebrates (Columbo and Belvedere, 1975), though it has yet to be proved that these steroids are secreted *in vivo* or that they play any role in the induction of ovulation.

Additionally, although testosterone has been identified in the testes of all vertebrates, it does not appear to have the ubiquitous role estrogen has in females. Other androgens equally as potent as testosterone occur in teleosts, and possibly urodeles. In fact, 11-ketotestosterone is probably the major androgen in many teleost species.

It appears, therefore, that the basic mammalian pattern of steroid hormone biosynthesis is present in the gonads of all vertebrates, with the possible exception of some teleost species. There are, however, many steroid metabolites produced by the gonads of nonmammalian vertebrates, which have yet to be identified in mammals. Reduction of progesterone via the 5β pathway does not occur in the gonads of mammals (Weist, 1963), but is often the predominant metabolic pathway in the ovaries of a number of poikilothermic vertebrates: cyclostomes (Hirose *et al.*, 1975), teleosts (Reinboth, 1975; Ungar *et al.*, 1977), amphibia (Schatz and Morrill, 1975), and reptiles (Lance and Lofts, 1977). Schatz and Morrill (1975) suggest that the 5β -reduced metabolites of progesterone may be important in oocyte maturation in the frog. Interspecific differences in the steroid metabolic profiles and seasonal changes in the steroidogenic capacity of the gonad are common, and should be taken into consideration when studying the reproductive cycles of cold-blooded vertebrates. It also is possible, as Reinboth (1975) pointed out, that some of these esoteric steroids may have important biological functions in the species in which they occur.

Reasons for the enormous differences in the levels of circulating sex steroids among several of the nonmammalian groups require more investigation. Cyclostomes appear to have low to undetectable plasma steroid levels in contrast to teleosts, which often have levels in excess of 100 ng/ml. Skates and rays generally have circulating androgen concentrations in excess of 40 ng/ml., whereas in the Squaliformes, testosterone levels are rarely greater than 6 ng/ml. A similar dichotomy occurs in the Amphibia, where steroid levels in the Anura range from less than one to approximately 25 ng/ml. in contrast to a range from 25 to 200 ng/ml. in urodeles. Whether these differences in hormone levels are attributable to differences in clearance rate, to differences in plasma steroid hormone binding proteins, or to differences in secretory rates remains to be determined. In addition, tissue sensitivity (cytosol receptor proteins) may exhibit differences in these species with high steroid levels. It is possible that these steroids found in such high levels in

teleosts and amphibians are not acting as hormones but function as precursor pools for some as-yet-unidentified biologically active metabolite, or as a precursor for estrogen formation in the central nervous system of the amphibian (Callard *et al.*, 1978) or reptile as suggested by Callard *et al.* (1977).

Gonadal steroidogenesis in all vertebrate species, with the possible exception of the Myxinoidei, is dependent on pituitary gonadotropins. The evidence also suggests that the gonadotropins are chemically alike in all vertebrates. All are glycoproteins of relatively similar amino acid composition (Table 3), and all appear to be composed of two separate subunits. Furthermore, the gonadotropins of all vertebrates probably stimulate gonadal steroidogenesis via a cell membrane-linked adenylate cyclase.

There are, however, no data as yet on two of the most ancient extant vertebrate groups, the cyclostomes and the elasmobranchs, but it is expected that they too probably will fit into the basic vertebrate pattern. Biologically active fractions of the ventral lobe of dogfish pituitary were extracted using techniques identical to those used to extract human gonadotropins, suggesting that elasmobranch gonadotropins too are glycoprotein in nature (Scanes *et al.*, 1972). It also is possible that the PAS-positive cells in lamprey pituitary are producing a glycoprotein gonadotropin. Thus, the evidence favors an extremely ancient origin of the glycoprotein gonadotropins. What is not yet certain, however, is whether the gonadotropins evolved from a TSH glycoprotein molecule as suggested by Fontaine (1969) or if the three pituitary glycoproteins evolved from a common ancestral molecule.

It is likely that one of the most ancient functions of gonadotropin was the stimulation of steroid secretion by the gonads. Evidence is accumulating to suggest that an LH-like molecule specifically stimulates steroid secretion in amphibians and reptiles. The gonadotropic control of steroidogenesis in cyclostomes, elasmobranchs, and teleosts does not appear to have been studied. *Tilapia* gonadotropin, however, is able to stimulate testosterone secretion in rat Leydig cells in a manner similar to mammalian LH (Farmer and Papkoff, 1977). Moreover, equally ancient gonadotropic functions must have been initiation of ovulation and spermiation in seasonally breeding species and possibly uptake of vitellogenin by the developing oocyte. Whether these different functions were all carried out by a single gonadotropic molecule as appears to be the case in some teleosts or require separate LH and FSH-like molecules remains to be determined.

Before we can draw any conclusions as to the nature and biological roles of gonadotropins in nonmammalian vertebrates, rigorous bioassays, preferably in the same or closely related species, from which the hormones were obtained must be performed. Ideally, each fraction should be tested for its activity on: 1) gonadal maturation; 2) steroidogenesis, including estrogen synthesis, which may be an FSH-dependent process; 3) vitellogenin uptake by the developing oocyte, especially important inasmuch as Campbell and Idler (1976) found that a nonglycoprotein teleost pituitary fraction was active in this regard in *Pseudopleuronectes*; 4) spermatogenesis and Sertoli

cell function; 5) ovulation and spermiation. Various other bioassays such as cAMP production by the gonads and ^{32}P uptake by the gonads are useful, especially when tested in the same species, but do not really define the biological activity of the gonadotropin.

As pure gonadotropins from nonmammalian species become available, gonad membrane receptor studies also will be important in defining gonadotropic action, as will studies to determine the particular cell type within the gonad that binds the hormone. Gonadal cell culture systems and cell separation techniques should also contribute much to the solution of the problem of gonadotropic function in nonmammalian vertebrates, especially with regard to Sertoli cell function and its proposed role in steroidogenesis. Only when studies such as these, and more *in vivo* experiments are performed on animals at different stages in the reproductive cycle, can any meaningful evolutionary scheme emerge. In the absence of data on the amino acid sequence of any nonmammalian vertebrate gonadotropin, all theories on the evolution of the hormonal control of reproduction must remain speculative.

In a thoughtful review on pituitary hormones, Wallis (1975) pointed out "that we have to think not only of the evolution of the hormones themselves, but also of their receptors, if we are to understand fully the phylogeny of the pituitary hormones. We are considering, in fact, the evolution of a pair of proteins, which interact in a specific fashion." He suggested that such pairs of proteins probably evolved by means of polyploidy, in which an entire genome may have been duplicated, leading to the appearance of both a new pituitary protein hormone and its tissue receptor protein. Haider and Blum (1977), however, suggested that the evolution of gonadotropins could have evolved in a stepwise manner leading to five possible combinations: evolving from a primitive state in which the pituitary possesses a single gonadotrope secreting a single gonadotropin and the gonad with a single receptor protein, and culminating in an advanced state in which the pituitary possesses two gonadtropes each secreting a distinct gonadotropin and a gonad with two distinct receptor proteins. The three possible intermediate combinations need not necessarily proceed from primitive to advanced.

The available data do not lend full support to either thesis, and, at our present state of knowledge, both should be useful in stimulating new lines of research.

ACKNOWLEDGMENTS

The authors would like to thank Drs. R. Etches and C. Muller for the use of unpublished material and Dr. D. Gapp, Janice Bolaffi, and Jill Rosset for their help in the preparation of the manuscript, which was typed by Shirley Manditch. Support from the National Science Foundation (grant number BMS 75-18910) is gratefully acknowledged.

LITERATURE CITED

- ALPERT, L. C., J. R. BRAWER, I. M. D. JACKSON, AND S. REICHLIN. 1976. Localization of LHRH in neurons in frog brain (*Rana pipiens* and *Rana catesbeiana*). *Endocrinology*, 98:910-921.
- ANDERSON, J. W., AND M. B. YATVIN. 1970. Metabolic and ultrastructural changes in the frog ovarian follicle in response to pituitary stimulation. *J. Cell Biol.*, 46:491-504.
- ANDRIEUX, B., A. COLLENOT, G. COLLENOT, AND C. PERGRALE. 1973. Aspects morphologiques de l'action d'hormones gonadotropes mammaliennes sur l'activité testiculaire du *Triton pleurodeles* nature hypophysectomisé. *Ann. Endocrinol. (Paris)*, 34:711-712.
- AOKI, A., R. VITALE-CALPE, AND A. PISANO. 1969. The testicular interstitial tissue of the amphibian *Physalaemus fuscumaculatus*. *Z. Zellforsch. Mikrosk. Anat.*, 98:9-16.
- ARAI, R. 1967. Androgenic effects of 11-ketotestosterone on some sexual characteristics in the teleost, *Oryzias latipes*. *Annot. Zool. Japonica*, 40:1-5.
- ARAI, R., AND B. I. TAMAOKI. 1967. Steroid biosynthesis *in vitro* by testes of rainbow trout, *Salmo gairdneri*. *Gen. Comp. Endocrinol.*, 8:305-313.
- ARVY, L. 1962. Présence d'une activité stéroïde- 3β -ol-déhydrogénasique chez quelques Saurapsides. *C. R. Hebd. Séanc. Acad. Sci., Paris*, 255:1803-1804.
- BALL, J. N., AND B. I. BAKER. 1969. The pituitary gland: anatomy and histophysiology. Pp. 1-110, in *Fish physiology* (W. S. Hoar and D. S. Randall, eds.), 2:xiv+ 1-465 pp.
- BARA, G. 1965. Histochemical localization of Δ^5 - 3β -hydroxysteroid dehydrogenase in ovaries of a teleost fish, *Scorpaenopsis scorpaenoides* L. *Gen. Comp. Endocrinol.*, 5:638-640.
- . 1969. Histochemical demonstration of 3β , 3α , 11β and 17β -hydroxysteroid dehydrogenases in the testis of *Fundulus heteroclitus*. *Gen. Comp. Endocrinol.*, 13:189-200.
- BARNES, K., AND M. W. HARDISTY. 1972. Ultrastructural and histochemical studies on the testis of the river lamprey, *Lampetra fluviatilis* (L.). *J. Endocrinol.*, 53:59-69.
- BARR, W. A. 1968. Patterns of ovarian activity. Pp. 164-238, in *Perspectives in endocrinology: hormones in the lives of lower vertebrates* (E. J. W. Barrington and C. B. Jørgensen, eds.), Academic Press, New York, xvi+583 pp.
- BARRINGTON, E. J. W. 1964. *Hormones and evolution*. Van Nostrand, Princeton, New Jersey, viii+154 pp.
- . 1968. Phylogenetic perspectives in vertebrate endocrinology. Pp. 1-46, in *Perspectives in endocrinology: Hormones in the lives of lower vertebrates* (E. J. W. Barrington and C. B. Jørgensen, eds.), Academic Press, New York, xvi+583 pp.
- BELLAIRS, A. 1969. *The life of reptiles*. Weidenfeld and Nicolson, London, 1:xii+ 1-282 pp.
- BELLERBY, C. W. 1934. A rapid test for the diagnosis of pregnancy by ovulation of the South African clawed toad after pregnant urine administration. *Nature*, 133:494.
- BENTLEY, P. J. 1976. *Comparative vertebrate endocrinology*. Cambridge Univ. Press, Cambridge, xi+415 pp.
- BERN, H. A., AND G. CHIEFFI. 1968. Bibliography on the steroid hormones of fishes. *Publ. Staz. Zool. Napoli*, 36:287-320.
- BERN, H. A., AND J. NANDI. 1964. Endocrinology of poikilothermic vertebrates. Pp. 199-298, in *The hormones* (G. Pincus, K. V. Thimann, and E. B. Astwood, eds.), Academic Press, New York, 4:x+1-966 pp.
- BERRILL, N. J. 1955. *The origin of the vertebrates*. Clarendon Press, Oxford, viii + 257 pp.
- BETZ, T. W. 1963. The ovarian histology of the diamond-backed water snake, *Natrix rhombifera*, during the reproductive cycle. *J. Morphol.*, 113:245-260.
- BICKNELL, R. J., AND B. K. FOLLETT. 1975. A quantitative assay for luteinizing hormone releasing hormone (LHRH) using dispersed pituitary cells. *Gen. Comp. Endocrinol.*, 26:141-152.
- BILLARD, R. 1970. The spermatogenesis of the guppy (*Poecilia reticulata*). III. Fine structure of the Sertoli cells. *Ann. Biol. Anim. Biochem. Biophys.*, 10:37-50.
- BISWAS, N. M. 1969. Δ^5 - 3β -Hydroxysteroid dehydrogenase in toad testis: synergistic action of ascorbic acid and luteinizing hormone. *Endocrinology*, 85:981-983.

- BOCK, W. J. 1969. The origin and radiation of birds. *Ann. New York Acad. Sci.*, 167: 147-155.
- BOLAFFI, J. L., AND I. P. CALLARD. 1976. *In vivo* gonadotropic stimulation of plasma steroids in male mud puppies, *Necturus maculosus*. *Abst. 4th New England Endocrinol. Conf.*, Boston.
- BOTTE, V. 1964. I lipidi e la Δ^5 - 3β -idrossisteroide deidrogenasi nel tessuto interstiziale del testicolo di *Rana esculenta* durante il ciclo sessuale. *Ricerche istochimiche. Atti Soc. Peloritana Sci. Fis. Mat. Nat.*, 10:521-528.
- BOTTE, V., AND E. COTTINO. 1964. Ricerche istochimiche sulla distribuzione del colesterolo e di alcuni enzimi della steroidogenesi nei follicoli ovarici e post-ovulatori di *Rana esculenta* e *Triturus cristatus*. *Boll. Zool.*, 31:491-500.
- BOTTE, V., AND G. DELRIO. 1967. Effect of estradiol- 17β on the distribution of 3β -hydroxysteroid dehydrogenase in the testes of *Rana esculenta* and *Lacerta sicula*. *Gen. Comp. Endocrinol.*, 9:110-115.
- BOTTE, V., AND C. LUPO DI PRISCO. 1965. The Δ^5 - 3β -hydroxysteroid dehydrogenase of the amphibian testicular tissue. *Gen. Comp. Endocrinol.*, 5:665-666.
- BOTTICELLI, C. R., F. L. HISAW, AND W. D. ROTH. 1963. Estradiol- 17β , estrone, and progesterone in the ovaries of lamprey (*Petromyzon marinus*). *Proc. Soc. Exp. Biol. Med.*, 114:255-257.
- BOUCEK, R. J., AND K. SAVARD. 1970. Steroid formation by the avian ovary *in vitro* (*Gallus domesticus*). *Gen. Comp. Endocrinol.*, 15:6-11.
- BOURNE, A. R., AND R. F. SEAMARK. 1975. Seasonal changes in 17β -hydroxysteroids in the plasma of a male lizard (*Tiliqua rugosa*). *Comp. Biochem. Physiol.*, 50B:535-536.
- BREDER, C. M., AND D. E. ROSEN. 1966. Modes of reproduction in fishes. *Natural History Press*, Garden City, New York, xv+941 pp.
- BRETON, B., AND C. WEIL. 1973. Effects du LH/FSH-RH synthétique et d'extraits hypothalamique de Carpe sur la sécrétion d'hormone gonadotrope *in vivo* chez la carpe (*Cyprinus carpio* L.). *C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D*, 277:1061-1064.
- BRETON, B., G. KANN, E. BURZAWA-GERARD, AND R. BILLARD. 1971. Dosage radioimmunologique d'une hormone gonadotrope de Carpe *Cyprinus carpio* L. *C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D*, 272:981-983.
- BRETON, B., R. BILLARD, B. JALABERT, AND G. KANN. 1972. Dosage radio-immunologique des gonadotropines plasmatiques chez *Carassius auratus* au cours du nycté-mère et pendant l'ovulation. *Gen. Comp. Endocrinol.*, 18:463-468.
- BRETON, B., R. BILLARD, AND B. JALABERT. 1973. Spécificité d'action et relations immunologiques des hormones gonadotropes de quelques téléostéens. *Ann. Biol. Anim. Biochem. Biophys.*, 13:347-362.
- BRETON, B., B. JALABERT, AND P. REINAUD. 1976. Purification of gonadotropin from rainbow trout (*Salmo gairdnerii* Richardson) pituitary glands. *Ann. Biol. Anim. Biochem. Biophys.*, 16:25-36.
- BROKELMANN, J. 1964. Über die Stütz- und Zwischenzellen des Froschodens Während des spermatogenetischen Zyklus. *Z. Zellforsch. Mikrosk. Anat.*, 64:429-461.
- BROWN, N. L., J. D. BAYLÉ, C. G. SCANES, AND B. K. FOLLETT. 1975. Chicken gonadotrophins: their effects on the testes of immature and hypophysectomized quail. *Cell Tissue Res.*, 156:499-520.
- BURNS, J. M. 1972. The influence of chicken and mammalian luteinizing hormones on ovarian estrogen synthesis in the pullet (*Gallus domesticus*). *Comp. Biochem. Physiol.*, 42A:1011-1017.
- BUSSON-MABILLOT, S. 1967. Structure ovarienne de la lamproie adulte, *Lampetra planeri* (Bloch). II. Les enveloppes de l'ovocyte: cellules folliculaires et stroma ovarien. *J. Microscopie*, 6:807-838.
- BURZAWA-GERARD, E. 1971. Purification d'une hormone gonadotrope hypophysaire d'un poisson téléostéen, la Carpe (*Cyprinus carpio* L.). *Biochimie*, 53:545-552.

- . 1974. Séparation et réassociation des sous-unités de l'hormone gonadotrope d'un Poisson Téléostéen, la Carpe (*Cyprinus carpio* L.). C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D, 279:1681-1684.
- BURZAWA-GERARD, E., AND Y. A. FONTAINE. 1972. The gonadotropins of lower vertebrates. Gen. Comp. Endocrinol., suppl. 3:715-728.
- . 1976. Formation d'une molécule hybride douée d'une activité gonadotrope sur la Grenouille, à partir de la sous-unité α de l'hormone lutéinisante bovine et d'une sous-unité de l'hormone gonadotrope d'un Poisson téléostéen. C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D, 282:97-100.
- BURZAWA-GERARD, E., B. F. GONCHAROV, AND Y. A. FONTAINE. 1975a. L'hormone gonadotrope hypophysaire d'un Poisson Chondrostéen, l'Esturgeon (*Acipenser stellatus* Pall.). I. Purification. Gen. Comp. Endocrinol., 27:289-295.
- . 1975b. L'Hormone gonadotrope hypophysaire d'un Poisson Chondrostéen l'Esturgeon (*Acipenser stellatus* Pall.). II. Propriétés biochimique. Gen. Comp. Endocrinol., 27:296-304.
- BURZAWA-GERARD, E., B. GONCHAROV, A. DUMAS, AND Y. A. FONTAINE. 1976. Further biochemical studies on carp gonadotropin (c-GTH); biochemical and biological comparison of c-GTH and a gonadotropin from *Acipenser stellatus* Pall. (Chondrostei). Gen. Comp. Endocrinol., 29:498-505.
- CALLARD, G. V., AND K. J. RYAN. 1977. Gonadotropin action and androgen synthesis in enzyme dispersed testicular cells of the turtle (*Chrysemys picta*). Gen. Comp. Endocrinol., 31:414-421.
- CALLARD, G. V., Z. PETRO, AND K. J. RYAN. 1978a. Identification of aromatase in the reptilian brain. Endocrinology, 100:1214-1218.
- . 1978b. Androgen metabolism in the brain and non-neural tissues of the bullfrog, *Rana catesbeiana*. Gen. Comp. Endocrinol., 34:18-25.
- CALLARD, I. P. 1967. Testicular steroid synthesis in the snake, *Natrix sipedon pictiventris*. J. Endocrinol., 37:105-106.
- CALLARD, I. P., AND G. V. CALLARD. 1978. The reptilian adrenal cortex. Pp. 370-448, in General, clinical and comparative endocrinology of the adrenal cortex (I. Chester-Jones and I. W. Henderson, eds.), Academic Press, New York, 2:xi+ 1-431 pp.
- CALLARD, I. P., AND J. H. LEATHEM. 1964. *In vitro* synthesis of progesterone by ovaries and adrenals of snakes. Proc. Soc. Exp. Biol. Med., 115:567-569.
- . 1965. *In vitro* steroid synthesis by the ovaries of elasmobranchs and snakes. Arch. Anat. Microsc. Morph. Exp., 54:35-48.
- . 1966. Steroid synthesis in amphibian ovarian tissue. Gen. Comp. Endocrinol., 7:80-84.
- CALLARD, I. P., AND V. LANCE. 1977. The control of reptilian follicular cycles. Pp. 199-210, in Reproduction and evolution (J. H. Calaby and C. H. Tyndale-Briscoe, eds.), Proc. 4th Int. Symp. Comp. Biol. Reprod., Canberra.
- CALLARD, I. P., AND H. ZEIGLER. 1970. Inhibitory effects of prolactin upon gonadotropin-stimulated ovarian growth in the iguanid lizard, *Dipsosaurus dorsalis*. J. Endocrinol., 47:131-132.
- CALLARD, I. P., J. DOOLITTLE, W. L. BANKS, AND S. W. CHAN. 1972a. Recent studies on the control of the reptilian ovarian cycle. Gen. Comp. Endocrinol., suppl., 3:65-75.
- CALLARD, I. P., C. G. BAYNE, AND W. F. MCCONNELL. 1972b. Hormones and reproduction in the female lizard, *Sceloporus cyanogenys*. Gen. Comp. Endocrinol., 18:175-194.
- CALLARD, I. P., G. V. CALLARD, V. LANCE, AND S. ECCLES. 1976a. Seasonal changes in testicular structure and function and the effects of gonadotropins in the freshwater turtle, *Chrysemys picta*. Gen. Comp. Endocrinol., 30:347-356.
- CALLARD, I. P., I. MCMHESNEY, C. SCANES, AND G. V. CALLARD. 1976b. The influence of mammalian and avian gonadotropins on *in vitro* ovarian steroid synthesis in the turtle (*Chrysemys picta*). Gen. Comp. Endocrinol., 28:2-9.

- CAMPBELL, C. M., AND D. R. IDLER. 1976. Hormonal control of vitellogenesis in hypophysectomized winter flounder, *Pseudopleuronectes americanus*. Gen. Comp. Endocrinol., 28:143-150.
- CAMPBELL, C. M., J. M. WALSH, AND D. R. IDLER. 1976. Steroids in the plasma of the winter flounder (*Pseudopleuronectes americanus* Walbaum). A seasonal study and investigation of steroid involvement in oocyte maturation. Gen. Comp. Endocrinol., 29:14-20.
- CAMPER, P. M., AND W. H. BURKE. 1977. Serum estradiol and progesterone levels of the laying turkey hen following acute treatment with mammalian luteinizing hormone or follicle stimulating hormone. Gen. Comp. Endocrinol., 31:224-232.
- CARROLL, R. L. 1969. Origin of reptiles. Pp. 1-44, in Biology of the Reptilia (C. Gans, ed.), Academic Press, New York, 1:xv+1-373 pp.
- . 1970. Problems on the origin of reptiles. Biol. Rev., 44:393-432.
- CÉDARD, L., AND R. OZON. 1962. Teneur en oestrogènes du sang de la grenouille rousse (*Rana temporaria*). C. R. Séanc. Soc. Biol., 156:1805-1806.
- CERTAIN, P., G. COLLENOT, A. COLLENOT, AND R. OZON. 1964. Mise en évidence biochimique et histochimique d'une Δ^5 - 3β -hydroxystéroïde déshydrogénase dans le testicules du Triton, *Pleurodeles waltii* Michah. C. R. Séanc. Soc. Biol., 158:1040-1043.
- CHAN, K. M. B., AND B. LOFTS. 1974. The testicular cycle and androgen biosynthesis in the tree sparrow, *Passer montanus saturatus*. J. Zool. (London), 172:47-66.
- CHAN, S. W. C., AND I. P. CALLARD. 1974. Reptilian ovarian steroidogenesis and the influence of mammalian gonadotropins (follicle-stimulating hormone and luteinizing hormone) *in vitro*. J. Endocrinol., 62:267-275.
- CHAN, S. W. C., S. ZIEGEL, AND I. P. CALLARD. 1973. Plasma progesterone in snakes. Comp. Biochem. Physiol., 44A:631-637.
- CHARNIAUX-COTTON, H., AND L. H. KLEINHOLZ. 1964. Hormones in invertebrates other than insects. Pp. 135-198, in The hormones (G. Pincus, K. V. Thimann, and E. B. Astwood, eds.), Academic Press, New York, 4:x+1-966 pp.
- CHESTER JONES, I. 1976. Evolutionary aspects of the adrenal cortex and its homologues. J. Endocrinol., 71:3P-30P.
- CHESTER JONES, I., D. BELLAMY, D. K. O. CHAN, B. K. FOLLETT, I. W. HENDERSON, J. G. PHILLIPS, AND R. S. SNART. 1972. Biological actions of steroid hormones in non-mammalian vertebrates. Pp. 414-480, in Steroids in nonmammalian vertebrates (D. R. Idler, ed.), Academic Press, New York, xii+504 pp.
- CHESTER JONES, I., J. N. BALL, I. W. HENDERSON, T. SANDOR, AND B. I. BAKER. 1974. Endocrinology of fishes. Chem. Zool., 8:523-593.
- CHIEFFI, G. 1967. The reproductive system of elasmobranchs: developmental and endocrinological aspects. Pp. 553-580, in Sharks, skates and rays (P. W. Gilbert, R. F. Mathewson, and D. P. Rall, eds.), Johns Hopkins Press, Baltimore, xii+504 pp.
- CHIEFFI, G., AND V. BOTTE. 1962. II tessuto intersiziale del testicolo dei ciclostomi. Ricerche istologiche ed istochimiche in *Lampetra fluviatilis* e *Lampetra planeri*. Rend. Ist. Sci. Camerino, 3:90-95.
- CHIEFFI, G., AND C. LUPO. 1961. Identification of oestradiol-17 β , testosterone and its precursors from *Scyliorhinus stellaris* testes. Nature, 190:169-170.
- . 1963. Identification of sex hormones in the ovarian extracts of *Torpedo marmorata* and *Bufo vulgaris*. Gen. Comp. Endocrinol., 3:149-152.
- CLARK, R. C., AND I. VAN ZYL. 1976. The composition of phosvitin from crocodile eggs. Int. J. Biochem., 7:229-233.
- CLEMENS, H. P., AND W. W. JOHNSON. 1964. Specificity of gonadal hydration factor in the pituitary of some freshwater fishes. Copeia, 1964:389-398.
- COLLENOT, C., AND R. OZON. 1965. Mises en évidence biochimique et histochimique d'une Δ^5 - 3β -hydroxystéroïde déshydrogénase dans le testicule de *Scyliorhinus canicula* L. Bull. Soc. Zool. France, 89:577-587.
- COLOMBO, L., AND P. C. BELVEDERE. 1975. Gonadal location of the enzymes 11 β - and 21-hydroxylases. Boll. Zool., 42:263-269.

- COLOMBO, L., AND Z. YARON. 1976. Steroid 21-hydroxylase activity in the ovary of the snake *Storeria dekayi* during pregnancy. Gen. Comp. Endocrinol., 28:403-412.
- COLOMBO, L., S. PESAVENTO, AND D. W. JOHNSON. 1972. Patterns of steroid metabolism in teleost and ganoid fishes. Gen. Comp. Endocrinol., suppl., 3:245-253.
- COLOMBO, L., H. A. BERN, J. PIEPRZYK, AND D. W. JOHNSON. 1973. Biosynthesis of 11-deoxycorticosteroids by teleost ovaries and their possible role in oocyte ovulation and maturation. Gen. Comp. Endocrinol., 21:168-178.
- COLOMBO, L., Z. YARON, E. DANIELS, AND P. BLEVEDERE. 1974. Biosynthesis of 11-deoxycorticosterone by the ovary of the Yucca night lizard, *Xantusia vigilis*. Gen. Comp. Endocrinol., 24:331-337.
- COLOMBO, L., P. C. BELVEDERE, AND L. BREDI. 1977. Progesterone metabolism by the gonads of the ascidian *Styela plicata* LeSueur. Comp. Biochem. Physiol., 56B:49-54.
- CONNELL, C. J. 1972. The effect of luteinizing hormone on the ultrastructure of the Leydig cell of the chick. Z. Zellforsch. Mikrosk. Anat., 128:139-151.
- CREASER, C. W., AND A. GORBMAN. 1939. Species specificity of the gonadotropic factors in vertebrates. Quart. Rev. Biol., 14:311-331.
- CREWS, D., AND P. LICHT. 1975a. Stimulation of *in vitro* steroid production in turtle tissue by reptilian, amphibian, and mammalian gonadotropins. Gen. Comp. Endocrinol., 27:71-83.
- . 1975b. Site of progesterone production in the reptilian ovarian follicle. Gen. Comp. Endocrinol., 27:553-556.
- CRIM, L. W., AND D. M. CLUETT. 1974. Elevation of plasma gonadotropin concentration in response to mammalian gonadotropin releasing hormone treatment of the male brown trout as determined by radioimmunoassay. Endocrinol. Res. Comm., 1:101-110.
- CRIM, L. W., R. K. MEYER, AND E. M. DONALDSON. 1973. Radioimmunoassay estimates of plasma gonadotropin levels in the spawning pink salmon. Gen. Comp. Endocrinol., 21:69-76.
- CRIM, L. W., E. G. WATTS, AND D. M. EVANS. 1975. The plasma gonadotropin profile during sexual maturation in a variety of salmonid fishes. Gen. Comp. Endocrinol., 27:62-70.
- CRIM, L. W., R. E. PETER, AND R. BILLARD. 1976. Stimulation of gonadotropin secretion by intraventricular injection of hypothalamic extracts in the goldfish *Carassius auratus*. Gen. Comp. Endocrinol., 30:77-82.
- CURRIE, C., AND H. L. TAYLOR. 1970. A histochemical study of the circumtesticular Leydig cells of a Teiid lizard, *Cnemidophorus tigris*. J. Morphol., 132:101-108.
- DAHL, E. 1971a. Studies of the fine structure of ovarian interstitial tissue I. A comparative study of the fine structure of the ovarian interstitial tissue in rat and the domestic fowl. J. Anat., 108:275-290.
- . 1971b. The fine structure of the granulosa cells in the domestic fowl and the rat. Z. Zellforsch. Mikrosk. Anat., 119:58-67.
- DALE, E., AND R. I. DORFMANN. 1967. Conversion of progesterone-4-C¹⁴ to testosterone by testicular tissue of the American bullfrog. Gen. Comp. Endocrinol., 9:313-318.
- DARROW, D. C., AND G. L. FLETCHER. 1972. Quantification of testosterone and testosterone glucuronide in testicular and peripheral plasma of mature thorny skate (*Raja radiata*). Gen. Comp. Endocrinol., 19:373-375.
- DAVIES, D. T., AND R. J. BICKNELL. 1976. The effect of testosterone on the responsiveness of the quail's pituitary to luteinizing hormone-releasing hormone (LH-RH) during photo-periodically induced testicular growth. Gen. Comp. Endocrinol., 30:487-499.
- DEERY, D. J. 1974. Determination by radioimmunoassay of the luteinizing hormone-releasing hormone (LHRH) content of the hypothalamus of the rat and some lower vertebrates. Gen. Comp. Endocrinol., 24:280-285.
- DELLA CORTE, F., V. BOTTE, AND G. CHIEFFI. 1961. Ricerca istochimica della steroide β -olodeidrogenasi nel testicolo di *Torpedo marmorata* Risso e di *Scyliorhinus stellaris* (L.). Atti Soc. Pelorit. Sci. Fis. Mat. Nat., 7:393-397.

- DELLA CORTE, F., M. GALGANO, AND L. COSENZA. 1962. Su alcune reazioni istochimiche per gli steroidi nel testicolo di *Triturus cristatus carnifex* (Laur.). Arch. Zool. Italiano, 47:353-363.
- DEVILAMING, V. L. 1974. Environmental and endocrine control of teleost reproduction. Pp. 13-83, in Control of sex in fishes (C. B. Schreck, ed.), Virginia Polytechnic Institute, Blacksburg, 106 pp.
- DEWOLFE, B. B., AND S. R. TELFORD. 1966. Lipid positive cells in the testis of the lizard, *Cnemidophorus tigris*. Copeia, 1966:590-592.
- D'ISTRIA, M., A. VALENTINO, AND R. GUASCO. 1973. Dosaggio radioimmunologico di ormoni sessuali nel plasma di *Serranus cabrilla* durante il ciclo riproduttivo. Atti Soc. Pelorit. Sci. Fis. Mat. Nat., 19:241-244.
- D'ISTRIA, M., G. DELRIO, V. BOTTE, AND G. CHIEFFI. 1974. Radioimmunoassay of testosterone, 17 β -oestradiol, and oesterone in the male and female plasma of *Rana esculenta* during sexual cycle. Steroids Lipids Res., 5:42-48.
- DODD, J. M. 1955. The hormones of sex and reproduction and their effects in fish and lower chordates. Mem. Soc. Endocrinol., 4:166-187.
- . 1960. Gonadal and gonadotrophic hormones in lower vertebrates Pp. 417-582, in Marshall's physiology of reproduction (A. S. Parkes, ed.), Longmans Green, London, 1(2):xx+1-877 pp.
- . 1972a. Ovarian control in cyclostomes and elasmobranchs. Amer. Zool., 12:325-339.
- . 1972b. The endocrine regulation of gametogenesis and gonad maturation in fishes. Gen. Comp. Endocrinol., suppl., 3:675-687.
- . 1975. The hormones of sex and reproduction and their effects in fish and lower chordates: twenty years on. Amer. Zool., 15(suppl. 1):137-171.
- DODD, J. M., P. J. EVENNETT, AND C. K. GODDARD. 1960. Reproductive endocrinology in cyclostomes and elasmobranchs. Symp. Zool. Soc. London, 1:77-103.
- DOERR-SCHOTT, J. 1964. Étude au microscope électronique des cellules interstitielles de la grenouille rousse *Rana temporaria* L. C. R. Hebd. Séanc. Acad. Sci., Paris, 258:2896-2898.
- . 1976a. Immunohistochemical detection by light and electron microscopy of pituitary hormones in cold-blooded vertebrates. I. Fish and amphibians. Gen. Comp. Endocrinol., 28:487-512.
- . 1976b. Immunohistochemical detection by light and electron microscopy of pituitary hormones in cold blooded vertebrates. II. Reptiles. Gen. Comp. Endocrinol., 28:513-529.
- DOERR-SCHOTT, J., AND M. P. DUBOIS. 1976. LHRH-like system in the brain of *Xenopus laevis* Daud. Immunohistochemical demonstration. Cell Tissue Res., 172:477-486.
- DONALDSON, E. M. 1973. Reproductive endocrinology of fishes. Amer. Zool., 13:909-928.
- DONALDSON, E. M., F. YAMAZAKI, H. DYE, AND W. W. PHILLEO. 1972. Preparation of gonadotropin from salmon (*Oncorhynchus tshawytscha*) pituitary glands. Gen. Comp. Endocrinol., 18:469-481.
- DUFAURE, J. P. 1970. L'ultrastructure du testicule de lézard vivipare (Reptile, Lacertilien). I. Les cellules interstitielles. Z. Zellforsch. Mikrosk. Anat., 109:33-45.
- DUFAURE, J. P., AND J. P. TURCHINI. 1966. Mise en évidence histochimique d'une activité stéroïdo- β -ol-déshydrogénasique dans le testicule du Lézard vivipare et du Lézard des murailles. C. R. Séanc. Soc. Biol., 160:1874.
- ECKSTEIN, B., AND Y. KATZ. 1971. Steroidogenesis in post- and pre-spawned ovaries of a cichlid fish, *Tilapia aurea*. Comp. Biochem. Physiol., 38A:329-338.
- EDDY, J. M. P. 1969. Metamorphosis and the pineal complex in the brook lamprey, *Lamprocyba planeri*. J. Endocrinol., 44:451-452.
- . 1971. The functions of the pineal complex of lampreys. J. Endocrinol., 51: xxii-xxiii.
- ELEFTHERIOU, B. E., K. W. BOEHLKE, AND O. W. TIEMEIER. 1966. Free plasma estrogens in the channel catfish. Proc. Soc. Exp. Biol. Med., 121:85-88.

- EMMERSEN, B., AND K. KJAER. 1974. Seasonal and hormonal changes in the serum level of the precursor protein vitellogenin in relation to ovarian vitellogenic growth in the toad *Bufo bufo bufo* (L.). Gen. Comp. Endocrinol., 22:261-267.
- ERNST, C. H. 1971. Sexual cycles and maturity of the turtle, *Chrysemys picta*. Biol. Bull., 140:191-200.
- ETCHES, R. J., AND F. J. CUNNINGHAM. 1976a. The effect of pregnenolone, progesterone, deoxycorticosterone or corticosterone on the time of ovulation and oviposition in the hen. British J. Poult. Sci., 17:637-642.
- . 1976b. The interrelationship between progesterone and luteinizing hormone during the ovulatory cycle of the hen (*Gallus domesticus*). J. Endocrinol., 71:51-58.
- . 1977. The plasma concentrations of testosterone and LH during the ovulation cycle of the hen (*Gallus domesticus*). Acta Endocrinol., 84:354-366.
- EVENNETT, P. J., AND J. M. DODD. 1963. Endocrinology of reproduction in the river lamprey. Nature, 197:715-716.
- FALKMER, S., N. W. THOMAS, AND L. BOQUIST. 1974. Endocrinology of the cyclostomata. Chem. Zool., 8:195-257.
- FARMER, S. W., AND H. PAKOFF. 1977. A teleost (*Tilapia mossambica*) gonadotropin that resembles luteinizing hormone. Life Sci., 20:1227-1232.
- FARMER, S. W., M. PAKOFF, AND P. LICHT. 1975. Purification of turkey gonadotropins. Biol. Reprod., 12:415-422.
- FARNER, D. L., AND R. A. LEWIS. 1971. Photoperiodism and reproductive cycles in birds. Pp. 325-370, in Photophysiology (F. C. Giese, ed.), Academic Press, New York, 6:vi+1-514 pp.
- FEDER, H. H., A. STOREY, D. GOODWIN, C. REBOULLEAU, AND R. SILVER. 1977. Testosterone and "5 β -dihydrotestosterone". Levels in peripheral plasma of male and female ring doves (*Streptopelia risoria*) during the reproductive cycle. Biol. Reprod., 16:666-677.
- FERNHOLM, B. 1969. A third embryo of *Myxine*. Considerations on hypophysial ontogeny and phylogeny. Acta Zool., 52:169-177.
- . 1972. Is there any steroid hormone formation in the ovary of the hagfish, *Myxine glutinosa*? Acta Zool., 53:235-242.
- FERNHOLM, B., AND R. OLSSON. 1965. Effects of gonadectomy and estrogen treatment on *Myxine* adenohipophysis. Gen. Comp. Endocrinol., 5:676.
- . 1969. A cytopharmacological study of the myxine adenohipophysis. Gen. Comp. Endocrinol., 13:336-356.
- FEVOLD, H. R., AND K. B. EIK-NES. 1963. Progesterone metabolism by testicular tissue of the English sparrow (*Passer domesticus*). Gen. Comp. Endocrinol., 3:335-345.
- FIRTH, J. A., AND L. VOLLRATH. 1973. Determination of the distribution of luteinizing hormone-like activity within the dogfish pituitary gland by means of the *Xenopus* oocyte meiosis assay. J. Endocrinol., 58:347-348.
- FLETCHER, G. L., D. C. HARDY, AND D. R. IDLER. 1969. Testosterone production rates and metabolic clearance rates in sexually mature male and female skate (*Raja radiata*). Endocrinology, 85:552-560.
- FOLLENIUS, E. 1968. Cytologie et cytophysiologie des l'épinoche: *Gasterosteus aculeatus* L. Étude au microscope électronique. Gen. Comp. Endocrinol., 11:198-219.
- FOLLETT, B. K. 1976. Plasma follicle stimulating hormone during photoperiodically induced sexual maturation in male Japanese quail. J. Endocrinol., 69:117-126.
- FOLLETT, B. K., AND D. T. DAVIES. 1975. Photoperiodicity and the neuroendocrine control of reproduction in birds. Symp. Zool. Soc. London, 35:199-224.
- FOLLETT, B. K., AND M. R. REDSHAW. 1974. The physiology of vitellogenesis. Pp. 219-308, in Physiology of the Amphibia (B. Lofts, ed.), Academic Press, New York, 2:xi+1-592 pp.
- FOLLETT, B. K., T. J. NICHOLLS, AND M. R. REDSHAW. 1968. The vitellogenic response in the South African clawed toad (*Xenopus laevis* Daudin). J. Cell. Physiol., 72(suppl.): 91-102.

- FOLLETT, B. K., C. G. SCANES, AND F. J. CUNNINGHAM. 1972. A radioimmunoassay for avian luteinizing hormone. *J. Endocrinol.*, 52:359-378.
- FONTAINE, Y. A. 1969. La spécificité zoologique des protéines hypophysaires capable de stimuler la thyroïde. *Acta Endocrinol. (suppl. 1)*, 136: 1-154.
- FOX, W. 1965. A comparison of the male urogenital systems of blind snakes Leptotyphlopidae and Typhlopidae. *Herpetologia*, 21:241-256.
- FRTZ, I. B., M. D. GRISWOLD, G. LOUIS, AND J. DORRINGTON. 1976. Similarity of response of cultured Sertoli cells to cholera toxin and FSH. *Mol. Cell. Endocrinol.*, 5:289-294.
- FURR, B. J. A., R. C. BONNEY, R. J. ENGLAND, AND F. J. CUNNINGHAM. 1973a. Luteinizing hormone and progesterone in peripheral blood during the ovulatory cycle of the hen *Gallus domesticus*. *J. Endocrinol.*, 57:159-169.
- FURR, B. J. A., G. I. ONUORA, R. C. BONNEY, AND F. J. CUNNINGHAM. 1973b. The effect of synthetic hypothalamic releasing factors on plasma levels of luteinizing hormone in the cockerel. *J. Endocrinol.*, 59:495-502.
- GABE, M., AND H. SAINT GIRONS. 1964. Contribution à l'histologie de *Sphenodon punctatus* Gray. Editions du C.N.R.S., Paris, 231 pp.
- GAILLI-MAININI, C. 1947. Pregnancy testing using the male toad. *J. Clin. Endocrinol.*, 7:653-658.
- GALLIEN, L., AND M. CHALUMEAU-LE FOULGOC. 1960. Mise en évidence de stéroïdes oestrogènes dans l'ovaire juvénile de *Xenopus laevis* Daudin, et cycles des oestrogènes au cours de la ponte. *C. R. Hebd. Séanc. Acad. Sci., Paris*, 251:460-462.
- GARNIER, D. H. 1971. Variations de la testostérone du plasma périphérique chez le Canard Pekin au cours du cycle annuel. *C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D*, 272:1665-1668.
- GAVAUD, J. 1975. Étude expérimentale du rôle des facteurs externes sur la spermatogenèse et la stéroïdogénese des mâles de *Nectophrynoides occidentalis* Angel. *J. Physiol. (Paris)*, 70:549-559.
- . 1976. La gamétogenèse du mâle de *Nectophrynoides occidentalis* Angel. (Amphibien anoure vivipare). *Ann. Biol. Anim. Biochem. Biophys.*, 16:1-12.
- GEORGES, D. 1976. Ovarian control of neuronal secretion in *Ciona intestinalis* (Tunicata). *Gen. Comp. Endocrinol.*, 29:299.
- GODDEN, P. M. M., AND C. G. SCANES. 1975. Studies on the purification and properties of avian gonadotrophins. *Gen. Comp. Endocrinol.*, 27:538-542.
- GOLDBERG, S. R., AND C. H. LOWE. 1966. The reproductive cycle of the western whiptail lizard (*Cnemidophorus tigris*) in southern Arizona. *J. Morphol.*, 118:543-548.
- GOOS, H. J. T., P. J. M. LIGTENBERG, AND P. G. W. J. VAN OORDT. 1976. Immunofluorescence studies on gonadotropin releasing hormone (GRH) in fore-brain and the neurohypophysis of the green frog, *Rana esculenta* L. *Cell Tissue Res.*, 168:325-333.
- GORBMAN, A. 1941. Comparative anatomy and physiology of the anterior pituitary. *Quart. Rev. Biol.*, 16:294-310.
- . 1965. Vascular relations between the neurohypophysis and adenohypophysis of cyclostomes and the problem of evolution of hypothalamic neuroendocrine control. *Arch. Anat. Microsc. Morph. Exp.*, 54:163-194.
- GORBMAN, A., H. KOBAYASHI, AND H. UEMURA. 1963. The vascularization of the hypophysial structures of the hagfish. *Gen. Comp. Endocrinol.*, 3:505-514.
- GOSWAMI, S. V., B. I. SUNDARARAJ, AND E. M. DONALDSON. 1974. *In vitro* maturation response of oocytes of the catfish, *Heteropneustes fossilis* (Bloch) to salmon gonadotropin in ovary-head kidney co-culture. *Canadian J. Zool.*, 52:745-748.
- GOTTFRIED, H. 1964. The occurrence and biological significance of steroids in lower vertebrates. A review. *Steroids*, 3:219-242.
- GRABER, J. W., AND A. V. NALBANDOV. 1976. Peripheral estrogen levels during the laying cycle of the hen. (*Gallus domesticus*). *Biol. Reprod.*, 14:109-114.
- GURAYA, S. S. 1976. Recent advances in the morphology, histochemistry, and biochemistry of steroid-synthesizing cellular sites in the nonmammalian vertebrate ovary. *Int. Rev. Cytol.*, 44:365-409.

- GUSTAFSON, A. W., AND M. SHEMESH. 1976. Changes in plasma testosterone levels during the annual reproductive cycle of the hibernating bat, *Myotis lucifugus*, with a survey of plasma testosterone levels in adult male vertebrates. *Biol. Reprod.*, 15:9-24.
- HAGEDORN, H. H. 1974. Juvenile hormone induction of the insect yolk protein precursor. *Amer. Zool.*, 14:1207-1218.
- HAIDER, S. G., AND V. BLÜM. 1977. On the evolution of pituitary gonadotropin system in lower vertebrates. *Invest. Pesq.*, 41:185-204.
- HARDISTY, M. W. 1972. Gonadogenesis, sex differentiation and gametogenesis. Pp. 295-359, in *The biology of lampreys* (M. W. Hardisty and I. C. Potter, eds.), Academic Press, New York, 2:xiv+1-466 pp.
- HARDISTY, M. W., AND K. BARNES. 1968. Activity of 3β -ol-dehydrogenase in cyclostome gonad. *Nature*, 218:880.
- HATTINGH, J., AND P. J. DU TOIT. 1973. Partial separation of the pituitary proteins of *Labeo umbratus*, Smith (mudfish). *J. Fish Biol.*, 5:41-47.
- HAYNES, N. B., K. J. COOPER, AND M. J. KAY. 1973. Plasma progesterone in the hen in relation to the ovulatory cycle. *British J. Poult. Sci.*, 14:349-357.
- HIGHFILL, D. R., AND R. A. MEAD. 1975a. Sources and levels of progesterone during pregnancy in the garter snake, *Thamnophis elegans*. *Gen. Comp. Endocrinol.*, 27:389-400.
- . 1975b. Function of corpora lutea of pregnancy in the viviparous garter snake, *Thamnophis elegans*. *Gen. Comp. Endocrinol.*, 27:401-407.
- HIGHNAM, K. C., AND L. HILL. 1969. The comparative endocrinology of the invertebrates. Edward Arnold, London, ix+270 pp.
- HINDE, R. A., AND E. STEEL. 1975. The dual role of daylength in controlling canary reproduction. *Symp. Zool. Soc. London*, 35:245-259.
- HIROSE, K., B. I. TAMAOKI, B. FERNHOLM, AND H. KOBAYASHI. 1975. *In vitro* bioconversion of steroids in the hagfish, *Eptatretus burgeri*. *Comp. Biochem. Physiol.*, 51B:403-408.
- HISAW, F. L. 1961. The evolution of endocrine adaptations of the ovarian follicle. Pp. 1-16, in *Physiology of reproduction* (F. L. Hisaw, Jr., ed.), Oregon State Univ. Press, Corvallis, 105 pp.
- HISHIDA, T., AND N. KAWAMOTO. 1970. Androgenic and male-inducing effects of 11-ketotestosterone on a teleost (*Oryzia latipes*). *J. Exp. Zool.*, 173:1053-1054.
- HOAR, W. S. 1969. Reproduction. Pp. 1-72, in *Fish physiology* (W. S. Hoar and D. J. Randall, eds.), Academic Press, New York, 3:xvi+1-485 pp.
- HOLMES, R. L., AND J. N. BALL. 1974. The pituitary gland. A comparative account. Cambridge Univ. Press, Cambridge and New York, x+397 pp.
- HOLSTEIN, A. F. 1969. Zur frage der lokalen Steuerung der Spermatogenese beim Dornhai (*Squalus acanthias*). *Z. Zellforsch. Mikrosk. Anat.*, 93:265-281.
- HORSTMANN, E., AND H. BRUCKER. 1972. Spezielle Mitochondrien in den Leydig-Zellen eines Knochenfisches *Trichogaster leeri*. (Bleeker). *Z. Zellforsch. Mikrosk. Anat.*, 134:97-103.
- HOUSSAY, B. A. 1954. Hormonal regulation of sexual functions of the male toad. *Acta Physiol. Latinoamerica*, 4:1-41.
- HUBERT, J. 1973. Les cellules piriformes du follicule ovarien de certains reptiles. *Arch. Anat. Histol. Embryol.*, 56:5-18.
- . 1976. Étude ultrastructurale des cellules piriformes du follicule ovarien chez 5 sau-riens. *Arch. Anat. Microsc. Morph. Exp.*, 65:47-58.
- HUBBS, C. L., AND I. C. POTTER. 1971. Distribution, phylogeny and taxonomy. Pp. 1-65, in *The biology of lampreys* (M. W. Hardisty and I. C. Potter, eds.), Academic Press, New York, 1:xiv+1-423 pp.
- HUBRECHT, A. A. W. 1887. The relation of the Nemertea to the Vertebrata. *Quart. J. Microsc. Sci.*, 27:605-644.
- HUMPHREY, R. R. 1921. The interstitial cells of the urodele testis. *Amer. J. Anat.*, 29:213-278.
- . 1922. The multiple testis in urodeles. *Biol. Bull.*, 43:45-67.
- . 1926. The multiple testis in *Diemyctylus*. *J. Morph. Physiol.*, 41:283-309.

- . 1977. Factors influencing ovulation in the Mexican axolotl as revealed by induced spawnings. *J. Exp. Zool.*, 199:209-214.
- HUMPHREYS, P. N. 1975. Ultrastructure of the budgerigar testis during a photoperiodically induced cycle. *Cell Tissue Res.*, 159:541-550.
- ISHII, S., AND D. S. FARNER. 1976. Binding of follicle-stimulating hormone by homogenates of testes of photostimulated white-crowned sparrows, *Zonotrichia leucophrys gambelli*. *Gen. Comp. Endocrinol.*, 30:443-450.
- IDLER, D. R., AND H. C. MACNAB. 1967. The biosynthesis of 11-ketotestosterone and 11-hydroxytestosterone by Atlantic salmon tissues *in vitro*. *Canadian J. Biochem.*, 45: 581-589.
- IDLER, D., AND B. TRUSCOTT. 1966. Identification and qualification of testosterone in peripheral plasma of skate. *Gen. Comp. Endocrinol.*, 7:375-383.
- IDLER, D. R., P. J. SCHMIDT, AND A. P. RONALD. 1959. Biochemical studies on sockeye salmon during spawning migration. VIII. Steroid hormones in plasma. *Canadian J. Biochem. Physiol.*, 37:1227-1238.
- IDLER, D. R., P. J. SCHMIDT, AND A. P. RONALD. 1960. Isolation and identification of 11-ketotestosterone in salmon plasma. *Canadian J. Biochem. Physiol.*, 38:1053-1057.
- IDLER, D. R., H. C. FREEMAN, AND B. TRUSCOTT. 1964. Steroid hormones in the plasma of spawned Atlantic salmon (*Salmo salar*) and a comparison of their determination by chemical and biological assay methods. *Canadian J. Biochem.*, 42:211-218.
- IDLER, D. R., B. TRUSCOTT, AND H. C. STEWART. 1968. Some distinctive aspects of steroidogenesis in fish. *Excerpta Med. Found. Int. Congr. Ser.*, 184:724-729.
- IDLER, D. R., D. A. HORNE, AND G. B. SANGALANG. 1971. Identification and quantification of the major androgens in testicular and peripheral plasma of Atlantic salmon (*Salmo salar*) during sexual maturation. *Gen. Comp. Endocrinol.*, 16:257-261.
- IDLER, D. R., L. S. BAZAR, AND S. J. HWANG. 1975a. Fish gonadotropin(s) *in vitro* with immature trout gonads. *Endocrine Res. Comm.*, 2:199-213.
- . 1975b. Fish gonadotropin(s) II. Isolation of gonadotropin(s) from chum salmon pituitary glands using affinity chromatography. *Endocrine Res. Comm.*, 2:215-235.
- . 1975c. Fish gonadotropin(s) III. Evidence for more than one gonadotropin in chum salmon pituitary glands. *Endocrine Res. Comm.*, 2:237-249.
- IDLER, D. R., R. REINBOTH, J. M. WALSH, AND B. TRUSCOTT. 1976. A comparison of 11-hydroxytestosterone and 11-ketotestosterone in blood of ambisexual and gonochoristic teleosts. *Gen. Comp. Endocrinol.*, 30:517-521.
- JARVIK, E. 1968. Aspects of vertebrate phylogeny. Pp. 497-527, in *Current problems of lower vertebrate phylogeny* (T. Orvig, ed.), Wiley (Interscience), New York, 539 pp.
- JEFFCOATE, S. L., P. J. SHARP, H. M. FRASER, D. T. HOLLAND, AND A. GUNN. 1974. Immunological and chromatographic similarity of rat, rabbit, chicken, and synthetic luteinizing hormone releasing hormone. *J. Endocrinol.*, 62:85-89.
- JOLY, J. 1965. Mise en évidence histochimique d'une $\Delta^3\beta$ -hydroxystéroïde-déshydrogénase dans l'ovaire de l'Urodele *Salamandra salamandra* (L.) à différents stades du cycle sexuel. *C. R. Hebd. Séanc. Acad. Sci., Paris*, 261:1569-1571.
- . 1971. Les cycles sexuels de *Salamandra* (L.). I. Cycle sexuel des mâles. *Ann. Sci. Nat., Paris, Zool. Biol. Anim.*, 13:451-504.
- JOLY, J., AND B. PICHERAL. 1972. Ultrastructure, histochimie et physiologie du follicule pré-ovulatoire et du corps jaune de l'urodèle ovo-vivipare *Salamandra salamandra* (L.). *Gen. Comp. Endocrinol.*, 18:235-259.
- JONES, R. E., N. B. SEDGLEY, F. M. GERRARD, AND J. J. ROTH. 1974. Endocrine control of clutch size in reptiles. III. $\Delta^5\beta$ -hydroxysteroid dehydrogenase activity in different-sized ovarian follicles of *Anolis carolinensis*. *Gen. Comp. Endocrinol.*, 22:448-453.
- JOSS, J. M. P. 1973. Pineal-gonad relationships in the lamprey, *Lampetra fluviatilis*. *Gen. Comp. Endocrinol.*, 21:118-122.
- KALT, M. R. 1976. Morphology and kinetics of spermatogenesis in *Xenopus laevis*. *J. Exp. Zool.*, 195:393-408.

- KATZ, Y., AND B. ECKSTEIN. 1974. Changes in steroid concentration in the blood of female *Tilapia aurea* (Teleostei, Cichlidae) during initiation of spawning. *Endocrinology*, 95:963-967.
- KATZ, Y., B. ECKSTEIN, R. ELKAN, AND R. GOTTLIEB. 1971. Estrone and estradiol-17 β in the ovaries of *Tilapia aurea* (Teleostei, Cichlidae). *Comp. Biochem. Physiol.*, 40B: 1005-1010.
- KELLEY, D. B., AND D. W. PFAFF. 1976. Hormone effects on male sex behavior in adult South African clawed frogs, *Xenopus laevis*. *Horm. Behav.*, 7:159-182.
- KEMP, N. E. 1956. Electron microscopy of growing oocytes of *Rana pipiens*. *J. Biophys. Biochem. Cytol.*, 2:281-292.
- KERLAN, J. T., AND R. B. JAFFE. 1974. Plasma testosterone levels during the testicular cycle of the Redwinged Blackbird (*Agelaius phoeniceus*). *Gen. Comp. Endocrinol.*, 22: 428-432.
- KERLAN, J. T., R. B. JAFFE, AND A. H. PAYNE. 1974. Sex steroid formation in gonadal tissue homogenates during the testicular cycle of the Redwinged Blackbird (*Agelaius phoeniceus*). *Gen. Comp. Endocrinol.*, 24:352-363.
- KESSEL, R. G., AND W. R. PANJE. 1968. Organization and activity in the pre-and post-ovulatory follicle of *Necturus maculosus*. *J. Cell Biol.*, 39:1-34.
- KETTERER, D., AND W. R. FORBES. 1972. Induction of spawning in the Mexican axolotl (*Ambystoma mexicanum*) by luteinizing hormone. *J. Endocrinol.*, 55:457-458.
- KJAER, K., AND C. B. JORGENSEN. 1971. Effects of mammalian gonadotropes on ovary in the toad *Bufo bufo* (L.). *Gen. Comp. Endocrinol.*, 17:424-431.
- KLICKA, J., AND I. Y. MAHMOUD. 1972. Conversion of pregnenolone-4¹⁴C to progesterone-4¹⁴C by turtle corpus luteum. *Gen. Comp. Endocrinol.*, 19:367-369.
- . 1973. Conversion of cholesterol to progesterone by turtle corpus luteum. *Steroids*, 21:483-495.
- KNOWLES, F., L. VOLLRATH, AND P. MEURLING. 1975. Cytology and neuroendocrine relations of the pituitary of the dogfish, *Scyliorhinus canicula*. *Proc. Roy. Soc. London, ser. B*, 191:507-525.
- KOBAYASHI, H., T. ICHIKAWA, H. SUZUKI, AND M. SEKIMOTO. 1972. Seasonal migration of the hagfish, *Eptatretus burgeri*. *Japanese J. Ichthyol.*, 19:191-194. [Japanese with English summary].
- KUMAGAI, S., AND K. HOMMA. 1974. High estrogen production in the medium-sized follicles during follicular growth and ovulation in the laying quail. *Endocrinol. Japonica*, 21:349-354.
- LAGUE, P. C., A. VAN TIENHOVEN, AND F. J. CUNNINGHAM. 1975. Concentrations of estrogens, progesterone and LH during the ovulatory cycle of the laying chicken (*Gallus domesticus*). *Biol. Reprod.*, 12:590-598.
- LAM, F., D. S. FARNER. 1976. The ultrastructure of the cells of Leydig in the Whitecrowned sparrow (*Zonotrichia leucophrys gambelii*) in relation to plasma levels of luteinizing hormone and testosterone. *Cell Tissue Res.*, 169:93-109.
- LAMBERT, J. G. D. 1970. The ovary of the guppy, *Poecilia reticulata*. The granulosa cells as sites of steroid synthesis. *Gen. Comp. Endocrinol.*, 15:464-476.
- LAMBERT, J. G. D., AND M. G. E. POT. 1975. Steroidogenesis in ovarian tissue of a viviparous teleost, the guppy *Poecilia reticulata*. *Comp. Biochem. Physiol.*, 50B:585-589.
- LAMBERT, J. G. D., J. H. H. THIJSEN, AND U. EYLATH. 1971. Conversion of ³H-androstenedione and ³H-testosterone to ³H-oestradiol-17 β *in vitro* by the ovary of the guppy, *Poecilia reticulata*. *Proc. K. Nederlandse Akad. Wet., ser. C*, 74:52-59.
- LANCE, V. 1975. Studies on the annual reproductive cycle of the female cobra, *Naja naja*-I. seasonal variation in plasma cholesterol. *Comp. Biochem. Physiol.*, 52A:519-525.
- . 1976. Studies of the annual reproductive cycle of the female cobra, *Naja naja*. Seasonal variation in plasma inorganic ions. *Comp. Biochem. Physiol.*, 53A:285-289.
- LANCE, V., AND I. P. CALLARD. 1969. A histochemical study of ovarian function in the oviparous elasmobranch, *Squalus acanthias*. *Gen. Comp. Endocrinol.*, 13:255-267.

- LANCE, V., AND I. P. CALLARD. 1978. Steroidogenesis by enzyme dispersed turtle (*Chrysemys picta*) ovarian cells in response to ovine gonadotropins (FSH and LH). Gen. Comp. Endocrinol., 34:304-311.
- LANCE, V., AND B. LOFTS. 1977. Studies on the reproductive cycle of the female cobra, *Naja naja* III. *In vitro* ovarian steroid synthesis. Gen. Comp. Endocrinol., 32:279-288.
- LANCE, V., C. SCANES, AND I. P. CALLARD. 1977. Plasma testosterone levels in male turtles, *Chrysemys picta*, following single injections of mammalian, avian and teleostean gonadotropins. Gen. Comp. Endocrinol., 31:435-441.
- LARSEN, L. O. 1969. Hypophyseal functions in river lampreys. Gen. Comp. Endocrinol., suppl. 2:522-527.
- . 1973. Development in adult, freshwater lampreys and its hormonal control. Unpublished thesis, Univ. Copenhagen, 212 pp.
- LARSEN, L. O., AND B. ROTHWELL. 1972. Adenohypophysis. Pp. 1-67, in The biology of lampreys (M. W. Hardisty and I. C. Potter, eds.), Academic Press, New York, 2:xiv+1-466 pp.
- LAZARD, L. 1976. Spermatogenesis and 3-HSDH activity in the testis of the axolotl. Nature, 264:796-797.
- LICHT, P. 1972. Actions of mammalian pituitary gonadotropins (FSH and LH) in reptiles. II. Turtles. Gen. Comp. Endocrinol., 19:282-289.
- . 1973. Induction of spermiation in anurans by mammalian pituitary gonadotropins and their subunits. Gen. Comp. Endocrinol., 20:522-529.
- . 1974a. Reptilian endocrinology. The pituitary system. Chem. Zool., 9:399-448.
- . 1974b. Induction of sperm release in frogs by mammalian gonadotropin-releasing hormone. Gen. Comp. Endocrinol., 23:352-354.
- . 1974c. Luteinizing hormone in the reptilian pituitary gland. Gen. Comp. Endocrinol., 22:463-469.
- LICHT, P., AND D. CREWS. 1976. Gonadotropin stimulation of *in vitro* progesterone production in reptilian and amphibian ovaries. Gen. Comp. Endocrinol., 29:141-151.
- LICHT, P., AND A. R. MIDGLEY, JR. 1976a. *In vitro* binding of radioiodinated follicle-stimulating hormone to reptilian and avian gonads: radioligand studies with mammalian hormones. Biol. Reprod., 15:195-205.
- . 1976b. Competition for the *in vitro* binding of radio-iodinated human follicle-stimulating hormone in reptilian, avian, and mammalian gonads by nonmammalian gonadotropins. Gen. Comp. Endocrinol., 30:364-371.
- . 1977. Autoradiographic localization of binding sites for human follicle-stimulating hormone in reptilian testes and ovaries. Biol. Reprod., 16:117-121.
- LICHT, P., AND H. PAPKOFF. 1974a. Separation of two distinct gonadotropins from the pituitary gland of the bullfrog, *Rana catesbeiana*. Endocrinology, 94:1587-1594.
- . 1974b. Phylogenetic survey of the neuraminidase sensitivity of reptilian gonadotropins. Gen. Comp. Endocrinol., 23:415-420.
- . 1976. Species specificity in the response of an *in vitro* amphibian (*Xenopus laevis*) ovulation assay to mammalian luteinizing hormones. Gen. Comp. Endocrinol., 29:552-555.
- LICHT, P., H. PAPKOFF, B. D. GOLDMAN, B. K. FOLLETT, AND C. D. SCANES. 1974. Immunological relatedness among reptilian, avian, and mammalian pituitary luteinizing hormones. Gen. Comp. Endocrinol., 24:168-176.
- LICHT, P., S. W. FARMER, AND H. PAPKOFF. 1975. The nature of the pituitary gonadotropins and their role in ovulation in a urodele amphibian (*Ambystoma tigrinum*). Life Sci., 17:1049-1054.
- LICHT, P., C. H. MULLER, AND H. W. TSUI. 1976a. Effects of mammalian and nonmammalian gonadotropins on androgen production by minced rabbit testis. Biol. Reprod., 14:194-201.
- LICHT, P., S. W. FARMER, AND H. PAPKOFF. 1976b. Further studies on the chemical nature of reptilian gonadotropins: FSH and LH in the American alligator and green sea turtle. Biol. Reprod., 14:222-232.

- LILEY, N. R. 1969. Hormones and reproductive behavior in fishes. Pp. 73-116, in *Fish physiology* (W. S. Hoar and D. J. Randall, eds.), Academic Press, New York, 3:xvi+1-485 pp.
- LIVNI, N. 1971. Ovarian histochemistry of the fishes *Cyprinus carpio*, *Mugil capito* and *Tilapia aurea* (Teleostei). *Histochem. J.*, 3:405-414.
- LOFTS, B. 1961. The effects of follicle-stimulating and luteinizing hormone on the testis of hypophysectomized frogs (*Rana temporaria*). *Gen. Comp. Endocrinol.*, 1:179-189.
- . 1968. Patterns of testicular activity. Pp. 239-304, in *Perspectives in endocrinology: Hormones in the lives of lower vertebrates* (E. J. W. Barrington and C. B. Jorgensen eds.), Academic Press, New York, xvi+583 pp.
- . 1972. The Sertoli cell. *Gen. Comp. Endocrinol.*, suppl., 3:636-648.
- . 1974. Reproduction. Pp. 107-218, in *Physiology of the Amphibia* (B. Lofts, ed.), Academic Press, New York, 2:xi+1-592 pp.
- . 1975. Environmental control of reproduction. *Symp. Zool. Soc. London*, 35:177-197.
- . 1977. Patterns of spermatogenesis and steroidogenesis in male reptiles. In *Reproduction and evolution* (C. H. Tyndale-Biscoe, ed.), *Proc. 4th International Symposium on Comparative Biology of Reproduction*, Canberra, Australia, 1976. In Press [?]
- LOFTS, B., AND H. A. BERN. 1972. The functional morphology of steroidogenic tissues. Pp. 37-126, in *Steroids in non-mammalian vertebrates* (D. R. Idler, ed.), Academic Press, New York, xii+504 pp.
- LOFTS, B., AND C. BOSWELL. 1961. Seasonal changes in the distribution of the testis lipids of the caspian terrapin *Clemmys caspica*. *Proc. Zool. Soc. London*, 136:581-592.
- LOFTS, B., AND R. K. MURTON. 1968. Photoperiodic and physiological adaptations regulating avian breeding cycles and their ecological significance. *J. Zool. (London)*, 155: 327-394.
- . 1973. Reproduction in birds. Pp. 1-107, in *Avian biology* (D. S. Farner and J. R. King, eds.), Academic Press, New York, 3:xix+1-573 pp.
- LOFTS, B., AND H. W. TSUI. 1977. Histological and histochemical changes in the gonads and epididymides of the male softshelled turtle, *Trionyx sinensis*. *J. Zool. (London)*, 181: 57-68.
- LOYEZ, M. 1906. Recherches sur le développement ovarien des oeufs meroblastiques à vitellus nutritif abondant. *Arch. Anat. Microsc.*, 8:69-397.
- LUPO, C., AND G. CHIEFFI. 1963. Ormoni sessuali e attività steroide- β -olo-deidrogenasica del testicolo di *Morone labrax*. *Atti Accad. Naz. Lincei Rend. Cl. Sci. Fis. Mat. Nat.*, ser. 8, 34:443-446.
- LUPO DI PRISCO, C., V. BOTTE, AND G. CHIEFFI. 1965. Differenze nella capacità di biosintesi degli ormoni steroidei tra follicoli postovulatori ed atresici in *Torpedo marmorata* e *Scyliorhinus stellaris*. *Boll. Zool.*, 32:185-190.
- LUPO DI PRISCO, C., C. VELLANO, AND G. CHIEFFI. 1967. Steroid hormones in the plasma of the elasmobranch *Torpedo marmorata* at various stages of the sexual cycle. *Gen. Comp. Endocrinol.*, 8:325-331.
- LUPO DI PRISCO, C., G. DELRIO, AND G. CHIEFFI. 1968. Sex hormones in the ovaries of the lizard *Lacerta sicula*. *Gen. Comp. Endocrinol.*, 10:292-295.
- LUPO DI PRISCO, C., C. BASILE, G. DELRIO, AND G. CHIEFFI. 1972. *In vitro* metabolism of cholesterol-4- 14 C and testosterone-4- 14 C in testes and fat bodies of *Triturus cristatus carnifex*. *Comp. Biochem. Physiol.*, 41B:245-249.
- MARSHALL, A. J., AND B. LOFTS. 1956. The Leydig cell homologue in certain teleost fishes. *Nature*, 177:706.
- MASHALY, M. M., G. P. BIRRENKOTT, M. M. EL-BEGEARMY, AND B. C. WENTWORTH. 1976. Plasma LH and progesterone concentrations in the turkey hen during the ovulatory cycle. *Poult. Sci.*, 55:1226-1234.
- MATTY, A. J., K. TSUNEMI, W. W. DICKHOFF, AND A. GORBMAN. 1976. Thyroid and gonadal function in hypophysectomized hagfish, *Eptatretus stouti*. *Gen. Comp. Endocrinol.*, 30:500-516.
- MAUNG, Z. W. 1976. Effect of LH and cAMP on steroidogenesis in interstitial cells isolated from the testis of the Japanese quail. *Gen. Comp. Endocrinol.*, 29:254.

- MAZZI, V., C. VELLANO, D. COLUCCI, AND A. MERLO. 1974. Gonadotropin stimulation by chronic administration of synthetic luteinizing hormone releasing hormone in hypophysectomized pituitary grafted male newts. *Gen. Comp. Endocrinol.*, 24:1-9.
- MELLINGER, J. 1972. Types cellulaires et fonctions de l'adénohypophyse de la Torpille (*Torpedo marmorata*). *Gen. Comp. Endocrinol.*, 18:608.
- MELLINGER, J., AND M. P. DUBOIS. 1973. Confirmation par l'immunofluorescence de la fonction corticotrope du lobe rostral et de la fonction gonadotrope du lobe ventral de l'hypophyse d'un Poisson cartilagineux, la Torpille marbrée (*Torpedo marmorata*). *C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D*, 276:1879-1881.
- MEIER, A. H., AND J. S. DUSSEAU. 1968. Prolactin and photoperiodic gonadal response in several avian species. *Physiol. Zool.*, 41:95-103.
- MILLER, M. R., AND M. E. ROBBINS. 1954. The reproductive cycle in *Taricha torosa* (*Triturus torosus*). *J. Exp. Zool.*, 125:415-446.
- MITCHELL, M. E. 1970. Treatment of hypophysectomized hens with partially purified avian FSH. *J. Reprod. Fertil.*, 22:233-241.
- MOLL, E. O. 1973. Latitudinal and interspecific variation in reproduction of the painted turtle *Chrysemys picta*. *Herpetologica*, 29:307-318.
- MOLL, E. O., AND J. M. LEGLER. 1971. The life history of a neotropical slider turtle, *Pseudemys scripta* (Schoepff) in Panama. *Sci. Bull. Nat. Hist. Mus., Los Angeles Co.* 11:1-102.
- MOON, Y. S., J. H. DORRINGTON, AND D. T. ARMSTRONG. 1975. Stimulatory action of follicle stimulating hormone on estradiol-17 β secretion by hypophysectomized rat ovaries in organ culture. *Endocrinology*, 97:244-247.
- MOORE, F. L. 1975. Spermatogenesis in larval *Ambystoma tigrinum*: positive and negative interactions of FSH and testosterone. *Gen. Comp. Endocrinol.*, 26:525-533.
- MORAT, M. 1969. Contribution à l'étude de l'activité Δ^5 -3 β -hydroxystéroïde-déshydrogénasique chez quelques reptiles du massif central. *Ann. Sta. Biol. Besse-en-Chandesse.*, 4:1-55.
- MOUS, J., B. PEETERS, F. HAUSTRAETE, AND W. ROMBAUTS. 1976. Synthesis of human placental lactogen in *Xenopus* oocytes. *Mol. Biol. Rpts.*, 2:407-412.
- MULLER, C. H. 1976. Steroidogenesis and spermatogenesis in the male bullfrog, *Rana catesbeiana*: regulation by purified bullfrog gonadotropins. Unpublished Ph.D. Thesis, Univ. California, Berkeley, 197 pp.
- . 1978a. Plasma 5 α -dihydrotestosterone and testosterone in the bullfrog, *Rana catesbeiana*: stimulation by bullfrog LH. *Gen. Comp. Endocrinol.*, (in press).
- . 1978b. *In vitro* stimulation of 5 α -dihydrotestosterone and testosterone secretion from bullfrog testis by nonmammalian and mammalian gonadotropins. *Gen. Comp. Endocrinol.*, (in press).
- MURTON, R. K. 1975. Ecological adaptation in avian reproductive physiology. *Symp. Zool. Soc. London*, 35:149-175.
- NAKAMURA, T., AND Y. TANABE. 1972. Pathways for androgen synthesis *in vitro* by the testes of Japanese quail (*Coturnix coturnix japonica*). *J. Endocrinol.*, 55:499-506.
- NALBANDOV, A. V. 1945. A study of prolactin on broodiness and on cock testes. *Endocrinology*, 36:251-258.
- NANDI, J. 1967. Comparative endocrinology of steroid hormones in vertebrates. *Amer. Zool.*, 7:115-134.
- N'DIAYE, A., D. SANDOZ, E. BOISVIEUX-ULRICH, AND R. OZON. 1974. Action des androgènes chez l'amphibien anoure *Discoglossus pictus* (Oth.) III. Effets de la castration et action des hormones androgènes sur les ultrastructures de la vésicule séminale. *Gen. Comp. Endocrinol.*, 24:286-304.
- NEAL, J., AND B. D. MURPHY. 1977. Response of immature, mature nonbreeding and mature breeding ferret testis to exogenous LH stimulation. *Biol. Reprod.*, 16:244-248.
- NEAVES, W. B. 1971. Intercellular bridges between follicle cells and oocyte in the lizard, *Anolis carolinensis*. *Anat. Rec.*, 170:285-302.

- . 1972. The passage of extracellular tracers through the follicular epithelium of lizard ovaries. *J. Exp. Zool.*, 179:339-364.
- . 1976. Structural characterization and rapid manual isolation of a reptilian testicular tunic rich in Leydig cells. *Anat. Rec.*, 186:553-564.
- NELSON, G. J. 1969. Origin and diversification of teleostean fishes. *Ann. New York Acad. Sci.*, 167:18-30.
- NICHOLLS, T. J., AND G. P. GRAHAM. 1972. The ultrastructure of lobule boundary cells and Leydig cell homologs in the testis of the cichlid fish, *Cichlasoma nigrofasciatum*. *Gen. Comp. Endocrinol.*, 19:133-147.
- O'HALLORAN, M. J., AND D. R. IDLER. 1970. Identification and distribution of the Leydig cell homologue in the testis of sexually mature Atlantic salmon (*Salmo salar*). *Gen. Comp. Endocrinol.*, 15:361-364.
- OLIVEREAU, M. 1976. Les cellules gonadotropes hypophysaires du saumon de l'atlantique: unicité ou dualité. *Gen. Comp. Endocrinol.*, 28:82-95.
- OLSSON, R. 1969. General review of the endocrinology of the protochordata and myxinoidea. *Gen. Comp. Endocrinol.*, suppl., 2:485-499.
- OTSUKA, S. 1956. Comparative studies on the pituitary hormones of vertebrates. IV. Effects of crude LH and FSH on the ovulation of hypophysectomized newts. *Endocrinol. Japonica*, 3:181-185.
- OZON, R. 1966. Isolement et identification de stéroïdes chez les Vertébrés inférieurs et les Oiseaux. *Ann. Biol. Anim. Biochim. Biophys.*, 6:537-551.
- . 1967. Synthèse, *in vitro*, des hormones stéroïdes dans le testicule et l'ovaire de l'Amphibien Urodèle *Pleurodeles waltlii* Michah. *Gen. Comp. Endocrinol.*, 8:214-227.
- . 1972a. Androgens in fishes, amphibians, reptiles and birds. Pp. 328-389, in *Steroids in nonmammalian vertebrates* (D. R. Idler, ed.), Academic Press, New York, xii+504 pp.
- . 1972b. Estrogens in fishes, amphibians, reptiles, and birds. Pp. 390-413, in *Steroids in nonmammalian vertebrates* (D. R. Idler, ed.), Academic Press, New York, xii+504 pp.
- OZON, R., AND H. BREUER. 1964. Untersuchungen über den Stoffwechsel von Steroidhormonen bei Vertebraten. IV. Aromatisierung von Testosteron zu Östrogenen im Ovar des Frosches (*Rana temporaria*). *Hoppe Seylers Z. Physiol. Chem.*, 337:61-65.
- OZON, R., AND C. STOCKER. 1974. Formation *in vitro* de 5 α -dihydrotestosterone par le testicule de *Discoglossus pictus*. *Gen. Comp. Endocrinol.*, 23:224-236.
- OZON, R., AND F. XAVIER. 1968. Biosynthèse *in vitro* des stéroïdes par l'ovaire de l'Anoure vivipare *Nectophrynoides occidentalis* au cours du cycle sexuel. *C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D*, 226:1173-1175.
- OZON, R., H. BREUER, AND B. P. LISBOA. 1964. Étude du métabolisme des hormones stéroïdes chez les Vertébrés inférieurs. III. Métabolisme *in vitro* de la testostérone par l'ovaire et le testicule de la grenouille *Rana temporaria*. *Gen. Comp. Endocrinol.*, 4:577-583.
- PACKARD, G. C., C. R. TRACY, AND J. J. ROTH. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. *Biol. Rev.*, 52:71-105.
- PALKA, Y. S., AND A. GORBMAN. 1973. Pituitary and testicular influenced sexual behavior in male frogs, *Rana pipiens*. *Gen. Comp. Endocrinol.*, 21:148-151.
- PAPKOFF, H., AND M. EKBLAD. 1970. Ovine follicle stimulating hormone: preparation and characterization of its subunits. *Biochem. Biophys. Res. Comm.*, 40:614-621.
- PAPKOFF, H., S. W. FARMER, AND P. LICHT. 1976a. Isolation and characterization of luteinizing hormone from amphibian (*Rana catesbeiana*) pituitaries. *Life Sci.*, 18:245-250.
- . 1976b. Isolation and characterization of follicle stimulating hormone and luteinizing hormone and its subunits from snapping turtle (*Chelydra serpentina*) pituitaries. *Endocrinology*, 98:767-777.
- PATZNER, R. A. 1975. Die fortschreitende Entwicklung und Reifung der Eier von *Myxine glutinosa* L. (Cyclostomata). Licht- und elektronenmikroskopische Untersuchungen. *Norwegian J. Zool.*, 23:111-120.

- PESONEN, S., AND J. RAPOLA. 1962. Observations on the metabolism of adrenal and gonadal steroids in *Xenopus laevis* and *Bufo bufo*. Gen. Comp. Endocrinol., 2:425-432.
- PETER, R. E. 1973. Neuroendocrinology of teleosts. Amer. Zool., 13:743-755.
- PICHERAL, M. B. 1966. Sur l'ultrastructure des mitochondries des cellules du tissu glandulaire du testicule de *Pleurodeles waltlii* Michah (Amphibian urodèle). C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D, 262:1769-1772.
- . 1968. Les tissus élaborateurs d'hormones stéroïdes chez les amphibiens urodèles. I. Ultrastructure des cellules du tissu glandulaire du testicule de *Pleurodeles waltlii* Michah. J. Microsc., 7:115-134.
- . 1970. Tissues secreting steroid hormones in urodele amphibians. IV. Electron and light microscopic studies of the glandular tissues of the testis and the interrenal gland in hypophysectomized *Pleurodeles waltlii* Michah. Z. Zellforsch. Mikrosk. Anat., 197:68-86.
- PICKERING, A. D. 1976a. Effects of gonadectomy, oestradiol and testosterone on the migrating river lamprey, *Lampetra fluviatilis* (L.). Gen. Comp. Endocrinol., 28:473-480.
- . 1976b. Stimulation of intestinal degeneration by oestradiol and testosterone implantation in the migrating river lamprey, *Lampetra fluviatilis* L. Gen. Comp. Endocrinol., 30:340-346.
- PICKERING, A. D., AND G. J. DOCKRAY. 1972. The effects of gonadectomy on osmoregulation in the migrating sea lamprey: *Lampetra fluviatilis* L. Comp. Biochem. Physiol., 41A:139-147.
- PICKFORD, G. E., AND J. W. ATZ. 1957. The physiology of the pituitary gland of fishes. New York Zoological Society, New York, 613 pp.
- PIERCE, J. G., M. R. FAITH, AND E. M. DONALDSON. 1976. Antibodies to reduced S-carboxymethylated alpha subunit of bovine luteinizing hormone and their application to study of the purification of gonadotropin from salmon (*Oncorhynchus tshawytscha*) pituitary glands. Gen. Comp. Endocrinol., 30:47-60.
- POLZONETTI-MAGNI, A., C. LUPO, R. K. RASTOGI, L. BELLINI-CARDELLINI, AND G. CHIEFFI. 1970. Estrogens in the plasma of females of *Rana esculenta* during the annual cycle and following ovariectomy. Gen. Comp. Endocrinol., 14:212-213.
- RAI, B. P. 1972. Gonadotropes in agnatha fishes and amphibia. Acta Anat., 81:42-52.
- RAO, G. S., H. BREUER, AND E. WITSCHI. 1969. *In vitro* conversion of 17α -hydroxyprogesterone to androstenedione by mashed gonads from metamorphic stages of *Xenopus laevis*. Gen. Comp. Endocrinol., 12:119-123.
- RASTOGI, R. K., L. IELA, P. K. SAXENA, AND G. CHIEFFI. 1976. The control of spermatogenesis in the green frog *Rana esculenta*. J. Exp. Zool., 196:151-166.
- REDSHAW, M. 1972. The hormonal control of the amphibian ovary. Amer. Zool., 12:289-306.
- REDSHAW, M. R., AND T. J. NICHOLLS. 1971. Oestrogen biosynthesis by ovarian tissue of the South African clawed toad *Xenopus laevis* Daudin. Gen. Comp. Endocrinol., 16:85-96.
- REINBOTH, R. 1972. Hormonal control of the teleost ovary. Amer. Zool., 12:307-324.
- . 1975. *In vitro* studies on steroid metabolism of testicular tissue in ambisexual teleost fish. J. Steroid Biochem., 6:341-344.
- RIVAROLA, M. A., C. A. SNIPES, AND C. J. MIGEON. 1968. Concentration of androgens in systemic plasma of rats, guinea pigs, salamanders, and pigeons. Endocrinology, 82:115-121.
- ROMER, A. S. 1967. Major steps in vertebrate evolution. Science, 158:1629-1637.
- ROOS, J., AND C. B. JØRGENSEN. 1974. Rates of disappearance from blood and biological potencies of mammalian gonadotropins (HCG and LH) in the toad *Bufo bufo* (L.). Gen. Comp. Endocrinol., 23:432-437.
- ROUY, S. 1972. Observations sur la structure du testicule adulte chez l'Urodèle pyrénéen *Euproctus asper* Duges et ses variations au cours du cycle sexuel chez une population de basse altitude. Bull. Soc. Zool. France, 97:653-675.

- . 1974. Observations cytologiques sur deux éléments caractéristiques de la structure du testicule multiple des amphibiens urodèles: Spermatogonies de réserve et épithélium columnaire. *Arch. Anat. Microsc.*, 63:183-202.
- RUSSELL, G. J., AND J. H. SUBAK-SHARPE. 1977. Similarity of the general design of protochordates and invertebrates. *Nature*, 266:533-536.
- SAGE, M., AND H. A. BERN. 1971. Cytophysiology of the teleost pituitary. *Int. Rev. Cytol.*, 31:339-368.
- SAIDAPUR, S. K., AND V. B. NADKARNI. 1973. Histochemical localization of Δ^5 - 3β -hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase in the testis of Indian skipper frog *Rana cynaophlyctis* (Schneider). *Gen. Comp. Endocrinol.*, 21:225-230.
- . 1974. Steroid synthesizing cellular sites in the amphibian ovary: a histochemical study. *Gen. Comp. Endocrinol.*, 22:459-462.
- SANDOR, T., S. SONEA, AND A. MEHDI. 1975. The possible role of steroids in evolution. *Amer. Zool.*, 15(suppl. 1):227-253.
- SAYLER, A., A. J. DOWD, AND A. WOLFSON. 1970. Influence of photoperiod on the localization of Δ^5 - 3β -hydroxysteroid dehydrogenase in the ovaries of maturing Japanese Quail. *Gen. Comp. Endocrinol.*, 15:20-30.
- SCANES, C. G., AND B. K. FOLLETT. 1972. Fractionation and assay of chicken pituitary hormones. *British J. Poult. Sci.*, 13:603-610.
- SCANES, C. G., B. K. FOLLETT, AND H. J. TH. GOOS. 1972a. Cross-reaction in a chicken LH radioimmunoassay with plasma and pituitary extracts from various species. *Gen. Comp. Endocrinol.*, 19:596-600.
- SCANES, C. G., S. DOBSON, B. K. FOLLETT, AND J. M. DODD. 1972b. Gonadotropic activity in the pituitary gland of the dogfish (*Scyliorhinus canicula*). *J. Endocrinol.*, 54:343-344.
- SCANES, C. G., P. J. SHARP, AND A. CHADWICK. 1977. Changes in plasma prolactin concentration during the ovulatory cycle of the chicken. *J. Endocrinol.*, 72:401-402.
- SCHAEFFER, B. 1969. Adaptive radiation of the fishes and the fish-amphibian transition. *Ann. New York Acad. Sci.*, 167:5-17.
- SCHATZ, F., AND G. MORRILL. 1975. 5β -reductive pathway of progesterone metabolism in the amphibian ovarian cytosol. *Biol. Reprod.*, 13:408-414.
- SCHEIB, D., AND K. HAFFEN. 1969. Apparition et localisation des hydroxystéroïdes déshydrogénases (Δ^5 - 3β et 17β) dans les gonades de la Caille (*Coturnix coturnix japonica*) et du Poulet (*Gallus gallus domesticus*). Étude histo-enzymologique comparative. *Gen. Comp. Endocrinol.*, 12:586-597.
- SCHMIDT, P. J., AND D. R. IDLER. 1962. Steroid hormones in the plasma of salmon at various stages of maturation. *Gen. Comp. Endocrinol.*, 2:204-214.
- SCHREIBMAN, M. P., J. F. LEATHERLAND, AND B. A. McKEOWN. 1973. Functional morphology of the teleost pituitary gland. *Amer. Zool.*, 13:719-742.
- SCHULZ, C. 1973. Veränderungen in der Morphologie der Leydigzellen von *Rana esculenta*. *Z. Zellforsch. Mikrosk. Anat.*, 142:367-386.
- SHAHABI, N. A., H. W. NORTON, AND A. V. NALBANDOV. 1975a. Steroid levels in follicles and plasma of hens during the ovulatory cycle. *Endocrinology*, 96:962-968.
- SHAHABI, N. A., J. M. BAHR, AND A. V. NALBANDOV. 1975b. Effect of LH injection on plasma and follicular steroids in the chicken. *Endocrinology*, 96:969-972.
- SHANI, J., B. J. A. FURR, AND I. A. FORSYTH. 1973. A reexamination of the antigonal effect of prolactin in the male fowl *Gallus domesticus*. *J. Reprod. Fert.*, 34:167-170.
- SHAPIRO, H. A., AND H. ZWARENSTEIN. 1934. A rapid test for pregnancy on *Xenopus laevis*. *Nature*, 133:339 and 762.
- SHODONO, M., T. NAKAMURA, Y. TANABE, AND K. WAKABAYASHI. 1975. Simultaneous determinations of oestradiol- 17β , progesterone and luteinizing hormone in the plasma during the ovulatory cycle of the hen. *Acta Endocrinol.*, 78:565-573.
- SIMON, N., AND R. REINBOTH. 1966. Juvenile Anuren als Testobjekt für gonadotrope Hormone. *Verh. Deutschen Zool. Ges. (Zool. Anz.)*, 30:254-264.

- SIMPSON, T. H., AND C. S. WARDLE. 1967. A seasonal cycle in the testis of the spurdog, *Squalus acanthias*, and the sites of 3-hydroxysteroid dehydrogenase activity. J. Mar. Biol. Assoc. United Kingdom, 47:699-708.
- SIMPSON, T. H., R. S. WRIGHT, AND S. V. HUNT. 1963. Sex hormones in fish. Part III: The oestrogens of *Scyliorhinus caniculus*. J. Endocrinol., 26:499-507.
- . 1964. Steroid biosynthesis in the testis of dogfish (*Squalus acanthias*). J. Endocrinol., 31:29-38.
- . 1968. Steroid biosynthesis *in vitro* by the ovary of the dogfish *Scyliorhinus caniculus* L. J. Endocrinol., 42:519-527.
- . 1969a. Biosynthesis of androgens by the testis of *Scyliorhinus caniculus*, identification in blood plasma. Gen. Comp. Endocrinol., 13:532.
- SIMPSON, T. H., R. S. WRIGHT, S. V. HUNT, AND C. S. WARDLE. 1969b. *In vitro* biosynthesis of steroids by the gonads of the lemon sole, *Microstomus kitt*. Gen. Comp. Endocrinol., 13:532.
- SMITH, C. L. 1955. Reproduction in female Amphibia. Mem. Soc. Endocrinol., 4:39-56.
- STANLEY, H., G. CHIEFFI, AND V. BOTTE. 1965. Histological and histochemical observations on the testis of *Gobius paganellus*. Z. Zellforsch. Mikrosk. Anat., 65:350-362.
- STEINBERGER, E. 1976. Biological actions of gonadotropins in the male. Pharmacol. Ther., 2:771-786.
- STENSIO, E. 1968. The cyclostomes with special references to the diphyletic origin of the Petromyzontida and Myxinoidea. Pp. 13-71, in Current problems of lower vertebrate phylogeny (T. Orvig, ed.), Wiley (Interscience), New York, 539 pp.
- STRAHAN, R. 1959. Pituitary hormones of *Myxine* and *Lampraea*. Trans. Asia Oceanic Reg. Congr., 1:24.
- SUNDARARAJ, B. I., AND S. V. GOSWAMI. 1969. Role of interrenal in luteinizing hormone-induced ovulation and spawning in the catfish, *Heteropneustes fossilis* (Bloch). Gen. Comp. Endocrinol., 8:403-416.
- SZARSKI, H. 1968. The origin of vertebrate foetal membranes. Evolution, 22:211-214.
- TADDEI, C. 1972. Significance of pyriform cells in ovarian follicle of *Lacerta sicula*. Exp. Cell. Res., 72:562-566.
- THORNTON, V. F. 1971. A bioassay for progesterone and gonadotropin based on the meiotic division of *Xenopus* oocytes *in vitro*. Gen. Comp. Endocrinol., 16:599-605.
- THORNTON, V. F., AND P. J. EVENNETT. 1973. Changes in the fine structure of the ovarian follicle of the toad (*Bufo bufo*) prior to induced ovulation. Gen. Comp. Endocrinol., 20:413-423.
- THORNTON, V. F., AND I. I. GESCHWIND. 1974. Hypothalamic control of gonadotropin release in Amphibia: evidence from studies of gonadotropin release *in vitro* and *in vivo*. Gen. Comp. Endocrinol., 23:294-301.
- TROTTIER, T. M., AND J. B. ARMSTRONG. 1975. Hormonal stimulation as an aid to artificial insemination in *Ambystoma mexicanum*. Canadian J. Zool., 53:171-173.
- TSO, E. C. F., AND B. LOFTS. 1977a. Seasonal changes in the newt, *Trituroides hongkongensis*, testis. I. A histological and histochemical study. Acta Zool. (Stockholm), 58:1-8.
- . 1977b. Seasonal changes in the newt, *Trituroides hongkongensis* testis. II. A ultrastructural study on the lobule boundary cell. Acta Zool. (Stockholm), 58:9-15.
- TSUI, H. W. 1976. Stimulation of androgen production by the lizard testis: site of action of ovine FSH and LH. Gen. Comp. Endocrinol., 28:386-394.
- TSUNEMI, K., A. URANO, AND H. KOBAYASHI. 1974. Monoamine oxidase and acetylcholine esterase in the neurohypophysis of the hagfish, *Eptatretus burgeri*. Gen. Comp. Endocrinol., 24:249-256.
- TURNER, C. D., AND J. T. BAGNARA. 1976. General Endocrinology. Sixth Edition. W. B. Saunders, Philadelphia, x+659 pp.
- UNGAR, F., R. GUNVILLE, B. I. SUNDARARAJ, AND S. V. GOSWAMI. 1977. Formation of 3α -hydroxy-5 β -pregnan-20-one in the ovaries of catfish, *Heteropneustes fossilis* (Bloch). Gen. Comp. Endocrinol., 31:53-59.

- UNSICKER, K. 1975. Fine structure of the male genital tract and kidney in the Anura *Xenopus laevis* Daudin, *Rana temporaria* L. under normal and experimental conditions. I. Testicular interstitial tissue and seminal efferent ducts. *Cell Tissue Res.*, 158:215-240.
- UPADHYAY, S. N., AND S. S. GURAYA. 1971. Histochemical observations on the interstitial tissue of fish testis. *Gen. Comp. Endocrinol.*, 16:504-510.
- URIST, M. R., AND A. O. SCHJEIDE. 1961. The partition of calcium and protein in the blood of oviparous vertebrates during estrus. *J. Gen. Physiol.*, 44:743-756.
- VAN DEN HURK, R., AND J. G. D. LAMBERT. 1977. Biosynthesis of 11β -hydroxy and 11 -ketoandrogens in the testis of the black molly *Poecilia* (= *Mollienisia*) *latipinna*. *Comp. Biochem. Physiol.*, 56B:117-118.
- VAN KEMENADE, J. A. M., AND W. J. VAN DOGEN. 1968. Seasonal changes in endocrine organs of the male common frog *Rana temporaria*. II. The interrenal tissue. *Z. Zellforsch. Mikrosk. Anat.*, 91:96-111.
- VAN OORDT, G. J., AND P. G. W. J. VAN OORDT. 1955. The regulation of spermatogenesis in the frog. *Mem. Soc. Endocrinol.*, 4:25-38.
- VAN OORDT, P. G. W. J. 1974. Cytology of the adenohypophysis. Pp. 53-106, in *Physiology of the Amphibia* (B. Lofts, ed.), Academic Press, New York, 2:xi+1-592 pp.
- VAN OORDT, P. G. W. J., AND F. BRANDS. 1970. The Sertoli cells in the testis of the common frog, *Rana temporaria*. *J. Endocrinol.*, 48:1.
- VAN OORDT, P. G. W. J., AND E. J. M. DE KORT. 1969. Functions of gonadotropins in adult Amphibia. La spécificité zoologique des hormones hypophysaires et de leurs activités. *Colloq. Int. Centre Nat. Rech. Sci.*, 177:345-350.
- VEITH, W. J. 1974. Reproductive biology of *Chamaelo pumilus pumilus* with special reference to the role of the corpus luteum and progesterone. *Zool. Africana*, 9:161-183.
- VELLANO, C. 1968. Alcuni dati istochimici sulla steroidogenesi nel testicolo di un anfibio urodelo. *Boll. Soc. Italiana Biol. Sper.*, 44:2062-2063.
- VELLANO, C., A. BONA, V. MAZZI, AND D. COLUCCI. 1974. The effect of synthetic luteinizing hormone releasing hormone on ovulation in the crested newt. *Gen. Comp. Endocrinol.*, 24:338-340.
- WADA, M., J. C. WINGFIELD, AND A. GORBMAN. 1976. Correlation between blood level and sexual behavior in male leopard frogs, *Rana pipiens*. *Gen. Comp. Endocrinol.*, 29:72-77.
- WALLIS, M. 1975. The molecular evolution of pituitary hormones. *Biol. Rev.*, 50:35-98.
- WALVIG, F. 1963. The gonads and the formation of the sexual cells. Pp. 530-580, in *The biology of myxine* (A. Brodal and R. Fänge, eds.), Universitetsforlaget, Oslo, Norway, xii+588 pp.
- WARD, D. N., L. E. REICHERT, W. K. LIU, H. S. NAHM, J. HSIA, W. N. LAMKIN, AND N. S. JONES. 1973. Chemical studies of luteinizing hormone from human and ovine pituitaries. *Rec. Prog. Horm. Res.*, 29:533-561.
- WEIL, C., B. BRETON, AND P. REINAUD. 1975. Étude de la réponse hypo-physaire à l'administration de Gn-RH exogène au cours du cycle reproducteur annuel chez la carpe, *Cyprinus carpio* L. *C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D*, 280:2469-2472.
- WENTWORTH, B. C., W. H. BURKE, AND G. P. BIRRENKOTT. 1976. A radioimmunoassay for turkey luteinizing hormone. *Gen. Comp. Endocrinol.*, 29:119-128.
- WERNER, Y. L., AND K. DROOK. 1967. The multipartite testis of the snake *Leptotyphlops phillipsi*. *Copeia*, 1967: 159-163.
- WIEBE, J. P. 1969. Steroid dehydrogenases and steroids in the gonads of the viviparous sea perch *Cymatogaster aggregata* Gibbons. *Gen. Comp. Endocrinol.*, 12:267-175.
- WILLMER, E. N. 1974. Nemertines as possible ancestors of the vertebrates. *Biol. Rev.*, 49:321-363.
- . 1975. The possible contribution of the nemertines to the problem of the phylogeny of the protochordates. *Symp. Zool. Soc. London*, 36:319-345.
- WILSON, S. C., AND P. J. SHARP. 1973. Variations in plasma LH levels during the ovulatory cycle of the hen, *Gallus domesticus*. *J. Reprod. Fert.*, 35:561-564.

- . 1975. Changes in plasma concentrations of luteinizing hormone after injection of progesterone at various times during the ovulatory cycle of the domestic hen (*Gallus domesticus*). J. Endocrinol., 67:59-70.
- . 1976a. Effects of androgens, oestrogens and deoxycorticosterone acetate on plasma concentrations of luteinizing hormone in laying hens. J. Endocrinol., 69:93-102.
- . 1976b. Induction of luteinizing hormone release by gonadal steroids in the ovariectomized domestic hen. J. Endocrinol., 71:87-98.
- WINGFIELD, J. C., AND D. S. FARNER. 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. Steroids, 26:311-327.
- WINGFIELD, J. C., AND A. S. GRIMM. 1976. Preliminary identification of plasma steroids in the plaice, *Pleuronectes platessa* L. Gen. Comp. Endocrinol., 29:78-83.
- . 1977. Seasonal changes in plasma cortisol, testosterone and oestradiol-17 β in the plaice, *Pleuronectes platessa* L. Gen. Comp. Endocrinol., 31:1-11.
- WOODS, J. E., AND L. V. DOMM. 1966. A histochemical identification of the androgen-producing cells in the gonads of the domestic fowl and albino rat. Gen. Comp. Endocrinol., 7:559-570.
- WOTIZ, H. H., C. BOTTICELLI, F. L. HISAW, AND L. RINGLER. 1958. Identification of estradiol-17 β from dogfish ova (*Squalus suckleyi*). J. Biol. Chem., 231:589-592.
- WOTIZ, H. H., C. R. BOTTICELLI, F. L. HISAW, JR., AND A. OLSEN. 1960. Estradiol-17 β , estrone and progesterone in the ovaries of dogfish (*Squalus suckleyi*). Proc. Nat. Acad. Sci., 46:580-583.
- YAMAZAKI, F., AND E. M. DONALDSON. 1968. The effects of partially purified salmon pituitary gonadotropin on spermatogenesis, vitellogenesis and ovulation in hypophysectomized goldfish (*Carassius auratus*). Gen. Comp. Endocrinol., 11:292-299.
- YARON, Z. 1966. Demonstration of 3-hydroxysteroid dehydrogenase in the testis of *Tilapia mossambica* (Cichlidae, Teleostei). J. Endocrinol., 34:127-128.
- . 1971. Observations on the granulosa cells of *Acanthobrama terraesanctae* and *Tilapia nilotica* (Teleostei). Gen. Comp. Endocrinol., 17:247-252.
- . 1972. Endocrine aspects of gestation in reptiles. Gen. Comp. Endocrinol., suppl., 3:663-674.

ROLE OF THE THYROID IN VERTEBRATE EVOLUTION

WILLIAM ETKIN AND YOUNG SO KIM

In the *Handbook of Physiology*, Etkin and Gona (1974) reviewed the literature through 1969 dealing with the evolution of thyroid function among lower vertebrates. The present review considers the basic concepts developed there in the light of studies published subsequently. What follows is a summary of pre-1970 concepts and an assessment of the impact later studies have had on earlier conclusions. However, rather than deal in detail with the technical literature as Etkin and Gona, we shall emphasize basic issues and seek an overall pattern in the evolution of thyroid function. References to be found in the 1974 review generally will not be repeated here.

The theoretic point of view developed by Etkin and Gona, around which the complex literature of the field was organized, may be summarized in this manner. There is evidence of a widespread presence of cellular mechanisms for specialized metabolism of iodine among tissues of chordates. This suggests that, in addition to an endocrine or signal-giving function, the thyroid gland may serve to store and distribute this anion and make it available for metabolic uses in other tissues. This may be the basis for its persistence in all vertebrates even though in some it seems to have minimal, if any, endocrine role. Persistence of the gland through the vertebrate series has made it possible for its products, the thyroid hormones, to have been incorporated in diverse and unrelated physiological adaptations as these arose in various lines of vertebrate evolution. In short, the thyroid performed a metabolic function continuously through vertebrate history, but it acquired many endocrine roles as independent specializations in different evolutionary lines.

Perhaps the distinction between metabolic and endocrine function as used here requires further clarification. Vitamins and micronutrients serve metabolic or constitutive functions in the sense that they are incorporated into functionally important chemicals and therefore are continuously essential for particular tissues. As these metabolic substances are generally available from the environment, they usually are not stored in specialized depots. However, in some instances (for example, vitamin D and calcium), they seem to be sequestered against periods of high demand. True hormones, on the other hand, function primarily as signal-giving or informational molecules. They activate receptor molecules thereby bringing about changes in cellular function that are adaptive to altering developmental, ecological, or behavioral requirements of the organism. Actions of the sex hormones in mammalian reproduction and of thyroid hormones in amphibian metamorphosis are among the most familiar examples of endocrine activity.

We thus propose the hypothesis that the thyroid gland has a dual role, metabolic and endocrine, in vertebrate evolution. It sequesters iodine, stor-

ing it in the colloid, and releases it primarily in the form of tri and tetraiodothyronine (T_3 and T_4). In this form the iodide anion can enter the cell and be released there by deiodination of T_3 - T_4 , a possibility suggested as essential to its function by Taurog (1974a). Within the cell, the anion may act either as a micronutrient, entering in some continuous metabolic function in mitochondria or elsewhere, or it may serve as a hormone by binding to a protein receptor (Oppenheimer *et al.*, 1976; Samuels *et al.*, 1974) and activating a portion of the genome. This theory regards the metabolic function as general throughout vertebrates and accounts for the presence of the gland in all evolutionary lines within the group. The endocrine function is seen as spasmodic, a recruitment of the thyroid products T_3 and T_4 for the timing of adaptive responses that arose independently in separate lines of evolution. This endocrine function is, of course, closely related to the governance of thyroid hormone release through the hypothalamic-pituitary-thyroid axis because this function requires a sensor to determine the requisite adjustments.

In our view, the value of this hypothesis lies in its ability to give structural coherence to the available information and to suggest approaches that may be tested experimentally. Two critical questions raised by this hypothesis, and on which we will concentrate our attention, are: 1) Is the special metabolism of iodine confined to thyroid cells? and 2) Is there a common basis for the endocrine actions of the thyroid hormones throughout the vertebrates?

IODINE METABOLISM IN RELATION TO THE ORIGIN OF THE THYROID

Textbooks of vertebrate evolution derive the thyroid gland from the endostylar organ found in urochordates and cephalochordates and the comparable subpharyngeal gland of the ammocoete larva of the lamprey. These organs, like the vertebrate thyroid, represent an evagination of the midventral floor of the pharynx. In ammocoetes, cells from the subpharyngeal gland form the thyroid follicles during the transformation of the larva to the adult. Consequently, when the central role of iodine in thyroid function was opened to detailed comparative study with the introduction of radioiodine-tracer technique (Gorbman and Evans, 1941), many workers were quick to show that endostylar cells metabolize iodine in the characteristic thyroid pattern. Special tracts of cells in the endostyle of tunicates and amphioxus concentrate this ion. In ammocoetes, these are the cells that give rise to the thyroid follicles of the adult (Wright and Youson, 1976). Indeed, much or all of the biochemical pathway characteristic of the metabolism of iodine by the thyroid was found in these antecedents of the gland. A recent study by Dunn (1975) concludes, however, that though concentration and binding of iodine of tyrosines occurs in the tunicate endostyle the final coupling of tyrosines to form the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) does not appear until the cyclostomes.

Although iodine studies clearly support the homology of some cells of the prevertebrate endostyle with those of the thyroid gland, they also have revealed a surprisingly widespread capacity of other tissues in these animals to metabolize iodine in a comparable manner. In fact, in tunicates and amphioxus many tissues such as notochord, branchial sac, tunic, gonads and blood, concentrate far more of the radioiodine supplied than does the endostyle. Many reports indicate the presence of much or even the complete sequence of iodine binding to the formation of T_3 and T_4 in these tissues (see 1974 review and Suzuki and Kondo, 1971). It would appear, therefore, that even though the thyroid cells may have evolved from endostylar components the basic capacity to pump atoms and incorporate them into organic components was a widespread capacity in prevertebrate tissues and was not unique to the thyroid precursor tissues.

Among marine invertebrates, iodine binding to proteins is found among algae, corals, mollusks, and others. Often it is associated with hard tissues and has been thought to have a role in effecting the cross-linkage of collagen in such tissues. In many lower vertebrates, iodine binding to tyrosine and proteins is common in notochord, ovaries, and pigment cells. The epithelium of parts of the digestive tract in vertebrates generally has a highly efficient mechanism for pumping iodine to and from the gut (Shaham and Lewitus, 1971).

Iodine is secreted by salivary and mammary glands though not in organic form. Soluble proteins *in vitro* can be iodized and T_3 and T_4 formed under appropriate conditions (Underwood, 1971). In thyroidectomized rats and amphibian larvae, elemental iodine or salts may replace thyroid hormone action to some extent (Chapman *et al.*, 1944; Morton *et al.*, 1943).

It has long been known that thyroid hormones are extensively deiodinated in many vertebrate tissues. The iodine thus released is strongly conserved by an enterohepatic circulation, being secreted by the liver into the bile and reabsorbed from the gut (Ashley and Frieden, 1972; Robinson and Galton, 1976). As an inference from studies of the deiodination of thyroid hormones, Taurog (1974a) suggested the possibility that the essential function of the thyroid hormones may be to introduce this element into the cell through the membrane which they, but not the iodide ion, can penetrate.

It would appear reasonable to speculate from the extensive capacity of vertebrate tissues to pump and metabolize iodine that this anion may play a significant role in physiology by acting in a metabolic role. To our knowledge, no definitive evidence for such a role has been adduced (Underwood, 1971). It may, indeed, be that its role in the thyroid hormones in mammals has obscured any metabolic function it may have as a micronutrient.

In summary, the evidence published in recent years supports the concept of a widespread participation of iodine in the metabolism of vertebrate tissues. To this extent our original suggestion remains viable. The ubiquity of the thyroid gland among vertebrates can be best understood in terms of a role in sequestering and storing iodine and making it available for some cel-

lular function as yet unknown. In that case, its endocrine role in different groups may have arisen independently in various lines of evolution. We may now examine the two principal areas of acknowledged endocrine action of the gland to ascertain whether or not they differ in fundamental mechanisms. If so, the notion of independent origin for these endocrine functions in evolution would be supported.

THYROID AND METABOLIC RATE

The increase in metabolic rate measured as oxygen consumption or heat production under basal conditions long has been recognized as the most characteristic action of thyroid hormones in mammals. Basal conditions in the intact animal imply a minimum of physical work, unstimulated resting conditions for digestive, circulatory, nervous, respiratory, and muscular systems. Many tissues isolated from animals previously injected with thyroid hormone show increased oxygen consumption, the principal exception being the central nervous system of the adult. Quantitatively, the muscles and liver contribute most to the overall result in the intact animal. Tissues from normal or hypothyroid animals generally do not respond when treated by thyroid hormones *in vitro* (however, see Tsai and Chen, 1976). *In vivo* there is a considerable delay, usually 12 to 40 hours, before the effects of hormone injection become effective. This suggests, as is to be expected, that the increase in metabolic rate is the result of a complex of changes in tissue and organ activities. Many of these, such as tachycardia, increased irritability, and growth, generally are regarded as derivative of or related to the effect on metabolic rate. The effect of thyroid action in birds, the other homeothermal group of vertebrates, appears to be very similar to that in mammals, although there are less data available.

The basic biochemical changes induced by thyroid action in responsive mammalian tissues have been much sought after and many have been described. Changes in mitochondria and mitochondrial enzymes have been identified but the concept that mitochondria are the primary seat of thyroid action has not won widespread acceptance. In recent years as a result of work of Edelman and his collaborators (Ismael-Beigi and Edelman, 1971; Edelman and Ismael-Beigi, 1974), a primary source of calorogenic action of thyroid hormones has been pinpointed as a stimulation of a membrane NaK-ATPase in the liver and muscle cell membrane. This enzyme, which is specifically inhibited by ouabain, has been shown to respond under physiological conditions by increased pumping of the sodium ion thereby accounting for the increase of oxygen consumption of the cells.

The recognition that the calorogenic influence of the thyroid is the primary adaptational function of the gland in homeotherms comes from studies of the activation of the thyroid in mammals under exposure to low environmental temperatures. Laboratory rats exposed to cold (4°C) initiate increased heat production by shivering but within a few days their thyroids are activated and the level of hormone in the blood rises. Eventually other

mechanisms take over and the level of thyroid hormone in the blood drops. This thyroid response seems to be a general one in mammals, although less apparent in man under mild cold stress (Rall 1974).

This signal-giving response arises from temperature sensors in the hypothalamus and operates by way of a hypothalamic thyrotropin releasing factor (TRF). This activates the release of thyrotropin by the pituitary, which produces the consequent activation of the thyroid. It has been shown recently (Guernsey and Stevens, 1977) that in the rat the response on exposure to cold is ouabain sensitive and operates through the NaK-ATPase system. Our present understanding of thyroid function in mammals is that the hormones T_3 and T_4 act primarily to increase the rate of metabolism of many tissues. This action serves an adaptive role in acclimation to low environmental temperatures and perhaps on other occasions. Thyroid function is, therefore, viewed as an essential component in the evolution of homeothermism in mammals and birds.

If the thyroid hormones serve as informational molecules with a similar action throughout the course of vertebrate evolution, we would expect that their influence on metabolic rate would be a general characteristic of the gland. Because amphibian metamorphosis is recognized as a response to thyroid hormone, it would be expected that the level of thyroid activity could be inferred from the increase in metabolic rate during this process. In testing this idea we were surprised that no such increase was found (Etkin 1934). An examination of the published literature showed that in previous studies the contrary conclusion had been based upon the oxygen consumption per unit wet weight. In normal metamorphosis and under thyroid treatment, tadpoles lose considerable weight. However, this weight loss consists almost entirely of water and intestinal contents (Etkin 1932). It could be seen that the older interpretation of an increase in metabolic rate in thyroid treated larvae was unjustified because it did not vary per unit protein. Our results clearly showed that the rate did not change per individual animal during normal metamorphosis; neither did it change during prometamorphosis when the animal continued to grow under the influence of low thyroid levels nor during climax when it underwent desiccation and intestinal evacuation at a time when the thyroid was at maximal activity. We were led, therefore, to doubt the common interpretation that thyroidization increases metabolic rate in these poikilothermic animals. We went on to test this negative view on fish and found that neither in goldfish nor in toadfish injected with thyroxin could an increase in metabolic rate be demonstrated, despite the views espoused in older literature (Root and Etkin, 1937; Etkin *et al.* 1940). Subsequent literature brought confirmatory evidence in regard to metamorphosis (Fletcher and Myant, 1959) and both confirmatory and contradictory evidence for various other poikilotherms. We felt justified, on reviewing the evidence to 1969, in favoring the view that thyroid plays no role in regulating metabolic rate in poikilotherms. We pointed out, however, that an exception might be seen in some reptiles where studies by Wil-

hoft (1966) clearly indicated an increase in oxygen consumption when the animals were treated with thyroid while maintained in a temperature gradient permitting them to position themselves at relatively high temperatures. Maher (1967) reported increases at higher but not lower temperatures in toads and frogs. We suggested in our earlier review that this phenomenon deserves more investigation because it presents a possible physiological change related to the origin of the homeothermal condition. We may now ask what the evidence published after 1969 adds to our knowledge of this problem.

A considerable number of studies published in the 1970s have reported thyroidal influence on oxygen consumption both *in vivo* and *in vitro* in amphibians and reptiles when the animals were tested at higher but not at lower temperatures (Turner and Tipton, 1972; Packard and Packard, 1973, 1975; Packard *et al.*, 1974; Packard, 1976; Thaphyal *et al.*, 1975; May and Packer, 1976). Contrary evidence was reported by Walker (1973). To these studies we must add work done on the neonatal rat in which the homeostatic mechanisms are known to be poorly developed. Such rats were found to respond to thyroid treatment when maintained at 35° but not at 29.5° (Hemon 1966). Relevant at this point also is the finding of Valcana and Timiras (1969) that brain tissue of infant rats does respond by increased metabolic rate to thyroid treatment though that of the adult does not. They also reported reduced NaK-ATPase activity after thyroidectomy in the brains of these rats.

These results compel us to reevaluate our concepts and accept the notion that in terrestrial poikilotherms, reptiles and adult amphibians, thyroidization of the animals leads to increased metabolic rate but only if the animals were kept at high environmental temperatures, that is temperatures from 25° to 32°C. At temperatures near 15° to 20° the different studies agree no effect of thyroid is found.

From the standpoint of evolution, we may note that most aquatic habitats are cooler than 25°C. It is only as vertebrates developed terrestrial forms that they found themselves often exposed to environments where temperatures are maintained continuously above this level. Because of the high specific heat of water, it would be extremely difficult for an animal with gills to conserve heat sufficiently to permit the evolution of the homeothermal condition. Just when in reptilian evolution homeothermism developed is unknown. It is possible that the reptilian lines that gave rise independently to birds and mammals (and perhaps to homeothermal dinosaurs) carried the preadaptive capacity to respond to thyroid activation by increases in metabolism into terrestrial habitats. By parallel evolution of hypothalamic temperature sensors, these lines of vertebrates could have incorporated this capacity into the development of homeothermal mechanisms. Under external cold, the system would be activated so that increased heat would be produced before body temperature dropped.

In this view, however, the capacity of the thyroid to raise the metabolic rate at higher temperatures in poikilotherms must be regarded as a coincidental preadaptation of lower vertebrates because it comes into play not, as in homeotherms, when they are exposed to cold but only at higher temperatures. A physiologic role for the high temperature response in poikilotherms might possibly be seen in the "sunning" behavior common in lizards and bullfrog tadpoles (Bogert 1959). In any case, we know of only one study, that of Lagerspetz *et al.* (1974) that suggests, indirectly, that the metabolic role of the thyroid in a poikilotherm may be related to temperature acclimation. It has long been known that thyroid action in amphibian metamorphosis is quite ineffective at temperatures below 10°C (Frieden *et al.* 1965, Norris and Platt 1974). Though a sharply rising temperature coefficient for thyroid action is clearly indicated in all animals by such data, we know of no detailed exploration of this problem in either its metabolic rate or metamorphic action aspects.

MODE OF ACTION OF THYROID HORMONES

The methods made available by modern molecular biology have been exploited extensively in the last ten years in analyzing the mode of action of thyroid hormones. As would be expected, the most favorable subjects have been rats, in which the action of hormones on liver cells has been examined, and tadpoles, in which the effect of hormones on various tissues during metamorphosis have been studied. A number of workers have described proteins that bind T_3 or T_4 specifically in a saturatable fashion indicating that they act as receptors for these hormones. Such proteins have been described in the cytosol and nucleus of rat liver cells (for literature see Samuels *et al.*, 1974; Oppenheimer *et al.*, 1976). In metamorphosing tadpoles, an activation of the genetic mechanism following nuclear binding of thyroid hormones, including the origin of new RNAs by transcription and new proteins by translation, has been shown by a number of workers (Cohen, 1970; Tata, 1970; Bennett *et al.*, 1970; Solursh and Gorbman, 1971; Eaton and Frieden, 1971; Tata, 1974; Kistler *et al.*, 1975; Yoshizato *et al.*, 1975). Wang and Frieden (1973) found reason to doubt a causal connection between new protein synthesis (cathepsin C) and tissue resorption. Although the evidence is not clear in detail, it appears likely that, in both rat and tadpole, thyroid acts by activating the genome through nuclear receptor proteins.

This may suggest a common basis for thyroid action at the molecular level in rats and tadpoles, but from an evolutionary viewpoint this evidence can hardly be interpreted as significant of common origin. Genome activation must be viewed as a basic mode of cellular action common to all cells and, therefore, not indicative of particular lines of evolution (Etkin and Livingston, 1947). Significant evidence of evolutionary connection between physiological processes must be sought in processes distinctive to different groups, that is, in modes of action following gene activation. For that rea-

son, we regard the question of the action of the sodium pump mechanism in mammalian liver cells as a better indication of an evolutionary connection between modes of thyroid action in different groups. Packard (1976) has tested the ouabain sensitivity of the response of tadpole liver cells to thyroxine, but found no effect comparable to that seen in the rat liver. This must be taken as suggestive of different and, therefore, independently evolved mechanisms of response in mammals and tadpoles. Another approach to this problem is suggested by the study of later events in the metamorphic response of tadpole tissues. Kim *et al.* (1977) have shown that tadpole tailfin tissue prior to undergoing reduction *in vitro* releases a diffusible substance that rapidly induces shrinkage in adjacent untreated tissue. A comparison of such later events in thyroid action may prove helpful in tracing common elements in the action of the hormones in different groups. However, we do not believe that the evidence now available justified an interpretation for or against a common basis for thyroid action among different vertebrate groups.

CONTROL OF THE THYROID

A comparison of the control of the activity of the thyroid gland among vertebrates may shed light upon the question of the roles played by that gland in vertebrate evolution. In accordance with the view proposed here, a signal-giving or endocrine function presupposes a control mechanism sensitive to physiological demands from the environment or from within the body whereas a purely sequestering function for the gland does not. For example, the control of thyroid activity by the hypothalamic-pituitary-thyroid axis, as this is understood in mammalian physiology, clearly implies signal-giving function. A sensor mechanism in the hypothalamus monitors the core temperature of the body and activates or restrains thyroid activity by the production rate of thyrotropin releasing factor (TRF). This controls the pituitary production of thyrotropin (TSH), which, in turn, regulates the rate of thyroid hormone production. A negative feedback of thyroid hormone on TSH production serves to maintain a steady state in the system appropriate to current demands. This has been called the "thyrostat action" (Reichlin *et al.*, 1972). The thyrostat can be set at different levels according to circumstances. The mechanism regulating thyroid activity in the metamorphosis of the tadpole appears to be entirely parallel to that of mammals involving a TRF-TSH-thyroid pathway with inhibitory feedback (Etkin, 1970). However, in the tadpole the link connecting the hypothalamus to the pituitary, which consists of the median eminence and the pituitary-portal blood vessels, depends for its development upon a positive feedback action whereby thyroid hormone stimulates its differentiation and thus initiates metamorphosis (Etkin, 1963). Reichlin *et al.* (1972) confirmed positive feedback during metamorphosis by determination of TRF in tadpole brain. They also reported increased TRF in rat brain after thyroidization. The parallelisms between the two systems so wide apart in vertebrate evolution suggest that

the basic control mechanisms for thyroid function may be common to all vertebrates and indicative of a common informational function throughout the group. We may now consider to what extent that inference is supported by more recent evidence.

Pituitary control of thyroid activity appears to be lacking in the cyclostomes (Larsen and Rosenkilde, 1971; Pickering, 1972, 1976) although some indication of response to TSH was reported in the hagfish (Kerkof *et al.*, 1972). In the higher vertebrate groups investigated, TSH control of the thyroid appears at least in some species of most groups (Buckingham, 1970; Chan and Eales, 1976). However, related species within a group may show differences in the degree of thyroid dependence upon the pituitary (Rosenkilde, 1972; Compher and Dent, 1973; Taurog, 1974b; Regard, 1974). The presence of the hypothalamic-pituitary-thyroid axis also is indicated by effectiveness of goitrogens (Subhedar and Rao, 1973). Variability further characterizes the role of the hypothalamus in TSH control. In frogs, a hypothalamic thyrotropic center in the hypothalamus was identified by Vandesande and Dierick (1971), but Peter (1970) found hypothalamic control to be inhibitory in the goldfish. Mammalian TRF, which has been identified chemically and synthesized, appears not to be effective as a releaser of TSH in any of the lower vertebrates studied though it is present in their brains. This has been reported by Etkin and Gona (1968) and Gona and Gona (1974) in the tadpole, by Taurog *et al.* (1974) in a salamander, by Vandesande and Aspeslagh (1974) in the adult frog and Gorbman and Hyder (1973) in lungfish. We can only conclude from the scanty knowledge available that even though the same basic elements of control are operative throughout the vertebrate groups above cyclostomes, considerable variation in detail occurs suggesting that the mammalian pattern is not a necessary condition for thyroid control.

With regard to the positive feedback action of thyroid hormones on the development of the median eminence in tadpoles, supporting evidence in neotenic salamanders has been adduced by Norris and Gern (1976). They reported that thyroid doses without effect elsewhere induced metamorphosis when injected into the hypothalamus. On the other hand, Van Oordt *et al.* (1972) found positive feedback with respect to differentiation of hypothalamic neurones in tadpoles and concluded that secretory activity of these neurones is inhibited by thyroid feedback. Dodd and Dodd (1976) questioned the importance of a positive feedback relation in tadpole metamorphosis, offering an alternative explanation based on the development of thyroid receptors during prometamorphosis. In the rat, the hypothalamic control mechanism on the pituitary develops during the first few postnatal days of life (Florsheim, 1974). A study by Kikuyama and Etkin (1964) failed to find any influence of the level of thyroid activity in the rat fetus and neonate on the differentiation of the median eminence. Apparently positive thyroid feedback, present in the tadpole, is absent in the rat.

AN EVOLUTIONARY HYPOTHESIS

We do not believe the present evidence allows a clear interpretation of the evolution of thyroid control. We offer the following suggestions as a tentative theory, consistent with the concept of multiple roles for the thyroid. A hypothalamic-pituitary connection was present in the earliest vertebrates. It presumably functioned in regulating reproduction or other activities but not the thyroid. The thyroid functioned purely in sequestering and binding iodine organically as in the protochordates and not as an endocrine organ. In the evolution of fish, the hypothalamic-pituitary mechanism developed a thyroid controlling activity coordinating it in an endocrine role with particular environmental demands, especially relating to aspects of reproduction (Sage, 1973). Each evolutionary line that developed such a true endocrine (signal-giving) function may have evolved its own TRF and TSH. In this way, the thyroid may have been drawn into adaptations involving reproduction, migration, development, and other specializations in different lines of evolution although in some it remained limited to its sequestering function.

In the amphibians, a primary adaptational change to which the thyroid became associated was the development of metamorphosis. This permitted the specialization of the larva for fresh-water habitats while the adult evolved terrestrial mechanisms (Etkin, 1970). As a mechanism for activation of the thyroid in controlling metamorphosis, a positive feedback response of thyroid hormone on hypothalamic development was evolved. This appears to be unique to amphibians. On the other hand, the necessary thyrotropin was developed from some previously existing glycoprotein hormone. Negative feedback control of TSH was evolved to cut off the spurt of thyroid activity after metamorphosis and to maintain any adult endocrine function, such as the control of molting, where this evolved as a terrestrial adaptation.

In mammals, the thyroid was drawn into the homeothermal mechanism as that evolved. This participation may have been founded upon the capacity of the thyroid to raise the metabolic rate of tissues in animals exposed to higher temperatures. Although this capacity in itself was of doubtful utility in aquatic poikilotherms, it was preadaptive for the fully terrestrial reptiles that were exposed to higher environmental temperatures. If that evolutionary process began in the stem reptiles, it would be expected to have evolved in parallel paths in several reptilian lines, including those leading to birds and mammals.

Of course, such a speculative reconstruction founded as it is on scanty and uncertain evidence cannot be expected to last long under the probing of energetic investigators. But that is where the excitement of comparative endocrinology lies, in the vastness of the unexplored realms it offers. It was our ambition in this presentation to stimulate new approaches to experimental analyses based on the basic issues of evolutionary thinking.

LITERATURE CITED

- ASHLEY, H., AND E. FRIEDEN. 1972. Metabolism and distribution of triiodothyronine and thyronine in the bullfrog tadpole. *Gen. Comp. Endocrinol.*, 18:22-31.
- BENNETT, T. P., J. S. GLENN, AND H. SHELDON. 1970. Changes in the fine structure of tadpole (*Rana catesbeiana*) liver during thyronine-induced metamorphosis. *Develop. Biol.*, 22:232-248.
- BOGERT, E. 1959. How reptiles regulate temperature. *Sci. American*, 200:105-111.
- BUCKINGHAM, M. 1970. Effect of TSH and thiourea upon thyroid functions in the lizard (*Xantus hinshawii*). *Gen. Comp. Endocrinol.*, 14:178-183.
- CHAN, H., AND J. G. EALES. 1976. Influence of bovine TSH on plasma thyronine levels and thyroid functions in brook trout (*Salvelinus fontinalis*). *Gen. Comp. Endocrinol.*, 28:461-472.
- CHAPMAN, A., G. HIGGINS, AND F. MANN. 1944. Additional studies of the extra thyroidal metabolism of iodine. *J. Endocrinol.*, 3:392-396.
- COHEN, P. P. 1970. Biochemical differentiation during amphibian metamorphosis. *Science*, 168:533-543.
- COMPHER, M. K., AND J. DENT. 1973. Effect of hypothalamic extirpations on thyroid and intermedial activity in the red spotted newt. *Gen. Comp. Endocrinol.*, 20:29-40.
- DODD, M., AND J. DODD. 1976. The biology of metamorphosis. Pp. 467-599, in *Physiology of the Amphibia* (B. Lofts, ed.), Academic Press, New York, 3:xiv+ 1-644.
- DUNN, A. 1975. Iodine metabolism in the ascidian, *Molgula manhattensis*. *Gen. Comp. Endocrinol.*, 25:83-95.
- EATON, S., AND E. FRIEDEN. 1971. Triiodothyronine-induced RNA synthesis and turnover in bullfrog tadpole liver. Pp. 240-260, in *Hormones in development* (M. Hamburgh and E. Barrington, eds.), Appleton Century Crofts, New York, xvii+854 pp.
- EDELMAN, I., AND F. ISMAEL-BEIGI. 1974. Thyroid thermogenesis and active sodium transport. *Rec. Prog. Hormone Res.*, 30:235-254.
- ETKIN, W. 1932. The phenomena of anuran metamorphosis. I. Growth and resorption phenomena in anuran metamorphosis. *Physiol. Zool.*, 5:275-300.
- . 1934. The phenomena of anuran metamorphosis. II. Oxygen consumption during normal metamorphosis. *Physiol. Zool.*, 7:129-148.
- . 1963. The metamorphosis activating system of the frog. *Science*, 139:810-814.
- . 1970. The endocrine mechanism of amphibian metamorphosis, an evolutionary achievement. Pp. 137-155, in *Hormones and the environment* (G. K. Benson and J. G. Phillips, eds.), The University Press, Cambridge, England, xvi+ 629 pp.
- ETKIN, W., AND A. GONA. 1968. Failure of mammalian thyrotropin releasing factor preparation to elicit metamorphic response in tadpoles. *Endocrinology*, 82:1067-1068.
- . 1974. Evolution of thyroid function in poikilothermic vertebrates. Pp. 5-20, in *Handbook of physiology*, Section 7, Endocrinology (R. O. Greep and E. B. Astwood, section eds.), Thyroid (M. A. Greer and D. H. Solomon, volume eds.), Amer. Physiol. Soc., Washington, D.C., 3:vii+1-491 pp.
- ETKIN, W., AND L. LIVINGSTON. 1947. A probability interpretation of the concept of homology. *Amer. Nat.*, 81:468-473.
- ETKIN, W., R. W. ROOT, AND B. P. MOFSHIN. 1940. The effect of thyroid feeding on oxygen consumption of the goldfish. *Physiol. Zool.*, 13:415-429.
- FLETCHER, K., AND N. B. MYANT. 1959. Oxygen consumption of tadpoles during metamorphosis. *J. Physiol. (London)*, 145:353-368.
- FLORSHEIM, W. 1974. Control of thyrotropin secretion. Pp. 449-467, in *Handbook of physiology*, Section 7, Endocrinology (R. O. Greep and E. B. Astwood, section eds.), The pituitary gland and its neuroendocrine control, Pt. 1 (E. Knobil and W. H. Sawyer, volume eds.), Amer. Physiol. Soc., Washington, D.C. 4:v+ 1-601 pp.
- FRIEDEN, E., A. WAHLBORG, AND E. HOWARD. 1965. Temperature control of the response of tadpoles to triiodothyronine. *Nature*, 205:1173-1176.

- GONA, A., AND O. GONA. 1974. Failure of synthetic TRF to elicit metamorphosis in frog tadpoles or spotted newts. *Gen. Comp. Endocrinol.*, 24:223-225.
- GORBMAN, A., AND H. EVANS. 1941. Correlation of histological differentiation with beginning of function of developing thyroid gland of frog. *Proc. Soc. Exp. Biol. Med.*, 47:103-106.
- GORBMAN, A., AND M. HYDER. 1973. Failure of TRH to stimulate thyroid function in the lungfish. *Gen. Comp. Endocrinol.*, 20:588-589.
- GUERNSEY, D., AND E. STEVENS. 1977. The cell membrane sodium pump as a mechanism for increasing thermogenesis during cold acclimation in rats. *Science*, 196:908-910.
- HEMON, P. 1966. Effect de la thyrosine sur la consommation d'oxygène du rat blanc au cours du développement post-natal. II. Importance de la température externe. *J. Physiol. (Paris)*, 58:40-48.
- ISMAEL-BEIGI, F., AND I. S. EDELMAN. 1971. The mechanism of the calorogenic action of thyroid hormone stimulation of Na^+K^+ -activated adenosinetriphosphatase activity. *J. Gen. Physiol.*, 57:710-722.
- KERKOF, P., D. BOSCHWITZ, AND A. GORBMAN. 1972. The response of hagfish thyroid tissue to thyroid inhibitors and to mammalian thyroid stimulatory hormone. *Gen. Comp. Endocrinol.*, 21:231-240.
- KIKUYAMA, S., AND W. ETKIN. 1964. Thyroid influence on the development of hypothalamic-hypophyseal system in the rat. *Amer. Zool.*, 4:326.
- KIM, Y., M. HAMBURGH, H. FRANKFORT, AND W. ETKIN. 1977. Reduction in the latent period of the response to thyroxine by tadpole tail discs fused to discs pretreated with thyroxine. *Devel. Biol.*, 55:387-391.
- KISTLER, A., K. YOSHIZATO, AND E. FRIEDEN. 1975. Binding of thyroxine and triiodothyronine by nuclei of isolated tadpole liver cells. *Endocrinology*, 97:1036-1042.
- LAGERSPEZ, K., N. HARRI, AND R. OKSLAHTI. 1974. Role of thyroid in temperature acclimation of the oxidative metabolism in the frog. *Gen. Comp. Endocrinol.*, 22:169-176.
- LARSEN, L. O., AND P. ROSENKILDE. 1971. Iodine metabolism in normal, hypophysectomized and thyrotropin-treated river lampreys, *Lamprocyba fluviatilis* L. (Cyclostomata). *Gen. Comp. Endocrinol.*, 17:94-104.
- MAHER, M. J. 1967. Response to thyroxine as a function of environmental temperature in the toad, *Bufo woodhousii* and the frog, *Rana pipiens*. *Copeia*, 2:261-365.
- MAY, T., AND R. PACKER. 1976. Thyroid hormones stimulate in vivo oxygen consumption of adult *Rana pipiens berlandieri* at high environmental temperatures. *Gen. Comp. Endocrinol.*, 30:525-527.
- MORTON, M., T. CHAIKOFF, W. REINHARDT, AND E. ANDERSON. 1943. The formation of thyroxine and di-iodothyrosine by the completely thyroidectomized animal. *J. Biol. Chem.*, 147:357-165.
- NORRIS, D., AND W. GERN. 1976. Thyronine-induced activation of hypothalamo-hypophyseal axis in neotenic salamander larvae. *Science*, 194:525-526.
- NORRIS, D. O., AND S. PLATT. 1974. T_3 and T_4 induced rates of metamorphosis in immature and sexually mature larvae of *A. tigrinum*. *J. Exp. Zool.*, 189:303-310.
- OPPENHEIMER, J. H., H. SCHWARTZ, M. SURKS, D. KOERNER, AND W. DILLMAN. 1976. Nuclear receptors and the initiation of thyroid hormone action. *Recent Prog. Hormone Res.*, 32:529-565.
- PACKARD, G. 1976. Thyroxine-induced calorogenesis in frog liver: insensitivity to ouabain. *Gen. Comp. Endocrinol.*, 28:334-338.
- PACKARD, G., AND M. PACKARD. 1973. Preliminary study of the influence of thyroxine, temperature and sex on oxygen uptake to tissues from the spadefoot toad (*Scaphiopus bombifrons*). *Gen. Comp. Endocrinol.*, 20:530-533.
- . 1975. The influence of acclimation temperature on the metabolic response of frog tissue to thyroxine administered in vivo. *Gen. Comp. Endocrinol.*, 27:162-168.
- PACKARD, G. C., M. J. PACKARD, AND R. K. STIVERSON. 1974. The influence of thyroxine on oxygen consumption of tissues from the frog, *Rana pipiens*. *Gen. Comp. Endocrinol.*, 22:195-198.

- PETER, R. E. 1970. Inhibitory hypothalamic control of thyroid gland activity in the goldfish, *Carassius auratus*. Pp. 127-139, in *Aspects of neuroendocrinology* (W. Bargmann and B. Scharer, eds.), Springer-Verlag, Berlin, xi+380 pp.
- PICKERING, A. 1972. Effects of hypophysectomy on the activity of the endostyle and thyroid gland in larvae and adult river lamprey, *Lampetra fluviatilis* L. *Gen. Comp. Endocrinol.*, 18:335-343.
- . 1976. Iodide uptake by the isolated thyroid gland of river lamprey, *Lampetra fluviatilis* L. *Gen. Comp. Endocrinol.*, 28:358-364.
- RALL, J. E. 1974. The role of the thyroid in endocrine control mechanisms. *Perspec. Biol. Med.*, 17:218-226.
- REGARD, E. 1974. Étude de la glande thyroïde de l'Axolotl, *Ambystoma mexicanum*. *Gen. Comp. Endocrinol.*, 23:191-211.
- REICHLIN, S., S. MARTIN, M. MITNICK, R. BOSHANS, V. GRIMM, J. BALLINGER, J. GORDON, AND J. MALACARA. 1972. The hypothalamus in pituitary-thyroid regulation. *Recent Prog. in Hormone Res.*, 28:229-286.
- ROBINSON, H., AND V. GALTON. 1976. Studies on the deiodination of thyroid hormones in *Xenopus laevis* tadpoles. *Gen. Comp. Endocrinol.*, 30:83-90.
- ROOT, R. W., AND W. ETKIN. 1937. Effect of thyroxine on oxygen consumption of the toadfish. *Proc. Soc. Exp. Biol. Med.*, 37:174-175.
- ROSENKILDE, P. 1972. Hypothalamic control of thyroid function in Amphibia. *Gen. Comp. Endocrinol.*, suppl. 3:32-40.
- SAGE, M. 1973. The evolution of thyroidal function in fishes. *Amer. Zool.*, 13:899-905.
- SAMUELS, H. H., J. S. TSAI, AND J. CASANOVA. 1974. Thyroid hormone: in vitro demonstration of putative receptors in isolated nuclei and soluble nuclear extracts. *Science*, 184:1188-1191.
- SHAHAM, T., AND Z. LEWITUS. 1971. Radio-iodine metabolism in the thyroidal gland of the lizard, *Agama stalloi*. *Gen. Comp. Endocrinol.*, 17:142-148.
- SOLURSH, M., AND A. GORBMAN. 1971. The role of transcriptional events in tissue-specific responses to thyroxine in induced anuran metamorphosis. Pp. 219-231, in *Hormones in development* (M. Hambrugh and E. Barrington, eds.), Appleton Century Crofts, New York, xvii+854 pp.
- SUBHEDAR, N., AND P. D. PRASADA RAO. 1973. Effects of some antithyroid drugs on the corpuscles of Stannius and thyroid gland of the catfish, *Heteropneustes fossilis* (Block). *Gen. Comp. Endocrinol.*, 26:327-333.
- SUZUKI, S., AND Y. KONDO. 1971. Demonstration of thyroglobulin-like iodinated proteins in the branchial sac of tunicates. *Gen. Comp. Endocrinol.*, 17:402-406.
- TATA, J. R. 1970. Simultaneous acquisition of metamorphic response and hormone binding of *Xenopus* larvae. *Nature*, 227:686-689.
- . 1974. Growth and developmental action of thyroid hormones at the cellular level. Pp. 469-478, in *Handbook of physiology*, Section 7, Endocrinology (R. O. Greep and E. B. Astwood, section eds.), Thyroid (M. A. Greer and D. H. Solomon, volume eds.), Amer. Physiol. Soc., Washington, D.C., 3:vii+1-491 pp.
- TAUROG, A. 1974a. Biosynthesis of iodoamino acids. Pp. 101-133, in *Handbook of physiology*, Section 7, Endocrinology (R. O. Greep and E. B. Astwood, section eds.), Thyroid (M. A. Greer and D. H. Solomon, volume eds.), Amer. Physiol. Soc., Washington, D.C., 3:vii+1-491 pp.
- . 1974b. Effect of TSH and long-acting thyroid stimulator on thyroid I—metabolism and metamorphosis of the Mexican axolotl, *Ambystoma mexicanum*. *Gen. Comp. Endocrinol.*, 24:257-266.
- TAUROG, A., C. OLIVER, R. ESKAY, J. PORTER, AND J. MCKENZIE. 1974. The role of TRH in the neoteny of the Mexican axolotl, *Ambystoma mexicanum*. *Gen. Comp. Endocrinol.*, 24:267-279.
- THAPHYAL, J. P., P. S. KUMAR, AND O. V. OOMMEN. 1975. Variations in thyroid and respiratory rate during a 24-hour period and role of testosterone and thyroxine on oxidative metabolism of the water snake, *Natrix piscator*. *Gen. Comp. Endocrinol.*, 26:100-106.

- TSAI, J., AND A. CHEN. 1976. Thyroid hormones: effect of physiological concentrations on cultured cardiac cells. *Science*, 194:202-204.
- TURNER, J., AND S. TIPTON. 1972. Metabolic response to temperature acclimation and T_4 in the water snake. *Gen. Comp. Endocrinol.*, 18:98-101.
- UNDERWOOD, E. J. 1971. Trace elements in human and animal nutrition. Academic Press, New York, xii+545 pp.
- VALCANA, T., AND P. S. TIMIRAS. 1969. Effect of hypothyroidism on ionic metabolism and Na-K activated ATP phosphorhydrolase activity in the developing rat brain. *J. Neurochem.*, 16:935.
- VANDESANDE, F., AND M. ASPESLAGH. 1974. Failure of thyrotropin releasing hormone to increase I uptake by the thyroid in *Rana temporaria*. *Gen. Comp. Endocrinol.*, 23: 355-356.
- VANDESANDE, F., AND K. DIERICKX. 1971. Experimental identification of a hypothalamic thyrotrope center in *Rana temporaria*. *Acta Endocrinol.*, 155(suppl.):33.
- VAN OORDT, P. G. W. J., H. J. T. GOOS, J. PEUTE, AND M. TERLOU. 1972. Hypothalamohypophysial relations in amphibian larvae. *Gen. Comp. Endocrinol.*, suppl. 3:41-50.
- WALKER, R. 1973. Temperature effects on thyroid function in the lizard, *Sceloporus cyanogenys*. *Gen. Comp. Endocrinol.*, 20:137-143.
- WANG, V., AND E. FRIEDEN. 1973. Changes in cathepsin C activity during spontaneous and induced metamorphosis of the bullfrog. *Gen. Comp. Endocrinol.*, 21:381-389.
- WILHOFT, D. C. 1966. The metabolic response to thyroxine of lizards maintained in a thermal gradient. *Gen. Comp. Endocrinol.*, 7:445-451.
- WRIGHT, G., AND J. YOUSON. 1976. Transformation of the endostyle of the anadromous sea lamprey, *Petromyzon marinus* L., during metamorphosis. *Gen. Comp. Endocrinol.*, 30:243-257.
- YOSHIZATO, K., A. KISTLER, AND E. FRIEDEN. 1975. Binding of thyroid hormones by nuclei of cells from bullfrog tadpole tail fins. *Endocrinology*, 97:1030-1035.

EVOLUTION OF THE GASTROINTESTINAL ENDOCRINE SYSTEM

SUSAN VAN NOORDEN, JULIA M. POLAK, AND A. G. E. PEARSE

It has been 75 years since the discovery of the first hormone, secretin (Bayliss and Starling, 1902), which marked the beginning of gastrointestinal endocrinology. We now know that there are at least 15 different hormone-like peptides in the mammalian gastroenteropancreatic system and yet we are only beginning to understand the complexity of the nervous and hormonal interactions that control it. Knowledge of the system in lower vertebrates is even less, but comparative studies are gradually filling the gaps. This paper is a survey of what is currently known about the evolution of the gastrointestinal endocrine system and reveals the vast areas open for future research. [Since this review was compiled much new information has become available. For a more recent survey the reader is referred to S. Van Noorden and S. Falkmer. Gut-islet endocrinology—some evolutionary aspects. Invest. Cell Pathol., in press.]

The endocrine pancreas must be considered at the same time as the gastrointestinal tract both on physiological and morphological grounds. Physiologically, the endocrine and exocrine secretions of the pancreas influence and are influenced by secretions from the rest of the gut. Morphologically, the embryonic development of the mammalian and avian pancreas proceeds by an evagination of the primitive gut epithelium; the budding tissue develops into exocrine pancreas containing zymogen cells and endocrine pancreatic islets with their various hormones. The early embryonic source of the pancreatic endocrine cells is still a matter for discussion, but it has been shown that both insulin and glucagon are present in the avian dorsal pancreatic rudiment at the beginning of the third day of development (Dieterlen-Lièvre and Beaupain, 1974; Beaupain and Dieterlen-Lièvre, 1974).

In this field as in many others, cyclostomes have attracted much attention and have been the object of considerable research on account of their key position in vertebrate phylogeny. These creatures, unlike all other vertebrates, have endocrine "islets" but no separate exocrine pancreas—the zymogen cells are scattered along the gut epithelium (Barrington, 1945). On the principle that ontogeny recapitulates phylogeny, it is satisfactory to find that the islet tissue can be seen budding out from the intestine and bile duct epithelium in the larval lamprey (Titlbach and Kern, 1969) and from the bile duct in the hagfish, which has no larval stage (Östberg *et al.*, 1976). In an even more primitive animal, *Amphioxus* (*Branchiostoma*) *lanceolatus*, an insulin-like substance seems to be produced by cells within the epithelium of the anterior intestine that apparently never reach the stage of forming a separate organ (Van Noorden and Pearse, 1976). It is thus evident that the pancreas and intestine cannot be considered separately for they form part of

a complex gastroenteropancreatic system and have many hormones in common.

Hormone-like peptides of the gastroenteropancreatic (GEP) system are produced in cells either scattered along the gut epithelium or grouped into islets in the pancreas. The cells are of the same general type throughout the chordates and similar ones also can be found among the invertebrates (Fritsch and Sprang, 1977). The cells are classified as either "open," resting on the basement membrane of the gut epithelium and extending into the lumen with microvilli or some other "taste receptor," or "closed," basally situated and presumably receiving local or blood-borne stimuli (Fujita, 1976). Under an electron microscope, GEP endocrine cells can be seen to contain electron-dense secretory granules, which in any one species are typical in size and shape for the particular peptide produced by the cell.

An hypothesis linking peptide hormone-producing cells with the nervous system was put forward in 1968 (Pearse, 1968). It was based on the presence of certain cytochemical and ultrastructural characteristics in both peptide-secreting endocrine cells and particular types of nerve cells. These characteristics included a high content of esterase, or cholinesterase, and α -glycerophosphate dehydrogenase, and either the presence of a biogenic amine or the ability to take up and decarboxylate the amino acid precursors of such amines. An acronym, APUD, was coined from this last property (Amine Precursor Uptake and Decarboxylation), and the cells producing peptide hormones came to be known as the APUD series. It was suggested that the properties these cells had in common, regardless of their position in the body, were due to a common embryological derivation from a cell of neural origin, perhaps from the neural crest. The theory, which was subsequently modified to include specialized neural or placodal ectoderm as well as the definitive neural crest (Pearse and Takor Takor, 1976), has received support from some recent discoveries. A number of peptides have been found in both the brain or nervous system and the gut where they are localized in typical epithelial endocrine cells. The peptides that make up this rapidly growing group are Vasoactive Intestinal Peptide (VIP), Substance P, Somatostatin, Neurotensin, Bombesin, Enkephalin, and Cholecystokinin (CCK). It is possible that these compounds act as neurotransmitters, or neuromodulators in the nervous system and as local or circulating hormones in the gut. We already know that many of these newly-discovered "dual" peptides are present in lower vertebrates in at least one of these systems. They thus can be regarded as "phylogenetically old". Because many of them also are capable of powerful actions on gut processes in mammals, they certainly ought to be considered in the context of the evolution of the GEP endocrine system. A classical hormonal status has not been assigned yet to some of these peptides, which are at present thought to have a local influence (paracrine) rather than an effect on a distant target (endocrine). This would correspond with their supposed neural origin. The modern form of the APUD concept regards peptide hormone-producing cells as a third division of the

nervous system with activities slower in onset but of longer duration than those of the nerve cells.

Diffuse GEP endocrine cell distribution is presumably advantageous for the continuous monitoring and timing of the various processes of digestion and absorption, but it has made it impossible to apply conventional methods for studying an endocrine organ (for example, by observing the effects of extirpation). It has also been difficult to extract and purify hormones of the gut because of their low concentration and susceptibility to proteolytic enzymes. Until recently, the only way of correlating a particular cell type with a secretory product was by inference from the known distribution of the hormone and possibly by association of the cell type with a particular pathological condition. The great breakthrough in the study of the gastrointestinal hormones came with the discovery by Mutt and his colleagues at the Karolinska Institute that the gut peptides are heat-stable. They developed new techniques for the isolation and purification of gut peptides using large quantities of tissue that were boiled to inactivate the proteases. Secretin was purified and sequenced in the 1960s (Jorpes and Mutt, 1970), followed by CCK (Mutt and Jorpes, 1968); in the 1970s several new peptides were assayed, VIP (Said and Mutt, 1972), Gastric Inhibitory Peptide (GIP) (Brown *et al.*, 1970), and motilin (Brown *et al.*, 1971).

Great advances were then made in elucidation of the molecular structure and amino acid sequences of the purified peptides. Synthetic substances could be made and structure/activity relationships worked out. The availability of pure hormones allowed the production of specific antibodies and the development of radioimmunoassays. It was then possible to study the normal physiology of the gut hormones in man and experimental animals and their involvement in pathological conditions. Antibodies could be used also for immunocytochemical localization of the hormones within their cells of origin, using techniques based on the original fluorescent antibody method of Coons (Coons *et al.*, 1955). In many instances, we can now confidently assign a peptide in the mammalian gut to a particular cell type although there are still some peptides in search of a cellular source and some unassigned cell types. One of the problems of immunostaining methods has been finding a suitable fixative to preserve the antigenicity of the peptide and the structure of the tissue. An important advance has been made in the use of new cross-linking reagents (p-benzoquinone and diethylpyrocarbonate) for the fixation of freeze-dried tissue in the vapor phase (Pearse and Polak, 1975). This preserves the antigenicity of several gut hormonal peptides, which is lost after formalin fixation, and allows many hormones to be immunostained in a single, small tissue sample. Another innovation has been the use of 1-micron resin-embedded sections so that in adjacent sections immunostained for different hormones the same cells can be identified (Polak *et al.*, 1975b). Immunostained sections also can be compared with their adjacent ultra thin sections conventionally stained for electron microscopy or even immunostained using either peroxidase-labeled

antibody or the more sensitive unlabeled antibody/peroxidase-anti-peroxidase method of Sternberger (Sternberger, 1974). The only disadvantage of immunocytochemistry at the electron microscopical level is that current methods of fixation, which preserve the antigenicity of the peptide, are sub-optimal for ultrastructural morphology.

These methods have been developed for mammals, where most of the work has been done, but it has been found that antibodies to mammalian hormones can be used to identify related substances in nonmammalian vertebrates. Immunological cross-reactivity can take place when the structure of a hormone has remained stable during the course of evolution. An antibody to mammalian insulin, for example, will cross-react with lamprey insulin in spite of the 500 million years since the cyclostomes diverged from the main line of vertebrate evolution (Van Noorden *et al.*, 1972).

Hagfish insulin has been well studied because of the key position cyclostomes occupy in vertebrate phylogeny. The molecule is identical to mammalian insulin in 20 of its 53 amino acids, and these invariant residues seem to be the same in all species examined (Peterson *et al.*, 1974; Steiner *et al.*, 1973). They are also the residues that are essential for biological activity and, in view of the cross immunoreactivity, are presumably the ones containing the antigenic sites. Little is known of the factors conferring antigenicity on hormone molecules except that the sites are not necessarily those required for biological activity. Structure/activity studies involving controlled chemical splitting of the molecule and the determination of the biological activity and receptor binding capacity of the different sequences compared with the whole molecule are of greatest importance in understanding of the way in which hormones work and how they have evolved. For a complete and ideal understanding, these studies should be carried out for every hormone in every animal. Admittedly this is not very realistic, but fortunately some hormones have retained adequate antigenicity so that we can carry out interspecific immunological cross-reactions and end the era of purely descriptive anatomy and histology.

It should now be possible for us to begin to fill in the speculations of the physiologists and morphologists with information on the endocrine cell types and the peptides they contain. It must be pointed out, however, that immunostaining and radioimmunoassay are not infallible. Species specificity and evolutionary alteration of the structure of a peptide may prevent a reaction so that a negative result means very little. Although a properly controlled positive result can be trusted, interclass cross-reaction and even interspecific cross-reaction is often weak when compared with results obtained using homologous antiserum. An antiserum to bovine insulin was used at a dilution of 1/1000 to stain mammalian pancreatic islets but had to be used at 1/50 to stain lamprey islets. It is obviously better if the animal's own hormones can be extracted and purified, but if cross-reactivity occurs it is more convenient to use an available antibody.

It must be remembered that immunological cross-reactivity in itself does not necessarily give any information about the role of the immunostained

hormone in its native species, which may be far removed on the evolutionary scale from the donor of the hormone antigen. The mammalian bias has naturally prompted workers to seek the same actions for similar molecules in different species. Although this may be a good starting point for investigations, it is now suggested that hormone function may have evolved separately from hormone structure through modification of the target organ response and cellular receptor sites. Prolactin, for example, is osmoregulatory in fishes and lactogenic in mammals (Nicoll, 1974).

Comparative studies on the GEP hormones of lower vertebrates are still a new venture. Most of the information available comes from bioassays of extracts with a few histological and electron microscopical studies. Only recently have radioimmunoassay and immunostaining techniques been employed.

HORMONES

Hormones of the mammalian gut and pancreas can be divided into various groups, which overlap to some extent.

Hormone families.—There are two groups of gut hormones that are considered related because of similarities of molecular structure. Similar structure suggests that they have all arisen from a common ancestral molecule. One of these groups contains glucagon, VIP, secretin, gastric inhibitory peptide (GIP), and probably another glucagon-like substance known as glucagon-like immunoreactive peptide or enteroglucagon. The other group contains gastrin, CCK, and motilin.

Peptides common to gut and nervous system.—There are some hormones found not only in the gut but also in the brain or nervous system. These hormones are CCK, VIP, somatostatin, neurotensin, bombesin-like activity, enkephalin, and substance P.

Peptides related to amphibian skin peptides.—Many biologically active peptides have been isolated from the skin of various amphibian species (Erspamer and Melchiorri, 1973) and have been shown to have counterparts in the mammalian gut. This group consists of gastrin and CCK (related to caerulein-like peptides), substance P (related to physalmine-like peptides), bombesin-like activity, and neurotensin (resembling Xenopsin) (Araki *et al.*, 1975; Carraway and Leeman, 1975).

Non-gut relationships.—Some of the gut hormones resemble peptides or proteins from other parts of the body. For example, insulin apparently resembles nerve growth factor in some structural aspects (Frazier *et al.*, 1972). Enkephalin is, in fact, a sequence of amino acids contained in the β -LPH molecule, and neurotensin has some structural and biological resemblance to LH-RH and vasopressin.

Molecular evolution of hormones.—The finding that there are "families" of peptides with structural resemblances and overlapping properties in the gut has given rise to the theory that peptides in a family all evolved from an ancestral molecule (Adelson, 1971; Weinstein, 1972). The gene responsible for the ancestral peptide is assumed to have undergone duplication and

mutation so that two slightly different forms of the peptide would be present. Natural selection would ensure that one form would retain its original role while the other would continue to change by mutation and perhaps acquire a different selectional advantage by becoming associated with a particular event or the emergence of a new target organ.

The existence of two slightly different forms of insulin in some species (rat, teleost fish; Smith, 1966) shows that this process of evolution is probably continuous.

A consequence of this theory would be that examination of animals lower than mammals on the evolutionary scale might provide evidence of molecules related to the ancestral forms of modern mammalian hormones. This in fact appears to be the case. We shall see that the study of the hormones of lower vertebrates gives some clues as to the evolutionary progress of the hormones and the pathway of evolution of the vertebrates themselves.

HORMONE FAMILIES

Gastrin Family

Gastrin and CCK occur in mammals in a variety of molecular forms that differ in chain length. Those that are biologically active contain the C-terminal pentapeptide sequence. This sequence is also present in the caeruleins and phyllocaeruleins from amphibian skin (Anastasi *et al.*, 1968). All these molecules also show a tyrosine residue at position 6 (gastrin) or 7 (CCK, caeruleins) from the C-terminal. Structure/activity relationships have been worked out for mammalian CCK and gastrin (Mutt and Jorpes, 1968; Gregory and Tracy, 1975). It has been shown that a tyrosine molecule at position 7 from the C-terminal confers the CCK-like activity with action on the gall bladder and exocrine pancreas and that a tyrosine molecule at position 6 implies gastrin-like action, promoting the secretion of acid from the stomach.

Antibodies tend to be formed to the C-terminal sequence. Inasmuch as this sequence is shared by the three peptides, immunostaining and radioimmunoassay cannot distinguish between CCK and gastrin unless antibodies to an unshared fragment of the molecules are used (Polak *et al.*, 1977b). The use of bioassays of extracts in combination with immunoreactivity has provided most information in nonmammalian vertebrates.

Some speculations on the evolution of gastrin and CCK can be made by considering data obtained on the primitive cyclostomes. Barrington and Dockray (1970) showed that an extract of lamprey intestine had CCK-like action when injected into the rat for it caused enzyme secretion from the pancreas and contraction of the gall bladder. Subsequently, it was shown that cells in the lamprey (Van Noorden and Pearse, 1974) and hagfish intestine (Östberg *et al.*, 1976) could be immunostained with antibodies to caerulein and gastrin. Lamprey intestinal extract has now been analyzed further, and it has been shown that the fraction responsible for the CCK-like activity resembles gastrin rather than CCK in its immunological properties and

cross-reacts with antisera specific for the C-terminal of the molecule. The extract nevertheless has CCK-like activity rather than gastrin-like activity. It is possible that cyclostomes produce a precursor of the later-evolved gastrin and CCK molecules (Dockray *et al.*, 1977).

Cyclostomes would have no need for gastrin as such because they lack a stomach and acid-producing cells (Barrington, 1972). On the other hand, they do have a gall bladder, and its contraction could conveniently be stimulated by a CCK-like hormone released from the gut in response to nutrients. However, recent experimental work by S. Vigna (personal communication, 1977) indicates that the gall bladder of the Pacific hagfish, *Eptatretus stouti*, cannot be made to contract either by mammalian CCK or by its own intestinal extract, but this extract will promote contraction of a guinea pig gall bladder. The physiological role of the CCK-like substance of cyclostomes is still in question.

The need for a stimulator of enzyme secretion from the zymogen cells is even less obvious for the cells are part of the intestinal epithelium and could receive stimuli directly from the gut lumen. However, the association of CCK with the zymogen cells could have arisen later at the stage when zymogen cells leave the gut to become a separate exocrine pancreas (Dockray, 1977).

Gastrin and CCK have been identified as separate hormones only in mammals. Gastrin-like and CCK-like activities have been found in a variety of nonmammalian vertebrate gastrointestinal tracts, but it is not known whether true gastrin and CCK exist outside the mammals, or whether these activities are due to one or more different substances possibly resembling caerulein. In amphibians, caerulein, which resembles CCK rather than gastrin in having a tyrosine residue at position 7 from the C-terminal instead of position 6, was found to be 1000 to 10,000 times more effective than mammalian gastrin and 25 times more effective than CCK in evoking acid secretion from the isolated stomach of *Rana catesbiana* (Negri and Erspamer, 1973; Davidson *et al.*, 1970). However, gastrin-like immunoreactivity with C-terminal specificity and molecular sizes corresponding to human gastrin has been shown in extracts of antrum and duodenum from three amphibian species (Gibson *et al.*, 1976). The mechanisms of acid stimulation in nonmammalian vertebrates have not been studied extensively, but in amphibians (Lechago *et al.*, 1978), birds (Erspamer *et al.*, 1978), and mammals (Walsh and Holmquist, 1976; Polak *et al.*, 1976) the question has been complicated by the discovery of gastrointestinal bombesin or bombesin-like immunoreactivity. Bombesin is apparently a pH-independent acid stimulator that may act via gastrin stimulation (Bertaccini *et al.*, 1974). Another complication, which has not been investigated, is that one hormone may act weakly on its own but can be enhanced by another hormone, as in the case of secretin and CCK (Solomon *et al.*, 1977).

Among the fishes, gastrin-like activity has been extracted from elasmobranch gastric mucosa (Hansen, 1975) and has been identified by immuno-

staining in the pyloric appendages of a teleost (Falkmer *et al.*, 1977). CCK-like activity has been found in intestinal extracts from a variety of fishes (Barrington and Dockray, 1972; Nilsson, 1970). Porcine gastrin, however, had no effect on acid secretion from isolated dogfish stomach although histamine did (Hogben, 1966). Dockray (1977) has suggested that acid secretion in fishes, as in amphibians (Burhol and Hirschowitz, 1971) and birds (Angelucci and Linari, 1970), may be stimulated by CCK-like peptides rather than gastrin-like peptides.

Gastrin-like activity has been identified even in the intestine and serum of some molluscan species (Straus *et al.*, 1975), but this is as yet an isolated study on invertebrates. Gregory (1966) noted that mammalian gastrin stimulated the secretion of an acid defense fluid from another species of mollusc.

In the nervous system, gastrin-like immunoreactivity was reported in a variety of vertebrates (dog, pigeon, frog, trout; Vanderhaegen *et al.*, 1975), but a more recent study suggests that this activity in mammals may be CCK-like rather than gastrin-like (Dockray, 1976). The functions of these nervous system peptides, their relationships with similar gut peptides, and their occurrence in the central nervous system of non-mammalian vertebrates have yet to be explored.

From the little we know of the gastrin and CCK-like hormones in the animal kingdom (which is actually more than we know about most of the other hormones of the GEP system), it seems that the original molecule might have been structurally like gastrin but with activity resembling that of CCK, possibly with a tyrosine residue at position 7 from the C-terminal. A gastrin-like function probably evolved together with an acid-secreting stomach, but the separation into two distinct hormones may not have occurred until quite late in evolution.

GLUCAGON FAMILY

The hormones glucagon, VIP, secretin, and GIP in mammals have structural resemblances and to some extent overlap in activity. Thus, all can act as stimulants to insulin secretion and can inhibit acid production by the stomach. VIP, GIP, and glucagon can stimulate intestinal juice flow. VIP is the only hormone of the four that is found unequivocally in both the central nervous system and gut and pancreatic islet cells. It also has other actions on gastrointestinal muscle and the cardiovascular system (see Pearse *et al.*, 1977 for review).

The main action of glucagon appears to be hyperglycemic, whereas that of secretin appears to be to promote secretion of alkaline pancreatic juice. It has been suggested recently that the primary role of GIP in mammals is to stimulate insulin release in response to food (Bloom and Polak, 1977). The physiological role of VIP is unknown, but in view of the association of this peptide with pancreatic tumours of the Verner-Morrison syndrome (watery diarrhea, hypokalemia, achlorhydria), one of its physiological actions in the gut may be to promote secretion of intestinal juice (Bloom and Polak, 1973,

1977). Information is trickling in about these peptides in lower vertebrates. Although we cannot yet say which are closest to the ancestral form, VIP and glucagon or substances related to them are the likeliest candidates.

Glucagon.—Glucagon-like activity has been found in protochordates and each chordate class, by extraction or immunostaining (Falkmer, 1975). It has even been described among invertebrates in the intestine of a crayfish (Maier *et al.*, 1975) and in the corpus cardiacum of an insect (Tager *et al.*, 1976), but there is, as yet, no report of the actions of these substances in their native species.

With regard to the cyclostomes, it has been known for some time that the islet tissues of *Myxine* and *Lampetra* do not contain glucagon even though glucagon-like immunoreactivity has been found in the gut epithelium of both the lamprey and the hagfish. The activity appears restricted to apparently endocrine cells with microvilli at the gut lumen and secretory granules at the base (Van Noorden and Pearse, 1974; Östberg *et al.*, 1976). Preliminary results on radioimmunoassay of glucagon-like material from the hagfish showed it resembled somewhat both mammalian gut glucagon and pancreatic glucagon (Bataille, personal communication 1976). It is known that injection of mammalian glucagon in the hagfish does not produce hyperglycemia and neither does isletectomy (Falkmer and Matty, 1966), although a rise in blood sugar was noted after total isletectomy in the lamprey, *L. fluviatilis* (Hardisty *et al.*, 1975). It seems that the structure and activity of the cyclostome glucagon-like material is somewhat different from that of the islet glucagon of higher vertebrates. It may bear greater resemblance to the mammalian intestinal glucagon-like activity, which has not yet been ascribed a function.

A further point of interest concerning the cyclostome glucagon-like activity is that immunostaining indicates it is also present in the same cell as the CCK-like activity. This is unusual for two peptides as dissimilar in their structure as glucagon and CCK. In mammals, several instances are known in which two peptides are secreted by the same cell, but generally the peptides either share molecular sequences or are obviously derived from the same molecule [LH and FSH in the pituitary gland and also the family of peptides that can be derived from the β -LPH molecule, β -LPH itself, ACTH, MSH, and endorphin (Polak *et al.*, 1978b)].

As mentioned above, an endocrine cell may secrete an amine as well as a peptide hormone (for example, motilin and 5-HT), and it has been found recently that other nervous system hormones or transmitters may be present with peptides in the same cell. In the rat thyroid gland, for example, some of the calcitonin-producing cells synthesize both somatostatin and calcitonin according to immunostaining results (Van Noorden *et al.*, 1977). Furthermore, production of enkephalin, a brain peptide with opiate activity, has been ascribed tentatively to the gastrin-producing G cells of the antrum, from which a large amount of enkephalin can be extracted (Polak *et al.*, 1977c).

It is possible that the lamprey and hagfish endocrine cells that exhibit CCK and glucagon-like activity represent the increasingly familiar situation of a nervous system hormone and a gut hormone in the same cell; both probably arose from the neuroectoderm. Studies of lamprey brain peptides may clarify this.

Another plausible explanation was suggested earlier by Weinstein's (1972) analysis of the similarities in amino acid sequences between the various hormonal polypeptides. He aligned the molecules to show the greatest similarities and found that the sequences of the gastrin family began where the sequences of the glucagon family ended. He postulated a "protoprothormone" consisting of a secretin-like sequence attached to a gastrin-like sequence, which he called "pro sec gas." This large peptide would have existed at some evolutionary stage and would have become split into two portions, one going on to develop the characteristics of the secretin family and the other of the gastrin family. We would prefer to call this hypothetical molecule "pro gluc CCK" in view of what we now know about the gut hormone-like activity of cyclostomes. It is possible that in cyclostomes we have a situation where the primitive molecule itself is still present—one end reacting with antisera to glucagon and the other with antisera to gastrin. Or, as is perhaps more likely, we may find that cyclostomes are at the stage where the original molecule has split into two, and the two portions with their specific activity are both contained in the same cell (Dockray, 1977). Further evolution might then separate the two activities into different cell types that respond to specific stimuli. At present all this is speculative but maybe in another ten years we shall be nearer the truth. The state of localization of gut hormone immunoreactivity is not yet known for any other cold-blooded vertebrates.

In support of the "protoprothormone" theory is the observation that many of the better investigated gut hormones of mammals (insulin, glucagon, gastrin) seem to be formed in the cell as precursors that are subsequently split by enzyme action to form the active smaller molecules to be secreted (Tager and Steiner, 1974).

There is no information on the presence of enteroglucagon in animals other than mammals. Glucagon has been localized by immunohistochemistry in the intestine of chicken and quail (Polak *et al.*, 1974a) and in the stomach of some frogs (Van Noorden, Negri, and Polak, unpublished data) but whether this is pancreatic or enteroglucagon cannot be determined yet.

Interspecific immunological cross-reaction seems to be very stable with glucagon, which has evidently undergone little change in structure during evolution. It has been suggested by Barrington and Dockray (1976) that strong selective pressure must have acted to cause the retention of so much of the structure, perhaps to preserve its identity from mutant forms that may have occurred through gene duplication to give rise to VIP, GIP, or secretin.

VIP, secretin, and GIP.—VIP, secretin, and GIP are less well studied hormones. Secretin-like activity has been noted in the intestine of represen-

tatives of all classes of vertebrates, including the cyclostomes, and also the octopus (Ledrut and Ungar, 1937). Some of these extracts may have been contaminated with a CCK-like substance, which has weak secretin-like activity (Barrington and Dockray, 1976), but recently a CCK-free preparation from pike intestine (Dockray, 1974) was shown to have strong secretin-like activity in bird (turkey) and weak reactivity in mammals (rat). Because porcine VIP is a better stimulant of bird pancreatic juice than porcine secretin, the action of the pike peptide is more VIP-like than secretin-like, suggesting that the fish peptide may be a VIP-like molecule. In birds, both secretin-like and VIP-like substances are present. This suggests that a common ancestral form existed a considerable time before the line leading to the birds diverged from reptiles. There is no information on reptilian secretin and VIP, and no reports of immunostaining of these hormones in fishes. Cyclostome intestine showed no reaction to an antibody to mammalian secretin (Van Noorden and Pearse, 1974), but the reaction to antibodies to VIP has not been investigated fully. VIP can occur in the islets and nerves in mammals as well as throughout the gastrointestinal tract (Polak *et al.*, 1974b, 1977a; Bryant *et al.*, 1976). However, it has not been looked for in these locations in lower vertebrates. There is a possibility that some of the unidentified islet cell types of fishes will turn out to contain VIP.

GIP has been found only in the mammalian gut to date although a recent report by Smith *et al.* (1977) suggests that it also can be present in pancreatic islets. No immunostaining was achieved in the gut of chick or frog with an antibody to mammalian GIP (Van Noorden, Negri, and Polak, unpublished results).

At present, then, it seems as if a molecule with glucagon-like activity is probably ancestral to the other three and that VIP developed second, followed by secretin and GIP. However, so little is known of the gut hormones of the poikilothermic vertebrates that much more work will have to be done before conclusions can be reached.

PEPTIDES IN GEP SYSTEM AND NERVOUS SYSTEM

Besides the VIP and gastrin/CCK-like substances already discussed, a number of other peptides have been found recently that occur in some part of the nervous system as well as the gut. There have even been a few studies on lower vertebrates, as well as mammals, and the results indicate that the nervous system/gut (neurointestinal) peptides may be phylogenetically ancient.

Somatostatin

This peptide was isolated originally from the hypothalamus as the growth hormone-release-inhibiting peptide (Brazeau *et al.*, 1973) and has since been found throughout the gut, in the pancreatic islets, and the nervous system of mammals. Its widespread hormone-release-inhibiting activities and rapid clearance from the blood indicate that somatostatin is probably a locally act-

ing rather than circulating hormone (Pearse *et al.*, 1977). In the pancreatic islets, it is localized in the D cells, which had not previously been assigned a hormone (Polak *et al.*, 1975a). Now that synthetic somatostatin has been produced and antibodies can be raised to it, somatostatin has been reported in the islets of representatives from most vertebrate classes, including fishes (Johnson *et al.*, 1976) and cyclostomes (Van Noorden *et al.*, 1976). Localization in the gut and brain has not been well studied. We have been able to immunostain somatostatin in bird (chick) and frog gut, and it has been shown by radioimmunoassay to be present in the brain, spinal cord, intestine, and islet body of a cyclostome, *Eptatretus stoutii* (Stewart *et al.*, 1977 as cited in Falkmer and Östberg, 1977). We have demonstrated recently by immunostaining that the second granulated cell type in *Myxine* islets and some granulated cells at the base of the bile duct mucosa contain somatostatin. In the lamprey, *L. fluviatilis*, it is the "light" lobules, which had previously been thought to be B cells in a stage of insulin secretion, that contain somatostatin. The epithelium of the adult lamprey intestine does not seem to contain a significant number of somatostatin cells, but in the metamorphosing larva the islet somatostatin cells bud out from the gut epithelium in the same way as the insulin cells albeit at a later stage of development. Insulin and somatostatin seem to be in separate cells from the first stage at which they can be stained. The function of the newly discovered cyclostome somatostatin has not been investigated yet. Its action is possibly inhibitory to insulin release in *Lampetra* where it is present in about the same number of islet lobules as insulin. In *Myxine*, however, where somatostatin cells are found mainly in the bile-duct epithelium, the number of somatostatin immunoreactive cells is relatively smaller. It is doubtful whether their sphere of influence could extend to the whole islet tissue.

Recent evidence has revealed the presence of somatostatin-like immunoreactivity in the gastrointestinal tract mucosa of a tunicate (*Ciona intestinalis*) but none was found in *Amphioxus*, echinoderms, arthropods, or molluscs (Falkmer *et al.*, 1977).

Somatostatin is thus a very ancient molecule and must have originated more than 500 million years ago. The immunological cross-reaction is extremely sensitive between the antibody to the synthetic mammalian peptide and the cyclostome immunoreactive substance. Consequently, somatostatin may prove a useful substance to look for in both gut and nervous systems of lower vertebrates and invertebrates with a view to charting the possible evolutionary relationships between groups of animals. It remains to be shown whether the hormone-release-inhibiting properties of somatostatin are present in lower vertebrates and invertebrates.

Neurotensin

This peptide was first isolated from the hypothalamus by Carraway and Leeman (1973) and was found subsequently in even larger amounts in

extracts of the rat intestine. A related peptide was found in the stomach (Carraway and Leeman, 1976).

Neurotensin is pharmacologically a potent hypotensive agent and causes contraction of smooth muscle preparations. It also produces a rise in plasma LH and FSH and a rapid hyperglycemia due to liver glycogenolysis. It can be measured in the plasma in concentrations needed for biological activity and therefore probably acts as a circulating hormone. Its chemical structure is unlike that of any known gut peptide but it has some resemblance to LH releasing factor and to vasopressin and might have a common ancestral origin with these substances. Like many other mammalian gut peptides it also resembles structurally a peptide extracted from frog skin, in this case Xenopsin from the skin of the toad *Xenopus laevis* (Carraway and Leeman, 1975). Immunostaining in our laboratory has demonstrated neurotensin in the human gut in the same areas as those in which it is found by radioimmunoassay (Polak *et al.*, 1977d). A recently published work has shown neurotensin distribution in chicken intestine (Sundler *et al.*, 1977b), and we have found it in the frog gut by immunostaining. No nonmammalian nervous system has been examined to date.

In mammals, neurotension can act potentially as a modulator of neural function in the central nervous system (where it is found in the synaptosomal fraction), a releasing factor in the hypothalamus, a promoter of muscle contraction in the stomach, or as a blood glucose regulator in the intestine.

It will be of particular interest to search among the less highly evolved vertebrates for possible ancestral forms of this peptide in view of its resemblance to vasopressin because the phylogeny of vasopressin/vasotocin/oxytocin is better known than many other cases of hormone variation (see P. J. Bentley, this symposium).

Enkephalin

This is another peptide that has been isolated from the brains of representatives of all vertebrates classes and is in fact found in greatest concentration in the brain of nonmammalian vertebrates. The hagfish, for example, contained a relatively large quantity (Simantov *et al.*, 1976).

In mammals, the endorphins and enkephalins are so-called opiate or opioid peptides with actions resembling those of morphine. Both have a molecular structure that can be derived from the β -LPH molecule, as can MSH. In the human pituitary, endorphin and ACTH are localized in the same cell.

Enkephalin has now been found by extraction and radioimmunoassay throughout the human gut, with largest concentrations occurring in the pyloric antrum (Polak *et al.*, 1977c). No enkephalin activity was found in the nervous system of invertebrates examined (flatworms, insects, spiders, crustaceans) (Simantov *et al.*, 1976). The guts of species other than mammals have not been investigated yet.

Substance P

This peptide resembles the physalaemin-like peptides of frog skins and is found in the mammalian nervous system, where it is thought to be a chemical transmitter, and also in one type of enterochromaffin cell in the gut. It has been reported also to stimulate salivary secretion and intestinal motility and to inhibit insulin release. Very little is known about Substance P in other vertebrate classes.

Motilin

This peptide too is known only in mammals and there seems to be species specificity here for interspecific cross-immunological reaction is not always achieved. Motilin has not been discovered in the nervous system but it probably belongs to this group as the motilin-type of enterochromaffin cell also contains 5-HT. As its name implies, this hormone causes contraction of the stomach and is found only in the upper small intestine.

Bombesin

Bombesin is one of two known gut hormones found first in nonmammalian species and subsequently in mammals; the other is pancreatic polypeptide. Bombesin is also one of the biologically active peptides found in extracts of amphibian skin. No mammalian counterpart was known but in view of its acid-stimulating activity in mammals a similar mammalian peptide was searched for and found (Walsh and Holmquist, 1976; Polak *et al.*, 1976). Bombesin-like activity has now been found in amphibian, chicken, and mammalian gut, and is present also in some nerves (Polak *et al.*, 1978a). In mammals, bombesin acts as a pH-dependent gastrin releaser and has CCK-like actions on gall bladder and pancreatic secretion. Structurally, bombesin seems to have some resemblance to VIP and may belong to the same family of peptides (Track, 1976). Work is only just beginning on these newly discovered peptides, and no conclusions as to their evolution can be drawn.

PANCREATIC POLYPEPTIDE (PP)

Avian PP was discovered by Kimmel in the course of purifying chicken insulin and was then purified and sequenced (Kimmel *et al.*, 1968); subsequently, a similar peptide was isolated from mammalian pancreas (Lin and Chance, 1972). The physiological action of PP has not been established yet in mammals or in birds, but a number of its effects are known. In man, it seems to have opposing actions to CCK, preventing gall bladder contraction and pancreatic enzyme output. In birds, the PP molecule has sequence similarities with glucagon and might be a distant relative of this family. PP can produce hyperglycemia in birds but its main effect appears to be stimulating secretion from the proventriculus. It may thus act on gastrin cells or directly on the proventriculus. PP is released by the presence of protein or acid in the gut.

PP has been localized in scattered cells in the exocrine parenchyma in the avian and mammalian pancreas (Larsson *et al.*, 1975; Heitz *et al.*, 1976) and at the borders of the islets. It has not been reported in other animals, but the pancreatic islets of many lower vertebrates contain a fourth cell type, in addition to the ubiquitous A, B, and D cells, that might well correspond to a PP-like hormone. Preliminary immunostaining experiments have indicated that PP is present in the islets (at the border and not in the exocrine pancreas) of a teleost fish (pike). PP was not found by immunostaining in the islets of the cyclostomes *Lampetra* and *Myxine*. Thus it could well be a peptide that would repay study in the lower vertebrates by providing a clue to phylogenetic relationships. A recent publication by Sundler *et al.* (1977a) shows PP-like immunoreactivity in the cerebral and subpharyngeal ganglia of an earthworm (*Lumbricus terrestris*). Further investigation of the occurrence of this substance should be very rewarding.

INSULIN

We have noted already that insulin is one of the well-studied GEP hormones because of its importance in carbohydrate metabolism in man. It seems to be present in the pancreatic islets of every vertebrate, has an extremely uniform structural conformation and sequence of amino acids, and is capable of lowering the blood sugar in every instance.

From experimental work that has been carried out in cyclostomes and fishes it appears that insulin in lower vertebrates is concerned with carbohydrate regulation and that it might also be involved in protein and lipid metabolism (Hardisty *et al.*, 1975; Thorpe and Ince, 1974; Ince and Thorpe, 1974; Lewander *et al.*, 1976). The full complexity of the gastroenteropancreatic system at this end of the evolutionary scale remains to be established.

Evidence is beginning to accumulate concerning the presence of insulin-like substances in invertebrates. In addition to *Amphioxus*, insulin appears to be in the gut of some molluscs (Fritsch *et al.*, 1976; Gomih and Grillo, 1976). A hypoglycemic effect of mammalian insulin in another mollusc also has been recorded. (Marques and Falkmer, 1976). Insulin-like immunoreactivity has been found in some insects, in the neurosecretory system (Tager *et al.*, 1976) as well as the gut (Ishay *et al.*, 1976), and insulin has even been shown to affect glucose uptake in a protozoan (Csaba and Lantos, 1975). This last study points to the immense antiquity of at least the receptors capable of responding to insulin-like molecules.

SUMMARY AND CONCLUSIONS

Gastroenteropancreatic hormones or hormone-like peptides are produced in basally granulated cells dispersed in the gut epithelium or gathered together in "islets." Ultrastructural characteristics of the granules are typical of the contained peptide but can vary greatly from species to species so that although the same secretory mechanism is found throughout the chordates, granule structure is not a sufficient guide to the type of peptide being pro-

duced by the cell. However, this may be identified by immunochemical methods in favorable cases where the hormone molecule has remained sufficiently stable over the course of evolution. In such cases, a lower vertebrate peptide may be identified by radioimmunoassay of a tissue extract or immunostaining with an antibody to a mammalian hormone. Use of the animal's own hormone would be preferable but this is not usually possible. Identification of a similar peptide in mammals and lower vertebrates does not necessarily imply similar function for the two substances because of the possibilities of convergence in peptide structure and divergence in specialization of receptor sites and target organs.

The mammalian gastroenteropancreatic system is regulated by complex interactions between circulating and locally acting hormonal and nervous influences and at least fifteen separate peptide hormone-like substances are involved in these processes. The division between neural and endocrine components of the GEP regulatory system is no longer distinct. Numerous recently discovered peptides have been found both in typical endocrine-like cells of the gut epithelium or pancreas and in the brain and nerves supplying the gut. Many of these peptides, which can have powerful actions on gut processes, have been identified in nonmammalian vertebrates and investigation of their full structure and function in these lower animals is essential for the understanding of the evolution of the gastrointestinal endocrine system. It also will be important to determine whether the first appearance of the "neurointestinal" peptides in phylogeny and during embryonic development is in the nervous or digestive system. It is becoming increasingly evident that the origins of many of the vertebrate hormones must be sought for among the invertebrates.

There are enormous gaps in our knowledge regarding the hormones of the gastroenteropancreatic system and endocrine systems in general of lower vertebrates in comparison with what we know about mammals. The mammalian work, and the exciting new discoveries linking the endocrine system with the nervous system, has provided an excellent basis for similar investigations in the lower vertebrates. Modern methods of peptide chemistry and immunochemistry combined with physiological and morphological studies should now be extensively used to fill in these gaps. Immunochemistry has given us a powerful tool with which to examine the occurrence and structure of hormone-like peptides, and interspecific cross-reactions can provide valuable information on the evolutionary relationships of peptides in various groups of animals.

There is obviously a great need for the extraction, purification, and sequencing of lower vertebrate hormones, as well as the identification and morphological localization of as many as possible of the known hormone-like peptides in key species of the evolutionary series. Such pioneering studies will be of scientific value to zoologists interested in evolution and will give researchers into the human condition greater insight into the ways in which the control systems of the body have evolved and how they may interact in health and disease.

LITERATURE CITED

- ADELSON, J. W. 1971. Enterosecretory proteins. *Nature*, 229:321-325.
- ANASTASI, A., V. ERSPAMER, AND R. ENDEAN. 1968. Isolation and amino acid sequence of caerulein, the active decapeptide of the skin of *Hyla caerulea*. *Arch. Biochem. Biophys.*, 125:57-68.
- ANGELUCCI, L., AND G. LINARI. 1970. The action of caerulein on gastric secretion of the chicken. *Eur. J. Pharmacol.*, 11:204-216.
- ARAKI, K., S. TACHIBANA, M. UCHIYAMA, T. NAKAJIMA, AND T. YASUHARA. 1975. Isolation and structure of a new active peptide "Xenopsin" on the smooth muscle, especially on a strip of fundus from a rat stomach, from the skin of *Xenopus laevis*. *Chem. Pharm. Bull.*, 21:2801-2804.
- BARRINGTON, E. J. W. 1945. The supposed pancreatic organs of *Petromyzon marinus* and *Myxine glutinosa*. *Quart. J. Microsc. Sci.*, 85:391-497.
- . 1972. The pancreas and intestine. Pp. 135-169, in *The biology of lampreys* (M. W. Hardisty and I. C. Potter, eds.), Academic Press, London, 2:xiv+ 1-466 pp.
- BARRINGTON, E. J. W., AND G. J. DOCKRAY. 1970. The effect of intestinal extracts of lampreys (*Lampetra fluviatilis* and *Petromyzon marinus*) on pancreatic secretion in the rat. *Gen. Comp. Endocrinol.*, 14:170-177.
- . 1972. Cholecystokinin-pancreozymin like activity in the eel, (*Anguilla anguilla* L.). *Gen. Comp. Endocrinol.*, 19:80-87.
- . 1976. Gastrointestinal hormones. *J. Endocrinol.*, 69:299-325.
- BAYLISS, W. M., AND E. H. STARLING. 1902. The mechanism of pancreatic secretion. *J. Physiol.*, 28:325-353.
- BEAUPAIN, D., AND F. DIETERLEN-LIÈVRE. 1974. Étude immunocytologique de la différenciation du pancréas endocrine chez l'embryon de Poulet. II. Glucagon. *Gen. Comp. Endocrinol.*, 23:421-431.
- BERTACCINI, G., V. ERSPAMER, P. MELCHIORRI, AND N. SOPRANZI. 1974. Gastrin release by bombesin in the dog. *British J. Pharmacol.*, 52:219-225.
- BLOOM, S. R., AND J. M. POLAK. 1973. Vasoactive intestinal peptide and the watery diarrhea syndrome. *The Lancet* II:14-16.
- . 1977. The new peptide hormones of the gut. *Prog. Gastroenterol.*, 3:109-115.
- BRAZEAU, P., W. VALE, R. BURGUS, B. LING, M. BUTCHER, J. RIVIER, AND R. GUILLEMIN. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science*, 179:77-79.
- BROWN, J. C., V. MUTT, AND R. A. PEDERSON. 1970. Further purification of a polypeptide demonstrating enteroglucagon activity. *J. Physiol.*, 209:57-64.
- BROWN, J. C., V. MUTT, AND J. R. DRYBURGH. 1971. The further purification of motilin, a gastric motor stimulating polypeptide from the mucosa of the small intestine of hogs. *Canadian J. Physiol. Pharmacol.*, 49:399-405.
- BRYANT, M. G., S. R. BLOOM, J. M. POLAK, R. H. ALBUQUERQUE, I. MODLIN, AND A. G. E. PEARSE. 1976. Possible dual role of vasoactive intestinal peptide as gastrointestinal hormone and neurotransmitter substance. *The Lancet*, 1:991-993.
- BURHOL, P. G., AND B. I. HIRSCHOWITZ. 1971. Gastric stimulation by subcutaneous infusion of cholecystokinin-pancreozymin in fistula chickens. *Scand. J. Gastroenterol.*, 6(Suppl. 11):41-48.
- CARRAWAY, R., AND S. E. LEEMAN. 1973. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalamus. *J. Biol. Chem.*, 248:6854-6861.
- . 1975. The amino acid sequence of a hypothalamic peptide, neurotensin. *J. Biol. Chem.*, 250:1907-1911.
- . 1976. Characterization of radioimmunoassayable neurotensin in the rat. Its differential distribution in the central nervous system, small intestine and stomach. *J. Biol. Chem.*, 251:7045-7052.
- COONS, A. H., E. H. LEDUC, AND J. M. CONNOLLY. 1955. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J. Exp. Med.*, 102:49-59.

- CSABA, G., AND T. LANTOS. 1975. Effects of insulin on the glucose uptake of Protozoa. *Experientia*, 31:1097-1098.
- DAVIDSON, W. D., B. D. W. CATHERWOOD, AND C. A. E. LEMMI. 1970. Action of caerulein, a gastrin-like decapeptide, on the isolated bullfrog mucosa. *Clin. Res.*, 18:173.
- DIETERLEN-LIÈVRE, F., AND D. BEAUPAIN. 1974. Étude immunocytoologique de la différenciation du pancréas endocrine chez l'embryon de Poulet. I. Ilôts à insuline. *Gen. Comp. Endocrinol.*, 22:62-69.
- DOCKRAY, G. J. 1974. Extraction of a secretin-like factor from the intestines of pike (*Esox lucius*). *Gen. Comp. Endocrinol.*, 23:340-347.
- . 1976. Immunocytochemical evidence of cholecystokinin-like peptides in brain. *Nature*, 264:568-570.
- . 1977. Molecular evolution of gut hormones: application of comparative studies on the regulation of digestion. *Gastroenterology*, 72:344-358.
- DOCKRAY, G. J., J. H. WALSH, AND A. L. HOLMQUIST. 1977. Immunochemical characterization of a gastrin-like factor in a primitive vertebrate, the lamprey (*Lampetra fluviatilis*). Abstract presented at First International Symposium on Gastrointestinal Hormones held at Asilomar, California, October 5th-8th 1976.
- ERSPAMER, V., AND P. MELCHIORRI. 1973. Active polypeptides of the amphibian skin and their synthetic analogues. *Pure Appl. Chem.*, 35:56-61.
- ERSPAMER, V., P. MELCHIORRI, C. FALCONIERI, ERSPAMER, AND L. NEGRI. 1978. Polypeptides of the amphibian skin active on the gut and their mammalian counterparts. Pp. 51-64, in *Gastrointestinal hormones and the pathology of the digestive system* (M. Grossman, V. Speranza, N. Basso, and E. Lezoché, eds.), Plenum Press, New York, 205 pp.
- FALKMER, S., AND A. J. MATTY. 1966. Blood sugar regulation in the hagfish *Myxine glutinosa*. *Gen. Comp. Endocrinol.*, 6:334-346.
- FALKMER, S., AND Y. ÖSTBERG. 1977. Comparative morphology of pancreatic islets in animals. Pp. 15-59, in *The diabetic pancreas* (B. W. Volk and K. F. Wellmann, eds.), Plenum Press, New York, xviii+599 pp.
- FALKMER, S., J. F. CUTFIELD, S. M. CUTFIELD, C. G. DODSON, S. GAMMELTOFT, M. MARQUES, J. D. PETERSON, D. F. STEINER, F. SUNDBY, S. O. EMDIN, N. HAVU, Y. ÖSTBERG, AND L. WINDBLADH. 1975. Comparative endocrinology of insulin and glucagon production. *Amer. Zool.*, suppl. 1:225-270.
- FALKMER, S., R. P. ELDE, C. HELLERSTRÖM, B. PETERSON, S. EFENDIC, J. FOHLMAN, AND J. B. SILJEVALL. 1977. Some phylogenetical aspects on the occurrence of somatostatin in the gastro-entero-pancreatic endocrine system. A histological and immunocytochemical study combined with quantitative radioimmunological assays of tissue extracts. *Arch. Histol. Japonica*, 40:99-118.
- FRAZIER, W. A., R. H. ANGELETTI, AND R. A. BRADSHAW. 1972. Nerve growth factor and insulin. *Science*, 176:482-488.
- FRITSCH, H. A. R., S. VAN NOORDEN, AND A. G. E. PEARSE. 1976. Cytochemical and immunofluorescence investigations on insulin-like producing cells in the intestine of *Mytilus edulis* L. (Bivalvia). *Cell Tiss. Res.*, 165:365-369.
- FRITSCH, H. A. R., AND R. SPRANG. 1977. On the ultrastructure of polypeptide hormone-producing cells in the gut of the ascidian, *Ciona intestinalis* L. and the bivalve, *Mytilus edulis* L. *Cell Tiss. Res.*, 177:407-413.
- FUJITA, T. 1976. The gastroenteric endocrine cell and its paraneuronic nature. Pp. 191-208, in *Chromaffin, enterochromaffin and related cells* (R. E. Coupland and T. Fujita, eds.), Elsevier, Amsterdam, xiii+400 pp.
- GIBSON, R. G., A. A. MIHAS, H. W. COLVIN, AND B. I. HIRSCHOWITZ. 1976. The search for submammalian gastrins: the identification of amphibian gastrin. *Proc. Soc. Exp. Biol. Med.*, 53:284-288.
- GOMIH, Y. K., AND T. A. I. GRILLO. 1976. Insulin-like activity of the extract of the digestive gland and the pylorus of the giant African snail, *Achatina fulica*. A preliminary report. Pp. 153-162, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epple, eds.), Pergamon Press, Oxford, xii+377 pp.

- GREGORY, R. A. 1966. The isolation and chemistry of gastrin. *Gastroenterology*, 51:953-959.
- GREGORY, R. A., AND H. J. TRACY. 1975. The chemistry of the gastrins, some recent advances. Pp. 13-24, in *Gastrointestinal hormones* (J. C. Thompson, ed.), Univ. Texas Press Austin, Texas, xxiv+366 pp.
- HANSEN, D. 1975. Evidence of a gastrin-like substance in *Rhinobatus productus*. *Comp. Biochem. Physiol.*, 52C:61-63.
- HARDISTY, M. W., P. R. ZELNIK, AND I. A. MOORE. 1975. The effects of subtotal and total isletectomy in the river lamprey, *Lampetra fluviatilis*. *Gen. Comp. Endocrinol.*, 20:179-192.
- HEITZ, P., J. M. POLAK, S. R. BLOOM, AND A. G. E. PEARSE. 1976. Identification of the D₁ cell as the source of human pancreatic polypeptide. *Gut*, 17:755-758.
- HOGBEN, C. A. M. 1966. Response of the isolated dogfish gastric mucosa to histamine. *Proc. Soc. Exp. Biol. Med.*, 124:890-893.
- INCE, B. W., AND A. THORPE. 1974. Effects of insulin and of metabolic loading on blood metabolites in the European silver eel (*Anguilla anguilla* L.). *Gen. Comp. Endocrinol.*, 23:460-471.
- ISHAY, J., S. GITTER, R. GALUN, M. DORON, AND Z. LARON. 1976. The presence of insulin in and some effects of exogenous insulin on Hymenoptera tissues and body fluids. *Comp. Biochem. Physiol.*, 54A:203-206.
- JOHNSON, D. E., J. L. TORRENCE, R. P. ELDE, E. BAUER, B. D. NOE, AND D. J. FLETCHER. 1976. Immunohistochemical localization of somatostatin, insulin and glucagon in the principal islets of the anglerfish (*Lophius americanus*) and the channel catfish (*Ictalurus punctatus*). *Amer. J. Anat.*, 147:119-124.
- JORPES, E., AND V. MUTT. 1970. On the biological activity and amino acid composition of secretin. *Acta chem. Scandinavia*, 15:513-519.
- KETTERER, H., H. J. RUOFF, AND K.-F. SEWING. 1973. Do chickens have gastrin-like compounds? *Experientia*, 29:1096.
- KIMMEL, J. R., H. G. POLLOCK, AND R. L. HAZELWOOD. 1968. Isolation and characterization of chicken insulin. *Endocrinology*, 83:1323-1330.
- LARSSON, L.-I., F. SUNDLER, AND R. HÅKANSON. 1975. Immunohistochemical localization of avian pancreatic polypeptide to a population of islet cells. *Cell Tiss. Res.*, 156:167-171.
- LECHAGO, J., A. L. HOLMQUIST, AND J. H. WALSH. 1977. Immunofluorescence localization of bombesin in cells of amphibian upper gastrointestinal mucosa. Abstract presented at First International Symposium on Gastrointestinal Hormones held at Asilomar, California, October 5-8th.
- LECHAGO, J., A. L. HOLMQUIST, G. L. ROSENQUIST, AND J. H. WALSH. 1978. Localization of bombesin-like peptides in frog gastric mucosa. *Gen. Comp. Endocrinol.*, 36:553-558.
- LEDRUT, J., AND G. UNGAR. 1937. Actions de la sécrétine chez l'*Octopus vulgaris*. *Arch. Int. Physiol.*, 44:205-211.
- LEWANDER, K., G. DAVE, M. L. JOHANSSON-SJÖBECK, Å. LARSSON, AND U. LIDMAN. 1976. Metabolic effects of insulin in the European eel, *Anguilla anguilla* L. *Gen. Comp. Endocrinol.*, 29:455-467.
- LIN, T. M., AND R. E. CHANCE. 1972. Spectrum gastrointestinal actions of a new bovine pancreatic polypeptide (BPP). *Gastroenterology*, 62:852.
- MAIER, V., A. KRODER, E. GRONER, R. KELLER, AND E. F. PFEIFFER. 1975. Glucagon-like activity (GLI) in the intestine of *Porcus domesticus* and *Astacus fluviatilis*. *Acta. Endocr. Suppl.*, 193:41.
- MARQUES, M., AND S. FALKMER. 1976. Effects of mammalian insulin on blood glucose level, glucose tolerance and glycogen content of musculature and hepatopancreas in a gastropod mollusc, *Strophocheilus oblongus*. *Gen. Comp. Endocrinol.*, 29:552-530.
- MUTT, V., AND E. JORPES. 1968. Structure of porcine cholecystokinin-pancreozymin. *European J. Biochem.*, 6:156-162.
- NEGRI, L., AND V. ERSFAMER. 1973. Action of caerulein and caerulein-like peptides on "short circuit current" and acid secretion in the isolated gastric mucosa of amphibia. *Naunyn Schmiedeberg, Arch. Pharmacol.*, 277:401-412.

- NICOLL, C. S. 1974. Physiological actions of prolactin. Pp. 253-292, in *Handbook of physiology*, Section 7, Endocrinology (R. O. Greep and E. B. Astwood, section eds.), The pituitary gland and its neuroendocrine control, Pt. 2 (E. Knobil and W. H. Sawyer, volume eds.), Amer. Physiol. Soc., Washington, D.C., 4:vi+ 1-601 pp.
- NILSSON, A. 1970. Gastrointestinal hormones in the holocephalian fish, *Chimaera monstrosa* L. *Comp. Biochem. Physiol.*, 32:387-390.
- ÖSTBERG, Y., S. VAN NOORDEN, A. G. E. PEARSE, AND N. W. THOMAS. 1976. Cyto-chemical, immunofluorescence and ultrastructural investigation on polypeptide hormone containing cells in the intestinal mucosa of a cyclostome, *Myxine glutinosa*. *Gen. Comp. Endocrinol.*, 28:213-227.
- PEARSE, A. G. E. 1968. Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (The APUD Series) and their relevance to thyroid and ultimobranchial C cells and calcitonin. *Proc. Roy. Soc., ser B*, 170:71-80.
- PEARSE, A. G. E., AND J. M. POLAK. 1975. Bifunctional reagents as vapour and liquid phase fixatives for immunohistochemistry. *Histochem. J.*, 7:179-186.
- PEARSE, A. G. E., AND T. TAKOR TAKOR. 1976. Neuroendocrine embryology and the APUD concept. *Clin. Endocrinol.*, 5(suppl.):229-244.
- PEARSE, A. G. E., J. M. POLAK, AND S. R. BLOOM. 1977. The newer gut hormones: cellular sources, physiology, pathology and clinical aspects. *Gastroenterol.*, 72:746-761.
- PETERSON, J. D., C. I. COULTER, D. F. STEINER, S. O. EMDIN, AND S. FALKMER. 1974. Structural and crystallographic observations on hagfish insulin. *Nature*, 251:239-240.
- POLAK, J. M., A. G. E. PEARSE, C. ADAMS, AND J.-C. GARAUD. 1974a. Immunohistochemical and ultrastructural studies on the endocrine polypeptide (APUD) cells of the avian gastro-intestinal tract. *Experientia*, 30:564-567.
- POLAK, J. M., A. G. E. PEARSE, J.-C. GARAUD, AND S. R. BLOOM. 1974b. Cellular localization of a vasoactive intestinal peptide in the mammalian and avian gastrointestinal tract. *Gut*, 15:720-724.
- POLAK, J. M., A. G. E. PEARSE, L. GRIMELIUS, S. R. BLOOM, AND A. ARIMURA. 1975a. Growth-hormone release-inhibiting hormone in gastrointestinal and pancreatic D Cells. *The Lancet*, 1:1220-1229.
- POLAK, J. M., A. G. E. PEARSE, AND C. M. HEATH. 1975b. Complete identification of endocrine cells in the gastrointestinal tract using semithin-thin sections to identify motilin cells in human and animal intestine. *Gut*, 16:225-229.
- POLAK, J. M., S. R. BLOOM, S. HOBBS, E. SOLCIA, AND A. G. E. PEARSE. 1976. Distribution of a bombesin-like peptide in human gastrointestinal tract. *The Lancet*, 1:1109-1110.
- POLAK, J. M., A. M. J. BUCHAN, C. M. TIMSON, S. R. BLOOM, AND A. G. E. PEARSE. 1977a. Two new cells in the human pancreatic islets. *Gut*, 18:A414.
- POLAK, J. M., A. G. E. PEARSE, M. SZELKE, S. R. BLOOM, D. HUDSON, P. FACER, A. M. J. BUCHAN, M. G. BRYANT, N. CHRISTOPHODES, AND I. MACINTYRE. 1977b. Specific immunostaining of CCK cells by use of synthetic fragment antisera. *Experientia*, 33:762-763.
- POLAK, J. M., S. N. SULLIVAN, S. R. BLOOM, P. FACER, AND A. G. E. PEARSE. 1977c. Enkephalin-like immunoreactivity in the human gastrointestinal tract. *The Lancet*, 1:972-974.
- POLAK, J. M., S. N. SULLIVAN, S. R. BLOOM, A. M. J. BUCHAN, P. FACER, M. R. BROWN, AND A. G. E. PEARSE. 1977d. Specific localization of neurotensin to the N cell in human intestine by radioimmunoassay and immunocytochemistry. *Nature*, 270:183.
- POLAK, J. M., A. M. J. BUCHAN, W. CZYKOWSKA, E. SOLCIA, S. R. BLOOM, AND A. G. E. PEARSE. 1978a. Bombesin in the gut. Pp. 540-542, in *Gut hormones* (S. R. Bloom, ed.), Churchill-Livingstone, New York, viii+649 pp.
- POLAK, J. M., S. N. SULLIVAN, A. M. J. BUCHAN, S. R. BLOOM, P. FACER, D. HUDSON, AND A. G. E. PEARSE. 1978b. Endorphins. Pp. 501-506, in *Gut hormones* (S. R. Bloom, ed.), Churchill Livingstone, New York, viii+649 pp.
- SAID, S. I., AND V. MUTT. 1972. Isolation from porcine intestinal wall of a vasoactive octacosapeptide related to secretin and glucagon. *European J. Biochem.*, 28:199-204.

- SIMANTOV, R., R. GOODMAN, D. APOSHIAN, AND S. H. SNYDER. 1976. Phylogenetic distribution of a morphine-like peptide, "enkephalin". *Brain Res.*, 111:204-211.
- SMITH, L. F. 1966. Species variations in the amino acid sequence of insulin. *Amer. J. Med.*, 40:662-666.
- SMITH, P. H., F. W. MERCHANT, D. G. JOHNSON, W. Y. FUJIMOTO, AND R. H. WILLIAMS. 1977. Immunocytochemical localization of a gastric inhibitory polypeptide-like material within A cells of the endocrine pancreas. *Anat. Rec.*, 187:714.
- SOLOMON, T., H. PETERSEN, AND M. I. GROSSMAN. 1977. Trophic effect of caerulein and secretin on the pancreas in rats—interaction and dose and time relationship. *Gastroenterology*, 72:824.
- STEINER, D. F., J. D. PETERSON, H. TAGER, S. EMDIN, Y. ÖSTBERG, AND S. FALKMER. 1973. Comparative aspects of proinsulin and insulin structure and biosynthesis. *Amer. Zool.*, 13:591-604.
- STERNBERGER, L. 1974. Immunocytochemistry. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, ix+246 pp.
- STRAUS, E., R. S. YALOW, AND H. GAINER. 1975. Molluscan gastrin; concentration and molecular forms. *Science*, 190:687-689.
- SUNDLER, F., R. HÅKANSON, J. ALUMETS, AND B. WALLE. 1977a. Neuronal localization of pancreatic polypeptide (PP) and vasoactive intestinal peptide (VIP) in the earthworm (*Lumbricus terrestris*). *Brain Res. Bull.*, 2:61-65.
- SUNDLER, F., R. HÅKANSON, R. A. HAMMER, J. ALUMETS, R. CARRAWAY, S. E. LEEMAN, AND E. A. ZIMMERMAN. 1977b. Immunohistochemical localization of neurotensin in endocrine cells of the gut. *Cell Tiss. Res.*, 178:311-321.
- TAGER, H. S., AND D. F. STEINER. 1974. Peptide hormones. *Ann. Rev. Biochem.*, 43:509-538.
- TAGER, H. S., J. MARKESE, K. J. KRAMER, R. D. SPIERS, AND C. N. CHILDS. 1976. Glucagon-like and insulin-like hormones of the insect neurosecretory system. *Biochem. J.*, 156:515-520.
- THORPE, A., AND B. W. INCE. 1974. The effects of pancreatic hormones, catecholamines and glucose loading on blood metabolites in the northern pike, (*Esox lucius* L.). *Gen. Comp. Endocrinol.*, 23:29-44.
- TITLBACH, M., AND H. KERN. 1969. Licht- und elektronenmikroskopische Untersuchungen am Inselorgan des Bachneunauges *Lampetra planeri* (Bloch). *Z. Zellforsch. Mikrosk. Anat.*, 97:403-415.
- TRACK, N. S. 1976. Bombesin and the human gastrointestinal tract. *The Lancet*, 2:148.
- VANDERHAEGEN, J. J., J. C. SIGNEAU, AND W. GEPTS. 1975. New peptide in the vertebrate CNS reacting with anti-gastrin antibodies. *Nature*, 257:604-605.
- VAN NOORDEN, S., AND A. G. E. PEARSE. 1974. Immunoreactive polypeptide hormones in the pancreas and gut of the lamprey. *Gen. Comp. Endocrinol.*, 23:311-324.
- . 1976. The localization of immunoreactivity to insulin, glucagon and gastrin in the gut of *Amphioxus* (*Branchiostoma*) *lanceolatus*. Pp. 163-178, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Eppe, eds.), Pergamon Press, Oxford, xii+377 pp.
- VAN NOORDEN, S., J. GREENBERG, AND A. G. E. PEARSE. 1972. Cytochemical and immunofluorescence investigations on polypeptide hormone localization in the pancreas and gut of the larval lamprey. *Gen. Comp. Endocrinol.*, 19:192-199.
- VAN NOORDEN, S., Y. ÖSTBERG, AND A. G. E. PEARSE. 1976. Localization of somatostatin-like immunoreactivity in the pancreatic islets of the hagfish, *Myxine glutinosa* and the lamprey, *Lampetra fluviatilis*. *Cell Tiss. Res.*, 177:281-285.
- VAN NOORDEN, S., J. M. POLAK, AND A. G. E. PEARSE. 1977. Single cellular origin of somatostatin and calcitonin in the rat thyroid gland. *Histochemistry*, 53:540-542.
- WALSH, J. H., AND A. L. HOLMQUIST. 1976. Radioimmunoassay of bombesin peptides: identification of bombesin-like immunoreactivity in vertebrate gut extracts. *Gastroenterology*, 70:A-90/948.
- WEINSTEIN, B. 1972. A generalized homology correlation for various hormones and proteins. *Experientia*, 28:1517-1522.

EVOLUTION OF PANCREATIC ISLET FUNCTIONS

AUGUST EPPLE, JACK E. BRINN, AND JEAN B. YOUNG

It has not been uncommon in pancreatic islet research for important histological discoveries to precede related physiological and biochemical findings by years, or even decades. This point is illustrated by the time span between Langerhans' (1869) description of the islets and their final recognition as the source of insulin more than 50 years later (see Wellmann and Volk, 1977); by a similar time interval between the discovery of the A-cells (Lane, 1907) and the general acceptance of glucagon as a second islet hormone (see Lefebvre and Unger, 1972); and by approximately 40 years of discussion concerning the nature of the D-cell (see Epple, 1963; Fujita, 1968; Falkmer and Patent, 1972; and contributions in Volk and Wellmann, 1977). It seems that only during the last decade have things changed; morphological, physiological, and biochemical islet research are at last running abreast in the race for new discoveries. This new era was initiated by new techniques and provocative ideas. Thus, the development of the radioimmunoassay, improved techniques of amino acid sequence analysis, hormone isolation, and immunohistology led to the discovery of prohormones (Lernmark *et al.*, 1976; Steiner, 1976) and the recognition of families of hormones with members occurring in nervous tissues, skin, digestive tract, and various endocrine glands, including the islets (see for example, Scharrer, 1978; Pearse, 1977b; Van Noorden *et al.*, this volume). On the other hand, Pearse's APUD theory (Pearse, 1977a, 1977b) and Fujita's concepts of the gastro-entero-pancreatic (GEP) endocrine system (Fujita, 1973; 1976) and of the paraneuron (Fujita, 1977) revolutionized our understanding of the pancreatic islets. As a consequence, it now is generally accepted that the islets of the gnathostomes regularly produce at least four hormones (see contributions in Volk and Wellmann, 1977). There is even evidence suggesting the occurrence of more than one hormone within a single islet cell (Epple, 1967; Erlandsen *et al.*, 1976; Smith *et al.*, 1977). Indeed, it seems that a long period of dogmatic skepticism is about to give way to a new openmindedness, with energies being used to explain interesting findings rather than to refute them.

At this point it appears useful to evaluate the state of knowledge of the evolution of the functions of the islet organ. Therefore, this review will attempt to reconstruct the early events in the evolution of the islet organ; identify evolutionary trends in the physiology of insulin and glucagon; compare islet ontogeny with islet phylogeny; and interpret the phylogenetic variations of the islet innervation.

ISLET EVOLUTION

The probable events that occurred during early islet evolution have been discussed in several recent publications (Falkmer and Patent, 1972; Fujita, 1973, 1976; Epple and Lewis, 1973; Epple and Brinn, 1975, 1976; Epple and Stetson, 1976; Falkmer and Östberg, 1977; Van Noorden *et al.*, this volume). However, inasmuch as the understanding of these events is fundamental to an evolutionary interpretation of the islet functions, they will be presented here in an admittedly subjective summary.

Diffuse endocrine cells, probably of neuroectodermal origin, seem to be a regular feature of the digestive tract of many invertebrates (see Falkmer and Östberg, 1977; Van Noorden *et al.*, this volume). Application of modern taxonomy suggests that these cells developed, at the latest, at the level of the ancestors of both the *Spiralia* (*Articulata*, molluscs, and several other groups) and the chordates. The phylogenetic prototype of these cells (see, for example, Fritsch and Sprang, 1977) is thought to be a receptor-secretor cell within the mucosal epithelium, the modified microvilli of which extend into the lumen of the digestive tract and the basal region of which contains endocrine granula. The microvilli appear to be chemoreceptors that monitor the contents of the digestive tube. Upon the appropriate stimulus (pH, nutrients, electrolytes), the cell releases the basal granula (see Fujita 1973; 1976). The resulting endocrine secretion is supposed to convey messages locally [Feyrter's (1953) concept of "paracrine" secretion] or to act on targets farther away, such as other regions of the digestive tube, gall bladder, exocrine and endocrine pancreas.

As far as we are aware, there is no definite proof yet of the paracrine concept in vertebrates, with the possible exception of the pancreas (see below). The so far well-established functional axes between the gastrointestinal receptor-secretors and target organs, involving the recognized hormones pancreaticozym, secretin, gastrin, and GIP (Grossman, 1976; Johnson, 1976; Dockray, 1977), all show the following feature: the cells are concentrated at a checkpoint away from their target. From here, their hormonal messages leave via the portal vein. When the hormones finally arrive by means of the general circulation at their target(s), they are homogeneously diluted. The advantages of this functional principle are: homogeneous dilution of the hormones prevents a local gradient that could result in an over or under-stimulation of the mucosal target cells, as might occur in a paracrine relationship; localization of the receptor-secretor cells away from their target tissues allows a better evaluation of the overall situation. Thus, for example, if the gastrin cells were located in the fundic glands rather than in the pyloric region they could hardly obtain a clear picture of the result of the digestive actions of the stomach; localization of secretin or pancreaticozym cells within their target tissues (pancreas and gall bladder) would totally defeat the purpose of their existence—to convey messages from the intestinal lumen.

However, a conceptual difficulty seems to arise with the question of why there are no circulatory shortcuts between the gastrointestinal receptor-

FIG. 1.—Two hypothetical steps in the evolution of the endocrine control mechanism of the gastrointestinal system, with exocrine cells as an example of a target of an intestinal hormone. Exocrine cells: large, dark apical granules; receptor-secretor cells: fingerlike, large microvilli extending into the gut lumen, and fine basal granulation; absorptive cells: small microvilli. (1a) Paracrine situation in a very small metazoan. The three mucosal cell types are randomly scattered; interstitial fluid flows in various directions between the mucosa (bottom) and other organs, depending on the body movements of the animal. (1b) Endocrine situation in an animal with a primitive circulation. Interstitial fluid ("hemolymph") has a directed flow (arrows) in primitive vessels. The secretion of the receptor-secretor cells is carried to their exocrine target cells, which are located "downstream".

secretor cells and their targets (see pages 402 and 403 in Fujita, 1976). It seems inefficient to send pancreaticozym on a long journey through the vascular system though the cells of its origin in the duodenum may be only millimeters away from its pancreatic targets. As in the case of other biological "misconstructions," the answer must be looked for in the phylogenetic past. We have to recall that most invertebrates have either an open circulatory system or none at all. Inasmuch as the phylogenetic distribution of the gastrointestinal receptor-secretors (cf. Falkmer and Östberg, 1977) suggests that they developed *before* the appearance of a closed system of blood vessels, it may be safe to assume that an original "paracrine" relationship between endocrine and exocrine gastrointestinal elements was abandoned when the increasing size of the animals required a more efficient circulatory system. Fig. 1 outlines the possible key events linking a hypothetical ancestral condition with the early intraintestinal pancreas situation as seen to some extent in lampreys (Barrington, 1972). If our hypothesis is correct in its main points, then the change from a paracrine to a vascular link must have been a very useful *preadaptation* for the following reasons: it allowed

FIG. 2.—Hypothetical stages in the evolution of “closed” endocrine cells from “open” receptor-secretors of the gastrointestinal mucosa. 1. “Open” cell (second from left) with receptor sites (asterisks) concentrated on modified microvilli, which extend into the intestinal lumen (Int Lu). 2. “Intermediate” receptor-secretor (center), located between absorptive cells. This cell responds to stimuli which are carried by pinocytotic vesicles of surrounding absorptive cells to the intercellular spaces below the tight junctions. 3. “Closed” cell (second from right) with receptors concentrated at the capillary pole. This cell responds to blood-borne stimuli from the capillary lumen (Cap Lu). The “closed cell’s” location between exocrine cells (large, apical granules) indicates that it no longer requires indirect luminal stimuli from absorptive cells.

the development of an extramural digestive gland without the creation of a new information link between mucosa and pancreas; extramural location allowed an enormous enlargement of the glandular tissue (which could be supplied by homogeneously diluted hormonal messages); because of the withdrawal of the exocrine tissue from the duodenal mucosa, space became available for the enlargement of the absorptive epithelium.

The “open” mucosa cells monitor intestinal contents, but the islet cells are specialists on changes in blood chemistry. A possible sequence from an “open” receptor-secretor to a “closed” ancestral islet cell is shown in Fig. 2. In essence, we propose that the well-known release of pinocytotic material by absorbing mucosa cells into the intercellular spaces of the intestinal epithelium and the gradual shift of the receptor sites of the receptor-secretor cell from the luminal to the capillary pole led to the development of the “closed” type. The evolutionary incentive to develop blood specialists of the closed type is understandable because the integration of messages from the digestive lumen with those from the circulation provides an excellent *dual humoral control system of the mucosal activities*—“open” cells monitor the process of digestion and “closed” cells coordinate this process with the state of the “milieu interne,” so that absorption, storage, and utilization of nutrients occur in optimal coordination. However, as long as “closed” cells are located within the absorptive regions of the intestinal mucosa, it may be difficult for them to distinguish between stimuli arriving from the general circulation and those resulting from intestinal uptake of metabolites. This

problem was largely solved when, after the appearance of submucosal glands and diverticuli, "closed" cells could be transferred to these locations. Here, they were supplied with unspoiled systemic arterial blood, the composition of which reflected the state of the post-hepatic "milieu interne." Later on, when pancreas and hepatic systems moved to an extramural position, the accompanying "closed" cells could proliferate, without interruption of the external muscle layers of the intestine, into many and/or large "islets." The trend to occupy extramural glands is exemplified still by the islet development of the cyclostomes (Barrington, 1972; Falkmer *et al.*, 1974; Falkmer and Östberg, 1977). In lampreys, a large cephalic islet is located in the submucosa of an intestinal caecum, and another large hepatic islet (caudal islet) is found at the base of the bile duct. The latter shows an enormous proliferation during the last phase of the animal's life cycle (Epple and Brinn, 1975; Brinn and Epple, 1976). In hagfishes, the islet tissue proliferates exclusively from the epithelium of the bile duct (Falkmer and Östberg, 1977).

While the *topographic* association of islets and exocrine pancreas is easy to understand, the intrapancreatic dispersion of the islets in mammals has made it tempting to postulate a *functional* relationship between the two (see, e.g., Ferner, 1957; Henderson, 1969). Indeed, Fujita and coworkers (Fujita *et al.*, 1976) have shown vascular connections in several mammals suggestive of a portal system; functional support of an insulo-acinar axis comes from several other studies (Malaisse-Lagae *et al.*, 1976; Ohara, 1976; Kanno *et al.*, 1976). As pointed out previously (Epple and Lewis, 1973; Epple and Brinn, 1975; 1976), the relatively homogeneous distribution of the islets in the mammalian pancreas may have been a preadaptation for an insulo-acinar axis. However, on a phylogenetic scale, the islet tissue tends to accumulate in certain pancreas regions rather than to disperse evenly. Prime examples of this trend are the Brockmann bodies of teleosts (Epple, 1969; Brinn, 1973) and the splenic lobe of birds (cf. Guha and Ghosh, 1978). Furthermore, the islet composition may vary greatly from one pancreas region to another as shown, for example, by the location of the A-islets of many birds (Mikami and Ono, 1962). Thus, a functionally significant insulo-acinar axis in mammals must be a phylogenetically rather special situation. The selachians are one of the few other groups whose intrapancreatic islet distribution is similar to that of mammals (Epple and Lewis, 1973). On the other hand, we have to recall that the developing exocrine pancreas uses the blood vessels and other organs of the portal vein-drainage system as a "trellis" (Epple and Brinn, 1975; 1976). As a consequence, the ubiquitous association of islet tissue and exocrine pancreas in gnathostomes assures that the islet hormones reach one of their most important targets and checkpoints, the liver, fast and in high concentrations. Probably, this explains why the gnathostome islet organ does not emancipate itself from the exocrine pancreas, although in light of its postulated neuroectodermal origin (Pearse, 1977a, 1977b), one might wonder why it does not normally settle in other locations.

In summary, the evolution of the gastrointestinal endocrine system probably began with the appearance of "open" receptor-secretor cells in the digestive mucosa of the common ancestors of both protostomians and deuterostomians. These cells may have been located between their epithelial target cells, serving a paracrine function. With the development of a circulatory system, certain "open" cells became concentrated at optimal checkpoints, from which their hormones were carried via the circulation to their, now distant, target cells. Development of the "closed" endocrine cells of the gastrointestinal mucosa from "open" cells was connected with a shift of receptor sites from the apical microvilli to the cell membrane bordering the intercellular spaces below the tight junctions, and from here to the basal surface close to a capillary. Thus, the "closed" cells became specialists on blood-borne messages. From this point on, "open" and "closed" mucosa cells form a dual control system of the gastrointestinal activities, which integrates messages from the digestive lumen with those from the "milieu interne." Withdrawal of the "closed" cells from the gastrointestinal epithelium and concomitant acquisition of a separate arterial blood supply further improved the recognition of the "milieu interne," the messages of which were now received without the interference of substances absorbed from the gut lumen. In addition, the subepithelial location provided space for the formation of "islets" by proliferation of "closed" cells. Association of the islets with the emerging extramural digestive glands provided further space for islet multiplication and guaranteed a fast and concentrated delivery of the islet hormones to the liver.

COMPARATIVE ISLET PHYSIOLOGY

Intermediary Metabolism

In the following section we will review the current state of knowledge of the phylogenetic distribution and functions of the four unequivocally identified islet secretions—insulin (Plisetskaya, 1975; Lernmark *et al.*, 1976; Ashcroft, 1976; Cahill, 1976; Plisetskaya *et al.*, 1976; Thorpe, 1976; Leisbon *et al.*, 1976b; Hazelwood, 1976a, 1976b; Gerich *et al.*, 1976; Bajaj, 1977), glucagon (Lefebvre and Unger, 1972; Lernmark *et al.*, 1976; Cahill, 1976; Gerich *et al.*, 1976; Hazelwood, 1976a, 1976b; Mialhe, 1976; Foa *et al.*, 1977; Unger *et al.*, 1977), pancreatic polypeptide (Hazelwood, 1976a; Larsson *et al.*, 1976; Floyd *et al.*, 1977), and somatostatin (Orci, *et al.*, 1975; Bloom, 1976; Guillemin and Gerich, 1976). Gastrin and vasoactive intestinal peptide (VIP) are frequently secreted by pancreatic tumors (Creutzfeldt, 1977) and, like gastric inhibitory peptide (GIP), may also occur in normal islets (Smith *et al.*, 1977; Erlandsen *et al.*, 1976; Larsson *et al.*, 1976; Dockray *et al.*, 1977). Because the status of these last two hormones as regular islet secretions requires further study, their functions will not be considered here. However, it must be emphasized that morphological data strongly suggest the existence of at least two unidentified islet hormones in a cyclostome (Epple and Brinn, 1975; Brinn and Epple, 1976; Falkmer and Östberg, 1977).

and at least one further islet hormone in the gnathostomes (see Epple and Brinn, 1975; Forssmann and Ito, 1976; Patent, 1976; Larsson *et al.*, 1976; Baetens *et al.*, 1977; Bergstrom *et al.*, 1977; Boquist, 1977; Falkmer and Östberg, 1977; LeCompte and Gepts, 1977). The occurrence of two secretions within a single type of islet cell was postulated originally on the basis of histological evidence in lower vertebrates (Epple, 1967), and this question has been raised again by immunohistological observations on gut cells in *Branchiostoma* and lamprey (see Van Noorden *et al.*, this volume), and on islet cells in mammals (Smith *et al.*, 1977; Erlandsen *et al.*, 1976). Recently, we discussed some relevant problems with respect to somatostatin (Epple and Brinn, 1976).

During the past three decades a number of cytotoxins have been used for the study of individual islet hormones, particularly insulin. However, the value of these cytotoxins as *physiological* tools in nonmammalian species is highly doubtful because careful histological evaluation in many species revealed either incomplete destruction of a given islet cell type or additional damage in other organs (see Miller, 1960, 1961; Lazarus and Volk, 1962; Epple and Farner, 1967; Epple, 1969; Winbladh, 1970; Kern, 1971; Brinn, 1973; Thorpe, 1976; Dulin and Soret, 1977). Therefore, "alloxan diabetes," "streptozotocin diabetes," and cytotoxin-induced "glucagon deficiency" will not be used in the following consideration of the islet functions in lower vertebrates.

Invertebrates

Gastro-entero-pancreatic hormones in invertebrates have been firmly established in recent years. These substances are present in both protostomians and deuterostomians (see Falkmer and Östberg, 1977), and, as in vertebrates, some of these substances have been identified in both the digestive and nervous system (Pearse, 1977a; Scharrer, 1978; Van Noorden *et al.*, this volume). Immunological evidence, recently reviewed by Falkmer and Östberg (1977) and Van Noorden *et al.* (this volume), suggests that of the islet hormones or closely related substances, insulin occurs in the digestive system of molluscs and in the intestine and nervous system of insects, glucagon in the intestine of crayfish and the nervous system of insects, pancreatic polypeptide in the nervous system of the earthworm and somatostatin in the intestine of a tunicate. Gastrin-like activity was found in both serum and intestine of molluscs and in the insect nervous system (Kramer *et al.*, 1977). There can be no question that this is only a preliminary list. Though the physiological role of these hormones in invertebrates is unknown, it is interesting that mammalian insulin has been found to affect glucose uptake in a protozoan (Csaba and Lantos, 1975) and in *Branchiostoma* (Leibson *et al.*, 1976b), and to lower the blood sugar in molluscs (Marques and Falkmer, 1976; Gomih and Grillo, 1976). However, the presence of at least some of these GEP hormones in "open" receptor-secreter cells of the digestive mucosa (Burighel and Milanesi, 1975; Fritsch and Sprang, 1977) leaves little doubt that they

carry messages from the intestinal lumen. Of particular importance for the understanding of the evolution of the islet organ are observations in *Branchiostoma*. Here, several types of endocrine mucosa cells have been identified (cf. Van Noorden and Pearse, 1976). Among these, an "open" argyrophil cell stains with antibodies to both mammalian glucagon and gastrin, and an aldehyde fuchsin positive cell stains with antibodies to mammalian insulin. Inasmuch as not all of these aldehyde fuchsin positive cells stain with insulin antiserum, it remains to be seen whether only one or both of these fractions are identical with the cells that release granula into the intestinal lumen when the animals are exposed to a glucose-containing medium (Polyakova and Plisetskaya, 1976). An ancestral B-cell as an exocrine cell of the intestine would suggest the possibility of a second evolutionary pathway from a mucosa cell to an islet cell, different from a transition of a receptor-secretor as outlined above. It also would revitalize the discussion of an endodermal origin of the B-cells, which has recently been considered in connection with mixed exocrine-endocrine pancreas cells (Gerlovin, 1976; Yaglov, 1976).

Vertebrates

In the sections that follow, the information currently available on the gastrointestinal endocrine system of each vertebrate class (from the primitive, jawless cyclostomes to the more advanced mammals) will be presented and discussed.

Class Agnatha

In both groups of extant cyclostomes, the hagfishes (*Myxiniidae*) and lampreys (*Petromyzontidae*), we find a well-developed islet organ in addition to a well-developed intramucosal endocrine system (Falkmer and Östberg, 1977; Van Noorden *et al.*, this volume). However, in contrast to the gnathostomes, the islet organ is completely separated from the presumptive equivalent of the exocrine pancreas, which occurs as individual cells in the intestinal mucosa (Epple, 1969; Barrington, 1972; Falkmer *et al.*, 1974). Before discussing the physiological role of the cyclostome islets it appears important to recall that the endocrine system of the extant cyclostomes may reflect the following evolutionary phenomena: primitive state; rudimentary stage; specialization; neoteny. The first three of these phenomena have been discussed by several authors (for references, see Wingstrand, 1966; Falkmer *et al.*, 1974; Falkmer and Östberg, 1977; Schreibman, this volume); neoteny has been considered only recently after we suggested that the appearance of new islet cells in the anadromous sea lamprey may reflect a neotenuous phenomenon (Epple and Brinn, 1975; Brinn and Epple, 1976). Specifically, this interpretation implies that extant lampreys are larvae of an ostracoderm-like ancestor that become sexually mature without undergoing a final metamorphosis, and that the appearance of new islet cells in the anadromous sea lamprey indicates an islet development that here advances farther towards the ances-

tral state of full maturation than is the case in other lampreys, where these cells have not been seen. Regardless of whether this interpretation is correct or not, at the current state of knowledge we cannot assume *a priori* that the islet biology of the extant cyclostomes shows the ancestral situation of the gnathostomes.

The common view that cyclostome islets produce only one hormone is no longer tenable. Although A-cells are lacking and all attempts to detect glucagon in the islets have failed, there is now ample evidence that the islets of both groups of cyclostomes produce insulin and a somatostatin-like substance (for lit., see Falkmer and Östberg, 1977; Van Noorden *et al.*, 1977). In the sea lamprey (*Petromyzon marinus*), there are at least two additional islet cells with unidentified secretions (Brinn and Epple, 1976). The *Myxinidae* do not have an ammocoete stage, and both somatostatin and insulin cells (B-cells) seem to occur throughout the animals' life (Falkmer *et al.*, 1974; Östberg *et al.*, 1976; Falkmer and Östberg, 1977). On the other hand, larval lampreys have only B-cells and an agranular precursor type (Ermisch, 1966; Titlbach and Kern, 1969; Falkmer and Patent, 1972; Barrington, 1972; Falkmer *et al.*, 1974; Falkmer and Östberg, 1977). The appearance of the somatostatin cells in the postmetamorphic lamprey islets is puzzling. Earlier speculations held that the somatostatin cells, then erroneously considered agranular, were a functional state of the B-cells (see Barrington, 1972; Falkmer and Patent, 1972). We now assume that the somatostatin cells have a specific endocrine function in both hagfishes and lampreys. Because they appear in *Lampetra fluviatilis* and *Petromyzon marinus* just before the parasitic or predatory phase of the life cycle, it is tempting to relate their function to the uptake of nutrition high in protein or lipids. However, their appearance also coincides with the catadramous migration to the oceans, and their proliferation continues during the anadromous fasting state preceding spawning and death; obviously, these observations invite further speculations. If our hypothesis of the neotenuous nature of lampreys is right, then the continued proliferation of somatostatin cells and the appearance of two other islet cells during the latest stage of the life cycle of *P. marinus* could be of no physiological significance at all. The nature and function of the somatostatin-like substance of cyclostome islets are at present unknown, and it could be that somatostatin occurs as a portion of a larger peptide (Van Noorden *et al.*, 1977).

The function of insulin in cyclostomes has received increasing attention during the past decade (see, for example, Leibson and Plisetskaya, 1973; Falkmer *et al.*, 1974; Plisetskaya, 1975; Hardisty *et al.*, 1975, 1976; Plisetskaya *et al.*, 1976; Larsen, 1976a, 1976b). In the myxinid *Eptatretus stouti*, isletectomy followed by ten weeks of fasting resulted in a significantly smaller drop of blood sugar than in controls (Matty and Gorbman, 1978). This is in contrast to observations in *Myxine glutinosa* where the operation had no effect on blood sugar level (Schirner, 1963; Falkmer and Matty, 1966). It remains to be seen if this difference is due to the observation time (which was shorter in the studies on *Myxine*), species differences, or other as

yet unidentified factors. However, the much greater sensitivity of *Eptatretus stouti* to mammalian insulin (Inui and Gorbman, 1977) suggests that a species difference may be involved. This would be consistent with Winbladh's (1976a, 1976b) conclusion that the peculiar islet follicles in *Myxine* indicate that in this genus insulin plays a less important role than in *Eptatretus*. Nevertheless, the very slow onset of insulin-induced hypoglycemia in both *Myxine glutinosa* and *Eptatretus stouti* (Falkmer and Matty, 1966; Inui and Gorbman, 1977), together with the failure of isletectomy to influence the glucose loading curve in *Myxine* (Falkmer and Matty, 1966), indicates that insulin plays only a minor role in the regulation of the blood sugar level of hagfishes. With respect to the insulin effect on glycogen, there is also a discrepancy between the findings in *Myxine* and *Eptatretus*. Matty and Falkmer (1965) found a decrease in liver glycogen and an increase in muscle glycogen in *Myxine*, whereas Inui and Gorbman (1977) found no effect at all in *Eptatretus*. Almost nothing seems to be known about the role of insulin in the lipid metabolism of hagfishes. Matty and Gorbman (1978) saw no effect of isletectomy on total plasma lipids in *Eptatretus stouti*. Isletectomy had no effect on total plasma proteins either (Matty and Gorbman, 1978); however, mammalian insulin at a dose as low as 0.1 IU/kg. markedly depressed the level of plasma amino acid nitrogen (Inui and Gorbman, 1977). The authors conclude "that the regulation of amino acid metabolism may be a primary function of insulin in the Pacific hagfish."

In contrast to the dearth of data on the role of insulin in hagfishes there are numerous pertinent investigations in lampreys. The function of insulin in the blood sugar regulation of the lampreys has been studied by various types of experiments: isletectomy (Barrington, 1942; Hardisty *et al.*, 1975), injection of insulin antibodies (Plisetskaya and Leibush, 1972; Plisetskaya *et al.*, 1976), insulin injections (Bentley and Follett, 1965a; Leibson and Plisetskaya, 1968, 1969; Plisetskaya and Prozorovskaya, 1971; Plisetskaya, 1975; Larsen, 1976a, 1976b), glucose loads followed by studies of blood sugar alterations (Bentley and Follett, 1965b; Hardisty *et al.*, 1975, 1976; Larsen, 1976a, 1976b; Plisetskaya *et al.*, 1976), changes in the islet cells (Barrington, 1942; Ermisch, 1966; Morris and Islam, 1969), and changes of the level of plasma immunoreactive insulin activity (Plisetskaya, 1975; Plisetskaya *et al.*, 1976). As in mammalian studies, isletectomy and injections of insulin antibodies caused hyperglycemia, whereas insulin injections had the opposite effect. Contrary to the isletectomized hagfish (Falkmer and Matty, 1966), the isletectomized pre and postmetamorphic lamprey becomes hyperglycemic (Barrington, 1942; Hardisty *et al.*, 1975). The latter authors estimate that the urine losses of glucose in postmetamorphic lampreys during the 24 hours following the operation amount to at least 60 per cent of the injected quantity, whereas intact controls lost during the same time approximately 0.5 per cent only. Injection of anti-insulin serum was followed by a hyperglycemia that lasted several days (Plisetskaya and Leibush, 1972; Plisetskaya *et al.*, 1976). The slow onset of this hyperglycemia, and the slow disappearance of both insulin-induced hypoglycemia and hyperglycemia after glucose injec-

tions (in the above-mentioned studies) are remarkable. As pointed out by Larsen (1976b), "the changes in blood glucose concentration after hypoglycemia induced by stress or by exogenous glucose...are so slow that one does not need to postulate hormonal control of the processes that return the blood glucose concentration to normal levels." Nevertheless, the above data show that insulin can effect the glycemia of lampreys; however, a mammalianlike control of the glycemia by insulin would require a response of the endogenous hormone to a glucose load, and this does not happen (Plisetskaya *et al.*, 1976).

Despite a large number of data (see Hardisty *et al.*, 1975; Plisetskaya, 1975; Leibson *et al.*, 1976b; Plisetskaya *et al.*, 1976), the physiological role of insulin in the regulation of glycogen still requires further investigation. A comparison of October-caught lampreys (*L. fluviatilis*) with lampreys collected during March showed a parallel decline of blood insulin (IRI), liver and myocardial glycogen; glycogen in the muscles of the body wall remained unchanged until a later date (Plisetskaya, 1975). After injection of insulin antibodies, there was a decrease in the glycogen content of liver, myocardium and glossohyal muscle, and no change in the glycogen of the body wall (Plisetskaya *et al.*, 1976). However, total isletectomy had no effect on liver glycogen (Hardisty *et al.*, 1975). During advanced insulin-induced hypoglycemia, there was a reduction of myocardial glycogen, an increase in liver glycogen, and no clear effect on the muscles of the body wall. In addition, the enormous glycogen stores of the lamprey brain were depleted (Leibson and Plisetskaya, 1968). An evaluation of these data is very difficult because the insulin effect varied in different organs at different times. Because large doses of insulin suppress the glucose-6-phosphatase activity of the liver *in vivo* (Plisetskaya and Ogorodnikova, 1967), and because glossohyal muscle responds to insulin *in vitro* with enhanced glucose uptake, activation of glycogen synthetase, and glycogen accumulation (Leibson *et al.*, 1976b), these tissues may be at least to some extent insulin-sensitive. However, it appears that the muscle of the body wall, the largest store of energy (Bentley and Follett, 1965a), is insulin-insensitive; the liver plays a modest role in insulin-induced changes of blood glucose (see Hardisty *et al.*, 1975; Larsen, 1978). The influence of large doses of insulin on the lipid metabolism of lampreys has been studied by Plisetskaya and Mazina (1969), who found a long-lasting decrease of nonesterified fatty acids (FFA) in the plasma. Interestingly, the FFA returned to starting levels before the hypoglycemia, which was simultaneously induced. A physiological role of insulin in the protein metabolism of lampreys is suggested by the strong increase of endogenous insulin after injections of amino acids (Plisetskaya *et al.*, 1976). Larsen (1976a, 1976b) observed a peculiar increase in body length after insulin injections; an understanding of the mechanisms responsible for this awaits further studies.

When compared with other groups of vertebrates, the islet physiology of the cyclostomes presents particular problems. Thus, the absence of A-cells could be due to degeneration or neoteny, and the physiological significance

of insulin could have decreased secondarily. Considerable differences in the glycemic effects of isletectomy between lampreys and *Myxine* on the one hand, and in the glycemic response to insulin between *Eptatretus* and *Myxine* on the other, if confirmed, might be related to the life style and habitat of these species. Lampreys are basically migratory animals with a marked life cycle, which includes originally both fresh and seawater phases (see Hardisty and Potter, 1971). *Eptatretus*, like all hagfishes, occurs only in the ocean where its species range from the surface to great depths; *Myxine* probably has been an inhabitant of the lower shelf for a very long time. This makes it tempting to draw a parallel between the presumable metabolic requirements of these animals and their insulin-sensitivity. However, the presently available data are too scarce to substantiate any of the above possibilities. We have to agree with Larsen (1976a, 1976b) that the physiological role of insulin in the lampreys is not clear and conclude that this also holds true for the hagfishes. However, it appears safe to state that both groups of cyclostomes differ from the gnathostomes in the following features: absence of typical A-cells, lack of hyperglycemic response to mammalian glucagon, and poor or absent glycemic response to hypophysectomy (Leibson and Plisetskaya, 1973; Falkmer *et al.*, 1974; Plisetskaya, 1975; Larsen, 1976a, 1976b; Plisetskaya *et al.*, 1976). Furthermore, very slow changes in blood glucose concentration after insulin or glucose injections are remarkable.

Gnathostomes

Many of the "jawed" vertebrates (gnathostomes) have been studied and all seem to possess both a well-developed gastrointestinal endocrine system (Fujita, 1973, 1976; Van Noorden *et al.*, this volume) and an islet organ. The latter is always associated with a usually extramural exocrine pancreas (Epple and Lewis, 1973; Epple and Brinn, 1975). Great variations in size, shape, and distribution of the islet tissue occur; however, the available evidence (Falkmer and Östberg, 1977) suggests that it produces in all gnathostomes insulin (B-cells), glucagon (A-cells), somatostatin (D-cells), and pancreatic polypeptide (PP-cells). A distinct fifth granular islet cell is so frequent in the holocephalians (Patent, 1976) and in *Lepisosteus* (Epple and Brinn, 1975) that we must assume that these species produce an additional regular islet hormone of so far unknown nature. More than four islet cell types also have been reported for various other species, belonging to all classes of gnathostomes with the exception of the lungfishes (Falkmer and Patent, 1972; Epple and Lewis, 1973; Watari, 1973; Falkmer and Östberg, 1977; J. B. Young, unpublished data). The apparent absence of a fifth cell type in the lungfishes may be due to the small number of investigations. Thus, it appears possible that a fifth islet hormone is a regular feature of all gnathostomes.

Class Chondrichthyes

Though the elasmobranch pancreas structurally represents the basic model for all gnathostomes (Epple, 1969), its endocrine functions are poorly

understood. It seems that no progress has been made since the review of Patent (1973), and the state of knowledge may be summarized as follows: exogenous insulin lowers the blood sugar level, which returns to normal more quickly after injections of crude extracts of shark insulin than after mammalian insulin. The effect of insulin on liver glycogen is difficult to evaluate because of great individual variations that may be related to the enormous quantities of liver lipids. *In vitro*, mammalian insulin increased the glucose uptake in the branchial muscle of the dogfish (Villem *et al.*, 1950). Mammalian glucagon increased the blood sugar (see Patent, 1973), but it has not yet been proven, if, or to what extent, this is due to hepatic glycogenolysis. Pancreatectomy (see Patent, 1973) showed varying results, and it seems that interference from surgical stress could be eliminated in only two out of four studies. In the shark, *Mustelus canis*, the operation resulted in blood sugar values ranging from hypo to hyperglycemia, with hyperglycemia prevailing (Abramowitz *et al.*, 1940). In the skate, *Raja erinacea*, the operation resulted in a hypoglycemic tendency (Grant *et al.*, 1969). In the Pacific ratfish, *Hydrolagus coliei*, injections of bovine, shark, and ratfish insulin all lowered the blood sugar. However, mammalian glucagon had no glycemic effect in this species, possibly because of very low levels of liver glycogen (Patent, 1970, 1973). From the data given here it is clear that the islet physiology of the cartilaginous fishes deserves much more attention than it has received so far.

Subclass Actinopterygii

This is the only group of fishes besides the chondrichthyes for which data on islet physiology exist. With the exception of one report on seasonal changes in the plasma insulin content in a sturgeon (Plisetskaya *et al.*, 1976), the data were all collected in teleosts, and in only a few species. As pointed out previously (Epple and Brinn, 1975; Lewis *et al.*, 1977), the widespread belief that the islet tissue of the teleosts is generally concentrated in a single Brockmann body is erroneous. There are enormous species-specific variations in shape and size of both exocrine pancreas and islet organ. Species with large Brockmann bodies usually have smaller islets in virtually surgically inaccessible pancreas regions. Inasmuch as the small islets may have a relatively high percentage of non-B-cells, removal of the Brockmann bodies only could result in a partially pancreatectomized animal in which the ratio between circulating insulin and glucagon is shifted in favor of the latter hormone (see Epple and Lewis, 1977).

In order to avoid this pitfall, we have studied the effects of total pancreatectomy in the American eel (*Anguilla rostrata*), the compact pancreas of which allows a virtually complete removal of all islet tissue. In a series of experiments, with one exception (Lewis and Epple, in preparation), we found no specific hyperglycemia. Rather, the values varied enormously from hypo to hyperglycemia. Liver glycogen was decreased temporarily or unaffected, and there were no clear alterations in muscle glycogen (Lewis and Epple, 1972; Lewis *et al.*, 1977; Epple and Lewis, 1977). These findings cor-

Yokote, 1975c). In cannulated European silver eels, a much lower dose of codfish insulin, but not bovine insulin, lowered plasma cholesterol (Ince and Thorpe, 1974). On the other hand, neither one of these preparations affected plasma cholesterol in the pike (Thorpe and Ince, 1974). Insulin injections in the pike increased the uptake of ^{14}C -glucose carbon into both liver and muscle lipids but had an opposite effect on ^{14}C -glycine carbon (Thorpe, 1976). On the other hand, isletectomy in *Myoxocephalus* (McCormick and Macleod, 1925), exposure to a glucose containing medium in cyprinids (Köhler, 1963; Sterne *et al.*, 1968), and glucose injections in *Clarias* (Yanni, 1964) all increased liver fat; this could be reversed by insulin injections. Similarly, an increase in muscle lipids after glucose injections was prevented by insulin (Yanni, 1964). *In vitro* insulin failed to alter fatty acid synthesis in liver, heart, and muscle preparations of *Opsanus tau* (Tashima and Cahill, 1964; 1968) but did stimulate hepatic lipogenesis in *Notemigonus chryssoleucas* (DeVlaming and Pardo, 1975).

The few reports on the effects of insulin on the protein metabolism of teleosts are rather consistent. Insulin injections lowered the plasma amino acid nitrogen in the European silver eel, Japanese eel, and pike (Ince and Thorpe, 1974; Thorpe and Ince, 1974; Inui and Yokote, 1975c). An analysis of individual plasma amino acids in the Japanese eel revealed that insulin decreased all amino acids with the exception of citrullin (Inui *et al.*, 1975). In the European yellow eel (Lewander *et al.*, 1976), a large dose of insulin was followed by an increase in liver protein (after 3-7 days) and muscle protein (after 3-10 days). In the pike, insulin injection strongly stimulated the incorporation of ^{14}C -glycine into muscle protein but had no effect on liver protein (Thorpe, 1976). Likewise, ^{14}C -glycine uptake into muscle protein was significantly increased after addition of insulin in *Opsanus tau* (Tashima and Cahill, 1968). In *Ophicephalus striatus* insulin injections lowered free amino acids and increased protein-bound amino acids in muscle (Seshadri, 1959). After pancreatectomy in the eel, there was a temporary increase in serum amino acids which may have been due to an acute insulin or glucagon deficiency (Epple and Lewis, 1977). Based on a large series of studies on intact, alloxanized, hepatectomized and/or insulin injected Japanese eels, it was concluded that insulin affects both plasma amino acids and hepatic gluconeogenesis by its anabolic action in peripheral tissues (Inui and Yokote, 1974, 1975a, 1975b, 1975c, 1975d; Inui *et al.*, 1975; Inui and Yokote, 1977).

It seems so far that the most promising findings on the function of insulin in teleosts have been obtained by the radioimmunological measurement of endogenous insulin, and of insulin released from islets *in vitro*. The data may be summarized as follows: plasma insulin levels vary with the season and reproductive activity. They are increased in spawning *Scorpaena* (Plisetskaya *et al.*, 1976) and goldfish (Singley and Chavin, 1976) but, interestingly, they drop during the spawning migration of the river lamprey and the sturgeon (Plisetskaya *et al.*, 1976). These differences may be related to

the differing feeding habits of these species inasmuch as starvation lowers and feeding increases plasma insulin. Glucose and certain amino acids have been found to be potent stimulators of insulin secretion in teleosts *in vivo* and *in vitro* (Watkins *et al.*, 1964; Tashima and Cahill, 1968; Patent and Foa, 1971; Ahmad and Matty, 1975; Plisetskaya *et al.*, 1976; Thorpe and Ince, 1976; Ince and Thorpe, 1977a, 1977b). However, in a comparison between fed and fasted rainbow trouts the correlation between plasma insulin and plasma amino acid nitrogen was more apparent than was the correlation between insulin and glucose (Thorpe and Ince, 1976). High glucagon injections raised the insulin level in *Scorpaena* (Plisetskaya *et al.*, 1976), but a smaller dose had no effect in the European silver eel even though it caused hyperglycemia. Ince and Thorpe (1977b) suggest that the latter result may be due to a stress-induced release of catecholamines.

The carp seems to be the only nonmammalian vertebrate known to develop a spontaneous type of diabetes mellitus, the so-called "Sekoke disease." Symptoms resemble in many ways those of severe longterm diabetes and include hyperglycemia, ketoacidosis, neuropathy, retinopathy, nephropathy, and muscle degeneration. However, the islets contain a considerable number of B-cells but, on the other hand, the animals respond poorly to insulin injections (Yokote, 1970a, 1970b; Nakamura *et al.*, 1971). From these data, it is difficult to draw any conclusions on the physiological role of insulin in teleosts.

The role of glucagon in teleost metabolism has been studied mostly with mammalian preparations, and it seems that no measurement of endogenous glucagon release has been performed. Usually, the injection of glucagon elicits a hyperglycemic response (see Epple, 1969; Plisetskaya, 1975; Bhatt and Khanna, 1976; Plisetskaya *et al.*, 1976; Inui and Yokote, 1977). However, *in vitro* studies in *Ictalurus nebulosus* and *Fundulus heteroclitus* (Umminger and Benziger, 1975; Umminger *et al.*, 1975) and observations in hepatectomized eels (Inui and Yokote, 1977) indicate that glucagon-induced hyperglycemia must be due mainly to hepatic glucose production though it is not necessarily accompanied by a decrease in liver glycogen concentration (Murat and Serfaty, 1975; Inui and Yokote, 1977; Murat and Plisetskaya, 1977). In the Japanese eel, muscle glycogen was not affected by glucagon injections (Inui and Yokote, 1977).

Data on the effects of glucagon on the lipid metabolism of teleosts are contradictory. No effect on plasma FFA was seen in the European eel (Larsson and Lewander, 1972). In the cannulated pike (*Esox lucius*), high doses caused an increase of plasma FFA, although they had no effect on plasma cholesterol (Thorpe and Ince, 1974; Ince and Thorpe, 1975). In *Lucioperca*, there was no effect on the release of FFA or glycerol from adipose tissue (Farkas, 1969).

Almost no data exist on the role of glucagon in the protein metabolism of teleosts. Single injections of this hormone in the pike (Thorpe and Ince, 1974) and in the Japanese eel (Inui and Yokote, 1977) had no effect on the

blood level of amino acid nitrogen. However, repeated injections in the Japanese eel lowered the AAN level. This effect was abolished by hepatectomy in both intact and alloxanized animals, which led Inui and Yokote (1977) to conclude that the hormone stimulates hepatic amino acid incorporation, probably resulting in gluconeogenesis.

Class Amphibia

Extant amphibians belong to three different orders: the *Urodela* (newts, salamanders), *Apoda* or *Gymnophiona* (caecilians), and *Anura* (toads, frogs). So far, only the islet physiology of the anurans and urodeles has been studied. Although only three types of islet cells were described in the caecilians (Gabe, 1968; Welsch and Storch, 1972), evidence from other studies suggests that all amphibians have at least the usual four types of islet cells (Epple and Brinn, 1976; Falkmer and Östberg, 1977). The pancreas is basically of the tetrapod type (Epple and Brinn, 1975), but size and cytological composition of the islets vary, particularly among the urodeles (Epple, 1966a; Epple and Brinn, 1975). A strong islet accumulation near the spleen has been described in the toad, *Bufo arenarum* (von Lawzewitsch, 1963). This inconspicuous "splenic lobe" has not been mentioned in any of the studies on the effects of pancreatectomy in other amphibians, and it remains to be seen if its possible neglect had an effect on the parameters measured. Another important factor is the strong cholinergic innervation of the frog islets (Trandaburu, 1976), the possible impact of which on the result of hormone injections seems to be unstudied.

Order Urodela

So far, pancreatectomy has been performed in only one urodele species, *Taricha torosa*. The operation included removal of part of the duodenum and of the bile duct, and blood sugar was measured only up to 24 hours after surgery (Wurster and Miller, 1960; Miller, 1961). This makes it impossible to decide whether the resulting hyperglycemia was due to an insulin deficiency or other disturbances. Injections of mammalian insulin lowered the already low normal blood sugar (9 to 58 mg. per cent; average, 25) to less than 8 mg. per cent (Wurster and Miller, 1960). The dosage of insulin affected the time of appearance and duration, but not the degree of hypoglycemia. With dosages greater than 50 units/kg., the blood sugar failed to return to normal, and the animals eventually died. With lower doses, the hypoglycemia lasted two to seven days. In *Necturus maculosus*, infusion of 25 mg. glucose per 100 g. body weight raised the plasma glucose level from approximately 9 to 69 mg. per cent within 30 minutes, followed by a return to normal within 24 hours. Simultaneous infusion of 10 IU insulin per 100 gm. body weight showed a distinct effect on all plasma glucose levels measured later than 30 minutes after glucose infusion, with values of less than 3 mg. per cent from 12 through 48 hours. Because animals without detectable plasma glucose did not show hypoglycemic symptoms, the authors con-

cluded that "plasma glucose is not a *required* source of energy in the mud puppy" (Copeland and DeRoos, 1971). However, in another neotenuous urodele (*Amphiuma means*), it was possible to enhance *in vitro* insulin release with 0.5 and 2.5 mg./ml. of glucose (Gater and Balls, 1977). In *Taricha torosa*, intraperitoneally injected amorphous glucagon failed to elicit any response in doses as high as 1000 μ g/kg. (Wurster and Miller, 1960). However, intravenous injections of 100 μ g/kg. of crystalline glucagon resulted in a threefold increase of blood sugar (see Miller, 1961).

Order Anura

In all anurans studied, pancreatectomy was followed by hyperglycemia (see Miller, 1961; Epple and Lewis, 1973; Penhos and Ramey, 1973). However, the specificity of this effect in older studies is difficult to assess for either "radical" or incomplete surgery may have effected the results (see Penhos and Ramey, 1973). With an improved technique, both pancreatectomized frogs (Penhos and Krahle, 1962) and toads (Penhos and Lavintman, 1964) developed a rapid and continuously increasing hyperglycemia and ketonemia. The average survival time for frogs was six days, for toads seven days. With a similarly improved technique, Dosne (1943) observed in subtotally operated *Bufo arenarum* a higher increase in blood sugar than in totally pancreatectomized animals. In this study, the average survival time was five days. Inasmuch as some regeneration of islet tissue and return to normal blood sugar levels was found in pancreatectomized animals 40 days after surgery, Penhos and Ramey (1973) suggested that in this study the islet tissue was incompletely removed. This raises the possibility that the higher glycemia in subtotally pancreatectomized toads may have been influenced by an increased percentage of persisting A-cells, as discussed above in connection with the removal of Brockmann bodies in teleosts. Insulin injections into anurans are followed by hypoglycemia, which, however, develops later and lasts longer than in mammals. Furthermore, anurans can survive at blood sugar levels that must be close to or actually be zero (see Miller, 1960, 1961; Rangneker and Sabnis, 1964; Penhos and Ramey, 1973; Hanke, 1974a, 1974b). In *Xenopus laevis*, the hypoglycemic response to insulin is stronger in postmetamorphic than in larval animals (Hanke and Neumann, 1972; Hanke, 1974c). This corroborates Frye's (1965) suggestion that larval amphibians may control their carbohydrate metabolism independently of insulin. The date on the effect of exogenous insulin on liver and muscle glycogen of intact anurans vary greatly, making it impossible to draw a general conclusion (see, for example, Hanke and Neumann, 1972; Leibson and Plisetskaya, 1973; Hanke, 1974b). *In vitro* insulin fosters the uptake of glucose from the incubation medium into liver, skeletal, and heart muscle of anurans and stimulates glycogen formation in skeletal muscle (see Penhos *et al.*, 1967b; Leibson and Plisetskaya, 1973; Leibson *et al.*, 1976b). Gourley *et al.* (1969) found seasonal differences in the effects of insulin on the carbohydrate metabolism of isolated frog muscle. Insulin, injected in September into

5°C-acclimated *Rana temporaria*, lowered the level of plasma free fatty acids but had no effect in 25°C-acclimated frogs; neither was there an effect in warm or cold-acclimated winter frogs. In the latter three experiments, there was no insulin effect on plasma glycerol, although this hormone increased plasma glycerol in cold-acclimated summer frogs. From these and additional findings, Harri and Puuska (1973) concluded that insulin lowered the level of plasma FFA by stimulation of reesterification and/or oxidation of FFA without affecting the lipid release from adipose tissue. This conclusion is in agreement with the suggestion of Farkas (1969) that the adipose tissue of the poikilotherms (unlike the situation in homeotherms) lacks a hormone-sensitive lipase. Insulin injections into larval and juvenile *Xenopus laevis* increased the total lipid content of the body and lowered the lipid content of the extracellular fluid. Insulin also lowered the FFA content of the extracellular fluid from stage 60 on. However, the differences, even though consistent, were statistically not significant (Gunesch, 1974). There seem to be only two studies on the effect of insulin on the protein metabolism in anurans. These studies show that insulin stimulates the incorporation of leucine into liver proteins of *Rana catesbeiana* (Penhos and Krahle, 1962; 1963).

Glucagon injections increase the blood sugar level of anurans (Miller, 1960, 1961; Leibson and Plisetskaya, 1973; Penhos and Ramey, 1973; Hanke, 1974a, 1974b), and this increase is obviously connected with hepatic glycogenolysis (Penhos and Lavintman, 1964). However, this hepatic effect is not always obvious, and restoration of normal levels of liver glycogen occurred in one experiment with *Rana temporaria* within 24 hours (Hanke, 1974b). Seasonal differences in the hyperglycemia response to exogenous glucagon have been observed in both *Rana temporaria* (Hanke and Neumann, 1972) and *Rana pipiens* (Farrar and Frye, 1977). Although the effective minimum dose of glucagon (mammalian preparations) is relatively higher than that of insulin, its actions appear faster and are of shorter duration (Hanke, 1974b). Glucagon has a definite glycemic effect in late larval stages of *Xenopus laevis*; however, the start of its glycogenolytic action in the liver could not be clearly determined, probably because the glycogen content of the liver of younger stages is too small (Hanke and Neumann, 1972). In *Rana temporaria*, mammalian glucagon had no effect on the level of plasma free fatty acids (Harri and Puuska, 1973). Using the perfused toad liver preparation, Penhos *et al.* (1967b) conclusively showed that glucagon decreased the level of circulating cholesterol and strongly stimulated ketogenesis. This is in agreement with its action in mammals (Unger *et al.*, 1977).

Class Reptilia

The extant reptiles belong to four different orders: Chelonia (turtles), Crocodylia (crocodiles, alligators), Squamata (lizards and snakes), and Rhynchocephalia (single living representative, *Sphenodon punctatus*). These groups represent a small sample of a once enormously varied and successful

group, species of which occupied all habitats from the ocean to the air; consequently, we may expect endocrine-metabolic similarities only between closely related groups. The Chelonia form a branch that left the main line of reptilian evolution rather early; they were followed by the Synapsida, ancestors of the mammals. Another side branch that developed later, the Archosauria, gave rise to both the Crocodilia and birds. Finally, the Lepidosauria gave rise to both the Squamata and Rhynchocephalia. For the comparative endocrinologist, it is somewhat disappointing that the islet biology of the birds resembles more that of the distant Squamata than that of the more closely related crocodiles, and that crocodiles and turtles resemble in some ways more the amphibians and mammals (see below). The Morphology of pancreas and islet organ has been reviewed by several authors during the past decade (Miller and Lagios, 1970; Gabe, 1970; Falkmer and Patent, 1972; Penhos and Ramey, 1973; Falkmer and Östberg, 1977). From the recent studies of Theret *et al.* (1975) and Grillo *et al.* (1976), it appears likely that the reptiles generally have at least four different cell types (the PP-cells have not yet been identified immunohistologically). A, B, and D-cells occur in all species so far studied, but their percentages seem to vary considerably. A particular problem exists in the Squamata, where special staining characteristics make it difficult to separate A and D-cells (see Gabe, 1970; Theret *et al.*, 1975). Perhaps, some of these unusual argyrophil cells will turn out to be PP-cells. Many Squamata have a preponderance of islet cells other than B-cells; however, the Amphisbaenidae (Gabe, 1970) and the water snake *Natrix piscator* (see Rangneker and Padgaonkar, 1972) seem to have a very high percentage of B-cells. A particular feature of the Squamata is the concentration of islet tissue in the pancreas region near the spleen; in snakes it may even invade the latter (see Miller, 1962; Falkmer and Patent, 1972; Theret *et al.*, 1975; Falkmer and Östberg, 1977). Most likely, this "splenic lobe" corresponds to the Brockmann bodies of the actinopterygians, the islet accumulation found in *Bufo arenarum*, and the splenic lobe of the birds. As shown by Rhoten (1974), this region of the squamate pancreas is ideal for *in vitro* studies, and it should be most useful for the extraction of islet hormones.

In the Chelonia, radical pancreatectomy results in hyperglycemia and glucosuria (Aldehoff, 1891; Nishi, 1910; Foglia *et al.*, 1955). It is noteworthy that Nishi (1910) observed identical increases in liver glycogen in both intact and pancreatectomized turtles. Because of the severe nature of the operation and early sacrifice in the studies of Aldehoff and Nishi, and because of the poor survival of the animals in the study of Foglia *et al.* (most animals died one to three days after surgery, and only one survived for 10 days), it is difficult to draw definite conclusions as to the effect of pancreatectomy in turtles. There is no indication of a hypoglycemic phase as in the Squamata, but the possible glycemic effect of postoperative stress requires further clarification. Turtles respond to mammalian insulin with hypoglycemia, and to mammalian glucagon with hyperglycemia (see Miller, 1961; Penhos and

Ramey, 1973). Chronic administration of glucagon lowered liver glycogen but had no effect on muscle glycogen (Marques, 1967).

Order Crocodilia

Pancreatectomy in *Alligator mississippiensis* resulted in a progressive increase in both blood sugar and ketonemia. Many animals survived for several weeks, and a single animal even for 15 weeks with a final blood sugar level of 589 mg. per cent and a ketonemia of 134 mg. per cent (Penhos *et al.*, 1967a). According to Penhos and Ramey (1973), the ketone levels found in this study are the highest reported for any vertebrate species. Alligators respond to mammalian insulin with hypoglycemia and to mammalian glucagon with hyperglycemia. In pancreatectomized alligators, glucagon is ketogenic. However, when compared with other reptiles and anurans, the glycemic response to both hormones is very slow, which is probably related to the slow rate of metabolism of the alligator (see Penhos and Ramey, 1973). In *Caiman latirostris*, insulin lowered the plasma amino acids and promoted protein synthesis within tissues (Hernandez and Coulson, 1968).

Order Squamata

Pancreatectomy in lizards resulted in an early hypoglycemia (see Miller, 1961; Rangneker and Sagnis, 1966; Penhos and Ramey, 1973). However, when the animals survived for one to two weeks, hyperglycemia developed; ketonemia showed a continuous increase from the beginning (Penhos *et al.*, 1967a). In the snake *Xenodon meremii*, the situation was the same but hypoglycemia developed earlier and was of shorter duration (Houssay and Penhos, 1960). In another water snake, *Natrix piscator*, there was no hypoglycemic phase but a continuous increase of blood sugar. Both liver and muscle glycogen were lower than in intact controls; however, these parameters underwent considerable variations during the 15 days of the experiment. After 15 days, plasma cholesterol was significantly increased (Rangneker and Padgaonkar, 1972). Both lizards and snakes respond to mammalian insulin with hypoglycemia; however, it appears that lizards are rather insensitive, and an avian-like insulin resistance has been reported (see Miller, 1961; Penhos and Ramey, 1973). Very high glucose concentrations caused a mammalian-like biphasic insulin release from lizard islets *in vitro* (Rhoten, 1974). *Natrix piscator* developed aglycemia 24 hours after injection of 40 IU/kg. of mammalian insulin, and the return to normal levels was not completed seven days after the injection. Pancreatectomized snakes were less insulin-sensitive than intact animals; hypophysectomized-pancreatectomized snakes were aglycemic within 3 hours (Padgaonkar and Rangneker, 1975). Glucagon injections provoked hyperglycemia in all squamata studied (see Miller, 1961; Suryawanshi and Rangneker, 1971; Penhos and Ramey, 1973). In the lizard *Uromastix hardwickii*, glucagon caused hypocholesterolemia, but insulin had no effect on this parameter (Suryawanshi and Rangneker,

1971). *In vitro*, amino acids stimulated insulin release from lizard islets (Rhoten, 1973).

Class Aves

The avian pancreas differs from that of the other gnathostomes in the peculiar arrangement of the islets. A and B-cells are largely separated in large A and small B-islets; both, however, contain D-cells (Guha and Ghosh, 1978). PP-cells occur at the periphery of the islets and as scattered elements within the exocrine tissue (Larsson *et al.*, 1974). Many islets are concentrated in an inconspicuous extension of the pancreas, the "splenic lobe." In the *Phasianidae*, the A-islets seem to be restricted to the splenic lobe and the so-called third lobe (Mikami and Mutoh, 1971; Smith, 1974). The high percentage of A and D-cells (see Falkmer and Östberg, 1977; Guha and Ghosh, 1978) explains the high pancreas content of glucagon (cf. Samols *et al.*, 1968; Hazelwood, 1976a, 1976b) and somatostatin (Weir *et al.*, 1976), when compared with mammals, and the high levels of circulating glucagon (see Hazelwood, 1976b; Mialhe, 1976; Laurent and Mialhe, 1976; Karmann and Mialhe, 1976; Sitbon and Mialhe, 1978). On the other hand, the low insulin content of the pancreas corresponds to the small percentage of B-cells. The glucagon/insulin ratio in blood is higher than in mammals, but it varies with the physiological state of the animal (see Langslow and Hales, 1971; Hazelwood, 1976a, 1976b; Mialhe, 1976; Karmann and Mialhe, 1976; Laurent and Mialhe, 1976).

Total pancreatectomy is possible, at least in some species. The greatest problem with this procedure is the complete removal of the splenic lobe, which is difficult to find especially when large amounts of adipose tissue are present. Failure to remove the splenic lobe with its many A-islets but relatively few B-islets may in part explain discrepancies between the reports on the effects of pancreatectomy. As shown by Mialhe (1958) in the duck, the functional state of the islet tissue in the splenic lobe of subtotally pancreatectomized ducks determines whether the operation is followed by normoglycemia (A and B-cells appear normal), hyperglycemia (exhaustion of B-cells), or hypoglycemia (exhaustion of A-cells). On the basis of these observations, it appears possible that the occurrence of "diabetes mellitus" in pancreatectomized birds of prey, and the greatly varying observations in pancreatectomized geese, ducks, pigeons, and chickens in older studies (cf. Mihail *et al.*, 1963; Langslow and Hales, 1971; Assenmacher, 1973; Hazelwood, 1976a, 1976b, Mialhe, 1976) may be a matter of the islet cell ratio in a pancreatic remnant rather than an indication of fundamental metabolic differences. According to Mialhe (1976), complete pancreatectomy in the duck leads inevitably to fatal hypoglycemia within hours after the operation unless glucose or glucagon is given. The same situation develops in the chicken after removal of most of the A-islets by partial pancreatectomy (Mikami and Ono, 1962). On the other hand, infusion of insulin antibodies into the duck (Mirsky *et al.*, 1964) and chicken (Bondareva, 1970) resulted in

hyperglycemia. In the pancreatectomized duck, there is a slow decrease of both plasma FFA and triglycerides (Desbals *et al.*, 1967), even if a normal level of glycemia is maintained (see Mialhe, 1976). In both the pancreatectomized duck (Samsel, 1973) and the 99 per cent pancreatectomized chicken, there is an increase in plasma AAN, but in the latter study it was not accompanied by hyperglycemia (Baker and Hazelwood, 1977). Based on findings in totally pancreatectomized chickens, it has been suggested that in this species an extrapancreatic source of insulin exists (Colca and Hazelwood, 1976). This may explain why measurable amounts of insulin can be found in what seem to be totally pancreatectomized birds (Sitbon and Mialhe, 1978).

Insulin injections provoke a mild hypoglycemia (see Langslow and Hales, 1971; Hazelwood, 1976a, 1976b; Groscolas and Bezard, 1977). However, avian tissues are more sensitive to avian than to mammalian insulin *in vivo* (Hazelwood *et al.*, 1968) but not *in vitro* (see Langslow, 1976; Leibson *et al.*, 1976b). On the other hand, birds show an enormous insulin resistance (see Hazelwood, 1976a) that is comparable only to that reported in *Squamata* (Miller, 1961). At least a reduced number of insulin binding sites (Simon *et al.*, 1977), a plasma binding factor, and a catecholamine response are involved in this phenomenon (see Turner and Hazelwood, 1974). Both *in vivo* and *in vitro* glucose is a poor stimulant of insulin release from the avian pancreas. There is only a transitory response to glucose even when hyperglycemia continues, and very high blood sugar levels are necessary to obtain a response. The second phase of insulin release after a glucose stimulus, typical for the mammalian pancreas and also seen in a lizard (Rhoten, 1974), is either very weak or absent (see Langslow and Freeman, 1972; Hazelwood, 1976a, 1976b; King and Hazelwood, 1976; Naber and Hazelwood, 1977). In the Gentoo penguin, injection of glucose (1 g./kg. body weight) did not stimulate insulin release at all (Chieri *et al.*, 1972). Thus, the glucose insensitivity of the avian B-cells is another similarity with the squamate pancreas (Rhoten, 1973; 1974). Insulin injections increase or decrease the glycogen content of liver, skeletal muscle, and heart muscle, depending on the nutritional state of the bird and the dose of the hormone (see Hazelwood, 1965; 1976a). Direct and indirect effects of insulin may be involved. As will be pointed out below, the embryonic chicken heart is ideally suited for *in vitro* studies on the effects of insulin on carbohydrate and protein metabolism. Exogenous insulin has a variable effect on lipid metabolism. In owls it has no effect at all on plasma FFA (Grande, 1970), whereas in other birds (goose, duck, fowl, Emperor penguin) it causes an increase (see Nir and Levy, 1973; Hazelwood, 1973, 1976a; Groscolas and Bezard, 1977). However, evidence from two types of experiments indicates that this insulin effect is due to a glucagon release triggered by hypoglycemia: it is absent in totally pancreatectomized ducks (Desbals, 1972); insulin has no antilipolytic effect on avian adipose tissue (Goodridge, 1968; Langslow and Hales, 1969; Grande, 1969). It remains to be seen whether the small

stimulating effect of large quantities of insulin on the fatty acid synthesis in isolated hepatocytes of neonatal chicks is of biological significance (Goodridge, 1973). There is little information on the role of insulin in avian protein metabolism. Insulin injections increased the uptake of amino acids in the heart of the chicken embryo (Guidotti *et al.*, 1969), and tolbutamide lowered plasma AAN in the chicken, an effect most likely due to stimulation of insulin release (Baker and Hazelwood, 1977). In young fowls, oral administration of amino acids resulted in an increase in both plasma insulin and glucose, the latter probably due to stimulation of glucagon release (Langslow *et al.*, 1970). In the Gentoo penguin, arginine infusion resulted in an immediate release of insulin. An increase of plasma urea and uric acid in the same experiment suggests that some of the arginine was metabolized through the urea cycle (Farina *et al.*, 1975).

Glucagon injections induce a hyperglycemia that involves hepatic glycogenolysis (Langslow and Hales, 1971; Hazelwood, 1973, 1976a, 1976b; Anderson and Langslow, 1975; Mialhe, 1976; Laurent and Mialhe, 1976; Sitbon and Mialhe, 1978). However, because glucagon-induced hyperglycemia is less marked but lasts longer than the lipolytic effect of the hormone, it seems that it is partly caused by a decreased glucose utilization in the presence of high levels of circulating FFA (see Grande and Prigge, 1970; Freeman and Manning, 1974; Groscolas and Bezard, 1977). Glucagon injections increase the glycogen content of both heart and liver in fed, but not in fasted, chickens in the presence of hyperglycemia but absence of increased insulin secretion (see Langslow and Hales, 1971). This indicates a gluconeogenic activity of glucagon in birds (see also Hazelwood, 1976a). Glucagon plays a particularly important role in the lipid metabolism of birds. *In vitro*, avian adipose tissue is highly sensitive to the lipolytic action of glucagon, as originally shown in the studies of Goodridge and Ball and subsequently confirmed by various other investigators (Goodridge and Ball, 1967; Langslow and Hales, 1971; Krug and Mialhe, 1971; Hazelwood, 1973, 1976a). *In vivo*, glucagon injections provoke a sharp rise in circulating FFA (see Langslow and Hales, 1971; Hazelwood, 1973, 1976b; Mialhe, 1976). The study of De Oya *et al.* (1971) in geese strongly suggests that the liver incorporates these fatty acids into triglycerides—their release into the blood stream is decreased by glucagon. On the other hand, high doses of glucagon inhibited the fatty acid synthesis in isolated hepatocytes of neonatal chicks (Goodridge, 1973). With respect to plasma amino acids, glucagon injections have the same effect as insulin injections, that is, they decrease the circulating levels of AAN (Samsel, 1973). Pancreatic glucagon secretion is decreased by high levels of blood sugar, and this reaction occurs faster than the response of the insulin secretion (see Mialhe, 1976; Laurent and Mialhe, 1976). Similarly, pancreatic glucagon is involved in a negative feedback system with plasma FFA (Gross and Mialhe, 1974). The extreme sensitivity of avian adipose tissue to glucagon and the lack of an antilipolytic effect of insulin in birds suggest that regulation of lipid metabolism may be the most

important function of avian glucagon (see Hazelwood, 1973, 1976a, 1976b; Gross and Mialhe, 1974; Mialhe, 1976). In this connection, it is noteworthy that the glucose-glucagon feedback, but not the FFA-glucagon feedback, mechanism of birds depends on the presence of insulin (Laurent and Mialhe, 1976). Arginine infusion stimulates the secretion of both pancreatic glucagon and insulin. Suggestions that glucagon is involved in both the thermoregulation (Palokangas *et al.*, 1973) and the stress response (Freeman and Manning, 1976) of birds require further studies. For a comparison with other groups of vertebrates, particularly the mammals, the most striking aspects of the physiology of insulin and glucagon in the avian metabolism may be summarized as follows: the glycemia depends much more on glucagon than on insulin, and this is reflected also in the ratio of circulating glucagon and insulin; insulin has no antilipolytic effect on avian adipose tissue but instead indirectly enhances the lipolytic activity of glucagon.

Pancreatic polypeptide was first discovered as a contaminant during the purification of avian insulin by Kimmel *et al.* (1968) and therefore was named "avian pancreatic polypeptide" (AAP). In further studies, Hazelwood, Kimmel, and co-workers conclusively showed that this substance is a true pancreatic hormone that has a powerful, direct action on the avian stomach (see Hazelwood, 1973, 1976a, 1976b). Despite its gastrin-like action, it shows no structural similarity to this hormone. In the chicken, APP strongly increases the total volume of proventricular secretions as well as the release of pepsin, acid, and protein. Inasmuch as this result is obtained with low doses of the hormone, it is likely that gastric stimulation is the major physiological function of APP. This suggestion is supported by the finding that during fasting the level of circulating APP drops and during refeeding it increases. The latter effect is more obvious in older than in younger chickens (Hazelwood, 1976a). Another possible physiological effect of APP is suggested by findings with isolated chicken adipocytes. Here, the hormone is specifically bound to receptors, and reduces glucagon-stimulated lipolysis (McCumbee and Hazelwood, 1977). A number of other metabolic effects, seen after injection of high doses of APP may be either pharmacological or due to the stimulation of glucagon release (see Hazelwood, 1973; 1976a, 1976b; McCumbee and Hazelwood, 1977).

Class Mammalia

In mammals, pancreatectomy leads to diabetes mellitus. However, the severity of the symptoms varies greatly, and it remains to be seen whether these variations are due to biological differences or technical problems. A particularly severe diabetes has been observed in pancreatectomized dogs and cats (see Sirek, 1969). The main symptoms of severe mammalian diabetes include hyperglycemia, glucosuria, depletion of liver and muscle glycogen, hyperlipemia (triglycerides, FFA, cholesterol), depletion of adipose tissue, fatty liver, ketonemia and ketonuria, and breakdown of tissue proteins, particularly in skeletal muscle. These symptoms are virtually identical with

those of severe alloxan and streptozotocin diabetes and they can be prevented with insulin injections (see Pfeiffer 1969, 1971; Steiner and Freinkel, 1972; Volk and Wellmann, 1977). The mammalian B-cell is sensitive to changes in the levels of circulating glucose, FFA, and certain amino acids. In sheep, it is more sensitive to butyrate than to glucose. Increases in the levels of these metabolites are answered by an insulin release (see Lacy, 1977). Thus, there can be little doubt that insulin is the dominating hormone of mammalian intermediary metabolism. It remains to be seen if, or to what extent, in the pancreatectomized mammal, the symptoms of the removal of pancreatic glucagon, PP, and somatostatin are masked by the direct and indirect symptoms of insulin deficiency, compensated by gastrointestinal secretion of these substances, or not yet clearly identified. Pancreatic glucagon seems to act mainly at the liver, where it causes glycogenolysis, gluconeogenesis, and ketogenesis. As a result of the glycogenolytic and gluconeogenic actions, the level of blood sugar will increase (Gerich *et al.*, 1976; Unger *et al.*, 1977). However, it appears safe to state that so far there is no evidence that the lipolytic activity of glucagon in mammals is as important as it is in birds. Increased blood levels of amino acids stimulate the secretion of glucagon simultaneously with that of insulin (Lefebvre and Unger, 1972; Gerich *et al.*, 1976; Mialhe, 1976). The physiological significance of this process is obviously the maintenance of a relatively normal blood sugar level when insulin exerts its anabolic actions in the postprandial mammal. Due to the sensory mechanisms of A and B-cells, insulin and glucagon synergize in the maintenance of the mammalian glycemia in a degree of perfection that may be unparalleled in the animal kingdom.

The physiological role of mammalian PP requires further study. The gastric actions are the same as in birds, though it must be recalled that contrary to other vertebrates, in mammals gastric enzymes and acid are produced by separate cell types. It seems that the strongest stimulus for PP-release is the ingestion of a meal rich in proteins. An interesting observation of still unknown significance is the strong increase in circulating PP with age (Floyd *et al.*, 1977), and of PP-cells in juvenile diabetes (Gepts *et al.*, 1977).

The functions of pancreatic somatostatin also are poorly understood. It remains to be seen if and how its "paracrine" inhibitory effect on the mammalian A and B-cells (Guillemin and Gerich, 1976; Unger *et al.*, 1977) can be integrated into the general picture of the islet functions. Its simultaneous occurrence with gastrin in the D-cells of the human (Erlandsen *et al.*, 1976) raises the question of the functional significance of this association, as does the ubiquitous occurrence of this substance in various nervous, digestive, and endocrine organs (Van Noorden *et al.*, this volume).

Summary

The available data suggest that among the gnathostomes the importance of both insulin and glucagon greatly increases in the tetrapods—

pancreatectomy is either followed by diabetes mellitus (mammals, crocodilians, turtles, anurans) or a severe hypoglycemia (birds and at least some *Squamata*). It seems that the control of amino acid levels is an ancient function of insulin, whereas its glucostatic function is more newly evolved and best developed in mammals. In lower vertebrates, insulin seems to be more concerned with glycogen formation and its glycemic effect may have been originally of minor importance. The extreme sensitivity of mammalian adipose tissue to insulin and of avian adipose tissue to glucagon are specializations that developed, phylogenetically, rather late. Probably, lipolysis in the adipose tissue of lower vertebrates is controlled by factors other than islet hormones. The role of glucagon in exothermic vertebrates is poorly understood. So far, the only clearly identified physiological role of PP seems to be the stimulation of gastric secretions in birds and mammals. Data on the evolution of the functions of pancreatic somatostatin are still lacking.

ISLET HORMONES AND OSMOREGULATION

For several years there has been increasing direct and indirect evidence of a role of the islet organ in osmoregulation. In the myxinid *Eptatretus stouti*, isletectomy and subsequent starvation increased the level of plasma potassium but there were no changes in the levels of sodium and calcium (Matty and Gorbman, 1978). It is noteworthy that under similar conditions hypophysectomy had no effect on these three electrolytes (Matty *et al.*, 1976). Recently, we reported that pancreatectomized eels (*Anguilla rostrata*) survived well in freshwater, but very poorly in seawater, and that pancreatectomized-hypophysectomized eels survived only in 1/3 seawater but not in fresh or 2/3 seawater. Furthermore, a series of sham operations showed convincingly that the mortality in seawater was due to an islet hormone deficiency (Epplé and Lewis, 1975, 1977). Before death of the pancreatectomized eels in seawater occurs, their muscles, but not livers, are strongly dehydrated, while simultaneously the plasma osmolarity (Na^+ and Cl^-) is strongly increased. Taken together, these data suggest a direct or indirect effect of islet hormone deficiency at one or more sites of ion transport (Epplé, unpublished data). Free amino acids and related substances, particularly taurine, play an important role in the osmoregulatory tissue adaptation of marine invertebrates (Allen and Garrett, 1971), and possibly also of teleosts (Love, 1970) and elasmobranchs (Boyd *et al.* 1977). In mammals, (see Huxtable and Barbeau, 1976), high levels of taurine are found in heart and brain, and it has been shown that taurine increases membrane permeability to potassium and chloride but not to sodium (Gruener *et al.*, 1976). In addition, taurine seems to be involved in "isosmotic intracellular regulation" (Hoffman and Hendil, 1976). A recent study of this substance in the eel gave the following results: pancreatectomy in freshwater eels causes a highly significant fall in taurine levels in both myocardium and gills but has no effect on taurine levels in white muscle. Pancreatectomy and subsequent transfer to seawater lead to decreased taurine levels in the myocardium only (Epplé

and Kocsis, unpublished data). The above data together show that in the eel, islet hormones affect both the levels of electrolytes and the level of an important intracellular amino acid-type substance. Clearly, more work is needed to analyze the osmoregulatory effects of the individual islet hormones. It may be relevant that exogenous insulin decreased the free amino acids in the muscles of an Indian teleost (Seshadri, 1959) and decreased almost all plasma amino acids, including taurine, in the Japanese eel (Inui *et al.*, 1975). In amphibians, insulin effects the ion transport across several epithelia (see Katz and Lindheimer, 1977; Park and Hong, 1977). In mammals, insulin affects the ion transport in kidney, heart and skeletal muscle, and brain (see Zierler, 1972; Kones, 1974; Arief *et al.*, 1974; Katz and Lindheimer, 1977). Glucagon has been shown in numerous studies to either prevent absorption, or to increase secretion of ions and water in the intestine (Hubel, 1972; Mekhjian *et al.*, 1972; Barbezat, 1973; Rudo and Rosenberg, 1973; Hicks and Turnberg, 1974; Mallinson *et al.*, 1974). Glucagon also affects plasma electrolytes and renal function (Foà, 1968; Walser, 1973; Conte *et al.*, 1972; Katz and Lindheimer, 1977). Shlatz and Marinetti (1972) observed antagonistic effects of insulin and glucagon on the binding of calcium to membranes of liver cells. Several studies on the control of circulating potassium indicate that its level in mammals are controlled by a feedback system involving both insulin and glucagon (Hiatt *et al.*, 1972; Santeusano *et al.*, 1972; Kuzuya *et al.*, 1974). At present, it is difficult to decide which of the reported effects are physiological and due to a direct action of the islet hormones. However, from all of these studies it appears that an antagonism between insulin and glucagon may exist in that insulin conserves water and certain ions and glucagon opposes this action.

ONTOGENY OF ISLET FUNCTIONS

A discussion of comparative studies leads naturally to an examination of developmental studies, for valuable similarities often exist between the two which may help to further our understanding of how processes have evolved. Several reviews have examined extensively the early literature dealing with the comparative and developmental morphology of the pancreatic islets (Falkmer and Marques, 1972; Falkmer and Patent, 1972; Epple and Brinn, 1975). The literature concerning the ontogenic development of function, principally in mammalian islets, also has been reviewed in detail (Asplund *et al.*, 1975; Milner *et al.*, 1975; Shelley *et al.*, 1975). Until recent years, data concerning the embryonic development of the few species studied in detail (rat and human fetuses) suggested some strong parallels to the phylogenetic development of the islets. However, as methods of analyzing fetal metabolism have been refined, and as more species are investigated, the comparisons between phylogeny and ontogeny become less and less clear. Some interesting points remain, however, that suggest some directions for future research.

Work in the area of fetal metabolism and islet physiology has largely been limited to various mammals, primarily the rat, rabbit, sheep, and human

fetuses, and to chick embryos (for review, see Leibson *et al.*, 1976a). During the development of the mammalian fetus, the primary changes in islet involvement in metabolism can be separated into four stages. The following chronology of the development of islet control of metabolism, based loosely on that of Foà *et al.* (1976), will deal with findings from mammalian studies, except where noted.

Stage I

From the first appearance of the pancreatic rudiment in both mammals and birds, A cells are identifiable by immunoassay or by electron microscopy—at eight to nine weeks in the human fetus (Assan and Boillot, 1971; Like and Orci, 1972), at day 11 of gestation in the rat fetus (Pictet and Rutter, 1972), and at 3 to 3.5 days of incubation (stage 17-18 of Hamburger and Hamilton, 1951) in the chick embryo (Beaupain and Dieterlen-Lievre, 1974). Slightly later, and in fewer numbers, B cells appear—at 10.5 weeks in the human fetus (Like and Orci, 1972), at day 11.5 in the fetal rat (Pictet and Rutter, 1972), and at 3.5 days of incubation (stage 20) in the chick embryo (Dieterlen-Lievre and Beaupain, 1974). Beaumont (1970) describes the development, first of B, then of A cells in the tadpole of *Discoglossus pictus*.

At about the same time the B cells appear, or slightly before in the human (Like and Orci, 1972), the D cell becomes identifiable—at least by 10.5 weeks of gestation in the human fetus (Like and Orci, 1972), by day 15 in the fetal rat (Pictet and Rutter, 1972), and by 3.75 days of incubation (40-45 somites) in the chick embryo (Andrew, 1977). Dubois *et al.* (1975) found cells immunoreactive to somatostatin in the pancreas of an eleven week human fetus.

In the three species noted above, the A to B ratio is high during this stage, with a concomitantly high ratio of pancreatic glucagon to insulin (Assan and Boillot, 1971; Pictet and Rutter, 1972; Beaupain and Dieterlen-Lievre, 1974). It is conjectured that glucagon may be released at this very early stage and may operate in organogenesis, at least of the pancreas and possibly of the embryo at large (Like and Orci, 1972; Pictet and Rutter, 1972; Rall *et al.*, 1973), because at this stage most of the receptors and enzymes needed for its function in carbohydrate metabolism are not yet mature (Phillippidis *et al.*, 1972; Vinicor *et al.*, 1976).

Stage II

As hormone receptors are formed and as the applicable enzyme systems develop, synthesis and release of both insulin and glucagon occur in measurable quantities (Milner *et al.*, 1975; Schwartz *et al.*, 1975). In the case of insulin in the rat, the development of secretory capabilities appears to coincide with the appearance of the intracellular storage form (DeGasparo *et al.*, 1975). During this period, which comprises most of the time spent *in utero*, the mammalian B to A cell (and therefore the insulin to glucagon) ratio becomes increasingly high (Grigsby *et al.*, 1974; Watts *et al.*, 1976), stimulat-

ing the accumulation of liver glycogen (Gain and Watts, 1976) and fat deposits (Picon and Jost, 1963), and also favoring growth throughout the embryo (Lambert *et al.*, 1971; Rall *et al.*, 1973; Liggins, 1974; Pedersen, 1975).

There are several distinctions to be made between islet control of carbohydrate metabolism in the adult and that which occurs in the embryo of most mammalian species (Asplund *et al.*, 1975; Shelley *et al.*, 1975; Foà *et al.*, 1976). Most evidence suggests that insulin secretion is regulated differently in the mammalian embryo than it is in the adult. During early stages in rat islets, glucose must be coupled with caffeine, or a similar agent, to cause insulin secretion (Grasso *et al.*, 1970; deGasparo *et al.*, 1975). In the rat fetus, glucose exhibits the expectedly strong stimulatory effect on insulin synthesis, but appears to have at best a muted effect on the secretory mechanism until just after birth (Asplund *et al.*, 1975; Blazquez *et al.*, 1975), although some recent reports show a stronger effect than was previously noted (deGasparo *et al.*, 1975). Some authors report three types of agents that are capable of causing insulin secretion during this period, either on their own, or in conjunction with glucose: some amino acids (Lambert *et al.*, 1969); ionic stimulants such as potassium, ouabain, and barium (Milner, 1969); and direct and indirect stimulants of cyclic nucleotides, such as glucagon, caffeine, and theophylline (Milner, 1971; Chez, 1974).

There are exceptions to this pattern, however. Fetal lambs appear to exhibit quite different patterns of metabolic fuel utilization and show much earlier insulin sensitivity (Bassett and Madill, 1974; Girard, 1975; Shelley *et al.*, 1975). It also has been found that when a one hour infusion of glucose was administered to pregnant rats, levels of circulating fetal insulin increased (Kervran and Girard, 1974), suggesting to the authors that such extended exposure may cause more rapid maturation of glucoreceptors. Brinn *et al.* (1977) think that this response may be due to stimulation of parasympathetic centers and a resultant stimulation of insulin secretion by the cholinergic innervation of the islet cells in rats. In any case, the timing of B cell maturity appears to vary with the species as well as with metabolic conditions during gestation.

Mammalian embryonic regulation of glucagon secretion also differs from that of the adult (for review, see Girard *et al.*, 1974). The A cell shows a lack of sensitivity to acute changes in blood glucose (Edwards *et al.*, 1972; Girard *et al.*, 1974), responding more intensely to some amino acids (Wise *et al.*, 1973; Shelley *et al.*, 1975; Alexander *et al.*, 1976; Sperling *et al.*, 1976), except in the case of the fetal lamb, which is unresponsive to such stimulus (Fiser *et al.*, 1974).

In the chicken embryo, insulin reaches a low serum level and then maintains that level, rising slightly only after hatching (Leibson *et al.*, 1976a). Insulin is effective in increasing liver glycogen until the fourteenth day of incubation, at which time antihypoglycemic factors mature, ending this storage phase (Leibson, 1965/1966). Because the chick can rely on the yolk

sac for nutrition before, during, and just after hatching, there is little need for stored glycogen, perhaps accounting for the fact that insulin does not rise significantly until after the first month post-hatching (Leibson *et al.*, 1976a). Plasma insulin levels rise markedly in response to glucose at least by day 12-16 of incubation (Leibson *et al.*, 1976a). The few studies relating to avian glucagon show electron microscopic evidence of secretion by day eight of incubation (Machino, 1966). Thommes and Firling (1964) have shown that the six day embryo can respond to administration of exogenous glucagon, and Dieterlen-Lievre (1965) has reported glucagonlike action on the liver *in vitro* at day nine.

Stage III

At the time of birth in the mammal, the insulin to glucagon ratio reverses as levels of glucagon greatly increase (Bearn *et al.*, 1974; Girard *et al.*, 1974; Gain and Watts, 1976; Sperling *et al.*, 1976; Watts *et al.*, 1976). This condition, along with the maturation of hepatic responsiveness to glucagon (Devos and Hers, 1974; Vinicor *et al.*, 1976), favors the release of stored glycogen from the liver to maintain euglycemia during parturition and until milk is available (Gain and Watts, 1976). Such preparations are particularly necessary because PEP carboxykinase has not appeared at that point (Phillippidis and Ballard, 1970; Phillippidis *et al.*, 1972), resulting in little gluconeogenesis, and because the adjustment to free fatty acid metabolism is not mature (Schwartz *et al.*, 1975).

Stage IV

After birth, the neonate undergoes a slow adjustment to the high carbohydrate diet of the adult mammal. Serum insulin levels increase slowly, with glucagon remaining predominant during nursing (Foà *et al.*, 1976; Keret *et al.*, 1976). With the enzymes necessary for gluconeogenesis (Phillippidis and Ballard, 1970; Phillippidis *et al.*, 1972) and for lipolysis (Blazquez *et al.*, 1975) becoming operable just after birth, the high glucagon-insulin ratio during nursing allows the preservation of euglycemia, even though milk is high in triglycerides and protein but relatively low in carbohydrates. As insulin levels increase to a mature level and stabilize, the mammal switches from milk to a high carbohydrate diet (Foà *et al.*, 1976).

It should be obvious, even from the quick overview presented above, that prenatal metabolism and islet physiology are not clearly understood, even in species that have been well studied, such as the rat. The dearth of information regarding nonmammalian species makes it impossible to draw definite conclusions regarding ontogenetic and phylogenetic correlations.

ISLET INNERVATION

Innervation of the vertebrate endocrine pancreas has been discussed previously (Epple and Brinn, 1975), and detailed reviews have addressed the

functional aspects of islet innervation, particularly in mammals (Woods and Porte, 1974; Porte *et al.*, 1975; Smith and Porte, 1976). Because most of the function-related data regarding autonomic regulation of islet hormone release have been acquired from mammals, a group in which the metabolic dependence on insulin has become distorted in a phylogenetic sense, it is difficult to assess the evolutionary significance of neuroinsular function. Therefore, much of our present discussion will be restricted to the available anatomical data and possible types of neurotransmitter substances.

It appears that the cyclostomes lack direct neural input to their islet cells (Epple and Brinn, 1975; Östberg *et al.*, 1975; Brinn and Epple, 1976), and excepting most birds (Watanabe and Yasuda, 1977), a few reptiles (see, Epple and Brinn, 1975), and possibly the holocephalians (Patent, 1976), the gnathostomes generally possess innervated islets. The degree of innervation is especially great among the teleosts, suggesting a strong influence of the central nervous system on islet function. Based on morphological data, we had suggested earlier that teleost islets are supplied by nerves containing a neurotransmitter other than acetylcholine or catecholamines (Brinn, 1975; Epple and Brinn, 1975). Apparently without knowledge of our work, Klein and Streicher (1976) partially tested this hypothesis by injecting tritiated norepinephrine into *Xiphophorus helleri* and examining the tissues by electron microscopic radioautography. Morphologically typical adrenergic nerves in the extra-insular tissue demonstrated silver grains, but the islet nerve endings containing dense-cored vesicles took up no radioactivity. The authors suggested that these islet nerves are either cholinergic or nonadrenergic, aminergic. While we agree with the latter half of Klein and Streicher's conclusion, we do not agree that these unusual nerves are cholinergic. It is clear that additional cytochemical and radioautographic studies are necessary to resolve this issue. The appearance of nonadrenergic, noncholinergic nerves in the islets should not be surprising though, when one considers two basic factors: the ontogeny of the pancreas from a gut-derived duct system, and the presence of purinergic (Burnstock, 1972), somatostatinergic (Hökfelt *et al.*, 1975), and Substance P-containing nerves (Nilsson *et al.*, cited by Hökfelt *et al.*, 1975) in the gut wall. Vasoactive intestinal peptide (VIP)-containing nerves have been reported also in the pancreas (Sundler *et al.*, 1978). It is not inconceivable that a unique pancreas form, the actinopterygian type (Epple, 1969; Epple and Brinn, 1975), might incorporate into its developmental scheme any one of the above nerve types that would be in the vicinity of the forming pancreatic bud.

The origin of the mammalian pancreatic nerve supply is generally held to be via branches of the vagus and splanchnic nerves, which enter the pancreas with the arterial supply. This concept was recently extended to a teleost, *Gillichthys mirabilis* (Patent and Kechele, 1976). However, the common observation of ganglion cells in mammalian pancreas and the description of nerves in the cultured fetal rat pancreas (Pictet and Rutter, 1972) prompted the question of whether the islets are supplied with intrapancreatic nerves.

That suggestion was confirmed (Brinn *et al.*, 1977) along with the conclusion that the intrapancreatic nerves are islet-specific. For the rat pancreas at least, this study lends support to the concept of the neuroinsular complex suggested by Simard (1937) even though ganglion cells and islet tissue may not be in the immediate vicinity of each other. Such studies are yet to be systematically applied to lower vertebrates.

Although much definitive information is available regarding the morphological aspects of islet innervation, the situation is less than lucid from a functional viewpoint. Isolated mammalian islets respond quite readily to appropriate secretagogues, indicating, at least on a short-term basis, an independence from neural regulation. Metabolic substrates (for example, glucose) appear to be the primary regulators of islet function in mammals; however, superimposed on the direct metabolic regulation could be a long-term neuroendocrine set point control (Porte *et al.*, 1975). This hypothesis should be very attractive to the comparative islet physiologists, especially those working with highly innervated fish islets, when the seasonal influences of light, temperature, and osmolarity on metabolism are considered. However, the complexity of the autonomic supply to the islets in conjunction with slowly changing seasonal variations in the above-mentioned factors render the set point hypothesis difficult to test in the laboratory.

Along this line of thought, caution is probably advisable in attaching any functional significance to the phylogenetic variations in islet nerve ending types. Just as substrate regulation of islet secretion has changed in evolution (Epple and Lewis, 1973), so too might the neurochemical input to the islets have changed. The basic function would remain the same though—the set point regulation of islet function.

It has been suggested that the islets are innervated more heavily than the exocrine pancreas (Epple and Brinn, 1975; Brinn *et al.*, 1977). This should not be taken to mean that the islets are under greater neural control than the exocrine elements. For further discussion of this point, one must not only consider the endocrine/exocrine ratio of nerve endings, but also the types of neuroglandular relationships and the relative heterogeneity of the cell types in the endocrine and exocrine compartments. Phylogenetically, it appears that the neuroinsular relationships are much more intimate than the neuroacinar. Synaptic arrangements between nerve terminals and islet cells have been described in fish (Watari *et al.*, 1970; Klein, 1971; Brinn, 1975), and gap junctions have been reported between nerves and islet cells in the rat (Orci *et al.*, 1974). On the other hand, the adrenergic supply to the exocrine pancreas retains a close relationship with the blood vessels (Pascal and Vaysse, 1976) whereas cholinergic terminals are occasionally observed close to or between exocrine cells (Watari, 1968). However, the cytological complexity of the islets, which contain three to four cell types, may demand more cell-specific regulation than the relatively homogeneous population of acinar cells. The continuous epithelium of the exocrine pancreas, with its cells joined by junctional complexes, could conceivably respond uniformly

to low levels of a blood borne or even a localized release of neurotransmitter. Although some islet cells also are joined by junctional complexes and can presumably communicate with each other via electrotonic coupling, the profound metabolic effects of insulin, glucagon, and possibly other islet hormones in higher vertebrates probably demand more specific neural regulation of the hormone producing cells. Conversely, the apparent absence of nerves in the cyclostome islets could render these structures, especially the islets of *Petromyzon marinus* which contain several cell types (Brinn and Epple, 1976), to be good models of substrate-only regulation of islet secretion.

CONCLUSIONS

The first stages of the evolution of the islet hormones must have occurred early in metazoan history before the protostome-deuterostome dichotomy was realized. It appears very possible that all islet hormones found in vertebrates already existed at this stage. However, neither their molecular structure nor their physiological roles at this point in time are known. Inasmuch as some of these hormones are found in both nervous and digestive tissues, it appears reasonable to assume that they served various functions in the areas of the neurotransmission and endocrine control. This conclusion would be consistent with Pearse's revised concept of the "ectoblast" origin of the "APUD" cells (Pearse, 1977a) and Fujita's (1977) paraneuron concept. In accordance with our "dual control theory" of the gastrointestinal functions, we divide the functional realm of the islet organ into three different levels:

1. At the gastrointestinal level, hormones of intramucosal islet cell ancestors reach their gastrointestinal targets after homogeneous dilution in the systemic circulation. This condition is probably retained in the function of PP, the main target of which seems to be the secretory cells in the stomach.
2. At the hepatic level, islet hormones reach a newly evolving extramural digestive gland, the liver, in very high concentrations via the portal system. Probably, they acted here originally as secretagogues of the exocrine secretion, a function perhaps still shown by the increased bile flow in the chicken after insulin injection (Turner and Hazelwood, 1974). As the liver became more of a storage organ, its glycogen and lipid deposits came under the control of the islet secretions. Probably, glucagon with its profound hepatic actions (glycogenolysis, gluconeogenesis, ketogenesis) largely retained its role at this level.
3. At the peripheral level, islet hormones affect targets beyond the liver, such as muscle and adipose tissue. This level is best illustrated by the well-known actions of insulin in mammals. Perhaps, an evolutionary procession from the hepatic to the peripheral level explains why the embryonic chicken liver responds to insulin with glycogen formation earlier than chicken muscle (see Leibson *et al.*, 1976a). However, there is at present no proof that the islet hormones did not act already at the periphery (that is, in muscle) before

the liver evolved. Some gastrointestinal actions of insulin and glucagon, observed in mammals (Foà, 1968; Pfeiffer, 1969), suggest that these islet hormones still have an effect on their ancient target organ.

Although the data on glucagon, somatostatin, and PP in lower vertebrates and vertebrate embryos are still too scarce (or nonexistent) for a detailed discussion of the evolution of their functions, data on the physiology of insulin invite some considerations. In all vertebrates studied from the *Myxiniidae* to mammals, insulin shows a profound effect on the level of circulating amino acids, and wherever data on the effect of amino acids on the B-cells are available, they show a secretory response to increased levels of amino acids. On the other hand, insulin stimulates the uptake of amino acids into muscle, and their incorporation into proteins. Taken together, these data show that insulin changes the levels of free amino acids in both blood and muscle. However, because free amino acids and related compounds such as taurine are of particular importance as intracellular osmoregulatory substances in aquatic invertebrates and in fishes, we suggest that insulin must affect the intracellular osmoregulation of these animals. It remains to be seen how important the osmoregulatory effect of insulin actually is, but it is most likely that a response of insulin to postprandially increased nutrients also must have osmoregulatory consequences. This is why we interpret the extremely strong and specialized innervation of the teleost islets as a means to check a postprandial insulin surge at a time when the osmoregulatory effects of insulin might be harmful to the animal. Teleologically, it makes sense to have a very strong innervation of certain endocrines in aquatic species whose life entails a never ending fight to maintain a relatively constant "milieu interne" against an either hyper or hyposmotic environment. On the other hand, neither osmoconformers such as the *Myxiniidae* (which in general live in a rather constant environment), nor most birds (which can usually avoid unfavorable conditions by flight) seem to have an islet innervation (Epple and Stetson, 1976). Probably, the available data on osmoregulatory actions of islet hormones only present the "tip of an iceberg." It appears reasonable to speculate that the osmoregulatory effects of islet hormones will be stronger in aquatic forms than in terrestrial animals that are faced to a lesser degree by osmo and ionoregulatory challenges.

The preceding considerations suggest that the involvement of insulin in protein metabolism may be a very ancient function, and may originally have entailed a dual function in both intermediary metabolism and osmoregulation. Other data, however, suggest that this hormone may also have a long-standing involvement in carbohydrate metabolism. Thus, for example, injections of insulin in molluscs resulted in both a decrease in intracellular glucose and an increase in tissue glycogen. In the cyclostomes, insulin is clearly involved in the carbohydrate metabolism of the *Petromyzontidae*, and yet the B-cells of these animals do not seem to respond to increased blood sugar levels. In the teleosts, the B-cells respond to glucose stimuli, but it seems that amino acids are better stimulants. In lizards and birds, the B-cells

respond very poorly, if at all, to physiological levels of blood sugar. It seems that of all the species studied so far, the mammalian B-cells respond most readily to physiological changes of blood sugar. At a first glance, these data appear to present a paradox. However, if one separates the anabolic actions of insulin (transmembranous glucose transport and glycogen synthesis) from the glucostatic action it exerts in mammals, then we have a simple explanation for its major activities in the carbohydrate metabolism: in most species, its primary function is at the tissue level, and only in mammals and possible in some other groups (including certain teleosts?) does it extend its realm to include also the blood sugar level *per se*. Great fluctuations of the blood sugar level (seasonal, individual, or due to other reasons) are well known for most exothermic vertebrates, and a mammalian like glucostatic role of the B-cells in most cases may neither be needed nor desired. In this connection, it must be remembered that certain amphibians and fishes can even survive when their blood contains no measurable levels of glucose.

From the preceding discussion, it appears that during its physiological evolution, insulin may have been involved in the regulation of amino acid levels well before it controlled blood sugar.

Finally, the role of insulin in lipid metabolism deserves some consideration. Here, the evidence is still very fragmentary, and both the absence of adipose tissue in the selachians, and the insulin-insensitivity of the adipocytes reported for exothermic vertebrates and birds suggest that insulin became involved recently in the control of lipolysis in the adipose tissue. However, this does not preclude the possibility that the hormone played a role in lipid metabolism long before it took on the adipocyte as a target cell. The excessive influence of insulin on mammalian fat pads makes one wonder how the B-cell could acquire such a dangerously powerful position. The sequelae of juvenile-type diabetes mellitus illustrate that a power monger can be detrimental even when annihilated.

SUMMARY

In recent years, considerable progress has been made in our understanding of the evolution of structure and function of the pancreatic islets. There are at least two regular islet secretions (insulin and somatostatin) in the cyclostomes in general, and probably two more in adult *Petromyzon marinus*. All gnathostomes seem to have at least four regular islet secretions (insulin, glucagon, somatostatin, and pancreatic polypeptide); in the holocephalians and *Lepisosteus* there is strong evidence of a regular fifth islet hormone. Like some other gastrointestinal hormones, the islet hormones have a long evolutionary history in the invertebrates. The islet organ is a derivative of scattered endocrine cells of the gastrointestinal mucosa. Probably, it evolved as part of a *dual hormonal control system* of the digestive processes, which integrated messages from the gastrointestinal lumen with those from the blood. The functional realm of the islet hormones can be divided into three levels: the gastrointestinal level (main target of pancreatic polypeptide);

hepatic level (main target of glucagon); peripheral level, for example, muscle and adipose tissue (targets of insulin). Ontogenetic and phylogenetic data suggest that the control of free amino acids (uptake and incorporation into proteins) may be the most basic function of insulin in vertebrates. Because free amino acids are important in the regulation of intracellular water content in aquatic animals, it may have been through this mechanism that insulin became involved in osmoregulation. The homeostatic control of blood sugar by insulin is probably a polyphyletic phenomenon that evolved as a "byproduct" of other activities of insulin in carbohydrate metabolism (transmembranous glucose transport and glycogen synthesis). Insulin seems to be the dominating hormone of the intermediary metabolism of anurans, turtles, crocodiles, and mammals. The evolution of the functions of glucagon is still enigmatic. Probably, it reaches its greatest influence in birds, where it dominates both carbohydrate and fat metabolism. The results of pancreatectomy suggest that the islet hormones are metabolically less important in selachians and teleosts than in tetrapods. At least in some fishes, their physiological effects are more balanced than in the tetrapods. At present, uncertainties of the biological status of the cyclostomes do not allow an evaluation of the evolutionary stage of their islet physiology. The islet innervation varies strongly among the tetrapods and is absent in the cyclostomes. It reaches its peak in the teleosts, which may be due to a trend towards a strong nervous control of the endocrines involved in the integration of both intermediary metabolism and osmoregulation.

LITERATURE CITED

- ABRAMOWITZ, A. A., F. L. HISAW, E. BOETTIGER, AND D. N. PAPANDREA. 1940. The origin of the diabetogenic hormone in the dogfish. *Biol. Bull.*, 78:189-201.
- AHMAD, M. M., AND A. J. MATTY. 1975. Insulin secretion in response to high and low protein indigestion in rainbow trout, *Salmo gairdneri*. *Pakistan J. Zool.*, 7:1-6.
- ALDEHOFF, G. 1891. Tritt auch bei Kaltblütern nach Pankreasexstirpation Diabetes mellitus auf? *Z. Biol.*, 28:293-304.
- ALEXANDER, D. P., R. ASSAN, H. G. BRITTON, E. FENTON, AND D. RESTONE. 1976. Glucagon release in the sheep fetus. I. Effect of hypo- and hyperglycemia and arginine. *Biol. Neonate*, 30:1-10.
- ALLEN, J. A., AND M. R. GARRETT. 1971. Taurine in marine invertebrates. *Adv. Mar. Biol.*, 9:205-253.
- ANDERSON, C. E., AND D. R. LANGSLOW. 1975. Glucose production and its hormonal control in isolated chicken hepatocytes. *Biochem. Soc. Trans.*, 3:1037-1039.
- ANDREW, A. 1977. Pancreatic D cells in very young chick embryos. *Gen. Comp. Endocrinol.*, 31:463-465.
- ARIEFF, A. I., T. DOERNER, H. ZELIG, AND S. A. MASSRY. 1974. Mechanism of seizures and coma in hypoglycemia: evidence for a direct effect of insulin on electrolyte transport in brain. *J. Clin. Invest.*, 54:654-663.
- ASHCROFT, S. J. H. 1976. The control of insulin release by sugars. Pp. 117-139, in *Poly-peptide hormones: molecular and cellular aspects* (Ciba Foundation Symposium), Elsevier, Excerpta Medica, North Holland, Amsterdam, xii+388 pp.
- ASPLUND, K., A. ANDERSSON, C. JARROUSSE, AND C. HELLERSTRÖM. 1975. Function of the fetal endocrine pancreas. *Israel J. Med. Sci.*, 11:581-590.
- ASSAN, R., AND J. BOILLOT. 1971. Pancreatic glucagon and glucagon-like material in tissues and plasmas from human fetuses 6-26 weeks old. Pp. 210-219, in *Metabolic pro-*

- cesses in the foetus and newborn infant (J. H. P. Jonxis, H. K. A. Visser, and J. A. Troelstra, eds.), Williams and Wilkins, Baltimore, xii+307 pp.
- ASSENMACHER, I. 1973. The peripheral endocrine glands. Pp. 184-286, in *Avian biology* (D. S. Farner and J. R. King, eds.), Academic Press, New York, 3:xix+ 573 pp.
- BAETENS, D., J. DEMEY, AND W. GEPTS. 1977. Immunohistochemical and ultrastructural identification of the pancreatic polypeptide-producing cell (PP-Cell) in the human pancreas. *Cell. Tissue Res.*, 185:239-246.
- BAJAJ, J. S. 1977. Insulin and metabolism. *Excerpta Medica*, Amsterdam, xii+ 296 pp.
- BAKER, L. A., AND R. L. HAZELWOOD. 1977. Chicken cerebrospinal fluid as affected by fasting, sulfonyleurea administration and pancreatectomy. *Comp. Biochem. Physiol.*, 56A:385-393.
- BARBEZAT, G. O. 1973. Stimulation of intestinal secretion by polypeptide hormones. *Scandinavian J. Gastroenterol.*, 8(suppl. 22):1-21.
- BARRINGTON, E. J. W. 1942. Blood sugar and the follicles of Langerhans in the ammocoete larva. *J. Exp. Biol.*, 19:45-55.
- . 1972. The pancreas and intestine. Pp. 135-169, in *The biology of lampreys* (M. W. Hardisty and I. C. Potter, eds.), Academic Press, London, 2:xiv+466 pp.
- BASSETT, J. M., AND D. MADILL. 1974. Influence of prolonged glucose infusions on plasma insulin and growth hormone concentrations of foetal lambs. *J. Endocrinol.*, 63: 299-309.
- BEARN, J. G., S. R. BLOOM, E. S. BROCKLEHURST, A. G. COBB, AND C. R. MONKHOUSE. 1974. Plasma pancreatic glucagon, plasma insulin and blood glucose studies in the fetal rabbit. *J. Anat.*, 118:362.
- BEAUMONT, A. 1970. Apparition des cellules insulaires A dans le pancréas lavaire d'un Amphibien Anoure: *Discoglossus pictus*, Otth. *Hebd. Séanc. C. R. Acad. Sci., Paris*, ser. D, 271:1104-1106.
- BEAUPAIN, D., AND F. DIETERLEN-LIEVRE. 1974. Étude immunocytoologique de la différenciation du pancréas endocrine chez l'embryon de poulet. II. Glucagon. *Gen. Comp. Endocrinol.*, 23:421-431.
- BENTLEY, P. J., AND B. K. FOLLETT. 1965a. Fat and carbohydrate reserves in the river lamprey during spawning migration. *Life Sci.*, 4:2003-2007.
- . 1965b. The effects of hormones on the carbohydrate metabolism of the lamprey *Lamprolaima fluviatilis*. *J. Endocrinol.*, 31:127-137.
- BERGSTRÖM, B. H., S. LOO, H. J. HIRSCH, D. SCHUTZEN, AND K. H. GABBAY. 1977. Ultrastructural localization of pancreatic polypeptide in human pancreas. *J. Clin. Endocrinol.*, 44:795-798.
- BHATT, S. D., AND S. S. KHANNA. 1976. Histopathological effects of glucagon in a fresh water teleost, *Clarias batrachus* L. Part I—The endocrine pancreas, blood glucose and tissue glycogen. *Indian J. Exp. Biol.*, 14:145-149.
- BLAZQUEZ, E., L. A. LIPAHAW, M. BLAZQUEZ, AND P. P. FOA. 1975. The synthesis and release of insulin in fetal, nursing and young adult rats: studies *in vivo* and *in vitro*. *Pediatr. Res.*, 9:17-25.
- BLOOM, S. R. 1976. Hormones of the gastrointestinal tract. Pp. 95-103, in *Recent Advances in Medicine* (D. N. Baron, N. Comstom, and A. Dawson, eds.), Churchill Livingstone, London, vi+392 pp.
- BONDAREVA, V. M. 1970. Effect of insulin deficiency upon the carbohydrate metabolism in chick embryos. *Ontogenes*, 1:282-285.
- BOQUIST, L. 1977. Histochemistry and electron microscopy of islets. Pp. 129-169, in *The diabetic pancreas* (B. W. Volk and K. F. Wellman, eds.), Plenum Press, New York, xviii+599 pp.
- BOYD, T. A., C. J. CHA, R. P. FORSTER, AND L. GOLDSTEIN. 1977. Free amino acids in tissues of the skate, *Raja erinacea*, and the stingray, *Dasyatis sabina*: effects of environmental dilution. *J. Exp. Zool.*, 199:435-442.
- BRINN, J. E. 1973. The pancreatic islets of bony fishes. *Amer. Zool.*, 13:653-665.

- . 1975. Pancreatic islet cytology of Ictaluridae (Teleostei). *Cell Tissue Res.*, 162: 357-365.
- BRINN, J. E., AND A. EPPLER. 1976. New types of islet cells in a cyclostome *Petromyzon marinus*. *Cell Tissue Res.*, 171:317-329.
- BRINN, J. E., H. E. BURDEN, AND M. R. SCHWEISTHAL. 1977. Innervation of the cultured fetal rat pancreas. *Cell Tissue Res.*, 182:133-138.
- BURIGHEL, P., AND C. MILANESI. 1975. Fine structure of the gastric epithelium of the ascidian *Botryllus schlosseri*. Mucous, endocrine and plicated cells. *Cell Tissue Res.*, 158:481-496.
- BURNSTOCK, G. 1972. Purinergic nerves. *Pharmacol. Res.*, 24:509-581.
- CAHILL, G. F., JR. 1976. Insulin and glucagon. Pp. 85-103, in *Peptide hormones* (J. A. Parsons, ed.), University Park Press, Baltimore, xii+399 pp.
- CAPARELLI, A. 1894. Sur le diabete pancreatique experimental. *Arch. Italiennes Biol.*, 21:398.
- CHEZ, R. A. 1974. The development and function of the human endocrine pancreas. Pp. 43, in *The endocrine milieu of pregnancy, puerperium and childhood*. Report of the Third Ross Conference on Obstetrics Research. Ross Laboratories, Columbus, Ohio, 135 pp.
- CHIERI, R. A., J. C. BASABE, J. M. FARINA, AND V. G. FOGLIA. 1972. Studies on carbohydrate metabolism in penguins, *Pygoscelis papua*. *Gen. Comp. Endocrinol.*, 18:1-4.
- COLCA, J. R., AND R. L. HAZELWOOD. 1976. Pancreatectomy in the chicken: does an extra-pancreatic source of insulin exist? *Gen. Comp. Endocrinol.*, 28:151-162.
- CONTE, N., G. FEDERSPIEL, S. FREZZATO, A. TRISOTTO, C. SCANDELLARI, AND G. PIEMONTE. 1972. Glucagon effect on plasma Mg concentration. *Horm. Metab. Res.*, 4:48-50.
- COPELAND, D. L., AND R. DEROOS. 1971. Effect of mammalian insulin on plasma glucose in the mudpuppy, *Necturus maculosus*. *J. Exp. Zool.*, 178:35-44.
- CREUTZFELDT, W. 1977. Endocrine tumors of the pancreas. Pp. 551-590, in *The diabetic pancreas* (B. W. Volk and K. F. Wellmann, eds.), Plenum Press, New York, xviii+599 pp.
- CSABA, G., AND T. LANTOS. 1975. Effect of insulin on the glucose uptake of protozoa. *Experientia*, 31:1097-1098.
- DEGASPARO, M., R. L. PICTET, L. B. RALL, AND W. J. RUTTER. 1975. Control of insulin secretion in the developing pancreatic rudiment. *Dev. Biol.*, 47:106-122.
- DEOYA, M., W. F. PRIGGE, AND F. GRANDE. 1971. Suppression by hepatectomy of glucagon-induced hypertriglyceridemia in geese. *Proc. Soc. Exp. Biol. Med.*, 136:107-110.
- DESBALS, P. 1972. Effects de la pancreatectomie et de l'hypophysectomie sur la circulation des lipides chez le Canard. *These Sci. Nat.*, Toulouse, France, 242 pp.
- DESBALS, P., S. DESBALS, AND P. MIALHE. 1967. Variations des lipides plasmatiques et hepatiques chez le canard totalement depancreaté. *J. Physiol.*, 59:232.
- DEVLAMING, V. L., AND R. J. PARDO. 1975. *In vitro* effects of insulin on liver lipid and carbohydrate metabolism in the teleost, *Notemigonus crysoleucas*. *Comp. Biochem. Physiol.*, 51B:489-497.
- DEVOS, P., AND H. G. HERS. 1974. Glycogen metabolism in the liver of the foetal rat. *Biochem. J.*, 140:331-340.
- DIETERLEN-LIEVRE, F. 1965. Étude morphologique et experimentale de la différenciation du pancreas chez l'embryon de poulet. *Bull. Biol. France Belgique*, 99:3-116.
- DIETERLEN-LIEVRE, F., AND D. BEAUPAIN. 1974. Étude immunocytologique de la différenciation du pancréas endocrine chez l'embryon de poulet. I. Îlots à insulin. *Gen. Comp. Endocrinol.*, 22:62-69.
- DOCKRAY, G. J. 1977. Molecular evolution of gut hormones: application of comparative studies on the regulation of digestion. *Gastroenterology*, 72:344-358.
- DOCKRAY, G. J., L. BEST, AND I. L. TAYLOR. 1977. Immunochemical characterization of gastrin in pancreatic islets of normal and genetically obese mice. *J. Endocrinol.*, 72:143-151.

- DOSNE, C. 1943. Study of interrelationship of pancreatic diabetes with endocrine glands in the toad. *Endocrinology*, 33:224-228.
- DUBOIS, P. M., C. PAULIN, R. ASSAN, AND M. P. DUBOIS. 1975. Evidence for immunoreactive somatostatin in the endocrine cells of human foetal pancreas. *Nature*, 256:731-732.
- DULIN, W. E., AND M. G. SORET. 1977. Chemically and hormonally induced diabetes. Pp. 425-465, in *The diabetic pancreas* (B. W. Volk and K. F. Wellman, eds.), Plenum Press, New York, xviii+599 pp.
- EDWARDS, J. C., K. ASPLUND, AND G. LUNDQVIST. 1972. Glucagon release from the pancreas of the newborn rat. *J. Endocrinol.*, 54:493-504.
- EPPLE, A. 1963. Zur vergleichenden Zytologie des Inselorgans. *Verh. Deutschen Zool. Ges.*, pp. 461-470.
- . 1966a. Cytology of pancreatic islet tissue in the toad, *Bufo bufo* (L.). *Gen. Comp. Endocrinol.*, 7:191-196.
- . 1966b. Islet cytology of urodele amphibians. *Gen. Comp. Endocrinol.*, 7:207-214.
- . 1967. Further observations on amphiphil cells in the pancreatic islets. *Gen. Comp. Endocrinol.*, 9:137-142.
- . 1969. The endocrine pancreas. Pp. 275-319, in *Fish physiology* (W. S. Hoar and D. J. Randall, eds.), Academic Press, New York, 2:xiv+465 pp.
- EPPLE, A., AND J. E. BRINN. 1975. Islet histophysiology: evolutionary correlations. *Gen. Comp. Endocrinol.*, 27:320-349.
- . 1976. New perspectives in comparative islet research. Pp. 83-95, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Eppler, eds.), Pergamon Press, Oxford and New York, xii+377 pp.
- EPPLE, A., AND D. S. FARNER. 1967. The pancreatic islets of the white-crowned sparrow, *Zonotrichia leucophrys gambelii*, during its annual cycle and under experimental conditions. *Z. Zellforsch. Mikrosk. Anat.*, 71:185-197.
- EPPLE, A., AND T. LEWIS. 1973. Comparative histophysiology of the pancreatic islets. *Amer. Zool.*, 13:567-590.
- . 1975. The effect of pancreatectomy on the survival of *Anguilla rostrata* in different salinities. *J. Exp. Zool.*, 193:457-462.
- . 1977. Metabolic effects of pancreatectomy and hypophysectomy in the yellow American eel, *Anguilla rostrata* LeSueur. *Gen. Comp. Endocrinol.*, 32:294-315.
- EPPLE, A., AND M. H. STETSON. 1976. Einige endokrinologische Besonderheiten der Vögel. *J. Ornithol.*, 117:257-278.
- ERLANDSEN, S. L., O. D. HEGRE, J. A. PARSONS, R. C. McEVOY, AND R. P. ELDE. 1976. Pancreatic islet cell hormones, distribution of cell types in the islet and evidence for the presence of somatostatin and gastrin within the D cell. *J. Histochem. Cytochem.*, 24:883-897.
- ERMISCH, A. 1966. Beiträge zur Histologie und Topochemie des Inselsystems der Neunaugen unter natürlichen und experimentellen Bedingungen. *Zool. Jb. Anat.*, 83:52-106.
- FALKMER, S., AND M. MARQUES. 1972. Phylogeny and ontogeny of glucagon production. Pp. 343-361, in *Glucagon: molecular physiology, clinical and therapeutic implications* (P. J. Lefebvre and R. H. Unger, eds.), Pergamon Press, Oxford and New York, xiii+370 pp.
- FALKMER, S., AND A. J. MATTY. 1966. Blood sugar regulation in the hagfish, *Myxine glutinosa*. *Gen. Comp. Endocrinol.*, 6:334-346.
- FALKMER, S., AND Y. ÖSTBERG. 1977. Comparative morphology of pancreatic islets in animals. Pp. 15-59, in *The diabetic pancreas* (B. W. Volk and K. F. Wellmann, eds.), Plenum Press, New York, xviii+599 pp.
- FALKMER, S., AND G. PATENT. 1972. Comparative aspects of the pancreatic islets. Pp. 1-23, in *Handbook of physiology*, section 7 (R. O. Greep and E. B. Astwood, section eds.), The endocrine pancreas (D. F. Steiner and N. Freinkel, vol. eds.), Amer. Physiol. Soc., Washington, D.C., 1:x+1-721 pp.
- FALKMER, S., AND S. WILSON. 1967. Comparative aspects of the immunology and biology of insulin. *Diabetologia*, 3:519-528.

- FALKMER, S., N. W. THOMAS, AND L. BOQUIST. 1974. Endocrinology of the cyclostomata. Pp. 195-257, in *Chemical zoology* (M. Florkin and B. T. Scheer, eds.), Academic Press, New York, 8:xxiii+436 pp.
- FARINA, J., J. PINTO, J. C. BASABE, AND R. A. CHERI. 1975. Aspects of intermediary metabolism and insulin level in the penguin, *Pygoscelis papua*. *Gen. Comp. Endocrinol.*, 27:209-213.
- FARKAS, T. 1969. Studies of the mobilization of fats in lower vertebrates. *Acta Biochem. Biophys. Acad. Sci. Hungaricae*, 4:237-249.
- FARRAR, E. S., AND B. E. FRYE. 1977. Seasonal variation in the effects of adrenalin and glucagon in *Rana pipiens*. *Gen. Comp. Endocrinol.*, 33:76-81.
- FERNER, H. 1957. Die Dissemination der Hodenzwischenzellen und Langerhansschen Inseln als funktionelles Prinzip fuhr die Samenkanälchen und das exokrine Pankreas. *Z. Mikrosk. Anat. Forsch.*, 63:35.
- FEYRTER, F. 1953. Über die peripheren endokrinen (parakrinen) Drüsen des Menschen. 2. Aufl. Wilhelm Maudrich, Wien, 83 pp.
- FISER, R. H., JR., A. ERENBERG, M. A. SPERLING, W. OH, AND D. A. FISHER. 1974. Insulin-glucagon substrate interrelations in the fetal sheep. *Pediatr. Res.*, 8:951-955.
- FLOYD, J. C., S. S. FAJANS, S. PEK, AND R. E. CHANCE. 1977. A newly recognized pancreatic polypeptide plasma levels in health and disease. *Rec. Progr. Horm. Res.*, 33:519-570.
- FOÀ, P. P. 1968. Glucagon. *Ergeb. Physiol.*, 60:142-219.
- FOÀ, P. P., E. BLAZQUEZ, J. C. SODOYEZ, AND F. SODOYEZ-GOFFAUX. 1976. The ontogeny of mammalian insular function. Pp. 7-19, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Eppe, eds.), Pergamon Press, Oxford, xii+ 377 pp.
- FOÀ, P. P., J. S. BAJAJ, AND N. L. FOÀ. 1977. Glucagon: its role in physiology and clinical medicine. Springer Verlag, New York, xii+793 pp.
- FOGLIA, V. G., E. M. WAGNER, M. DEBARROS, AND M. MARQUES. 1955. La diabetes por pancreatectomia in la tortuga normal e hipofisopriva. *Rev. Soc. Argentina Biol.*, 31:87-95.
- FORSSMANN, W. G., AND S. ITO. 1976. Ultrastructural analysis of the GEP endocrine system in primates. Pp. 25-41, in *Endocrine gut and pancreas* (T. Fujita, ed.), Elsevier Amsterdam, xiv+411 pp.
- FREEMAN, B. M., AND A. C. C. MANNING. 1974. The prandial state and the glycaemic and lipolytic responses of *Gallus domesticus* to catecholamines and glucagon. *Comp. Biochem. Physiol.*, 47A:1145-1152.
- . 1976. Mediation of glucagon in the response of the domestic fowl to stress. *Comp. Biochem. Physiol.*, 53A:169-171.
- FRITSCH, H. A. R., AND R. SPRANG. 1977. Ultrastructure of polypeptide hormone-producing cells in gut of ascidians, *Ciona intestinalis* L. and the bivalve, *Mytilus edulis* L. *Cell Tissue Res.*, 177:407-413.
- FRYE, B. E. 1965. Hypertrophy of the islets of Langerhans of frog tadpoles after partial pancreatectomy. *J. Exp. Zool.*, 158:133-139.
- FUJITA, T. 1968. D cell, the third endocrine element of the pancreatic islet. *Arch. Histol. Japonicum*, 29:1-40.
- . 1973. Gastro-entero-pancreatic system. Igaku Shoin, Tokyo, viii+195 pp.
- . 1976. *Endocrine gut and pancreas*. Elsevier, Amsterdam, xiv+411 pp.
- . 1977. Concept of paraneurons. *Arch. Histol. Japonicum*, 40:1-12.
- FUJITA, P., Y. YANATORI, AND T. MURAKAMI. 1976. Insulo-acinar axis, its vascular basis and its functional and morphological changes caused by CCK-PZ and caerulein. Pp. 347-357, in *Endocrine gut and pancreas* (P. Fujita, ed.), Elsevier, Amsterdam, xiv+411 pp.
- GABE, M. 1968. Données histologiques sur le pancréas endocrine d'*Ichthyophis glutinosus* (L.) (Batracien gymnophione). *Arch. Anat. Histol. Embryol.*, 51:232-246.
- . 1970. Données histologiques sur le pancréas endocrine des Lepidosauriens (reptiles). *Ergebn. Anat.*, 42:7-63.
- GAIN, K., AND C. WATTS. 1976. Glucose homeostasis in the developing rat. II. Blood glucose, plasma insulin and hepatic glycogen in the newborn rat. *Biol. Neonate*, 30:35-39.

- GATER, S., AND M. BALLS. 1977. Amphibian pancreas function in long-term organ culture. Control of insulin release. *Gen. Comp. Endocrinol.*, 33:94-102.
- GEPTS, W., J. DEMEY, AND M. MARICHAL-PIPELEERS. 1977. Hyperplasia of "Pancreatic polypeptide"—cells in the pancreas of juvenile diabetics. *Diabetologia*, 13:27-34.
- GERICH, J. E., M. A. CHARLES, AND G. M. GRODSKY. 1976. Regulation of pancreatic insulin and glucagon secretion. *Ann. Rev. Physiol.*, 38:353-388.
- GERLOVIN, E. SH. 1976. Some principles of cytodifferentiation of pancreatic islets in vertebrata during onto- and phylogeny from the standpoint of molecular biology and genetics. Pp. 97-112, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epplé, eds.), Pergamon Press, Oxford and New York, xii+377 pp.
- GIRARD, J. R. 1975. Metabolic fuels of the fetus. *Israel J. Med. Sci.*, 11:591-600.
- GIRARD, J. R., E. B. MARLISS, AND R. ASSAN. 1974. Glucagon and perinatal metabolism in the rat. *Adv. Biosci.*, 13:5-16.
- GOMIH, Y. K., AND T. A. I. GRILLO. 1976. Insulin-like activity of the extract of the digestive gland and the pylorus of the Giant African Snail, *Achatina fulica*,—a preliminary report. Pp. 153-162, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epplé, eds.), Pergamon Press, Oxford, xii+377 pp.
- GOODRIDGE, A. G. 1968. Lipolysis *in vitro* in adipose tissue from embryonic and growing chicks. *Amer. J. Physiol.*, 214:897.
- . 1973. Regulation of fatty-acid synthesis in isolated hepatocytes prepared from livers of neonatal chicks. *J. Biol. Chem.*, 248:1924-1931.
- GOODRIDGE, A. G., AND E. G. BALL. 1967. Lipogenesis in the pigeon: *in vivo* studies. *Amer. J. Physiol.*, 213:245-249.
- GOURLEY, D. R. H., S. T. KYU, AND L. L. BRUNTON. 1969. Seasonal differences and the effect of insulin on pyruvate uptake oxidation and synthesis to glycogen by frog skeletal muscle. *Comp. Biochem. Physiol.*, 29:509-524.
- GRANDE, F. 1969. Lack of insulin effect on free fatty acid mobilization produced by glucagon in birds. *Proc. Soc. Exp. Biol. Med.*, 130:711.
- . 1970. Effects of glucagon and insulin on plasma free fatty acids and blood sugar in owls. *Proc. Soc. Exp. Biol. Med.*, 133:540-543.
- GRANDE, F., AND W. F. PRIGGE. 1970. Glucagon infusion, plasma FFA and triglycerides, blood sugar, and liver lipids in birds. *Amer. J. Physiol.*, 218:1406.
- GRANT, P. T., JR., F. T. HENDLER, P. M. BANKS. 1969. Studies on blood sugar regulation in the little skate, *Raja erinacea*. *Physiol. Zool.*, 42:231-247.
- GRASSO, S., A. MESSINA, N. SAPORITO, AND G. REITANO. 1970. Effect of theophylline, glucagon and theophylline plus glycogen on insulin secretion in the premature infant. *Diabetes*, 19:837-842.
- GRIGSBY, J. S., W. D. OXENDER, H. D. HAFS, D. G. BRITT, AND R. A. MERKEL. 1974. Serum insulin, glucose, and free fatty acids in the cow and fetus during gestation. *Proc. Soc. Exp. Biol. Med.*, 147:830-834.
- GRILLO, T. A. I., R. ITO, AND K. WATANABE. 1976. The endocrine pancreas of some West African reptiles—electron microscopy, histochemistry, and hormone content. Pp. 189-249, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epplé, eds.), Pergamon Press, Oxford and New York, xii+377 pp.
- GROSCOLAS, R., AND J. BEZARD. 1977. Effect of glucagon and insulin on plasma free fatty acids and glucose in the emperor penguin, *Aptenodytes forsteri*. *Gen. Comp. Endocrinol.*, 32:230-235.
- GROSS, R., AND P. MIALHE. 1974. Free fatty-acid-glucagon feed-back mechanism. *Diabetologia*, 10:277-283.
- GROSSMAN, M. I. 1976. Gastrointestinal hormones. Pp. 105-117, in *Peptide hormones* (J. A. Parsons, ed.), University Park Press, Baltimore, xii+399 pp.
- GRUENER, R., H. BRYANT, D. MARKOWITZ, R. HUXTABLE, AND R. BRESSLER. 1976. Ionic actions of taurine on nerve and muscle membranes: electrophysiologic studies. Pp. 225-242, in *Taurine* (R. Huxtable and A. Barbeau, eds.), Raven Press, New York, xiv+398 pp.

- GUHA, B., AND A. GHOSH. 1978. A cytomorphological study of the endocrine pancreas of some Indian birds. *Gen. Comp. Endocrinol.*, 34:38-44.
- GUIDOTTI, G. G., B. LUNEBURG, AND A. F. BORGHETTI. 1969. Amino acid uptake in isolated chick embryo heart cells. *Biochem. J.*, 114:97.
- GUILLEMIN, R., AND J. E. GERICH. 1976. Somatostatin: physiological and clinical significance. Pp. 379-388, in *Annual review of medicine* (W. P. Creger, C. H. Coggins, and E. W. Hancock, eds.), Annual Reviews Inc., Palo Alto, Calif., xi+412 pp.
- GUNESCH, K. D. 1974. Changes of the lipid metabolism in the clawed toad, *Xenopus laevis* Daudin, induced by ACTH, corticosteroids and insulin. *Zool. Jb. Physiol.*, 78:108-127.
- HAMBURGER, V., AND H. L. HAMILTON. 1951. A series of normal stages in the development of the chick embryo. *J. Morph.*, 88:49-92.
- HANKE, W. 1974a. Endocrinology of amphibia. Pp. 123-159, in *Chemical zoology* (M. Florin and B. T. Scheer, eds.), Academic Press, New York, 9:xxi+472 pp.
- . 1974b. Hormonal regulation of metabolism in amphibia. *Fortschr. Zool.*, 22:431-455.
- . 1974c. Überblick über den Aktivitätsbeginn hormonaler Systeme während der Amphibienentwicklung. *Fortschr. Zool.*, 22:468-470.
- HANKE, W., AND U. NEUMANN. 1972. Carbohydrate metabolism in amphibia. *Gen. Comp. Endocrinol.*, 3:198-208.
- HARDISTY, M. W., AND I. C. POTTER. 1971. General biology of adult lampreys. Pp. 127-206, in *The biology of the lamprey* (M. W. Hardisty and I. C. Potter, eds.), Academic Press, London, 1:xiv+1-423 pp.
- HARDISTY, M. W., P. R. ZELNIK, AND I. A. MOORE. 1975. The effects of subtotal and total islectomy in the river lamprey, *Lampetra fluviatilis*. *Gen. Comp. Endocrinol.*, 27:179-192.
- HARDISTY, M. W., P. R. ZELNIK, AND V. C. WARIGHT. 1976. The effects of hypoxia on blood sugar levels and on the endocrine pancreas, interrenal, and chromaffin tissues of the lamprey, *Lampetra fluviatilis* L. *Gen. Comp. Endocrinol.*, 28:184-204.
- HARRI, M. N. E., AND M. PUUSKA. 1973. Hormonal control of fat metabolism in the frog, *Rana temporaria*. *Gen. Comp. Endocrinol.*, 21:129-137.
- HAZELWOOD, R. L. 1965. Carbohydrate metabolism. Pp. 313-317, in *Avian physiology* (P. D. Sturkie, ed.), 2nd ed., Cornell University Press, New York, xiii+400 pp.
- . 1973. The avian endocrine pancreas. *Amer. Zool.*, 13:699-709.
- . 1976a. Carbohydrate metabolism. Pp. 210-232, in *Avian physiology* (P. D. Sturkie, ed.), 3rd ed., Springer Verlag, New York, xiii+400 pp.
- . 1976b. Three avian pancreatic hormones. Pp. 321-334, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epplé, eds.), Pergamon Press, Oxford, xiii+377 pp.
- HAZELWOOD, R. L., J. R. KIMMEL, AND H. G. POLLOCK. 1968. Biological characterization of chicken insulin activity in rats and domestic fowl. *Endocrinology*, 83:1331.
- HENDERSON, J. R. 1969. Why are the islets of Langerhans? *Lancet*, 2:469-470.
- HERNANDEZ, T., AND R. A. COULSON. 1968. Effect of insulin on free amino acids in caiman tissue and plasma. *Comp. Biochem. Physiol.*, 26:991-996.
- HIATT, N., M. B. DAVIDSON, AND G. BONORRIS. 1972. The effect potassium chloride infusion on insulin secretion *in vivo*. *Horm. Metab. Res.*, 4:64-68.
- HICKS, T., AND L. A. TURNBERG. 1974. The influence of secretion on ion transport in the human jejunum. *Gut*, 14:485-490.
- HOFFMAN, E. K., AND K. B. HENDIL. 1976. The role of amino acids and taurine in isosmotic intracellular regulation in Ehrlich ascites mouse tumor cells. *J. Comp. Physiol.*, 108:279-286.
- HÖKFELT, T., O. JOHANSSON, S. EFENDIC, R. LUFT, AND A. ARIMURA. 1975. Are there somatostatin-containing nerves in the rat gut? Immunohistochemical evidence for a new type of peripheral nerves. *Experientia*, 31:852-854.

- HOUSSAY, B. A., AND J. C. PENHOS. 1960. Pancreatic diabetes and hypophysectomy in the snake *Xenodon merremii*. *Acta Endocrinol.*, 35:313-323.
- HUBEL, K. A. 1972. Effects of secretion and glucagon on intestinal transport of ions and water in the rat. *Proc. Soc. Exp. Biol. Med.*, 139:656-658.
- HUXTABLE, R., AND A. BARBEAU. 1976. *Taurine*. Raven Press, New York, xiv+ 398 pp.
- INCE, B. W., AND A. THORPE. 1974. Effects of insulin and of metabolic loading on blood metabolites in the European silver eel, *Anguilla anguilla* L. *Gen. Comp. Endocrinol.*, 23:460-471.
- . 1975. Hormonal and metabolite effects on plasma free fatty acids in the Northern pike, *Esox lucius* L. *Gen. Comp. Endocrinol.*, 27:144-152.
- . 1977a. Glucose and amino acid-stimulated insulin release in the European silver eel (*Anguilla anguilla* L.). *Gen. Comp. Endocrinol.*, 31:249-256.
- . 1977b. Plasma insulin and glucose responses to glucagon and catecholamines in the European silver eel, *Anguilla anguilla* L. *Gen. Comp. Endocrinol.*, 33:453-459.
- INUI, Y., AND A. GORBMAN. 1977. Sensitivity of Pacific Hagfish, *Eptatretus stouti*, to mammalian insulin. *Gen. Comp. Endocrinol.*, 33:423-427.
- INUI, Y., AND M. YOKOTE. 1974. Gluconeogenesis in the eel—I. Gluconeogenesis in the fasted eel. *Bull. Freshwater Fish. Res. Lab.*, 24:33-46.
- . 1975a. Gluconeogenesis in the eel—II. Gluconeogenesis in the alloxanized eel. *Bull. Japanese Soc. Sci. Fish.*, 41:291-300.
- . 1975b. Gluconeogenesis in the eel—III. Effects of mammalian insulin on the carbohydrate metabolism of the eel. *Bull. Japanese Soc. Sci. Fish.*, 41:965-972.
- . 1975c. Gluconeogenesis in the eel—IV. Gluconeogenesis in the hydrocortisone-administered eel. *Bull. Japanese Soc. Sci. Fish.*, 41:973-981.
- . 1975d. Gluconeogenesis in the eel—V. Effects of alloxan and hydrocortisone administration on amino acid mobilization in the hepatectomized eel. *Bull. Japanese Soc. Sci. Fish.*, 41:1101-1104.
- . 1977. Effects of glucagon on amino acid metabolism in Japanese eels, *Anguilla japonica*. *Gen. Comp. Endocrinol.*, 33:167-173.
- INUI, Y., S. ARAI, AND M. YOKOTE. 1975. Gluconeogenesis in the eel—VI. Effects of hepatectomy, alloxan, and mammalian insulin on the behavior of plasma amino acids. *Bull. Japanese Soc. Sci. Fish.*, 41:1105-1111.
- JOHNSON, L. R. 1976. The trophic action of gastrointestinal hormones. *Gastroenterology*, 70:278-288.
- KANNO, T., N. UEDA, AND A. SAITO. 1976. Insulo-acinar axis: a possible role of insulin potentiating the effects of pancreaticozym in the pancreatic acinar cell. Pp. 335-345, in *Endocrine gut and pancreas* (T. Fujita, ed.), Elsevier, Amsterdam, xiv+ 411 pp.
- KARMANN, H., AND P. MIALHE. 1976. Glucose, insulin, and glucagon in the diabetic goose. *Horm. Metab. Res.*, 8:419-426.
- KATZ, A. I., AND M. D. LINDHEIMER. 1977. Actions of hormones on the kidney. *Ann. Rev. Physiol.*, 39:97-134.
- KERET, R., S. MATHIASH, M. BROWN, A. PERTZELAN, I. LEVY, AND A. PERTZELAN, I. LEVY, AND Z. LARON. 1976. Correlation between plasma growth hormone and insulin and blood glucose concentrations in premature infants. *Horm. Res.*, 7:313-318.
- KERVAN, A., AND J. R. GIRARD. 1974. Glucose-induced increase of plasma insulin in the rat foetus *in utero*. *J. Endocrinol.*, 62:545-551.
- KERN, H. F. 1971. Vergleichende Morphologie der Langerhansschen Inseln der Wirbeltiere. Pp. 1-70, in *Handbuch der experimentellen Pharmakologie* (E. Dörzbach, ed.), Springer Verlag, Berlin, 31:vi+1-387 pp.
- KIMMEL, J. R., H. G. POLLOCK, AND R. L. HAZELWOOD. 1968. Isolation and characterization of chicken insulin. *Endocrinology*, 83:1323-1330.
- KING, D. L., AND R. L. HAZELWOOD. 1976. Regulation of avian insulin secretion by isolated perfused chicken pancreas. *Amer. J. Physiol.*, 231:1830-1839.
- KLEIN, C. 1971. Innervation des cellules du pancréas endocrine du Poisson Téléostéen, *Xiphophorus helleri*. *Z. Zellforsch. Mikrosk. Anat.*, 113:564-580.

- KLEIN, C., AND D. STREICHER. 1976. Étude autoradiographique, au microscope électronique, de l'innervation des cellules du pancréas endocrine d'un poisson téléostéen, *Xiphophorus helleri*. C. R. Hebd. Séanc. Acad. Sci. Paris, ser. D, 183:635-638.
- KÖHLER, E. 1963. Versuche zur genese und Therapie der Fettleber bei Fischen. Z. gesamte inn. Med. ihre Grenzgeb., 18:936-942.
- KONES, R. F. 1974. Insulin, adenylyl cyclase, ions, and the heart. Trans. New York Acad. Sci., 36:738-774.
- KRAMER, K. J., R. D. SPEIRS, AND C. N. CHILDS. 1977. Immunochemical evidence for a gastrin-like peptide in insect neuroendocrine system. Gen. Comp. Endocrinol., 32:423-426.
- KRUG, E., AND P. MIALHE. 1971. Pancreatic and intestinal glucagon in the duck. Horm. Metab. Res., 3:24.
- KUZUYA, T., H. KAJINUMA, AND T. IDE. 1974. Effect of intrapancreatic injection of potassium and calcium on insulin and glucagon secretion in dogs. Diabetes, 23:55-60.
- LACY, P. E. 1977. The physiology of insulin release. Pp. 211-230, in The diabetic pancreas (B. W. Volk and K. F. Wellmann, eds.), Plenum Press, New York, xvii+ 599 pp.
- LAMBERT, A. E., B. JEANRENAUD, A. JUNOD, AND A. E. RENOLD. 1969. Organ culture of fetal rat pancreas. II. Insulin release induced by amino and organic acids, by hormonal peptides, by cationic alterations of the medium and by other agents. Biochem. Biophys. Acta, 184:540-553.
- LAMBERT, A. E., L. ORCI, Y. KANAZAWA, B. JEANRENAUD, AND A. E. RENOLD. 1971. Biochemical and morphological events associated with the development of fetal rat pancreas *in vivo* and *in vitro*: effect of glucagon and other agents. P. 35, in Current topics on glucagon (M. Austoni, C. Scandellari, G. Federspil, and A. Trisotto, eds.), Cedam, Padua, viii+226 pp.
- LANE, M. A. 1907. The cytological characters of the areas of Langerhans. Amer. J. Anat., 7:409-422.
- LANGERHANS, P. 1869. Beiträge zur mikroskopischen Anatomie der Bauchspeicheldrüse. Inaugural Dissertation. G. Lange, Berlin, 163 pp.
- LANGSLOW, D. R. 1976. The half-life of bovine and chicken insulin in chicken plasma. Gen. Comp. Endocrinol., 29:423-425.
- LANGSLOW, D. R., AND B. M. FREEMAN. 1972. Partial pancreatectomy and the role of insulin in carbohydrate metabolism in *Gallus domesticus*. Diabetologia, 8:206-210.
- LANGSLOW, D. R., AND C. N. HALES. 1969. Lipolysis in chicken adipose tissue *in vitro*. J. Endocrinol., 43:285.
- . 1971. The role of the endocrine pancreas and catecholamines in the control of carbohydrate and lipid metabolism. Pp. 521-547, in Physiology and biochemistry of the domestic fowl (D. J. Bell and B. Freeman, eds.), Academic Press, London, 1: vii+1-638 pp.
- LANGSLOW, D. R., E. J. BUTLER, C. N. HALES, AND A. W. PEARSON. 1970. The response of plasma insulin, glucose and nonesterified fatty acids to various hormones, nutrients and drugs in the domestic fowl. J. Endocrinol., 46:243.
- LARSEN, L. O. 1976a. Regulation of glucose in the river lamprey: the possible physiological role of insulin and hyperglycemic hormones. Pp. 285-290, in The evolution of pancreatic islets (T. A. I. Grillo, L. Leibson, and A. Eppele, eds.), Pergamon Press, Oxford, xii+377 pp.
- . 1976b. Blood glucose levels in intact and hypophysectomized river lampreys, *Lampetra fluviatilis* L., treated with insulin, "stress," or glucose, before and during the period of sexual maturation. Gen. Comp. Endocrinol., 29:1-13.
- . 1978. Role of the liver in blood glucose regulation and vitellogenesis, and regeneration of the liver after subtotal hepatectomy of intact or hypophysectomized river lampreys. Gen. Comp. Endocrinol., 34:94-95.
- LARSSON, A., AND K. LEWANDER. 1972. Effects of glucagon administration to eels (*Anguilla anguilla* L.), Comp. Biochem. Physiol., 43A:831-836.

- LARSSON, L. I., F. SUNDLER, R. HAKANSON, H. G. POLLOCK, AND J. R. KIMMEL. 1974. Localization of APP, a postulated new hormone, to a pancreatic endocrine cell type. *Histochemie*, 42:377-382.
- . 1976. Pancreatic polypeptide—a postulated new hormone: identification of its cellular storage site by light and electron microscopic immunocytochemistry. *Diabetologia*, 12:211-226.
- LAURENT, F., AND P. MIALHE. 1976. Insulin and the glucose-glucagon feedback mechanism in the duck. *Diabetologia*, 12:23-33.
- LAZARUS, S. S., AND B. V. VOLK. 1962. The pancreas in human and experimental diabetes. Grune and Stratton, New York, xii+279 pp.
- LECOMPTE, P. M., AND W. GEPTS. 1977. The pathology of juvenile diabetes. Pp. 325-363, in *The diabetic pancreas* (B. W. Volk and K. F. Wellmann, eds.), Plenum Press, New York, xviii+599 pp.
- LEFEBVRE, P. J., AND R. H. UNGER. 1972. Glucagon: molecular physiology clinical and therapeutic implications. Pergamon, Oxford, xiii+370 pp.
- LEIBSON, L. G. 1965/1966. The endocrine factors in the regulation of carbohydrate metabolism in the developing chick embryo. *Biol. Neonate*, 9:249-262.
- LEIBSON, L. G., AND E. M. PLISETSKAYA. 1968. Effect of insulin on blood sugar level and glycogen content in organs of some cyclostomes and fish. *Gen. Comp. Endocrinol.*, 11:381-392.
- . 1969. Hormonal control of blood sugar level in cyclostomes. *Gen. Comp. Endocrinol.*, 2:528-534.
- . 1973. Effects of hormones in poikilothermic vertebrates. Pp. 625-684, in *Internat. Encycl. Pharmacol. Ther. Section 85* (M. J. Michelson, ed.), Pergamon Press, Oxford, New York, xi+716 pp.
- LEIBSON, L. G., E. M. PLISETSKAYA, AND T. I. MAZINA. 1968. Concentration of non-esterified fatty acids in blood plasma of cyclostomes and fishes and its changes under the influence of adrenalin and insulin. *J. Evol. Biochem. Physiol.*, 4:121-127.
- LEIBSON, L. G., V. BONDAREVA, AND L. SOLTITSKAYA. 1976a. The secretion and the role of insulin in chick embryos and chickens. Pp. 69-79, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Eppe, eds.), Pergamon Press, Oxford, xii+377 pp.
- LEIBSON, L. G., E. PLISETSKAYA, AND B. LEIBUSH. 1976b. The comparative study of mechanism of insulin action on muscle carbohydrate metabolism. Pp. 345-362, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Eppe, eds.), Pergamon Press, Oxford, xii+377 pp.
- LERNMARK, A., S. J. CHAN, R. CHOY, A. NATHANS, R. CARROLL, H. S. TAGER, A. H. RUBENSTEIN, H. H. SWIFT, AND D. F. STEINER. 1976. Biosynthesis of insulin and glucagon: a review of the current state of the art. Pp. 7-30, in *Polypeptide hormones: molecular and cellular aspects* (Ciba Foundation Symposium), Elsevier, Excerpta Medica, North Holland, Amsterdam, xii+388 pp.
- LEWANDER, K., G. DAVE, M. L. JOHANSSON-SJÖBECK, A. LARSSON, AND U. LIDMAN. 1976. Metabolic effects of insulin in the European eel, *Anguilla anguilla* L. *Gen. Comp. Endocrinol.*, 29:455-467.
- LEWIS, T. L., AND A. EPPE. 1972. Pancreatectomy in the eel: effects on serum glucose and cholesterol. *Science*, 178:1286-1288.
- LEWIS, T. L., W. W. PARKE, AND A. EPPE. 1977. Pancreatectomy in a teleost fish, *Anguilla rostrata*, (American eel). *Lab. Anim. Sci.*, 27:102-109.
- LIGGINS, G. C. 1974. The influence of the fetal hypothalamus and pituitary on growth. Pp. 165-183, in *Size at birth* (K. Elliott and J. Knight, eds.), Ciba Foundation Symposium, No. 27. Associated Scientific Publ., Amsterdam, ix+408 pp.
- LIKE, A. A., AND L. ORCI. 1972. Embryogenesis of the human pancreatic islets: a light and electron microscopic study. *Diabetes*, 21(suppl. 2):511-534.
- LOVE, R. M. 1970. The chemical biology of fishes. Academic Press, New York, xv+547 pp.

- MACHINO, M. 1966. Electron microscope observations of pancreatic islet cells of the early chick embryo. *Nature*, 210:853-854.
- MALAISSÉ-LAGAE, F., M. RAVAZZOLA, P. ROBBERECHT, A. VANDERMEERS, W. J. MALAISSÉ, AND L. ORCI. 1976. Hydrolases content in peri-insular and tele-insular exocrine pancreas. Pp. 313-320, in *Endocrine gut and pancreas* (T. Fujita, ed.), Elsevier, Amsterdam, xiv+411 pp.
- MALLINSON, C. N., S. R. BLOOM, A. P. WARIN, P. R. SALMAN, AND B. COX. 1974. A glucagonoma syndrome. *Lancet*, 2:1.
- MARQUES, M. 1967. Effects of prolonged glucagon administration to turtles (*Chrysemys d'orbigny*). *Gen. Comp. Endocrinol.*, 9:102-109.
- MARQUES, M., AND S. FALKMER. 1976. Effects of mammalian insulin on blood glucose level, glucose tolerance, and glycogen content of musculature and hepatopancreas in a gastropod mollusk, *Strophocheilus oblongus*. *Gen. Comp. Endocrinol.*, 29:522-530.
- MATTY, A. J., AND S. FALKMER. 1965. Hypophysectomy and blood sugar regulation in a cyclostome, *Myxine glutinosa*. *Nature*, 207:533-534.
- MATTY, A. J., AND A. GORBMAN. 1978. The effect of isletectomy and hypophysectomy on some plasma constituents of the hagfish, *Eptatretus stouti*. *Gen. Comp. Endocrinol.*, 34:94.
- MATTY, A. J., K. TSUNEMI, W. W. DICKHOFF, AND A. GORBMAN. 1976. Thyroid and gonadal function in hypophysectomized hagfish, *Eptatretus stouti*. *Gen. Comp. Endocrinol.*, 30:500-516.
- MCCORMICK, N. A., AND J. J. R. MACLEOD. 1925. The effect on the blood sugar of fish of various conditions including removal of the principal islets (isletectomy). *Proc. Roy. Soc., ser. B*, 98:1-29.
- MCCUMBEE, W. D., AND R. L. HAZELWOOD. 1977. Biological evaluation of the third pancreatic hormone (APP): hepatocyte and adipocyte effects. *Gen. Comp. Endocrinol.*, 33:518-525.
- MEKHJIAN, H., D. KING, L. SANZENBACHER, AND R. ZOLLINGER. 1972. Glucagon and secretion inhibit water and electrolyte transport in the human jejunum. *Gastroenterology*, 62:782.
- MIALHE, P. 1958. Glucagon, insulin et régulation endocrine de la glycémie chez le canard. *Acta endocrinol.*, 36(suppl. 36):1-134.
- . 1976. The role of glucagon in birds and mammals. Pp. 291-300, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epplé, eds.), Pergamon Press, Oxford, xii+377 pp.
- MIHAIL, N., M. IONESCU, AND L. DUSA. 1963. Morphologische Auswirkungen der Pankreatektomie bei der Taube. *Anat. Anz.*, 112:97-100.
- MIKAMI, S., AND K. MUTOH. 1971. Light- and electron-microscopic studies of the pancreatic islet cells in the chicken under normal and experimental conditions. *Z. Zellforsch. Mikrosk. Anat.*, 116:205-225.
- MIKAMI, S., AND K. ONO. 1962. Glucagon deficiency induced by extirpation of alpha cells of the fowl pancreas. *Endocrinology*, 71:464.
- MILLER, M. R. 1960. Pancreatic islet histology and carbohydrate metabolism in amphibians and reptiles. *Diabetes*, 9:318-323.
- . 1961. Carbohydrate metabolism in amphibians and reptiles. Pp. 125-147, in *Comparative physiology of carbohydrate metabolism in heterothermic animals* (A. W. Martin, ed.), Univ. Washington Press, Seattle, 144 pp.
- . 1962. Observations on the comparative histology of the reptilian pancreatic islets. *Gen. Comp. Endocrinol.*, 2:407-414.
- MILLER, M. R., AND M. D. LAGIOS. 1970. The Pancreas. Pp. 319-346, in *Biology of the Reptilia* (C. Gans and T. Parsons, eds.), Academic Press, New York, xv+ 373 pp.
- MILNER, R. D. G. 1969. The secretion of insulin from foetal and postnatal rabbit pancreas *in vitro* in response to various substances. *J. Endocrinol.*, 44:267-272.
- . 1971. The development of insulin secretion in man. Pp. 193-207, in *Metabolic processes in the foetus and newborn infant* (J. H. P. Jonxis, H. K. A. Visser, and J. A. Troelstra, eds.), Williams and Wilkins, Baltimore, xii+307 pp.

- MILNER, R. D. G., F. N. LEACH, AND P. M. B. JACK. 1975. Reactivity of the fetal islet. Pp. 83-104, in *Carbohydrate metabolism in pregnancy and the newborn* (H. W. Sutherland and J. M. Stowers, eds.), Churchill-Livingstone, Edinburgh, 252 pp.
- MINICK, M. C., AND W. CHAVIN. 1972. Effects of vertebrate insulins upon serum FFA and phospholipid levels in the goldfish, *Carassius auratus* Linnaeus. *Comp. Biochem. Physiol.*, 41A:791-804.
- MIRSKY, I. A., R. JINKS, AND G. PERISUITTI. 1964. Production of diabetes mellitus in the duck by insulin antibodies. *Amer. J. Physiol.*, 206:133-135.
- MORRIS, R., AND D. S. ISLAM. 1969. The effects of hormones and hormone inhibitors on blood sugar regulation and the follicles of Langerhans in ammocoete larvae. *Gen. Comp. Endocrinol.*, 12:81-90.
- MURAT, J. C., AND E. M. PLISETSKAYA. 1977. Effects du glucagon sur la glycémie, le glycogène et la glycogène-synthetase hépatique chez la Carpe et la Lamproie. *C. R. Soc. Biol., ser. D (Paris)*, 171:1302.
- MURAT, J. C., AND A. SERFATY. 1975. Effects de l'adrénaline du glucagon et de l'insuline sur le métabolisme glucidique de la Carpe. *C. R. Soc. Biol., ser. D (Paris)*, 169:228-232.
- NABER, S. P., AND R. L. HAZELWOOD. 1977. *In vitro* insulin release from chicken pancreas. *Gen. Comp. Endocrinol.*, 32:495-504.
- NAKAMURA, M., K. YAMADA, AND M. YOKOTE. 1971. Ultrastructural aspects of the pancreatic islets in carps of spontaneous Diabetes mellitus. *Experientia*, 27:75-76.
- NIR, I., AND V. LEVY. 1973. Response of blood plasma glucose, free fatty acids, triglycerides, insulin and food intake to bovine insulin in geese and cockerels. *Poultry Sci.*, 52:886-892.
- NISHI, M. 1910. Über Glykogenbildung in der Leber pankreasdiabetischer Schildkroten. *Arch. Exp. Path. Pharmacol.*, 62:170.
- OHARA, H. 1976. Clinical and experimental studies on the entero-insular and insulo-acinar correlations. Pp. 321-333, in *Endocrine gut and pancreas* (T. Fujita, ed.), Elsevier, Amsterdam, xiv+411 pp.
- ORCI, L., A. PERRELET, M. RAVAZZOLA, F. MALAISSE-LAGAE, AND A. RENOLD. 1974. A specialized membrane junction between nerve endings and cells in islets of Langerhans. *European J. Clin. Invest.*, 3:443-445.
- ORCI, L., D. BAETENS, M. P. DUBOIS, AND C. RUFENER. 1975. Evidence for the D-cell of the pancreas secreting somatostatin. *Horm. Metab. Res.*, 7:400-402.
- ÖSTBERG, Y., S. VAN NOORDEN, AND A. G. E. PEARSE. 1975. Cytochemical, immunofluorescence, and ultrastructural investigations on polypeptide hormone localization in the islet parenchyma and bile duct mucosa of a cyclostome, *Myxine glutinosa*. *Gen. Comp. Endocrinol.*, 25:274-291.
- ÖSTBERG, Y., S. VAN NOORDEN, A. G. E. PEARSE, AND N. W. THOMAS. 1976. Cytochemical, immunofluorescence, and ultrastructural investigations on polypeptide hormone containing cells in the intestinal mucosa of a cyclostome, *Myxine glutinosa*. *Gen. Comp. Endocrinol.*, 28:213-227.
- PADGAONKAR, A. S., AND P. V. RANGNEKER. 1975. Insulin tolerance tests in the normal, pancreatectomized, hypophysectomized and hypophysopancreatectomized water-snake, *Natrix piscator* (Russell). *J. Anim. Morphol. Physiol.*, 22:38-42.
- PALOKANGAS, R., V. VIHKO, AND I. NUUJA. 1973. The effects of cold and glucagon on lipolysis, glycogenolysis and oxygen consumption in young chicks. *Comp. Biochem. Physiol.*, 45:489-495.
- PARK, Y. S., AND S. K. HONG. 1977. Effects of insulin on short-circuit current and Na-K-ATPase activity of the toad skin and urinary bladder. *Comp. Biochem. Physiol.*, 58A:155-158.
- PASCAL, J., AND N. VAYSSE. 1976. Action du système adrénergique sur le pancréas exocrine. *Biol. Gastroenterol. (Paris)*, 9:243-254.
- PATENT, G. J. 1970. Comparison of some hormonal effects on carbohydrate metabolism in an elasmobranch, *Squalus acanthias*, and a holocephalan, *Hydrolagus coliei*. *Gen. Comp. Endocrinol.*, 14:215-242.

- . 1973. The chondrichthyan endocrine pancreas: what are its functions? *Amer. Zool.*, 13:639-651.
- . 1976. The ultrastructure and innervation of the pancreatic islets of the holoccephalian ratfish, *Hydrolagus colliei*. Pp. 131-140, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Eppler, eds.), Pergamon Press, Oxford, New York, xii+377 pp.
- PATENT, G. J., AND P. FOÀ. 1971. Radioimmunoassay of insulin in fishes, experiments *in vivo* and *in vitro*. *Gen. Comp. Endocrinol.*, 16:41-46.
- PATENT, G. J., AND P. KECHELE. 1976. Innervation of the pancreatic islets of the teleost fish, *Gillichthys mirabilis*. *Amer. Zool.*, 16:236.
- PEARSE, A. G. E. 1977a. The diffuse neuroendocrine system and the APUD concept: related 'endocrine' peptides in brain, intestine, pituitary, placenta, and anuran cutaneous glands. *Med. Biol.*, 55:115-125.
- . 1977b. The diffuse neuroendocrine system and the "common peptides". Pp. 309-323, in *Molecular endocrinology* (I. MacIntyre and M. Szelke, eds.), Elsevier/North-Holland Biomedical Press, Amsterdam, v+401 pp.
- PEDERSEN, J. 1975. Fetal macrosomia. Pp. 127-139, in *Carbohydrate metabolism in pregnancy and the newborn* (H. W. Sutherland and J. M. Stowers, eds.), Churchill-Livingstone, Edinburgh, 252 pp.
- PENHOS, J. C., AND M. E. KRAHL. 1962. Insulin stimulus of leucine incorporation into frog liver protein. *Amer. J. Physiol.*, 203:687-689.
- . 1963. Stimulus of leucine incorporation into perfused liver protein by insulin. *Amer. J. Physiol.*, 204:140-142.
- PENHOS, J. C., AND N. LAVINTMAN. 1964. Total pancreatectomy in toads: effect of hypophysectomy and glucagon. *Gen. Comp. Endocrinol.*, 4:264-270.
- PENHOS, J. C., AND E. RAMEY. 1973. Studies on the endocrine pancreas of amphibians and reptiles. *Amer. Zool.*, 13:667-698.
- PENHOS, J. C., C. H. WU, M. REITMAN, E. SODERO, R. WHITE, AND R. LEVINE. 1967a. Effects of several hormones after total pancreatectomy in alligators. *Gen. Comp. Endocrinol.*, 8:32-43.
- PENHOS, J. C., B. UNO, AND B. A. HOUSSEY. 1967b. Glucose and lipid metabolism in the toad's perfused liver. *Gen. Comp. Endocrinol.*, 8:297-304.
- PFEIFFER, E. F. 1969. *Handbuch des Diabetes mellitus*. J. F. Lehmann, München, 1:xxxii +962 pp.
- PFEIFFER, E. F. 1971. *Handbuch des Diabetes mellitus*. J. F. Lehmann, München, 2:xxxii +1403 pp.
- PHILLIPPIDIS, H., AND F. J. BALLARD. 1970. The development of gluconeogenesis in rat liver: effects of glucagon and ether. *Biochem. J.*, 120:385-392.
- PHILLIPPIDIS, H., R. W. HANSON, L. RESHEF, M. F. HOPGOOD, AND F. J. BALLARD. 1972. The initial synthesis of proteins during development. *Biochem. J.*, 126:1127-1134.
- PICON, L., AND A. JOST. 1963. Teneur du corps en lipide et en protides chez le fœtus de rat et de lapin et chez le rat nouveau-né. *C. R. Séanc. Soc. Biol.*, 157:1368-1372.
- PICTET, R., AND W. RUTTER. 1972. Development of the embryonic endocrine pancreas. Pp. 25-66, in *Handbook of physiology*, section 7 (R. O. Greep and E. B. Astwood, section eds.), *The endocrine pancreas* (D. F. Steiner and N. Freinkel, vol. eds.), *Amer. Physiol. Soc.*, Washington, D.C., 1:x+1-721 pp.
- PLISETSKAYA, E. M. 1975. Hormonal regulation of carbohydrate metabolism in lower vertebrates. *Acad. Sci. USSR, Leningrad*, 215 pp.
- PLISETSKAYA, E. M., AND V. M. BONDAREVA. 1972. The effect of acute insulin insufficiency on glycemic level and glycogen content in organs of marine teleosts. *Physiol. J. USSR*, 58:1152-1156.
- PLISETSKAYA, E. M., AND B. N. LEIBUSH. 1972. Insulin-like activity and immunoreactive insulin in the blood of the lamprey, *Lampetra fluviatilis*. *J. Evol. Biochem. Physiol.*, 8:499-505.

- PLISETSKAYA, E. M., AND T. I. MAZINA. 1969. The influence of hormones on nonesterified fatty acids in the blood of the Baltic lamprey, *Lampetra fluviatilis*. J. Evol. Biochem. Physiol., 5:457-463.
- PLISETSKAYA, E. M., AND L. G. OGORODNIKOVA. 1967. The effect of insulin and cortisol acetate on the glucose-6-phosphatase activity of lamprey liver. J. Evol. Biochem. Physiol., 3:304-308.
- PLISETSKAYA, E. M., AND M. P. PROZOKOVSKAYA. 1971. Catecholamines in the blood and cardiac muscle of the lamprey, *Lampetra fluviatilis*, during insulin hypoglycemia. J. Evol. Biochem. Physiol., 7:101-103.
- PLISETSKAYA, E. M., B. N. LEIBUSH, AND V. BONDAREVA. 1976. The secretion of insulin and its role in cyclostomes and fishes. Pp. 251-269, in The evolution of pancreatic islets (T. A. I. Grillo, L. Leibson, and A. Epple, eds.), Pergamon Press, Oxford, xii+377 pp.
- POLYAKOVA, T. I., AND E. M. PLISETSKAYA. 1976. The effect of glucose on gut epithelium cells in the lancelet, *Branchiostoma lanceolatum*. J. Evol. Biochem. Physiol., 12:184-187.
- PORTE, D., JR., S. WOODS, M. CHEN, P. SMITH, AND J. ENSINCK. 1975. Central factors in the control of insulin and glucagon secretion. Pharmacol. Biochem. Behav., 3(suppl. 1): 127-133.
- RALL, L. B., R. L. PICTET, R. H. WILLIAMS, AND W. J. RUTTER. 1973. Early differentiation of glucagon-producing cells in embryonic pancreas: a possible developmental role of glucagon. Proc. Nat. Acad. Sci., 70:3478-3482.
- RANGNEKER, P. V., AND A. S. PADGAONKAR. 1972. Effects of total pancreatectomy in the snake, *Natrix piscator*, Russell. J. Anim. Morphol. Physiol., 19:63-76.
- RANGNEKER, P. V., AND P. B. SABNIS. 1964. Blood sugar responses to glucagon and insulin administration in the frog, *Rana tigrina* and the lizard, *Varanus monitor*. J. Anim. Morphol. Physiol., 11:173-177.
- . 1966. Effects of pancreatectomy, hypophysectomy and both on blood sugar levels in the frog, *Rana tigrina* (Daud). J. Anim. Morphol. Physiol., 13:78-84.
- RHOTEN, W. B. 1973. Insulin release from pancreatic islets of a lizard following glycine or arginine. J. Herpetol., 7:207-210.
- . 1974. Sensitivity of saurian pancreatic islets to glucose. Amer. J. Physiol., 227: 993-997.
- RUDO, N. H., AND I. H. ROSENBERG. 1973. Chronic glucagon administration enhances intestinal transport in the rat. Proc. Soc. Exp. Biol. Med., 142:521-525.
- SAMOLS, E., J. M. TYLER, V. MARKS, AND P. MIALHE. 1968. The physiological role of glucagon in different species. Excerpta Medica Int. Congr. Ser., 184:206-219.
- SAMSEL, J. 1973. Acides aminés et sécrétion des glucagons chez le Canard. Thèse de 3^{ème} cycle, Université Strasbourg, 274 pp.
- SANTEUSANIO, F., G. R. FALOONA, J. P. KNOCHEL, AND R. H. UNGER. 1972. The role of the islets of Langerhans in potassium homeostasis. J. Clin. Invest., 51:85a.
- SCHARRER, B. 1978. Peptidergic neurons: facts and trends. Gen. Comp. Endocrinol., 34:50-62.
- SCHIRNER, H. 1963. Das Pankreas von *Myxine glutinosa* und *Bdellostoma stouti*. Ein Beitrag zur Phylogenie des Pankreas. Nytt mag. zool., 11:5-18.
- SCHWARTZ, A. L., N. C. R. RAIHA, AND T. W. RALL. 1975. Hormonal regulation of glycogen metabolism in human fetal liver. I. Normal development and effects of dibutylryl cyclic AMP, glucagon and insulin in liver explants. Diabetes, 24:1101-1112.
- SESHADRI, B. 1959. Effect of insulin on the amino nitrogen content of free and protein-bound amino acids in the skeletal muscle of the murrel, *Ophiocephalus striatus* (Bloch). Current Sci. (India), 28:121-122.
- . 1964. Effect of insulin on blood glucose level in the fish *Ophiocephalus striatus*. J. Anim. Morph. Physiol., 11:302-308.
- SHELLEY, H. J., J. M. BASSETT, AND R. D. G. MILNER. 1975. Control of carbohydrate metabolism in the fetus and newborn. British Med. Bull., 31:37-43.

- SHLATZ, Z. L., AND G. V. MARINETTI. 1972. Hormone-calcium interactions with plasma-membrane of rat liver cells. *Science*, 176:175-177.
- SIMARD, L. C. 1937. Les complexes neuro-insulaires du pancréas humain. *Arch. Anat. Microsc. Morph. Exp.*, 33:49-64.
- SIMON, J., P. FREYCHET, AND G. ROSSELIN. 1977. A study of insulin binding sites in the chicken tissues. *Diabetologia*, 13:219-228.
- SINGLEY, J. A., AND W. CHAVIN. 1976. The diel rhythm of circulating ACTH titer in the goldfish, *Carassius auratus* L. *Comp. Biochem. Physiol.*, 53A:291-293.
- SIREK, A. 1969. Pancreatectomy and diabetes. Pp. 727-743, in *Handbuch des Diabetes* (E. F. Pfeiffer, ed.), J. F. Lehmann, München, 1:xxxii+962 pp.
- SITBON, G., AND P. MIALHE. 1978. Pancreatic hormones and plasma glucose: regulation mechanisms in the goose under physiological conditions. I. Pancreatectomy and replacement therapy. *Horm. Metab. Res.*, 10:12-16.
- SMITH, P. H. 1974. Pancreatic islets of *Coturnix* quail. *Anat. Rec.*, 178:567-586.
- SMITH, P. H., AND D. PORTE, JR. 1976. Neuropharmacology of the pancreatic islets. *Ann. Rev. Pharmacol. Toxicol.*, 16:269-285.
- SMITH, P. H., F. W. MERCHANT, D. G. JOHNSON, W. Y. FUJIMOTO, AND R. H. WILLIAMS. 1977. Immunocytochemical localization of a gastric inhibitory polypeptide-like material within A-cells of the endocrine pancreas. *Amer. J. Anat.*, 149:585-590.
- SPEGLING, M. A., L. A. GRAJWER, R. LEAKER, AND D. A. FISHER. 1976. Role of glucagon in perinatal glucose homeostasis. *Metabolism*, 25:1385-1387.
- STEINER, D. F. 1976. Peptide hormone precursors: biosynthesis, processing and significance. Pp. 49-65, in *Peptide hormones* (J. A. Parsons, ed.), Macmillan, New York, xii+399 pp.
- STEINER, D. F., AND N. FREINKEL (volume eds.). 1972. The endocrine pancreas. *Handbook of physiology*, Section 7 (R. O. Greep and E. B. Astwood, section eds.), *Amer. Physiol. Soc.*, Washington, D.C., 1:x+1-721 pp.
- STERNE, J., C. HIRSCH, AND M. F. PELE. 1968. Disorders of carbohydrate metabolism following feeding of glucose alone in the fish *Carassius auratus*. *Path. Biol.*, 16:639.
- SUNDLER, F., J. ALUMETS, R. HAKANSON, J. FAHRENKRUG, AND O. S. DE MUCKADELL. 1978. Peptidergic (VIP) nerves in the pancreas. *Histochemie*, 55:173-176.
- SURYAWANSHI, S. A., AND P. V. RANGNEKER. 1971. Effects of administration of glucagon and insulin in the lizard, *Uromastix hardwickii*, Gray. *J. Anim. Morphol. Physiol.*, 18:134-144.
- TASHIMA, L. S., AND G. F. CAHILL. 1964. Role of glucagon and insulin in the carbohydrate metabolism of the toadfish. *Excerpta Med. Int. Congr. Ser.*, 74:140.
- . 1968. Effects of insulin in the toadfish, *Opsanus tau*. *Gen. Comp. Endocrinol.*, 11:262-271.
- THERET, C., J. ALLIET, G. COMLAN, AND D. GOURDIER. 1975. Endocrine pancreas of *Varanus niloticus*—ultrastructural and cytochemical study with electron microscopy and x-ray microdiffraction. *C. R. Hebd. Séanc. Acad. Sci., Paris, ser. D*, 280:2125-2128.
- THOMMES, R. C., AND C. E. FIRLING. 1964. Blood glucose and liver glycogen levels in glucagon-treated chick embryos. *Gen. Comp. Endocrinol.*, 4:1-8.
- THORPE, A. 1976. Studies of the role of insulin in teleost metabolism. Pp. 271-284, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epplé, eds.), Pergamon Press, Oxford and New York, xii+377 pp.
- THORPE, A., AND B. W. INCE. 1974. The effects of pancreatic hormones, catecholamines and glucose loading on blood metabolites in the Northern Pike, *Esox lucius*. *Gen. Comp. Endocrinol.*, 23:29-44.
- . 1976. Plasma insulin levels in teleosts determined by a charcoal-separation radio-immunoassay technique. *Gen. Comp. Endocrinol.*, 30:332-339.
- TITLBACH, M., AND H. KERN. 1969. Licht- und elektronenmikroskopische Untersuchungen am Inselorgan des Bachneunauges *Lampetra planeri* (Bloch). *Z. Zellforsch. Mikrosk. Anat.*, 97:403-415.

- TRANDABURU, T. 1976. Intrinsic innervation, monoamines, and acetylcholinesterase activity in the pancreatic islets of some poikilothermic vertebrates and birds. Pp. 121-130, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epplé, eds.), Pergamon Press, Oxford, xii+377 pp.
- TURNER, S. D., AND R. L. HAZELWOOD. 1974. Biliary excretion of insulin in the adult chicken. *Comp. Biochem. Physiol.*, 47A:303-313.
- UMMINGER, B. L., AND D. BENZIGER. 1975. *In vitro* stimulation of hepatic glycogen phosphorylase activity by epinephrine and glucagon in the brown bullhead, *Ictalurus nebulosus*. *Gen. Comp. Endocrinol.*, 25:96-104.
- UMMINGER, B. L., D. BENZIGER, AND S. LEVY. 1975. *In vitro* stimulation of hepatic glycogen phosphorylase activity by epinephrine and glucagon in the killifish, *Fundulus heteroclitus*. *Comp. Biochem. Physiol.*, 51C:111-115.
- UNGER, R. H., P. RASKIN, C. B. SRIKANT, AND L. ORCI. 1977. Glucagon and the A-cells. *Rec. Progr. Horm. Res.*, 33:477-517.
- VAN NOORDEN, S., AND A. G. E. PEARSE. 1976. The localization of immunoreactivity to insulin, glucagon, and gastrin in the gut of *Amphioxus* (*Branchiostoma*) *lanceolatus*. Pp. 163-178, in *The evolution of pancreatic islets* (T. A. I. Grillo, L. Leibson, and A. Epplé, eds.), Pergamon Press, Oxford, xii+377 pp.
- VAN NOORDEN, S., J. M. POLAK, AND A. G. E. PEARSE. 1977. Single cellular origin of somatostatin and calcitonin in the rat thyroid gland. *Histochemie*, 53:243-247.
- VILLEE, C. A., R. LICHTENSTEIN, N. NATHANSON, AND B. ROLANDER. 1950. Studies on the carbohydrate metabolism of invertebrate tissue *in vitro*. *Biol. Bull.*, 99:365.
- VINICOR, F., G. HIGDON, J. F. CLARK, AND C. M. CLARK. 1976. Development of glucagon sensitivity in neonatal rat liver. *J. Clin. Invest.*, 58:571-578.
- VOLK, B. W., AND K. F. WELLMANN, EDS. 1977. *The diabetic pancreas*. Plenum Press, New York, xviii+599 pp.
- VON LAWZEWSCH, I. 1963. Topographical distribution of the islets of Langerhans in the toad, *Bufo arenarum*, Hensel. *Acta. Physiol. Latinoamerica* 13:382-384.
- WALSER, M. 1973. Divalent cations: physicochemical state in glomerular filtrate and urine and renal excretion. Pp. 555-586, in *Renal physiology, Handbook of physiology, section 8* (J. Orloff and R. W. Berliner, section eds.), Amer. Physiol. Soc., Washington, D.C., vii+1082 pp.
- WATANABE, T., AND M. YASUDA. 1977. Electron microscopic study on the innervation of the pancreas of the domestic fowl. *Cell Tissue Res.*, 180:453-465.
- WATARI, N. 1968. Fine structure of nervous elements in the pancreas of some vertebrates. *Z. Zellforsch. Mikrosk. Anat.*, 85:291-314.
- . 1973. The non-A and non-B cells of the endocrine pancreas. Pp. 71-89, in *Gastroentero-pancreatic endocrine system-A cell-biological approach* (T. Fujita, ed.), Igaku Shoin Ltd., Tokyo, viii+195 pp.
- WATARI, N., N. TSUKAGOSHI, AND Y. HONMA. 1970. The correlative light and electron microscopy of the islets of Langerhans in some lower vertebrates. *Arch. Histol. Japonicum*, 31:371-392.
- WATKINS, D., J. LEONARDS, P. K. DIXIT, S. J. COOPERSTEIN, AND A. LAZAROW. 1964. Glucose stimulation of insulin release from toadfish islet tissue *in vitro*. *Biol. Bull.*, 127: 395-396.
- WATTS, C., K. GAIN, AND P. L. SANDIN. 1976. Glucose homeostasis in the developing rat. I. Blood glucose and immunoreactive insulin in the later stages of gestation of the fetal rat. *Biol. Neonate*, 30:88-94.
- WEIR, G. C., P. C. GOLTSOS, E. P. STERNBERG, AND Y. C. PATEL. 1976. High concentration of somatostatin immunoreactivity in chicken pancreas. *Diabetologia*, 12:129-132.
- WELLMANN, K. F., AND B. W. VOLK. 1977. Historical review. Pp. 1-14, in *The diabetic pancreas* (B. W. Volk and K. F. Wellmann, eds.), Plenum Press, New York, xviii+599 pp.
- WELSCH, U. N., AND V. N. STORCH. 1972. The fine structure of the endocrine pancreatic cells of *Ichthyophis kohtaoensis* (*Gymnophiona, Amphibia*). *Arch. Histol. Japonicum*, 34:73-85.

- WINBLADH, L. B. 1970. Alloxan effects on blood glucose level and pancreatic islet tissue in *Lampetra fluviatilis*. Gen. Comp. Endocrinol., 15:43-51.
- . 1976a. Pancreatic islets of some myxinoide cyclostomes. Pp. 179-188, in The evolution of pancreatic islets (T. A. I. Grillo, L. Leibson, and A. Eppe, eds.), Pergamon Press, Oxford, New York, xii+377 pp.
- . 1976b. Follicles in the endocrine pancreas of some myxinoide species. Acta Zool., 57:7-11.
- WINGSTRAND, K. D. 1966. Comparative anatomy and evolution of the hypophysis. Pp. 58-126, in The pituitary gland (G. W. Harris and B. T. Donovan, eds.), Butterworths, London, 1:xiv+587 pp.
- WISE, J. K., S. S. LYALL, R. HENDLER, AND P. FELIG. 1973. Evidence of stimulation of glucagon secretion by alanine in the human fetus at term. J. Clin. Endocrinol. Metab., 37:345-348.
- WOODS, S., AND D. PORTE, JR. 1974. Neural control of the endocrine pancreas. Physiol. Rev., 54:596-619.
- WURSTER, D. H., AND M. R. MILLER. 1960. Studies on the blood glucose and pancreatic islets of the salamander, *Taricha torosa*. Comp. Biochem. Physiol., 1:101-109.
- YAGLOV, V. V. 1976. Evolutionary morphology and classification of pancreatic acinar-islet cells. Pp. 113-120, in The evolution of pancreatic islets (T. A. I. Grillo, L. Leibson, and A. Eppe, eds.), Pergamon Press, Oxford, New York, xii+377 pp.
- YANNI, M. 1964. Effect of insulin and excess glucose on the carbohydrates, water and fat contents of the tissues of *Clarias lazera*. Z. Physiol., 48:624-631.
- YOKOTE, M. 1970a. Sekoke disease, spontaneous diabetes in carp, *Cyprinus carpio*, found in fish farms. I. Pathological study. Bull. Freshwater Fish. Res. Lab., 20:39-72.
- . 1970b. Sekoke disease, spontaneous diabetes in carp found in fish farms. III. Response to mammalian insulin. Bull. Japanese Soc. Sci. Fish., 36:1219-1223.
- ZIERLER, K. L. 1972. Insulin, ions, and membrane potentials. Pp. 347-368, in Handbook of physiology, section 7 (R. O. Greep and E. B. Astwood, section eds.), The endocrine pancreas (D. F. Steiner and N. Freinkel, vol. eds.), Amer. Physiol. Soc., Washington, D.C., 1:x+1-721 pp.

EVOLUTION OF ENDOCRINE CONTROL OF CALCIUM REGULATION

PETER K. T. PANG, ALEXANDER D. KENNY, AND CHITARU OGURO

Although the endocrine control of calcium regulation has been studied extensively, most investigations have been conducted with mammals and comparative studies with lower vertebrates are incomplete. We still lack definite proof of the participation of some endocrine systems in lower vertebrate calcium regulation. It is, therefore, difficult to discuss the evolution of this regulation.

We shall first discuss the problems involved in analyzing this subject. Several important features of the evolution of the endocrine control of calcium regulation will then be outlined. The remainder of the paper is divided into two parts: hypercalcemic regulation and hypocalcemic regulation. In each section, the endocrine systems thought to be involved will be described briefly for each vertebrate class. The main discussion will center on evolutionary implications and speculation. Hopefully, this presentation will serve not only as a source of information but also as a stimulus for future investigations.

GENERAL CONSIDERATIONS

Problems inherent in evolutionary considerations

The main problem in evaluating the evolution of the endocrine control of calcium balance is the determination of the hormones involved. Participation of a hormone in physiological regulation must fulfill certain criteria. First, specific deficiency symptoms must be demonstrated to occur after the surgical removal of the tissue. Second, these symptoms must be correctable by replacement therapy. Third, the active principle must be demonstrated in the circulation. Fourth, circulating levels of the principle must be shown to change according to the physiological requirements of the organism. Some endocrine tissues of lower vertebrates do not satisfy even the first criterion. In most cases, deficiency symptoms have been described, but the principles have not been demonstrated in the circulation. In a few cases, the principle has been shown in the circulation but the levels have not been correlated with physiological demands. The main difficulty resides in the methodology used to measure the principle(s). Another problem in discussing the evolution of hormones is to pinpoint their physiological functions in lower vertebrates. Very often these hormones have known physiological functions in mammals, and investigators working with lower vertebrates frequently begin with a bias towards the mammalian function of the hormone. Such preconceptions can be misleading in the interpretation of data. Changes in the

functions of hormones have been demonstrated frequently in comparative studies of mammals and lower vertebrates.

Another obstacle to the study of the evolution of the endocrine control of calcium regulation is the diversity of vertebrate species. In most vertebrate classes, only a few species have been studied in detail. Nevertheless, it is tempting to generalize from limited data and speculate on evolutionary trends among different vertebrate groups. It is very difficult to determine whether the features observed in one or two species are characteristic of the entire taxon. Often, these features may represent a specialized adaptation of the species to its environment and, as such, do not have any bearing on the evolutionary history of the group as a whole. To understand this problem better, investigators must give more attention to phylogenetic relationships. This was discussed in the first chapter (Atz *et al.*, this volume). It is obvious that in order to determine evolutionary trends among vertebrate groups, large numbers of living species must be shown to possess similar features and these features must have been adaptive to ancestral ecological requirements. Very few studies of this nature have been undertaken. In addition to the large number of species desired, the choice of species is also important. This has been discussed by various investigators (Bern, 1967; Sawyer, 1972). Some species are obviously evolutionary dead ends or specializations and the information we obtain from them has little or no importance in understanding evolutionary trends. An example of this is urea retention in the coelacanth. It has been debated whether this interesting characteristic is merely a specialized feature (Pang *et al.*, 1977; Griffith and Pang, 1979). There are, nevertheless, species which offer good possibilities. These include the lungfishes and urodeles.

There are several interesting questions concerning the evolution of the endocrinel control of calcium regulation. First, do all vertebrates have similar endocrine systems for calcium regulation? This is clearly so for most tetrapods, but there are still some unsolved problems among the cyclostomes, elasmobranchs, and bony fishes. These primary aquatic vertebrates respire through gills that are in constant contact with the surrounding water and allow for the exchange of gases. Although the cells are not completely permeable, considerable movement of water and electrolytes takes place. When a fish inhabits seawater, the external calcium level is much higher than the internal and there is a tendency for calcium to enter the body. On the other hand, the lower calcium level of soft freshwater would lead to a loss of calcium from fish living in this medium. Thus, aquatic vertebrates face a constant environmental calcium challenge. To survive, they must maintain their calcium balance.

When the ancestral tetrapods moved out of water onto land, this particular osmoregulatory problem disappeared and the controlling mechanisms possessed by the aquatic organisms became obsolete. One would expect, therefore, drastic changes in the endocrine control of calcium regulation: changes in hormone functions and the appearance of new endocrine sys-

tems. At present very little is known about this aspect of calcium regulation. Studies on the endocrine control of other electrolytes seem to support the notion of changes in hormonal function during the water-to-land transition. The participation of arginine vasotocin in water and electrolyte regulation is a good example and has been explored in detail in other communications (Pang, 1977; Sawyer *et al.*, 1978).

Another interesting problem concerns the role played by bone and other calcified tissues in the evolution of calcium regulation. In higher vertebrates, such as mammals and birds, bone is an important reservoir for calcium regulation. Internal storage would be desirable for physiological regulation in animals dependent on food and drinking water as the main calcium source. On the other hand, animals that live in the marine environment are surrounded by an inexhaustible "storage" supply of calcium. In soft freshwater, the calcium level is very low. However, if the organism possesses a way to extract this calcium against the concentration gradient, this reservoir of calcium is readily available and virtually inexhaustible. We would then expect the endocrine controlling mechanisms in the primary aquatic vertebrates to be different from those of the terrestrial vertebrates because the target tissues would be very different. The use of the aquatic environment as a source of electrolytes is certainly not impossible. It is well known that fishes living in low sodium freshwater can extract it from the environment against a steep concentration gradient. In this paper, the relation between the aquatic environment and calcium balance in fishes will be explored in detail.

One of the greatest challenges that egg-laying vertebrates face is the mobilization of calcium during egg formation. This problem, common to the monotremes and most nonmammalian vertebrates, is more acute in those vertebrates that form eggs with calcareous shells. It is, therefore, interesting to analyze the hormonal involvement and to see if the same mechanism is present throughout these groups.

Endocrine Systems in Calcium Regulation

Almost every endocrine system has been implicated at one time or another in calcium regulation. Because it is impossible to describe all of them here, only those for which there is strong evidence will be considered. Table 1 summarizes the presence and absence of major calcium regulating endocrine systems in various vertebrate groups. The endocrine systems include the parathyroid glands, vitamin D metabolizing tissues, pituitary gland, ovaries, calcitonin producing cells, and Stannius corpuscles. The pituitary gland and ovaries are present in all vertebrate groups, and the calcitonin producing cells have been found in all vertebrates except the cyclostomes. A vitamin D metabolizing system has been reported in teleosts, lungfishes, amphibians, reptiles, birds, and mammals, but further studies are needed to identify this system in elasmobranchs and cyclostomes. Parathyroid glands, absent from all fishes, are found throughout the tetrapods with the excep-

TABLE. 1.—Distribution of calcium regulating endocrine system in vertebrates. ?, not known yet; +, present; —, absent; ±, absent in some species; (—), definitive studies lacking.

Vertebrates	Vitamin D metabolizing system	Parathyroids	Pituitary gland	Ovary	Calcitonin producing tissues	Stannius corpuscles
Cyclostomes	?	—	+	+	(—)	(—)
Elasmobranchs	?	—	+	+	+	(—)
Teleosts	+	—	+	+	+	+
Lungfishes	+	—	+	+	+	(—)
Amphibians	+	±	+	+	+	(—)
Reptiles	+	+	+	+	+	(—)
Birds	+	+	+	+	+	(—)
Mammals	+	+	+	+	+	(—)

tion of some aquatic urodeles. Stannius corpuscles are present only in some bony fishes; whether they are present in elasmobranchs and cyclostomes in a similar or a different form requires further careful investigation. The presence of these endocrine systems in a particular vertebrate group, however, does not necessarily mean that a physiological function has been demonstrated. Table 2 shows whether the endocrine systems have been assigned physiological actions in the various vertebrate groups.

Although there is little information available concerning cyclostomes or elasmobranchs, there has been considerable work done on teleosts. There is strong evidence for hypocalcemic action of the Stannius corpuscles and hypercalcemic action of estrogen and the pituitary gland in most teleostean species studied. Even though the vitamin D metabolizing system is present in fishes, its role in calcium regulation remains to be demonstrated. The participation of calcitonin in fish calcium regulation has been shown in eels, but it is still somewhat controversial for other teleosts. The hypercalcemic action of estrogen and the parathyroid principle have been demonstrated in all nonmammalian tetrapods. The physiological role of calcitonin in tetrapod calcium regulation still has not been established satisfactorily. Recent intensive studies on vitamin D metabolites have pointed to their importance in mammalian and avian calcium regulation. There is, however, little or no information concerning the action of vitamin D metabolites in lower tetrapods. There also have been recent investigations involving the pituitary gland and tetrapod calcium regulation; further investigations are badly needed.

From the simple analysis presented above, we can see some interesting features of the endocrine evolution of calcium regulation. Major changes in endocrine participation appear to have occurred during the water to land transition. The problems in calcium regulation facing animals in aquatic, as opposed to terrestrial, environments must be appreciated. It is extremely

TABLE 2.—*Effects of calcium regulating principles in vertebrates. Symbols are: +, positive effects observed; —, no effect; ±, mixed reports; (+), more work needed to confirm; ?, no clear studies performed.*

Vertebrates	Hypercalcemic principles				Hypocalcemic principles	
	PTH	Pituitary principles	Estrogen	Vitamin D	Calcitonin	Stannius corpuscles principles
Cyclostomes	—	?	+	?	—	?
Elasmobranchs	—	?	+	?	—	?
Teleosts	—	+	+	?	±	+
Lungfishes	—	?	+	?	—	?
Amphibians	+	(+)	+	(+)	±	?
Reptiles	+	?	+	?	—	?
Birds	+	(+)	+	+	±	?
Mammals	+	(+)	+	+	±	?

important to understand how various aquatic vertebrates, especially the elasmobranchs and the cyclostomes, regulate calcium. When this information is coupled with our knowledge of tetrapod calcium metabolism, we may eventually be able to understand the evolution of the endocrine control of calcium regulation in vertebrates.

HYPERCALCEMIC REGULATION

Parathyroid Gland

In view of the extensive literature on parathyroid glands, it is impossible for us to review much of it here so we refer interested individuals to the volume of the *Handbook of Physiology on the parathyroid gland* (Aurbach, 1976) and the proceedings of recent parathyroid conferences for extensive summaries on various aspects of the biology of this gland.

When the parathyroid glands are present, there are usually four. There are, however, vertebrates that have two or six. The glands are derivatives of the third and/or fourth branchial pouches (Roth and Schiller, 1976) and were considered to be endodermal in origin, but recent evidence indicates that they may actually come from the neural crest (Pearse and Takor Takor, 1976). Histologically, these glands are similar throughout the tetrapods and consist of cords of secretory chief cells surrounded by various amounts of fat and connective tissue. Vascularization may be different in different species. These glands often show substantial seasonal changes.

Hypercalcemic principles are present in all tetrapods possessing parathyroid glands. Most studies of the biochemistry of these principles have been conducted with mammalian species. Recent investigations show that these principles may be first synthesized as a pre-proparathyroid hormone with 115 amino acids. This molecule is then cleaved to form a parathyroid hormone that is further broken down to parathyroid hormone, having 84 amino acids, and released into the circulation (Habener *et al.*, 1976). Circulating parathyroid hormones consist of the whole molecule of 84 amino acids and various fragments. The activity of the molecule is found in the

fragment of 1-34 amino acids. The amino acid sequence of bovine and porcine parathyroid hormones have been determined. The human active 1-34 fragment also has been determined and synthesized. There is little or no data on the biochemistry of the parathyroid principles of nonmammalian tetrapods.

Parathyroid glands have not been identified in cyclostomes, elasmobranchs, or bony fishes (Pang, 1973). Although the ultimobranchial body was suggested as the parathyroid gland in *Astyanax mexicanum* (Rasquin and Rosenbloom, 1954), subsequent studies on the killifish, *Fundulus heteroclitus* (Pang, 1971b), failed to confirm this. Moreover, it is now well established that the ultimobranchial body is the source of calcitonin (see discussion below). Although a typical parathyroid gland has not been found in fishes, we do not yet know whether parathyroid-like substances are present in this group.

Mammalian parathyroid hormones have been tested in fishes under various conditions. In spite of a few studies that reported positive effects of the mammalian hormone in teleosts, the majority of the evidence indicates the opposite (see review by Pang, 1973). Mammalian parathyroid hormones do not seem to have any significant effect on plasma calcium levels. In view of the failure to locate the glands and the absence of clear effects with exogenous hormones, it is safe to conclude that the parathyroid glands cannot be considered as a hormonal regulatory mechanism in the calcium homeostasis of teleosts.

Data on other fishes are even more scarce. The presence of a parathyroid gland has not been reported for cyclostomes, elasmobranchs, or other bony fishes including lungfishes. Injections of parathyroid hormone into elasmobranchs did not have a hypercalcemic effect (Urist, 1962). Crude and purified mammalian parathyroid extracts and synthetic human (1-34) parathyroid hormone fragment also failed to elicit hypercalcemia in the South American lungfish, *Lepidosiren paradoxa* (Urist *et al.*, 1972; Pang and Sawyer, 1975).

Amphibians are probably the first tetrapods to possess parathyroid glands. Although all anurans thus far examined have parathyroid glands, they are absent from the urodeles *Cryptobranchus alleganiensis*, *Necturus maculosus*, and neotenic *Ambystoma tigrinum* (Oguro and Pang, unpublished data). The presence and absence of parathyroid glands in different amphibian groups are shown in Fig. 1. There are still groups that have not been studied carefully so that the picture is far from complete.

There are many studies on the physiological importance of parathyroid glands in amphibians, but most of these studies concern anurans (Waggener, 1930; Cortelyou *et al.*, 1960; Robertson, 1970, 1977; Oguro, 1971). It is generally accepted that parathyroidectomy elicits hypocalcemia in anurans and that parathyroid hormones have opposite effects. This is far from true for all the urodeles. Parathyroidectomy of *Cynops pyrrhogaster* resulted in hypocalcemia and tetanic convulsions (Oguro and Uchiyama, 1975). On the

FIG. 1.—Distribution of parathyroid glands in amphibians. Symbols are as follows: +, present; —, absent; +—, present in some species only; (—), absence requires confirmation with more definitive work; ?, not definitely known.

other hand, parathyroidectomy had no effect in *Hynobius nigrescens* or *Megalobatrachus davidianus* (Oguro, 1973; Oguro and Uchiyama, 1975). Furthermore, there are species of urodeles that do not possess parathyroid glands. How do these conduct their hypercalcemic regulation? Recent evidence suggests that the pituitary gland may be involved. This will be discussed in the pituitary section below.

Parathyroid glands are present in all reptilian species examined to date (Roth and Schiller, 1976) and the effects of parathyroidectomy have been studied. Hypocalcemia usually follows this surgery in lizards (Clark, 1968a; Clark *et al.*, 1969; McWhinnie and Cortelyou, 1968; Oguro, 1975), snakes (Oguro, 1970, 1972; Clark, 1971a), and the caiman (Oguro and Sasayama, 1976). In most species studied, hypocalcemia was accompanied by tetany, but injection of mammalian parathyroid hormone had a corrective effect in the hypocalcemic animals. However, hypocalcemia was not observed in two species of parathyroidectomized turtles, *Chrysemys picta* and *Pseudemys scripta* (Clark, 1965). In two other species of turtles, however, *Geoclemys reevesii* and *Testudo graeca*, parathyroidectomized animals showed plasma calcium levels of only 60 per cent those of control animals. Tetany was not observed in these species (Oguro and Tomisawa, 1972; Oguro *et al.*, 1974).

Because of the commercial value of birds, there have been more studies on these animals than on other lower vertebrates. However, in most cases the studies were conducted with domestic birds such as the chicken or duck, which can hardly be considered as evolutionarily important species. Nevertheless, parathyroidectomy has been reported repeatedly to produce hypocalcemia in chickens and ducks (Benoit *et al.*, 1941; Riddle and McDonald, 1945; Cherian and Cipera, 1968) and tetany was often associated with hypo-

calcemia (Polin and Sturkie, 1958; Anderson and Consuegra, 1970). Recently, Clark and Wideman (1977) studied the effect of parathyroidectomy in starlings and reported hypocalcemia one hour after removal of the glands.

Injections of mammalian parathyroid hormone have hypercalcemic effects in intact as well as parathyroidectomized birds (Candlish and Taylor, 1970; Mueller *et al.*, 1973; Gonnerman *et al.*, 1975; Clark and Wideman, 1977). In fact, young chicks (Parsons *et al.*, 1973) and young Japanese quail (Dacke and Kenny, 1973) are so sensitive to parathyroid hormone injections that they have been used in bioassays for parathyroid hormone.

In egg-laying hens, the calcium demand is great. The parathyroid glands have been shown to be hyperactive and these birds are more sensitive to injections of parathyroid hormone (see reviews by Simkiss, 1974; Kenny and Dacke, 1975). Such studies add to the physiological importance of these glands in calcium regulation.

The bird is the only lower vertebrate in which the biochemistry of parathyroid hormones has been studied. Chicken parathyroid hormone and pro-parathyroid hormone have been shown to contain 80 and 90 amino acids, respectively, although the amino acid sequence has not been determined (MacGregor *et al.*, 1973; Habener and Potts, 1976).

As stated above, most of the work on parathyroid glands has been done with mammals. These glands are present in all mammalian species studied (Roth and Schiller, 1976). There have been numerous investigations showing that the parathyroids are physiologically important in mammalian hypercalcemic regulation (see review by Aurbach, 1976) and this has prompted extensive study of the biochemistry of parathyroid hormones. There are, in addition, many methods available to assay for these hormones in the circulation, including *in vivo* and *in vitro* assays and radioimmunoassays. These studies have been instrumental in identifying the true hormonal status and physiological significance of mammalian parathyroid hormones in healthy and diseased states.

Although parathyroid hormones are important for hypercalcemic regulation in mammalian species, we cannot draw the same conclusion for many of the lower vertebrate groups. In birds, reptiles, and anurans, extirpation and injection experiments clearly indicate the hypercalcemic properties of these glands. In some species, it is true that parathyroidectomy had no effect on serum calcium levels, but this does not mean that these glands are not important in calcium regulation. Robertson (1977) reported a seasonal responsiveness of frogs to parathyroidectomy. Failure of certain species to respond may merely reflect the animal's physiological state at the time of the operation. However, assay methods have not been perfected to measure circulating levels of parathyroid hormones in lower vertebrates. Without such data, we cannot conclude that parathyroid hormone is a physiologically significant circulating hormone in lower vertebrates, although the parathyroid glands are certainly involved in calcium regulation in most species.

We see from the above discussion that the important step in the evolution of the parathyroid glands takes place during the water to land transition. The gland is absent in fishes, including the lungfishes (Oguro and Pang, unpublished data), and first appears in amphibians. We suggested above that in an aquatic environment the constant challenges through the exchange surfaces require specific endocrine regulation. Appearance of the parathyroid glands in the first vertebrate group to become adapted to land supports this hypothesis. Furthermore, absence of parathyroid glands in some aquatic urodeles also suggests that since these species do not have a terrestrial period in their life history, the parathyroid glands are not needed. If this is so, one would expect these species to regulate calcium in the same way as do the fishes. This will be discussed later.

Although the parathyroid glands appear first in the amphibians, this does not imply that the gland is necessarily a new invention of the tetrapods. It is possible that there is some kind of precursor among the fishes and ancestors of the amphibians. This will certainly be an important area of research for the future understanding of parathyroid evolution.

Pituitary Gland

As mentioned earlier, almost all vertebrate endocrine organs have been implicated at one time or another in calcium regulation. The pituitary is no exception. However, there was little clear-cut evidence that demonstrated the action of this gland in the physiological regulation of calcium. Recent studies on fishes, birds, and urodeles contain some exciting data about this endocrine system.

Fishes do not possess parathyroid glands but they are efficient in maintaining plasma calcium levels in a calcium-depleting environment. It is, therefore, logical to assume that they possess hypercalcemic endocrine organs, the identity of which have escaped endocrinologists for a long time. The early literature contains reports that hypophysectomy results in hypocalcemia (Fontaine, 1956; Oliverau and Chartier-Baraduc, 1965; Chan and Chester Jones, 1968; Chan *et al.*, 1968a). In addition to hypocalcemia, there are, in most cases, simultaneous decreases in other electrolytes. This makes it difficult to recognize the specific calcium controlling effect and to separate it from the known osmoregulatory effect of the pituitary gland (Chan and Chester Jones, 1968). However, in some studies on the killifish, *Fundulus heteroclitus*, the distinct control of calcium by the pituitary gland was demonstrated. When killifish were adapted to artificial calcium-deficient seawater, hypophysectomy elicited a significant decrease in plasma calcium but not in other electrolytes. In extreme cases, tetanic seizures were evident (Pang *et al.*, 1971a). When calcium was present in the environment, hypophysectomy did not have a hypocalcemic effect. Because the pituitary gland is involved in hypercalcemic regulation, high environmental calcium would inhibit the activity of this gland and its removal would have no effect. This strongly suggests a physiological function of the pituitary gland in calcium regulation. Furthermore, replacement therapy with a pituitary

transplant or injections of pituitary homogenate were both effective in correcting the hypocalcemia (Pang *et al.*, 1973b).

We have sought to identify the hypercalcemic pituitary hormone. In some preliminary studies, both prolactin and ACTH were shown to have a hypercalcemic effect in hypocalcemic hypophysectomized killifish (Pang *et al.*, 1973b). Recently, the hypercalcemic effect of prolactin was established more firmly and different parts of carp pituitary glands were tested also. A prolactin-free part did not have a hypercalcemic effect in the test fish (Pang *et al.*, 1978). We are, therefore, quite confident that the pituitary gland is an important hypercalcemic endocrine gland in killifish and that prolactin is a hypercalcemic hormone. The hypocalcemic effect of hypophysectomy has been reported also in another species of killifish, *Fundulus diaphanus* (Pang *et al.*, 1973a). Nevertheless, more species must be studied before a general conclusion can be reached.

Although these studies are preliminary, they provide a possible explanation for the ability of teleosts to regulate their calcium levels in the absence of parathyroid glands. According to our hypothesis, participation of the pituitary gland in hypercalcemic regulation may reflect constant environmental challenges to the organism through the exchange surfaces. If this is true, aquatic urodeles, such as the mud puppy and hellbender that lack parathyroid glands, should have a similar pituitary controlling mechanism. When the mud puppy, *Necturus maculosus*, was hypophysectomized, hypocalcemia was observed. Prolactin injections corrected the decrease in calcium (Pang, unpublished data). Similar operations were performed on hellbenders, and hypocalcemia was observed again (Oguro and Pang, unpublished data). In another urodele, *Hynobius nigrens*, which possesses parathyroid glands, hypophysectomy but not parathyroidectomy produced a significant fall in plasma calcium levels (Oguro and Uchiyama, 1975). These studies are still preliminary in nature and the role played by the pituitary gland and prolactin in amphibian calcium regulations remains to be elucidated.

There is very little information on the pituitary control of calcium regulation among other tetrapods. Injection of prolactin has been reported to produce hypercalcemia in bullfrogs, Japanese quail (Baksi *et al.*, 1978), and rats (Robinson *et al.*, 1975). Some recent studies on the regulation of vitamin D metabolism revealed that prolactin may be involved also. Prolactin injections stimulated the renal production of active metabolites of vitamin D₃ in birds (Spanos *et al.*, 1976a, 1976b; Baksi *et al.*, 1978). The relationship between vitamin D metabolism and prolactin and its evolutionary implications will be discussed later.

This is one of the most exciting areas of the comparative endocrinology of calcium regulation and much more work is necessary. Based on these preliminary studies, one can make the following interpretations and suggestions. It is possible that pituitary hypercalcemic control is a very old regulatory process. This mechanism would be an important adaptation for vertebrates invading low calcium fresh water environments. Taxonomic

groups such as the chondrosteans and holosteans should be studied in addition to other euryhaline and stenohaline freshwater teleosts. The invasion of the terrestrial environment would render this regulatory mechanism obsolete, but it appears that aquatic urodeles that do not possess a terrestrial phase in their life history still employ the pituitary controlling mechanism. The lungfishes should be studied carefully for they are the closest living relatives of the ancestors of the tetrapods. If they also possess pituitary control, it would give strong support to our hypothesis.

If prolactin is one of the pituitary hypercalcemic principles, it would present some interesting evolutionary problems. Is the prolactin mechanism in lower vertebrates related to the vitamin D system? To answer this we must determine whether prolactin stimulates vitamin D metabolism in all tetrapod species and whether the hypercalcemic effect of prolactin in fishes and aquatic urodeles is related to vitamin D stimulation. The prolactin-vitamin D system may be an old system that originated in fishes living in aquatic environments. On the other hand, it is equally possible that prolactin is not related to vitamin D metabolism in fishes and aquatic urodeles. During the tetrapod invasion of the terrestrial environment, the action of prolactin in hypercalcemic regulation is changed substantially and may then have become linked with vitamin D metabolism. A substantial change in hormonal function is not uncommon during the water to land transition. An example of this can be seen in the neurohypophyseal hormones (Pang, 1977).

Vitamin D

The history of vitamin D investigations may be conveniently divided into two periods: pre and post-1970. Prior to 1970, vitamin D was classified solely as a vitamin. In 1968, Blunt and his coworkers isolated a biologically active metabolite of vitamin D₃ and identified it as 25-hydroxyvitamin D₃. Ponchon and DeLuca (1969) suggested that the liver was the main site of formation of 25-hydroxyvitamin D₃ from vitamin D₃. The hormonal status of vitamin D₃ metabolites was established in 1970. It was reported that 25-hydroxyvitamin D₃ was further hydroxylated to 1,25-dihydroxyvitamin D₃ in the kidney and that this metabolic product is in fact the active form of the vitamin (Fraser and Kodicek, 1970). By 1971, largely due to the work of DeLuca, Kodicek, Norman, Haussler, and their associates, the renal-vitamin D endocrine system was recognized and 1,25-dihydroxyvitamin D₃ was identified as the hormonal form of vitamin D₃. For an historical review of vitamin D research, readers are referred to the review by Omdahl and DeLuca (1973).

Most of the research on vitamin D has been conducted with mammals and birds. In recent years, there has been intensive interest in certain areas such as the mechanism of action of the hormone, the physiological importance of several of the metabolites that have been recognized, and the control of this endocrine system. Here we shall discuss mainly the last aspect. Before we go

into this analysis any further, we will discuss the presence of this endocrine system in lower vertebrates.

The metabolism of vitamin D in poikilothermic vertebrates has received little attention. Since the recognition in the 1920s of its antirachitic activity, most of the pre-1970 reports concerning vitamin D in cold-blooded animals have dealt with its presence in various tissues, notably the liver (Duel, 1951; Urist, 1964). Tissue vitamin D contents are valuable data, but they reveal little concerning the physiological function of the vitamin. Oizumi and Monder (1972) studied the distribution and metabolism of vitamin D in goldfish and concluded that these animals could not metabolize vitamin D. In this study, the longest time interval after administration of vitamin D₃ or 25-hydroxyvitamin D₃ was 24 hours. This time interval, although adequate to demonstrate conversion in a warm-blooded animal such as the rat, may be too short for sufficient metabolism in a cold-blooded species. More recently, the plasma transport proteins for 25-hydroxyvitamin D₃ have been identified in fishes, amphibians, and birds (Hays and Watson, 1976). The most important vitamin D₃ study in lower vertebrates was that of Henry and Norman (1975). They surveyed 14 cold-blooded species for the presence of renal 25-hydroxyvitamin D₃-1-hydroxylase, the renal enzyme that converts 25-hydroxyvitamin D₃ to its dihydroxy hormonally active form. These included eight species of teleosts, two species of amphibians, and four species of reptiles. The enzyme was detected in all 14 species. We also have looked into the presence of this key renal enzyme and have detected it in bullfrogs (*Rana catesbeiana*), neotenic tiger salamanders (*Ambystoma tigrinum*), South American lungfish (*Lepidosiren paradoxa*), and killifish (*Fundulus heteroclitus*) (Kenny *et al.*, 1977). From these studies, it seems that the renal endocrine system for vitamin D metabolism is present in almost all vertebrates. At present, there is no information for cyclostomes, elasmobranchs, or bony fishes other than teleosts. This is an area that needs attention urgently.

In most studies of lower vertebrates, the renal enzyme activity is very low. Furthermore, until the physiological significance of the active metabolite can be elucidated and the hormone can be demonstrated in the circulation in response to physiological needs, the mere presence of the enzyme in the kidney does not necessarily mean the existence of a functional endocrine system. Unfortunately, there is very little work on the physiological effect of vitamin D₃ metabolites in lower vertebrates. Administration of large doses of vitamin D to horn sharks, cyclostomes (Urist 1964), and lungfish (Urist *et al.*, 1972) failed to raise serum calcium levels to any significant extent. Robertson (1975a, 1975b) reported that vitamin D increased intestinal absorption of calcium in frogs. Recently, 1,25-hydroxyvitamin D₃ was shown to elicit hyperphosphatemia and increased bone resorption in eels (MacIntyre *et al.*, 1976; Lopez *et al.*, 1977). At present, the dihydroxy active metabolite of vitamin D is not readily available for physiological studies. Nevertheless, work on lower vertebrates with this hormone is badly needed.

There has been more work on the physiological actions of vitamin D₃ metabolites on mammals and birds. This hormone is important in bone resorption and intestinal calcium transport. There are many studies leading to such conclusions and readers are referred to reviews by DeLuca (1974) and DeLuca and Schnoes (1978). The hormone probably acts on the nuclei of receptor organs (Brumbaugh and Haussler, 1974). There have been tremendous advances in the investigation of the physiological control of vitamin D₃ metabolism. In rats and chickens, the feed-back control of 1,25-dihydroxyvitamin D₃ production by serum calcium levels has been clearly indicated. Hypocalcemia stimulates production of the active metabolite whereas hypercalcemia inhibits it. This was tested in both *in vivo* (Boyle *et al.*, 1971) and *in vitro* (Omdahl *et al.*, 1972) systems. These studies have established the active metabolite as a physiologically important circulating hormone in some warm-blooded vertebrates.

The interrelationship between the renal vitamin D endocrine system and other calcium regulating endocrine systems has been studied in mammals and birds. Parathyroid hormone is involved in the regulation of the renal conversion of 25-hydroxyvitamin D₃ to the active hormone, 1,25-dihydroxyvitamin D₃ (Garabedian *et al.*, 1972; Rasmussen *et al.*, 1972; Fraser and Kodicek, 1973). It is believed that the intestinal calcium absorption effect of parathyroid hormone may be mediated through the active metabolite of vitamin D₃ (Garabedian *et al.*, 1974). Recently, prolactin was shown to stimulate the renal formation of the active vitamin D₃ metabolite in birds (Spanos *et al.*, 1976a, 1976b; Baksi *et al.*, 1978).

Estrogen also has been shown to stimulate the renal production of vitamin D₃ active metabolite in birds. This can be demonstrated in both sexes and can be correlated with egg laying in normal female birds (Tanaka *et al.*, 1976; Kenny, 1976; Baksi and Kenny, 1977a, 1977b; Sedrani and Taylor, 1977). Such a stimulation of 1,25-dihydroxyvitamin D₃ by estrogen was seen also in rats (Baksi and Kenny, 1977c). There is only one such study in poikilotherms. Baksi *et al.* (1978) reported estrogen stimulation of the renal 1-hydroxylase activity in bullfrogs.

Although there is little or no proof at this stage, the vitamin D metabolizing endocrine system may be one of the oldest hypercalcemic endocrine systems in vertebrates. We have discussed, in the previous section, the importance of pituitary principles and, perhaps, prolactin in the evolution of hypercalcemic regulation. Is there any relationship between this and the control of the vitamin D₃ system by prolactin as shown in birds and mammals? To answer this we must demonstrate the relationship between prolactin and vitamin D metabolism in fishes and lower tetrapods. If such a relationship is present among lower vertebrates, then perhaps these two hypercalcemic regulating endocrine systems have coexisted since an early stage of evolution.

Can parathyroid stimulation of renal vitamin D metabolism be demonstrated in other tetrapods? Is this control loop a specialty of the homeother-

mic tetrapods? Did this relationship appear at the same time as the first appearance of the parathyroid glands in amphibians? Comparative studies on vitamin D₃ metabolism may be able to answer some of these questions.

Estrogen has been shown to produce hypercalcemia in all egg-laying vertebrates. This has been related to the increase in calcium demand in the deposition of yolk in the developing eggs. Some of this increased need would no doubt be met with an increase in intestinal absorption of ingested calcium. Inasmuch as the active vitamin D metabolite is important in facilitating intestinal absorption of calcium, a close relationship between these two endocrine systems is desirable. This is clearly seen in birds and bullfrogs. Similar physiological events occur during egg formation in all vertebrates so that one would expect to see similar relationships between estrogen and vitamin D metabolism in all egg laying vertebrates. More experimental work is needed to establish this. However, once this is demonstrated, especially in cyclostomes, it will be established as the most primitive hypercalcemic regulating system.

Gonadal Hormones

The problem of calcium homeostasis in vertebrate reproduction has been studied extensively (see Simkiss, 1967). The relationship between calcium regulation and reproduction exists predominantly in females. This is understandable because the production of calcium rich eggs and the maintenance of embryos and young are performed by the females. However, there are some studies that suggest calcium regulation may be affected by male reproductive cycles. Such studies have been reviewed for the fishes (Pang, 1973). Recent investigations on the killifish did not support such an idea (Balbontin *et al.*, 1978; Pang and Balbontin, 1978). Similarly, there is little evidence that androgens play an important role in calcium regulation in other vertebrates. For this reason, the remainder of this discussion will concentrate on the effects of estrogens.

It appears that ovarian maturation in most fishes is closely associated with an increase in plasma bound calcium levels. Injections of estrogen can produce the same effect in both male and female fishes, although this could not be demonstrated in cyclostomes (Urist *et al.*, 1972). However, Pickering (1976) showed the hypercalcemic effect of estradiol in the river lamprey, *Lampetra fluviatilis*. The effect of estrogen is either absent or minimal in elasmobranchs (Urist and Schjeide, 1961; Woodhead, 1969). In teleosts and lungfishes, estrogen stimulates the hepatic synthesis of yolk protein, which contains high levels of bound calcium (Bailey, 1957; Urist *et al.*, 1972).

Amphibians show a similar mechanism. Estrogen induces hypercalcemia, which is associated with increased hepatic synthesis of yolk protein (Follett and Redshaw, 1968; Munday *et al.*, 1968; Wallace and Bergink, 1974). This is particularly interesting for estrogen has now been demonstrated to stimulate renal vitamin D metabolism in bullfrogs (Baksi *et al.*, 1978), and vitamin D increases intestinal absorption of calcium (Robertson, 1975a,

1975b). When estrogen stimulates yolk protein formation, calcium must be obtained to be incorporated into the yolk protein. A simultaneous increase in calcium absorption through the action of the active vitamin D metabolite can be beneficial. It is important to see if a similar mechanism exists in the fishes.

For reptiles and birds, the problem of calcium regulation during egg laying becomes more acute. Apart from the incorporation of calcium into yolk protein, the formation of the calcium rich egg shell makes strenuous demands on the calcium regulating systems in these species. It is, therefore, not surprising to find that estrogen stimulates yolk protein formation and hypercalcemia in reptiles (Dessauer *et al.*, 1956; Clark, 1967). There is no information as to whether estrogen can also stimulate the renal vitamin D metabolizing system.

The hypercalcemic effect of estrogen in birds is well known (Simkiss, 1967, 1975), and the stimulation of yolk protein has been well studied also (Bergink *et al.*, 1974). Kyes and Potter (1934) first noticed that egg-laying was associated with the development of medullary bone formation, which can be mobilized rapidly for egg formation. Urist (1967) concluded that medullary bone turn over was affected by estrogen. Although reptiles also form calcium rich egg shells, estrogen does not seem to induce medullary bone formation (Simkiss, 1961; Suzuki and Prosser, 1968). Estrogen and egg laying both stimulate renal vitamin D metabolism (see above section). This may be an important mechanism to maximize the absorption and mobilization of calcium during times of high calcium stress.

Mammalian viviparous reproduction is very different from that of the egg-laying vertebrates; estrogen injection does not increase plasma calcium or protein levels in mammals (Urist and Schjeide, 1961). The estrogen mechanism may be one of the oldest hypercalcemic regulating mechanisms among the vertebrates, evolving as an adaptation to meet high calcium demands during egg formation. It is unlikely that in the viviparous mammals, the estrogen mechanism becomes insignificant. Its presence in elasmobranchs is doubtful. This may be related to the marine habitat of this group and the substantially higher plasma calcium levels of elasmobranchs compared with the rest of the higher vertebrates (Pang *et al.*, 1977).

HYPOCALCEMIC REGULATION

Calcitonin

Since the naming of the hypocalcemic hormone, calcitonin, by Copp and his co-workers (1962), there has been much research on the presence and activity of this hormone in various vertebrate species. As far as the comparative endocrinology of calcium regulation is concerned, calcitonin is probably the best studied hormone. Its chemistry is also the most fully understood. However, most investigators believe that, even in mammals, the calcemic effect of this hormone in normal physiology is not fully established. There is a wealth of information about this hormone and it is not

our intention to review it here. In the proceedings of the third, fourth, fifth, and sixth Parathyroid Conferences, calcitonin is discussed at great length and readers are referred to these volumes.

At the beginning of calcitonin research, most investigations were conducted with mammals. Hirsch *et al.* (1963) reported the thyroid origin of calcitonin in rats. Pearse (1966) demonstrated that the hormone was not produced by the follicular cells but by the parafollicular "C" cells that originate embryologically from the ultimobranchial body (Pearse and Carnevali 1967). Inasmuch as the ultimobranchial body remains separate from the thyroid in lower vertebrates, this gland is more conveniently located for surgical removal. An exception to this is the fishes. Subsequently, calcitonin was identified in extracts of ultimobranchial bodies of all nonmammalian vertebrates (Copp and Parkes, 1968) with the exception of the cyclostomes (Copp *et al.*, 1970). Fish glands also proved to be a good source of calcitonin. Within the next few years, sufficient quantities of calcitonins were obtained from salmon and eels so that the amino acid sequences could be determined. Moreover, salmon calcitonin was synthesized. Fish calcitonins, like the mammalian hormone, contain 32 amino acids with an N-terminal seven amino acid ring. Potts and Aurbach (1976) compared the primary structure of eel, salmon, porcine, ovine, bovine, and human calcitonins and speculated on the molecular evolution of these hormones. Just like parathyroid hormone, calcitonin is first synthesized in a prohormone form, procalcitonin, which is subsequently broken down to be released as a smaller molecule (Roos *et al.*, 1974).

The hormonal status of most of the endocrine principles we have discussed in this paper cannot be established because we lack methods sensitive enough to determine the circulating levels of the principles. In most cases, however, the effects of these principles have been clearly demonstrated. Calcitonin has been found in the circulation by bioassays (Kenny, 1972) as well as radioimmunoassays (Deftos *et al.*, 1968; Tashjian *et al.*, 1970). However, until now a consistent physiological action of the hormone has not been demonstrated in any vertebrate group (see review by Copp, 1976). This hormone was first discovered as a hypocalcemic principle in a rat assay. Since then, much effort has been directed toward elucidating the hypocalcemic effect of this hormone in different target organs. Although it is most likely that this hormone will eventually be demonstrated as physiologically important in calcium regulation, it is also possible that calcitonin may have some yet unidentified physiological function unrelated to calcium metabolism. A good understanding of the evolution of this hormone is a very important tool to aid in ascertaining all of calcitonin's physiological actions.

When calcitonin was first discovered, the endocrine control of calcium regulation in fish was still not understood. Investigators hoped to establish a hypocalcemic role for calcitonin in fishes, especially because this hormone was abundant in fish ultimobranchial bodies. Calcitonin has, thus far, been detected in elasmobranchs, a number of species of teleosts, and the Austral-

ian and South American lungfishes (Copp *et al.*, 1970; Pang *et al.*, 1971*b*). Mammalian and salmon calcitonins have been tested in several species of fishes. A hypocalcemic response has not been shown in elasmobranchs (Urist, 1967; Louw *et al.*, 1969; Copp *et al.*, 1970) or lungfishes (Urist *et al.*, 1972; Pang and Sawyer, 1975). The picture is more complicated in teleosts. Many species of teleosts have been injected with mammalian and salmon calcitonin. Most failed to show hypocalcemia after treatment. These fishes include killifish, catfish, salmon, and trout (Pang and Pickford, 1967; Pang 1971*c*, 1973; Copp *et al.*, 1972; Lopez *et al.*, 1971). Other investigators reported hypocalcemia in catfish injected with crude mammalian calcitonin preparations (Louw *et al.*, 1967), but these effects were ascribed later to contaminants in the preparation rather than to calcitonin (Kenny, 1972). Hypocalcemia was reported also in calcitonin treated eels (Chan *et al.*, 1968*b*; Pang, 1971*c*; Lopez *et al.*, 1976). However, calcitonin injections failed at times to produce hypocalcemia in eels (Pang, 1971*c*; Orimo *et al.*, 1972; Hayslett *et al.*, 1971; Dacke, cited by Kenny, 1972). It has been suggested that plasma calcium levels may not be sufficiently sensitive to indicate calcitonin's effect in fishes (Fleming *et al.*, 1973). Detailed histophysiological studies of salmon and eel revealed profound effects of calcitonin on bone (Lopez *et al.*, 1971, 1976). Furthermore, ultimobranchiaectomy, which is a very difficult operation for complete removal of the gland in fishes, produced hypercalcemia in eels (Chan, 1970; Lopez *et al.*, 1976). Fenwick (1975) also observed hypercalcemia in partially ultimobranchiaectomized goldfish. However, Chan (1972) reported hypocalcemia in the ultimobranchiaectomized eel.

Circulating levels of calcitonin have been measured in fish and have been shown to be related to calcium regulation (Dacke *et al.*, 1971; Deftos *et al.*, 1972). On the other hand, circulating levels of calcitonin also have been shown to increase with sexual maturation in fishes (Deftos *et al.*, 1974; Watts *et al.*, 1972; Orimo *et al.*, 1977). Very high concentrations of calcitonin were observed in spawning salmon (see review by Copp, 1976). Histological studies further indicated that glandular activities were increased during gonadal maturation (Oguri, 1973; Yamane and Yamada, 1977).

Most studies suggest that calcitonin plays a role in calcium regulation in teleosts. However, in view of the inconsistency in the data from even the same species of fish, this role may be minimal when compared to other endocrine systems. It is also possible that the ultimobranchial body is involved in other physiological processes. For example, calcitonin is known to affect serum electrolytes other than calcium (Pang, 1973; Orimo *et al.*, 1972). Although calcitonin may eventually be convincingly demonstrated to act as an important hormone in fish calcium regulation, investigators must not have a biased view towards its hypocalcemic action. We need to clarify the physiological function of calcitonin among the primitive fishes.

The function of the ultimobranchial body and calcitonin in amphibian calcium regulation has been studied extensively by Robertson using the leop-

ard frog, *Rana pipiens*. Apart from his work, there are almost no other studies on amphibians. Boschwitz and Bern (1971) gave *Bufo marinus* a bolus injection of porcine calcitonin and reported hypocalcemia. However, in two other experiments, chronic injections of the hormone produced hypercalcemia. Robertson performed ultimobranchialectomy in *Rana pipiens* and observed hypercalcemia and increased osteoclastic activity (Robertson, 1969a), increased urinary excretion of calcium (Robertson, 1969b), disturbances in bone healing (Robertson, 1969c) and increased depletion of calcium deposits in the endolymphatic lime sac (Robertson, 1972). These data strongly suggest that the ultimobranchial body is involved in calcium regulation in this species of anuran. However, it is important to extend these studies to other amphibian species, especially the urodeles, which have not been investigated at all.

The role of the ultimobranchial body and calcitonin in reptilian calcium regulation is far from being understood. Ultimobranchialectomy has not been performed in this group. Injection of calcitonin has no effect on turtles (Clark, 1968b, 1971b), lizards (Dix *et al.*, 1970), or snakes (Clark, 1971b). Bélanger *et al.* (1973) observed inhibition of osteolytic osteolysis in calcitonin treated turtles. It seems possible that calcitonin may be important in reptilian calcium regulation, but more work is badly needed.

Extensive research has been done on calcitonin in birds. Earlier work showed that calcitonin administration has no effect on plasma calcium levels in intact birds (Urist, 1967; Candlish and Taylor, 1970; Kraintz and Inscher, 1967), but produced hypocalcemia in parathyroidectomized birds (Kraintz and Intscher, 1969). Brown *et al.* (1969, 1970) did not observe significant changes in plasma calcium levels in ultimobranchialectomized newly hatched chicks, but these animals are less able to combat a parathyroid hormone challenge. Similarly, Copp *et al.* (1970) reported the inability of ultimobranchialectomized young turkeys to correct the hypercalcemia produced by calcium infusion. Furthermore, chronic injections of calcitonin in young chicks increased growth and reduced bone resorption (Bélanger and Copp, 1972), and McWhinnie (1975) observed increased bone weight in chick embryos treated chronically with salmon calcitonin.

The relationship between calcium challenges and calcitonin secretion has been studied in birds. Calcium increased calcitonin secretion by chick ultimobranchial bodies in tissue culture (Feinblatt and Raisz, 1972; Nieto *et al.*, 1975). Hen ultimobranchial bodies perfused with calcium secreted more calcitonin than did controls (Ziegler *et al.*, 1969). Calcium infusion also increased plasma circulating levels of calcitonin in turkeys (Copp *et al.*, 1970).

Considering all the data together, the hormonal status of calcitonin in birds has been established. The strongest evidence comes from the demonstration of the hormone in the circulation in response to calcium challenges. Although the physiological effect of the hormone is not clear, the participation of calcitonin in combating hypocalcemia has been shown. The failure

of injections of calcitonin to produce an effect may reflect the high endogenous levels of the hormone. Perhaps, the hormone should be tested on ultimobranchialectomized birds or on birds with reduced circulating levels of calcitonin, such as those on a hypocalcemic diet.

This hormone was first discovered in mammals, and consequently they have been the object of most study. It is impossible to cover the literature in this area here. Recent reviews in this area by Gray *et al.* (1974), Potts (1976), and Munson (1976) are available. It seems that this hormone has hypocalcemic effects in young mammals and that it inhibits bone resorption. These actions have been discussed extensively in the above mentioned reviews. However, all authors agree that the physiological role of the hormone in adult mammals is far from being understood. There are indications that it may be involved in acute calcium regulation during increased calcium uptake after a calcium rich meal. Nevertheless, more studies are needed to clearly delineate the physiological function of calcitonin in mammals, especially in man.

After considering this hormone in all vertebrate groups, the conclusion we can draw is that calcitonin may be involved in calcium regulation throughout all the vertebrates, although more studies are needed to establish its precise physiological significance. In many vertebrate groups, considerable doubt exists as to whether or not the hormone plays a central role in calcium regulation. Investigators should study other possible non-calcemic roles for calcitonin. For example, this hormone is known to have significant effects on the renal excretion of water and sodium in mammals (see review by Munson, 1976). In lungfish, calcitonin has been reported as a diuretic (Pang and Sawyer, 1975). It is possible that actions like this may be as important as the calcemic effect of the hormone. Studies such as these, along evolutionary lines, may help us to determine which of the actions of calcitonin is most important physiologically.

Stannius Corpuscles

As indicated at the beginning of this paper, fishes in their aquatic habitat have problems in calcium regulation that are very different from those of tetrapods. In high calcium, hard freshwater and seawater, environmental calcium levels are much higher than that of the internal milieu. There are constant exchanges with the environment, especially at the respiratory surfaces. Fishes must therefore have efficient systems to combat the constant diffusional influx of calcium. The fact that most fishes are capable of maintaining stable plasma calcium levels regardless of environmental levels indicates that they must have an efficient hypocalcemic regulatory mechanism. From our discussion of the ultimobranchial body and calcitonin, it seems that calcitonin may participate in calcium regulation in some fishes, but that it may not play a very significant role. What, then, is the principle hypocalcemic regulatory mechanism in the fishes? Studies in recent years have shown that the Stannius corpuscles, first described in 1839 by Stannius,

may be the hypocalcemic endocrine system in fishes. So far, however, they are found in only some fishes, are absent from tetrapods, and have not been identified in cyclostomes or elasmobranchs. In teleosts, there are, in most cases, two or more of these whitish glandular bodies closely associated with kidneys. In *Amia* and the paddlefish, there are numerous corpuscles along the sides of the kidneys. Some of the earlier studies on the Stannius corpuscles have been reviewed by Pang (1973). Most of the early studies concerned histological features of the glands; work during the 1950s and 1960s suggested that the glands might be related to osmoregulation.

The Stannius corpuscles often have been misidentified as the interrenals. Although there have been suggestions that these two endocrine systems are related, they are, in fact, quite different. Histologically, cells of the Stannius corpuscles are typical of cells synthesizing and elaborating protein or peptides. In addition, the removal of these glands produces hypercalcemia. Vargas and Concha (1957), using the Chilean clingfish (*Sicyases sanguineus*), were among the first to remove the Stannius corpuscles surgically but they mistakenly reported that they had removed the interrenals. Recently, in collaboration with Dr. S. M. Galli-Gallardo of the University of Chile, we showed that the bodies removed from *S. sanguineus* by Vargas and Concha were actually the Stannius corpuscles (Galli-Gallardo *et al.*, 1977).

Since the discovery by Fontaine (1964) that Stanniectomy induced hypercalcemia in the European eel, this phenomenon has been reported in all species thus far investigated. These include the European eel, *Anguilla anguilla* (Fontaine, 1964, 1967; Chester Jones and Henderson, 1965; Chester Jones *et al.*, 1967; Chan *et al.*, 1967; Chan and Chester Jones, 1968; Chan *et al.*, 1969; Chan, 1970; Lopez, 1970; Fenwick and Forster, 1972), the Japanese eel, *Anguilla japonica* (Chan, 1970, 1972), the American eel, *Anguilla rostrata* (Butler, 1969; Fenwick, 1974), the goldfish, *Carassius auratus* (Ogawa, 1968), the killifish, *Fundulus heteroclitus* (Pang, 1971a), and the Chilean clingfish, *Sicyases sanguineus* (Galli-Gallardo *et al.*, 1977). The increase in serum calcium levels is the most consistent effect thus far observed in Stanniectomized fish. However, in some cases, other electrolyte levels also are affected by the surgical removal of these glands (Fontaine, 1964, 1967; Chan *et al.*, 1967; Chan *et al.*, 1969; Chan, 1970, 1972; Butler, 1969).

Calcium is the only electrolyte that we can relate to Stannius corpuscle physiology (Pang *et al.*, 1975) (Table 3). In addition, the effect of Stanniectomy in the killifish and eel is related to the environmental calcium challenge. Removal of the glands from fish adapted to high calcium environments produces distinct hypercalcemia whereas such an effect is reduced or absent in Stanniectomized fish adapted to low-calcium water (Fenwick and Forster, 1972; Pang *et al.*, 1973c).

Both light and electron microscopic studies have shown that the Stannius corpuscles are stimulated in fish adapted to high-calcium environment compared with those of fish adapted to low-calcium environments such as soft freshwater or calcium deficient seawater (Carpenter and Heyl, 1974; Cohen *et al.*, 1975).

TABLE 3.—Relationship between the Stannius corpuscles and plasma electrolyte levels. Symbols are: SW, seawater; FW+CA, calcium enriched freshwater; SW-CA, artificial calcium deficient seawater; FW, freshwater; C.S., Stannius corpuscles; 0, no effect; +, increase; —, decrease.

	CA ⁺⁺	NA ⁺	K ⁺	Cl ⁻
Clingfish (effects of Stanniectomy)				
SW	+	0	0	0
Killifish (effects of Stanniectomy)				
SW	+	0	0	0
FW+CA	+	0	0	0
SW-CA	0	0	0	0
FW	0	0	0	0
Killifish (effects of C.S. injections)				
Cod C.S.	—	0	0	0
Catfish C.S.	—	0	0	0
Carp C.S.	—	0	0	0
Salmon C.S.	—	0	0	0
Goosefish C.S.	—	0	0	0
Killifish C.S.	—	0	0	0

All the histological and physiological studies clearly indicate that the Stannius corpuscles are important in the hypocalcemic regulation of bony fishes. However, it is still possible that the Stannius corpuscles are involved in the regulation of other electrolytes, especially since two cell types have been described in the Stannius corpuscles of some species (Wendelaar Bonga and Greven, 1975; Wendelaar Bonga *et al.*, 1977). However, only one cell type has been seen in the killifish (Cohen *et al.*, 1975), which may account for the rather specific hypocalcemic function of these glands in this species.

There are two main areas in Stannius corpuscle physiology that deserve our particular attention: the chemical identity of the hypocalcemic substance(s) in these bodies and the mechanism of action of the hypocalcemic principle(s). There has been considerable controversy in the literature concerning the steroidal or proteinaceous nature of the Stannius corpuscles' end product. However, it is now clear that these glands mainly have the features necessary for protein synthesis (see review by Krishnamurthy, 1976). We designed a bioassay to study the hypocalcemic activities of the Stannius corpuscles. This rather crude assay depends on the ability of Stannius corpuscle preparations to lower blood calcium levels in assay killifish adapted to low calcium seawater, which reduces the endogenous activity of these glands (Pang *et al.*, 1974). Hypocalcin was suggested as the name of the hypocalcemic substances detected in the Stannius corpuscles of all species of fish thus far studied. These fishes include cod, two species of catfish, carp from the United States and Japan, salmon, the Japanese goosefish, and the killifish (Pang, unpublished data). Linear log dose related responses can be obtained with preparations of Stannius corpuscles from all these species. Preliminary studies suggest that hypocalcin is a heat labile protein that is stable in dilute acid (Pang and Pang, unpublished data).

Recently, another more convenient assay for Stannius corpuscle activity was reported (Ma and Copp, 1977). A large number of samples can be

determined at the same time because it does not depend on the response of whole living animals. In this assay, Stannius corpuscle preparations linearly decrease *in vitro* calcium-activated ATPase activity of the eel gill. Homogenates and chromatographic fractions of eel Stannius corpuscles were assayed in this *in vitro* system, and the molecular weight of an active fraction was determined to be around 4000.

One interesting feature of Stannius corpuscle biochemistry is its relation to the renin angiotensin system. Chester Jones *et al.* (1966) first demonstrated that the Stannius corpuscles contain a renin-like pressor substance. This was subsequently confirmed by Sokabe and his colleagues (Sokabe *et al.*, 1970; Nakajima *et al.*, 1971), who incubated Stannius corpuscles with plasma to produce an angiotensin-like pressor substance. These findings immediately raise the question of whether the renin-like substance in the Stannius corpuscles is related to hypocalcin, the hypocalcemic principle. Our recent studies in collaboration with Dr. H. Sokabe indicate that angiotensin from Stannius corpuscle incubation with homologous plasma possesses hypocalcin activity (Pang, Pang, and Sokabe, unpublished data). Akagi *et al.* (1977) reported that angiotensin generated from the Stannius corpuscles has a similar molecular weight (1300) but different chromatographic properties compared to that generated from the kidney. These findings are particularly interesting inasmuch as kidney preparations and mammalian angiotensin are not hypocalcemic in the killifish bioassay (Pang *et al.*, 1974).

All these findings point to the possibility that the Stannius corpuscles produce a protein that converts a plasma substrate to an angiotensin-like pressor principle. This principle has a hypocalcemic effect in bony fishes. Although this is a very attractive hypothesis, there are many problems to be solved. When Stannius corpuscle homogenates are injected into intact animals, as in the killifish and eel studies, the renin-like substance can act on the plasma substrate to produce the hypocalcemic angiotensin-like principle. However, the Ca-activated ATPase assay of Ma and Copp, the homogenates and chromatographic fractions of Stannius corpuscles were added directly to test tubes of buffer-suspended eel gill membrane. These preparations had to act directly on the Ca-activated ATPase system. It is, therefore, possible that two different substances are being tested, especially in view of the fact that two cell types have been described in the Stannius corpuscles of some fishes. The molecular weight of the active principle in Ma and Copp's studies (1977) is around 4000, but that of Sokabe's preparation is approximately 1000.

The second important area of Stannius corpuscle research concerns the mode and sites of action of this endocrine system. Earlier studies produced controversial results regarding the renal effects of the Stannius corpuscles. Stanniectomized European eels exhibited decreased renal excretion of calcium (Chan *et al.*, 1969; Chan, 1972). However, Büttler (1969) and Fenwick (1974) reported that renal excretion of calcium in Stanniectomized American

eels was either unaffected or increased. Lopez (1970, 1973), in her very elegant studies on eel bone morphology, described profound changes in this tissue after Stanniectomy. It is important to extend these studies to other species of fishes and to see, in addition, whether purified active principles from Stannius corpuscles can produce the same effects in these target tissues.

Hypercalcemia in Stanniectomized fishes may result from decreased excretion, increased release from internal sources, and increased uptake from the environment. Fontaine *et al.* (1972) reported increased calcium uptake in Stanniectomized European eels. More recent investigations with isolated perfused eel gills showed that Stanniectomy increased the active uptake of calcium from the environmental water (Fenwick and So, 1974; So and Fenwick, 1977). Fenwick (1976) further demonstrated that Stanniectomy stimulated gill Ca-activated ATPase in the eel. He suggested that this enzyme might be related to active branchial uptake of calcium. In some recent studies, we observed that Stanniectomy increased branchial permeability to calcium in killifish (Pang, Griffith, Pic, and Maetz, unpublished data). All the data from various laboratories indicate that teleostean gills may be a very important site of action for the Stannius corpuscles.

The above analysis of the Stannius corpuscles has demonstrated the hypocalcemic properties of these glands, and their participation in the physiological regulation of calcium is well established. The effects of surgical removal and replacement therapy are consistent in all species studied. The recent literature suggests that the glands produce a proteinaceous hypocalcemic principle and the glands from all species so far tested have hypocalcemic activity. However, this is still not sufficient to establish the hormonal status of hypocalcin. For this, we need the purification of the hormone, the chemical analysis of its structure, and the development of sensitive methods to measure its circulating levels in response to various physiological challenges.

It is interesting that these glands apparently disappeared when the vertebrates moved onto land. This would agree with our hypothesis that the aquatic environment imposed special challenges to the living organisms, which adapted by means of specialized regulatory mechanisms. When tetrapods moved onto land, these mechanisms became obsolete and either changed in function or disappeared. The Stannius corpuscles may be an example of this type of gland.

CONCLUSION

The participation of endocrine systems involved in calcium regulation has been considered. In view of the uncertainty of the endocrine status of most of the systems in lower vertebrates, it is difficult to delineate the evolution of the endocrine control of vertebrate calcium regulation. Nevertheless, certain trends can be depicted. First, the aquatic environment presents unique problems in calcium regulation. Constant exchanges within the environment require specialized regulatory mechanisms, which can be seen in fishes and

aquatic urodeles, especially in their hypercalcemic regulation. Such a hypothesis is not limited to calcium regulation. Studies on sodium regulation, for example, indicate that in both teleosts and aquatic urodeles, prolactin is important in the control of sodium efflux (Pang and Sawyer, 1974). Second, there are fundamental differences between the calcium requirements of primary aquatic vertebrates and terrestrial vertebrates. Environmental calcium, even in low calcium environments, can be utilized by fishes, and these reservoirs are inexhaustible. Terrestrial vertebrates, on the other hand, depend more on bone for calcium storage.

There are also several distinct features in the evolution of calcium regulating endocrine systems. First, one of the oldest hypercalcemic regulatory mechanisms is probably related to reproduction. The mobilization of calcium during egg formation can be seen in all vertebrate groups, including the cyclostomes. The participation of estrogen has been demonstrated consistently. Second, the vitamin D metabolizing system in the kidney has been shown in teleosts and all tetrapods. It is important to study such a system in the elasmobranchs and cyclostomes. Furthermore, we know very little at present about the physiological functions of this system in the lower vertebrates. The interrelationship between vitamin D and other endocrine systems also presents many challenging problems. This is a very exciting area and studies in lower vertebrates should be rewarding. Third, it is generally accepted that fishes do not have discrete parathyroid glands. The appearance of this endocrine tissue in the tetrapods may be related to the increased dependence on bone. Fourth, the hypercalcemic actions of the pituitary gland have been noted in various vertebrate groups. The precise role of prolactin and other pituitary hypercalcemic hormones remains to be demonstrated. The relationship between the pituitary hormone and other hypercalcemic hormones, such as parathyroid hormone and vitamin D, is not fully understood at present. Fifth, the physiological significance of the ultimobranchial body and calcitonin has been suggested for only a few species of vertebrates. More detailed studies are needed before we can clearly delineate the hypocalcemic action of this hormone. The evolution of this hormone may prove to be quite interesting. Sixth, the Stannius corpuscles are unique endocrine glands found in some fishes. These glands are physiologically important as a hypocalcemic system. They are still not fully understood and present many interesting problems. The reason for their disappearance in the tetrapods and the identity of the active substance are two of the many questions to be answered.

ACKNOWLEDGMENT

The unpublished studies described in this paper were supported by a National Science Foundation Grant No. TCM 76-82239 to P. K. T. Pang.

LITERATURE CITED

- AKAGI, H., T. NAKAJIMA, T. X. WATANABE, AND H. SOKABE. 1977. Teleostean angiotensins produced by renin in the corpuscles of Stannius. Proc. Japan-U.S. Co-operative Seminar on Comparative Studies of the Renin-Angiotensin System, Jichi Medical School, Tochigi-ken, Japan, Nov. 1977, Abstr. #204.
- ANDERSON, D. I., AND P. F. CONSUEGRA. 1970. Endocrine control of calcium homeostasis in the fowl. Poultry Sci., 49:849-869.
- AURBACH, G. D. (ED.). 1976. Parathyroid gland. Handbook of physiology, section 7 (R. O. Greep and E. B. Astwood, section eds.), Amer. Physiol. Soc., Washington, D.C., 7:vii+1-480 pp.
- BAILEY, R. E. 1957. The effect of estradiol on serum calcium, phosphorus and protein of goldfish. J. Exp. Zool., 136:455-469.
- BAKSI, S. N., AND A. D. KENNY. 1977a. The effects of administration of antiestrogen (tamoxifen) *in vivo* on the *in vitro* metabolism of 25-hydroxy-vitamin D₃ in Japanese quail. Biochem. Pharmacol., 26:2439-2443.
- . 1977b. Vitamin D₃ metabolism in immature Japanese quail: effects of ovarian hormones. Endocrinology, 101:1216-1220.
- . 1977c. Estradiol stimulation of 1,25-dihydroxyvitamin D₃ production in normal rats. Pharmacologist, 19:186.
- BAKSI, S. N., A. D. KENNY, S. M. GALLI-GALLARDO, AND P. K. T. PANG. 1978. Vitamin D metabolism in bullfrogs and Japanese quail: effects of estradiol and prolactin. Gen. Comp. Endocrinol., 35:258-262.
- BALBONTIN, F., X. ESPINOSA, AND P. K. T. PANG. 1978. Gonadal maturation and serum calcium levels in two teleosts, the hake and the killifish. Comp. Biochem. Physiol., 61A:617-621.
- BÉLANGER, L. F., AND D. H. COPP. 1972. The skeletal effects of prolonged calcitonin administration in birds, under various conditions. Pp. 41-50, in Calcium, parathyroid hormone and the calcitonins (R. V. Talmage and P. L. Munson, eds.), Excerpta Medica Foundation, Amsterdam, vii+560 pp.
- BÉLANGER, L. F., M. T. DIMOND, AND D. H. COPP. 1973. Histological observations on bone and cartilage of growing turtles treated with calcitonin. Gen. Comp. Endocrinol., 20:297-304.
- BENOIT, J., G. FABIANI, R. GRANGARD, AND J. CLAVERT. 1941. Suppression par la parathyroïdectomie de l'action hypercalcémiant du dipropionate d'estradiol chez le canard domestique. C. R. Soc. Biol., 135:1606-1609.
- BERGINK, E. W., R. A. WALLACE, J. A. VAN DE BERG, E. S. BOS, M. GRUBER, AND G. AB. 1974. Estrogen-induced synthesis of yolk proteins in roosters. Amer. Zool., 14:1177-1193.
- BERN, H. A. 1967. Hormones and endocrine glands of fishes. Science, 158:455-462.
- BLUNT, J. W., H. F. DELUCA, AND H. K. SCHNOES. 1968. 25-hydroxycholecalciferol. A biologically active metabolite of vitamin D₃. Biochemistry, 7:3317-3322.
- BOSCHWITZ, D., AND H. A. BERN. 1971. Prolactin, calcitonin, and blood calcium in the toads *Bufo boreas* and *Bufo marinus*. Gen. Comp. Endocrinol., 17:586-588.
- BOYLE, I. T., R. W. GRAY, AND H. F. DELUCA. 1971. Regulation by calcium of *in vivo* synthesis of 1,25-dihydroxycholecalciferol and 21,25-dihydroxycholecalciferol. Proc. Nat. Acad. Sci., 68:2131-2134.
- BROWN, D. M., D. Y. E. PEREY, P. B. DENT, AND R. A. GOOD. 1969. Effect of chronic calcitonin deficiency on the skeleton of the chicken. Proc. Soc. Exp. Biol. Med., 130:1001-1004.
- BROWN, D. M., D. Y. E. PEREY, AND J. JOWSEY. 1970. Effects of ultimobranchialectomy on bone composition and mineral metabolism in the chicken. Endocrinology, 87:1282-1291.
- BRUMBAUGH, P. F., AND M. R. HAUSSLER. 1974. 1 α ,25-dihydroxycholecalciferol receptors in intestine. II. Temperature-dependent transfer of the hormone to chromatin via a specific cytosol receptor. J. Biol. Chem., 249:1258-1262.

- BUTLER, D. G. 1969. Corpuscles of Stannius and renal physiology in the eel (*Anguilla rostrata*). J. Fish Res. Bd. Canada, 26:639-654.
- CANDLISH, J. K., AND T. G. TAYLOR. 1970. The response-time to the parathyroid hormone in the laying fowl. J. Endocrinol., 48:143-144.
- CARPENTER, S. J., AND H. L. HEYL. 1974. Fine structure of the corpuscles of Stannius of Atlantic salmon during the freshwater spawning journey. Gen. Comp. Endocrinol., 23:212-223.
- CHAN, D. K. O. 1970. Endocrine regulation of calcium and inorganic phosphate balance in fresh-water adapted teleost fish, *Anguilla anguilla* and *A. japonica*. Proc. Third Int. Congr. Endocrinol., Excerpta Medica Foundation, Int. Congr. Ser. No 184:709-715.
- . 1972. Hormonal regulation of calcium balance in teleost fish. Gen. Comp. Endocrinol., suppl. 3:411-420.
- CHAN, D. K. O., AND I. CHESTER JONES. 1968. Regulation and distribution of plasma calcium and inorganic phosphate in the European eel (*Anguilla anguilla* L.). J. Endocrinol., 32:109-117.
- CHAN, D. K. O., I. CHESTER JONES, I. W. HENDERSON, AND J. C. RANKIN. 1967. Studies on the experimental alterations of water and electrolyte composition of the eel (*Anguilla anguilla* L.). J. Endocrinol., 37:297-317.
- CHAN, D. K. O., I. CHESTER JONES, AND W. MOSLEY. 1968a. Pituitary and adrenocortical factors in the control of the water and electrolyte composition of the freshwater European eel (*Anguilla anguilla* L.). J. Endocrinol., 42:91-98.
- CHAN, D. K. O., I. CHESTER JONES, AND R. N. SMITH. 1968b. The effect of mammalian calcitonin on the plasma levels of calcium and inorganic phosphate in the European eel (*Anguilla anguilla* L.). Gen. Comp. Endocrinol., 11:243-245.
- CHAN, D. K. O., J. C. RANKIN, AND I. CHESTER JONES. 1969. Influence of the adrenal cortex and the corpuscles of Stannius on osmoregulation in the European eel (*Anguilla anguilla* L.) adapted to fresh water. Gen. Comp. Endocrinol., suppl. 2:342-353.
- CHERIAN, A. G., AND J. D. CIPERA. 1968. Effects of parathyroidectomy and of administration of parathormone on serum calcium in immature chicks. Poultry Sci., 47:76-82.
- CHESTER JONES, I., AND I. W. HENDERSON. 1965. Electrolyte changes in the European eel (*Anguilla anguilla* L.). J. Endocrinol., 32:iii-iv.
- CHESTER JONES, I., I. W. HENDERSON, D. K. O. CHAN, J. C. RANKIN, W. MOSLEY, J. J. BROWN, A. F. LEVER, J. I. S. ROBERTSON, AND M. TREE. 1966. Pressor activity of extracts of the corpuscles of Stannius from the European eel (*Anguilla anguilla* L.). J. Endocrinol., 34:570-572.
- CHESTER JONES, I., I. W. HENDERSON, D. K. O. CHAN, AND J. C. RANKIN. 1967. Steroids and pressor substances in bony fish with special reference to the adrenal cortex and corpuscles of Stannius of the eel (*Anguilla anguilla* L.). Proc. Int. Congr. Steroid Hormones, Excerpta Medica Foundation, Int. Congr. Ser. No. 132, 136-143.
- CLARK, N. B. 1965. Experimental and histological studies of the parathyroid glands of freshwater turtles. Gen. Comp. Endocrinol., 5:297-312.
- . 1967. Influence of estrogens upon serum calcium, phosphate and protein concentrations of fresh-water turtles. Comp. Biochem. Physiol., 20:823-834.
- . 1968a. Effect of parathyroidectomy in the lizard, *Anolis carolinensis*. Gen. Comp. Endocrinol., 10:99-102.
- . 1968b. Calcitonin studies in turtles. Endocrinology, 83:1145-1148.
- . 1971a. Function of the parathyroid gland of the snake, *Thamnophis sirtalis*. J. Exp. Zool., 178:9-14.
- . 1971b. The ultimobranchial body of reptiles. J. Exp. Zool., 178:115-124.
- CLARK, N. B., AND R. F. WIDEMAN, JR. 1977. Renal excretion of phosphate and calcium in parathyroidectomized starlings. Amer. J. Physiol., 223:F138-F144.
- CLARK, N. B., P. K. T. PANG, AND M. W. DIX. 1969. Parathyroid glands and calcium and phosphate regulation in the lizard, *Anolis carolinensis*. Gen. Comp. Endocrinol., 12:614-618.

- COHEN, R. S., P. K. T. PANG, AND N. B. CLARK. 1975. Ultrastructure of the corpuscles of Stannius of the killifish, *Fundulus heteroclitus*, and its relation to calcium regulation. *Gen. Comp. Endocrinol.*, 27:413-423.
- COPP, D. H. 1972. Evolution of calcium regulation in vertebrates. *Clinics Endocrinol. Metab.*, 1:21-32.
- . 1976. Comparative endocrinology of calcitonin. Pp. 431-442, in *Parathyroid gland* (G. D. Aurbach, ed.), *Handbook of physiology*, section 7 (R. O. Greep and E. B. Astwood, section eds.), Amer. Physiol. Soc., Washington, D.C., 7:vii+ 1-480 pp.
- COPP, D. H., AND C. O. PARKES. 1968. Extraction of calcitonin from ultimobranchial tissue. Pp. 74-84, in *Parathyroid hormone and thyrocalcitonin (Calcitonin)* (R. V. Talmage and L. F. Bélanger, eds.), *Excerpta Medica Foundation*, Amsterdam, viii+535 pp.
- COPP, D. H., E. C. CAMERON, B. A. CHENY, A. G. F. DAVIDSON, AND K. G. HENZE. 1962. Evidence for calcitonin—a new hormone from the parathyroid that lowers blood calcium. *Endocrinology*, 70:638-649.
- COPP, D. H., C. E. BROOKS, B. S. LOW, F. NEWSOME, R. K. O'DOR, C. O. PARKES, V. WALKER, AND E. G. WATTS. 1970. Calcitonin and ultimobranchial function in lower vertebrates. Pp. 281-294, in *Calcitonin* (S. Taylor, ed.), *Heinemann Medical Books*, London, xii+568.
- COPP, D. H., P. G. H. BYFIELD, C. R. KERR, F. NEWSOME, V. WALKER, AND E. G. WATTS. 1972. Calcitonin and ultimobranchial function in fishes and birds. Pp. 12-20, in *Calcium, parathyroid hormone and the calcitonins* (R. V. Talmage and P. L. Munson, eds.), *Excerpta Medica Foundation*, Amsterdam, viii+560 pp.
- CORTELYOU, J. R., A. HIBNER-OWERKO, AND J. MULROY. 1960. Blood and urine calcium changes in totally parathyroidectomized *Rana pipiens*. *Endocrinology*, 66:441-450.
- DACKE, C. G., AND A. D. KENNY. 1973. Avian bioassay method for parathyroid hormone. *Endocrinology*, 92:463-470.
- DACKE, C. G., W. R. FLEMING, AND A. D. KENNY. 1971. Plasma calcitonin levels in fish. *Physiologist*, 14:127.
- DEFTOS, L. J., M. R. LEE, AND J. T. POTTS, JR. 1968. A radioimmunoassay for thyrocalcitonin. *Proc. Nat. Acad. Sci.*, 60:293-299.
- DEFTOS, L. J., E. G. WATTS, AND D. H. COPP. 1972. Sex differences in calcitonin (CT) secretion by salmon. *Fourth Int. Congr. Endocrinol.*, *Int. Congr. Ser.*, No. 256:180.
- DEFTOS, L. J., E. G. WATTS, D. H. COPP, AND J. T. POTTS, JR. 1974. A radioimmunoassay for salmon calcitonin. *Endocrinology*, 94:115-160.
- DELUCA, H. F. 1974. Vitamin D: the vitamin and the hormone. *Fed. Proc.*, 33:2211-2219.
- DELUCA, H. F., AND H. K. SCHNOES. 1978. Recent advances in our understanding of the metabolism and mechanism of action of 1,25-dihydroxyvitamin D₃. Pp. 178-186, in *Endocrinology of calcium metabolism* (D. H. Copp and R. V. Talmage, eds.), *Excerpta Medica*, Amsterdam, xiv+462 pp.
- DESSAUER, H. C., W. FOX, AND N. L. GILBERT. 1956. Plasma calcium, magnesium and protein of viviparous colubrid snakes during estrous cycle. *Proc. Soc. Exp. Biol. Med.*, 92:299-301.
- DUEL, H. J., JR. 1951. The lipids: their chemistry and biochemistry. *Interscience Publishers, Inc.*, New York, 1:1-792 pp.
- DIX, M. W., P. K. T. PANG, AND N. B. CLARK. 1970. Lizard calcium metabolism: lack of effect of calcitonin on serum calcium or phosphate levels in normal or parathyroidectomized lizards, *Anolis carolinensis*. *Gen. Comp. Endocrinol.*, 14:243-247.
- FEINBLATT, J. D., AND L. G. RAISZ. 1972. Secretion of avian ultimobranchial calcitonin in organ culture. Pp. 51-54, in *Calcium, parathyroid hormone and the calcitonins* (R. V. Talmage and P. L. Munson, eds.), *Excerpta Medica Foundation*, Amsterdam, vii+560 pp.
- FENWICK, J. C. 1974. The corpuscles of Stannius and calcium regulation in the North American eel (*Anguilla rostrata* LeSueur). *Gen. Comp. Endocrinol.*, 23:127-135.

- . 1975. Effect of partial ultimobranchialectomy on plasma calcium concentration and on some related parameters in goldfish (*Carassius auratus* L.) during acute transfer from fresh water to 30% sea water. *Gen. Comp. Endocrinol.*, 93:112-116.
- . 1976. Effect of Stanniectomy on calcium activated adenosinetriphosphatase activity in the gills of fresh water adapted North American eels, *Anguilla rostrata* LeSueur. *Gen. Comp. Endocrinol.*, 29:383-387.
- FENWICK, J. C., AND M. E. FORSTER. 1972. Effects of Stanniectomy and hypophysectomy on total plasma cortisol levels in the eel (*Anguilla anguilla* L.). *Gen. Comp. Endocrinol.*, 19:184-191.
- FENWICK, J. C., AND Y. P. SO. 1974. A perfusion study of the effect of Stanniectomy on the net influx of calcium-45 across an isolated eel gill. *J. Exp. Zool.*, 188:125-131.
- FLEMING, W. R., J. BREHE, AND R. HANSON. 1973. Some complicating factors in the study of the calcium metabolism of teleosts. *Amer. Zool.*, 13:793-797.
- FOLLETT, B. K., AND M. R. REDSHAW. 1968. The effects of oestrogen and gonadotropins on lipid and protein metabolism in *Xenopus laevis* Daudin. *J. Endocrinol.*, 40:439-456.
- FONTAINE, M. 1956. The hormonal control of water and salt-electrolyte metabolism in fish. *Mem. Soc. Endocrinol.*, 5:69-81.
- . 1964. Corpuscles de Stannius et régulation ionique (Ca, K, Na) due milieu intérieur de l'Anguille (*Anguilla anguilla* L.). *C. R. Acad. Sci., ser D (Paris)*, 259:875-878.
- . 1967. Intervention des corpuscles de Stannius dans l'équilibre phosphocalcique du milieu intérieur d'un poisson téléostéen, l'Anguille. *C. R. Acad. Sci., ser. D (Paris)*, 264:736-737.
- FONTAINE, M., N. DELERUE, E. MARTELLY, J. MARCHELIDON, AND C. MILET. 1972. Rôle des corpuscles de Stannius dans les échanges de calcium d'un poisson téléostéen, l'Anguille (*Anguilla anguilla* L.), avec le milieu ambiant. *C. R. Acad. Sci., ser D (Paris)*, 275:1523-1528.
- FRASER, D. R., AND E. KODICEK. 1970. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature*, 228:764-766.
- . 1973. Regulation of 25-hydroxycholecalciferol-1-hydroxylase activity in kidney by parathyroid hormone. *Nature (New Biol.)*, 241:163-166.
- GALLI-GALLARDO, S. M., E. T. MARUSIC, AND P. K. T. PANG. 1977. Studies on the Stannius corpuscles of the Chilean clingfish, *Sicyases sanguineus*. *Gen. Comp. Endocrinol.*, 32:316-320.
- GARABEDIAN, M., M. F. HOLICK, AND H. F. DELUCA. 1972. Control of 25-hydroxycholecalciferol metabolism by the parathyroid glands. *Proc. Nat. Acad. Sci.*, 69:1673-1676.
- GARABEDIAN, M., Y. TANAKA, AND M. F. HOLICK. 1974. Response of intestinal calcium transport and bone calcium mobilization to 1,25-dihydroxyvitamin D₃ in thyroparathyroidectomized rats. *Endocrinology*, 94:1022-1027.
- GONNERMAN, W. A., W. K. RAMP, AND S. U. TOVERUD. 1975. Vitamin D, dietary calcium and parathyroid hormone interactions in chicks. *Endocrinology*, 96:275-281.
- GRAY, T. K., C. W. COOPER, AND P. L. MUNSON. 1974. Parathyroid hormone, thyrocalcitonin and the control of mineral metabolism. Pp. 239-276, in *Endocrine physiology* (S. M. McCann, ed.), M. T. P. Int. Rev. Sci., Physiol., ser. 1, 5:xii+ 1-348.
- GRIFFITH, R. W., AND P. K. T. PANG. 1979. Mechanisms of osmoregulation in the coelacanth, *Latimeria chalumnae*: evolutionary implications. *Proc. California Acad. Sci.*, in press.
- HABENER, J. F., AND J. T. POTTS, JR. 1976. Chemistry, biosynthesis, secretion, and metabolism of parathyroid hormone. Pp. 313-342, in *Parathyroid hormone* (G. D. Aurbach, vol. ed.), *Handbook of physiology*, section 7 (R. O. Greep and E. B. Astwood, section eds.), 7:vii+1-480 pp.
- HABENER, J. F., H. KRONENBERG, A. RICH, AND J. T. POTTS, JR. 1976. Molecular mechanisms in the cellular synthesis and transport of parathyroid hormone. Pp. 281-290, in *Endocrinology of calcium metabolism* (D. H. Copp and R. V. Talmage, eds.), *Excerpta Medica*, Amsterdam and Oxford, vi+496 pp.

- HAYS, A. W. M., AND G. WATSON. 1976. The plasma transport proteins of 25-hydroxycholecalciferol in fish, amphibians, reptiles and birds. *Comp. Biochem. Physiol.*, 53B:167-172.
- HAYSLETT, J. P., M. EPSTEIN, D. SPECTOR, J. D. MYERS, H. V. MURDAUGH, AND F. H. EPSTEIN. 1971. Effect of calcitonin on sodium metabolism in *Squalus acanthias* and *Anguilla rostrata*. *Bull. Mt. Desert Is. Biol. Lab.*, 11:33-35.
- HENRY, H., AND A. W. NORMAN. 1975. Presence of renal 25-hydroxyvitamin D-1-hydroxylase in species of all vertebrate classes. *Comp. Biochem. Physiol.*, 50B:431-434.
- HIRSCH, P. F., G. F. GAUTHIER, AND P. L. MUNSON. 1963. Thyroid hypocalcemic principle and recurrent laryngeal nerve injury as factors affecting the response to parathyroidectomy in rats. *Endocrinology*, 73:244-252.
- KENNY, A. D. 1972. Introductory remarks. Pp. 9-11, in *Calcium, parathyroid hormone and the calcitonins* (R. V. Talmage and P. L. Munson, eds.), Excerpta Medica Foundation, Amsterdam, viii+560 pp.
- . 1976. Vitamin D metabolism: physiological regulation in egg-laying Japanese quail. *Amer. J. Physiol.*, 230:1609-1615.
- KENNY, A. D., AND C. G. DACKER. 1975. Parathyroid hormone and calcium metabolism. *World Rev. Nutrit. Diet.*, 20:231-298.
- KENNY, A. D., S. N. BAKSI, S. M. GALLI-GALLARDO, AND P. K. T. PANG. 1977. Vitamin D metabolism in Amphibia and fish. *Fed. Proc.*, 36:1097.
- KRAINTZ, L., AND K. INTSCHER. 1969. Effect of calcitonin on the domestic fowl. *Canadian J. Physiol. Pharmacol.*, 47:313-315.
- KRISHNAMURTHY, V. G. 1976. Cytophysiology of corpuscles of Stannius. *Int. Rev. Cytol.*, 46:177-249.
- KYES, P., AND T. S. POTTER. 1934. Physiological marrow ossification in female pigeons. *Anat. Rec.*, 60:377-379.
- LOPEZ, E. 1970. L'os cellulaire d'un poisson téléostéen, *Anguilla anguilla* L. II. Action de l'ablation des corpuscles de Stannius. *Z. Zellforsch. Mikrosk. Anat.*, 109:566-672.
- . 1973. Étude morphologique et physiologique de l'os cellulaire des poissons téléostéens. *Mem. Mus. Nat. Hist.*, ser. A, 53:1-90.
- LOPEZ, E., M. M. CHARTIER-BARADUC, AND J. DEVILLE. 1971. Mise en évidence de l'action de la calcitonine porcine sur l'os de la truite *Salmo gairdnerii* soumise à un traitement déminéralisant. *C. R. Acad. Sci.*, ser. D (Paris), 272: 2600-2603.
- LOPEZ, E., J. PEIGNOUX-DEVILLE, F. LALLIER, E. MARTELLY, AND C. MILET. 1976. Effects of calcitonin and ultimobranchialectomy (UBX) on calcium and bone metabolism in the eel, *Anguilla anguilla* L. *Calcif. Tissue Res.*, 20:173-186.
- LOPEZ, E., J. PEIGNOUX-DEVILLE, F. LALLIER, K. W. COLSTON, AND T. MACINTYRE. 1977. Response of bone metabolism in the eel (*Anguilla anguilla*) to injections of 1,25-dihydroxyvitamin D₃. *Calcif. Tissue Res.*, 22(suppl.):19-23.
- LOUW, G. N., W. S. SUTTON, AND A. D. KENNY. 1967. Action of thyrocalcitonin in the teleost fish *Ictalurus melas*. *Nature*, 215:888-889.
- LOUW, G. N., P. D. STAHL, AND A. D. KENNY. 1969. Thyrocalcitonin studies in elasmobranch fish. *Zool. Africana*, 4:95-98.
- MACGREGOR, R. R., L. L. H. CHIU, J. W. HAMILTON, AND D. V. COHN. 1973. Partial purification of parathyroid hormone from chicken parathyroid glands. *Endocrinology*, 92:1312-1318.
- MACINTYRE, I., K. W. COLSTON, I. M. A. EVANS, E. LOPEZ, S. J. MACAULEY, J. PEIGNOUX-DEVILLE, E. SPANOS, AND M. SZELKE. 1976. Regulation of vitamin D: an evolutionary view. *Clin. Endocrinol.*, 5(suppl.):855-955.
- MCWHINNIE, D. J. 1975. *In vivo* effects of mammalian thyrocalcitonin on bone growth and alkaline phosphatase activity in the chick embryo. *Comp. Biochem. Physiol.*, 50A: 169-175.
- MCWHINNIE, D. J., AND J. R. CORTELYOU. 1968. Influence of parathyroid extract on blood and urine mineral levels in iguanid lizards. *Gen. Comp. Endocrinol.*, 11:78-87.

- MA, S. W. Y. 1976. Studies on the mechanism of branchial calcium transport in teleost fish, with special reference to a calcium-stimulated ATPase in the gill plasma membranes. Unpublished Ph.D. Dissertation, Univ. of British Columbia (Canada), 214 pp.
- MA, S. W. Y., AND D. H. COPP. 1977. Purification and assay of an active principle from the corpuscles of Stannius of salmon. *Endocrinology*, 100(suppl.):185A.
- MUELLER, W. J., K. L. HALL, C. A. MAURER, JR., AND I. G. JOSHUA. 1973. Plasma calcium and inorganic phosphate response of laying hens to parathyroid hormone. *Endocrinology*, 92:853-856.
- MUNDAY, K. A., A. Q. ANSARI, D. OLDROYD, AND M. AKHTAR. 1968. Oestrogen-induced calcium-binding protein in *Xenopus laevis*. *Biochem. Biophys. Acta*, 166:748-751.
- MUNSON, P. L. 1976. Physiology and pharmacology of thyrocalcitonin. Pp. 443-464, in Parathyroid gland (G. D. Aurbach, vol. ed.), Handbook of Physiology, section 7 (R. O. Greep and E. B. Astwood, section eds.), Amer. Physiol. Soc., Washington, D.C., 7:viii+1-480 pp.
- NAKAJIMA, T., T. NAKAYAMA, AND H. SOKABE. 1971. Examination of angiotensin-like substances from renal and extrarenal sources in mammalian and nonmammalian species. *Gen. Comp. Endocrinol.*, 17:458-466.
- NIETO, A., J. J. L-FANDO, AND J. L. R-CANDELA. 1975. Biosynthesis and secretion of calcitonin *in vitro* in chicken ultimobranchial gland. *Gen. Comp. Endocrinol.*, 25:259-263.
- OGAWA, M. 1968. Osmotic and ionic regulation in goldfish following removal of the corpuscles of Stannius or the pituitary gland. *Canadian J. Zool.*, 46:669-676.
- OGURI, M. 1973. Seasonal histologic changes in the ultimobranchial gland of goldfish. *Bull. Japan Soc. Sci. Fish.*, 39:851-858.
- OGURO, C. 1970. Parathyroid gland of the snake *Elaphe quadrivirgata* with special reference to parathyroidectomy. *Gen. Comp. Endocrinol.*, 15:313-319.
- . 1971. Parathyroidectomy in the toad *Bufo bufo japonicus*. *Japanese J. Zool.*, 16:177-180.
- . 1972. Parathyroidectomy in the snake *Rhabdophis tigrinus tigrinus*. *Gen. Comp. Endocrinol.*, 18:412-415.
- . 1973. Parathyroid gland and serum calcium concentration in the giant salamander, *Megalobatrachus davidianus*. *Gen. Comp. Endocrinol.*, 21:565-568.
- . 1975. Effects of parathyroidectomy on the serum calcium concentration of the gecko, *Gekko japonicus*. *Annot. Zool. Japonenses*, 48:9-14.
- OGURO, C., AND Y. SASAYAMA. 1976. Morphology and function of the parathyroid gland of the caiman, *Caiman crocodilus*. *Gen. Comp. Endocrinol.*, 29:161-169.
- OGURO, C., AND A. TOMISAWA. 1972. Effects of parathyroidectomy on the serum calcium concentration of the turtle, *Geoclemys reevesii*. *Gen. Comp. Endocrinol.*, 19:587-588.
- OGURO, C., AND M. UCHIYAMA. 1975. Control of serum calcium concentration by parathyroid gland in two species of urodele amphibians. *Gen. Comp. Endocrinol.*, 27:531-537.
- OGURO, C., A. TOMISAWA, AND N. MATUOKA. 1974. Effect of parathyroidectomy on the serum calcium and phosphorus concentrations in the tortoise, *Testudo graeca*. *Zool. Mag. (Tokyo)*, 83:201-202.
- OIZUMI, K., AND C. MONDER. 1972. Localization and metabolism of 1,2-³H-vitamin D₃ and 26,27-³H-25-hydroxycholecalciferol in goldfish (*Carassius auratus* L.). *Comp. Biochem. Physiol.*, 42B:523-537.
- OLIVEREAU, M., AND M. M. CHARTIER-BARADUC. 1965. Action de la prolactine chez l'anguille intacte et hypophysectomisée. II. Effets sur les électrolytes plasmatiques (sodium, potassium et calcium). *Gen. Comp. Endocrinol.*, 7:27-36.
- OMDAHL, J. L., AND H. F. DELUCA. 1973. Regulation of vitamin D metabolism and function. *Physiol. Rev.*, 53:327-372.
- OMDAHL, J. L., R. W. GRAY, AND I. T. BOYLE. 1972. Regulation of metabolism of 25-hydroxycholecalciferol by kidney tissue *in vitro* by dietary calcium. *Nature (New Biology)*, 237:63-64.

- ORIMO, H., T. FUJITA, M. YOSHIKAWA, J. ABE, S. WATANABE, AND K. ONTANI. 1972. Ultimobranchial calcitonin of *Anguilla japonica* (Japanese eel). Fourth Int. Congr. Endocrinol., Int. Congr. Ser., No. 256:181.
- ORIMO, H., H. YAMAUCHI, T. OHYAMA, M. MATUO, AND M. OTANI. 1977. Radioimmunoassay and immunological properties of eel calcitonin. Gen. Comp. Endocrinol., 31:482-486.
- PANG, P. K. T. 1971a. The relationship between corpuscles of Stannius and serum electrolyte regulation in killifish, *Fundulus heteroclitus*. J. Exp. Zool., 178:1-8.
- . 1971b. The effects of complete darkness and vitamin C supplement on killifish, *Fundulus heteroclitus*, adapted to sea water. I. Calcium metabolism and gonadal maturation. J. Exp. Zool., 178:15-22.
- . 1971c. Calcitonin and ultimobranchial glands in fish. J. Exp. Zool., 178:89-100.
- . 1973. Endocrine control of calcium metabolism in teleosts. Amer. Zool., 13:775-792.
- . 1977. Osmoregulatory functions of neurohypophyseal hormones in fishes and amphibians. Amer. Zool., 17:739-749.
- PANG, P. K. T., AND F. BALBONTIN. 1978. Effects of sex steroids on plasma calcium levels in male killifish, *Fundulus heteroclitus*. Gen. Comp. Endocrinol., 36:317-320.
- PANG, P. K. T., AND G. E. PICKFORD. 1967. Failure of hog thyrocalcitonin to elicit hypocalcemia in the teleost fish, *Fundulus heteroclitus*. Comp. Biochem. Physiol., 21:573-578.
- PANG, P. K. T., AND W. H. SAWYER. 1974. Effects of prolactin on hypophysectomized mud puppies, *Necturus maculosus*. Amer. J. Physiol., 226:458-462.
- . 1975. Parathyroid hormone preparations, salmon calcitonin, and urine flow in the South American lungfish, *Lepidosiren paradoxa*. J. Exp. Zool., 193:407-412.
- PANG, P. K. T., R. W. GRIFFITH, AND G. E. PICKFORD. 1971a. Hypocalcemia and tetanic seizures in hypophysectomized killifish, *Fundulus heteroclitus*. Proc. Soc. Exp. Biol. Med., 136:85-87.
- PANG, P. K. T., N. B. CLARK, AND K. S. THOMSON. 1971b. Hypocalcemic activities in the ultimobranchial bodies of lungfishes, *Neoceratodus forsteri* and *Lepidosiren paradoxa* and teleosts, *Fundulus heteroclitus* and *Gadus morhua*. Gen. Comp. Endocrinol., 17:582-585.
- PANG, P. K. T., R. W. GRIFFITH, AND M. P. SCHREIBMAN. 1973a. The pituitary gland and calcium metabolism in *Fundulus diaphanus* (Teleostei). Gen. Comp. Endocrinol., 20:358-361.
- PANG, P. K. T., M. P. SCHREIBMAN, AND R. W. GRIFFITH. 1973b. Pituitary regulation of serum calcium levels in the killifish, *Fundulus heteroclitus* L. Gen. Comp. Endocrinol., 21:536-542.
- PANG, P. K. T., R. K. PANG, AND W. H. SAWYER. 1973c. Effects of environmental calcium and replacement therapy on the killifish, *Fundulus heteroclitus*, after the surgical removal of the corpuscles of Stannius. Endocrinology, 93:705-710.
- . 1974. Environmental calcium and the sensitivity of the killifish (*Fundulus heteroclitus*) in bioassays for the hypocalcemic response to Stannius corpuscles from killifish and cod (*Gadus morhua*). Endocrinology, 94:548-555.
- PANG, P. K. T., R. K. PANG, AND R. W. GRIFFITH. 1975. Corpuscles of Stannius: lack of direct involvement in regulation of serum sodium, potassium and chloride in the teleost, *Fundulus heteroclitus*, after the surgical removal of the corpuscles of Stannius. Endocrinology, 93:705-710.
- PANG, P. K. T., R. W. GRIFFITH, AND J. W. ATZ. 1977. Osmoregulation in elasmobranchs. Amer. Zool., 17:365-377.
- PANG, P. K. T., M. P. SCHREIBMAN, F. BALBONTIN, AND R. K. PANG. 1978. Prolactin and pituitary control of calcium regulation in the killifish, *Fundulus heteroclitus*. Gen. Comp. Endocrinol., 36:306-316.
- PARSONS, J. A., B. REIT, AND C. J. ROBINSON. 1973. Bioassay for parathyroid hormone using chicks. Endocrinology, 92:454-462.

- PEARSE, A. G. E. 1966. The cytochemistry of the thyroid C cells and their relationship to calcitonin. *Proc. Roy. Soc. London, ser. B*, 164:478-487.
- PEARSE, A. G. E., AND A. F. CARVALHEIRA. 1967. Cytochemical evidence for an ultimobranchial origin of rodent thyroid C cells. *Nature*, 214:929-930.
- PEARSE, A. G. E., AND T. TAKOR TAKOR. 1976. Neuroendocrine embryology and the APUD concept. *Clin. Endocrinol., suppl.* 5:229S-244S.
- PICKERING, A. D. 1976. Effects of gonadectomy, oestradiol and testosterone on the migrating river lamprey, *Lampetra fluviatilis* (L.). *Gen. Comp. Endocrinol.*, 28:473-480.
- POLIN, D., AND P. D. STURKIE. 1958. Parathyroid and gonad relationship in regulating blood calcium fractions in chickens. *Endocrinology*, 63:177-182.
- PONCHON, G., AND H. F. DELUCA. 1969. The role of the liver in the metabolism of vitamin D. *J. Clin. Invest.*, 48:1273-1279.
- POTTS, J. T., JR. 1976. Parathyroid hormone and calcitonin. Pp. 119-144, in *Peptide hormones* (J. A. Parsons, ed.), University Park Press, Baltimore, xii+399 pp.
- POTTS, J. T., JR., AND G. C. AURBACH. 1976. Chemistry of the calcitonins. Pp. 423-430, in *Parathyroid gland* (G. D. Aurbach, vol. ed.), *Handbook of physiology*, section 7 (R. O. Greep and E. B. Astwood, section eds.), Amer. Physiol. Soc., Washington, D.C., 7:vii+1-480 pp.
- RASMUSSEN, H., H. DELUCA, AND C. ARNAUD. 1972. The relationship between vitamin D and parathyroid hormone. *J. Clin. Invest.*, 42:1940-1946.
- RASQUIN, P., AND L. ROSENBLUM. 1954. Endocrine imbalance and tissue hyperplasia in teleosts maintained in darkness. *Bull. Amer. Mus. Nat. Hist.*, 104:359-426.
- RIDDLE, O., AND M. R. McDONALD. 1945. The partition of plasma calcium and inorganic phosphorus in estrogen-treated normal and parathyroidectomized birds. *Endocrinology*, 36:48-52.
- ROBERTSON, D. R. 1969a. The ultimobranchial body of *Rana pipiens*. VIII. Effects of extirpation upon calcium distribution and bone cell types. *Gen. Comp. Endocrinol.*, 12:479-490.
- . 1969b. The ultimobranchial body of *Rana pipiens*. IX. Effects of extirpation and transplantation on urinary calcium excretion. *Endocrinology*, 84:1174-1178.
- . 1969c. The ultimobranchial body of *Rana pipiens*. X. Effects of glandular extirpation on fracture healing. *J. Exp. Zool.*, 172:425-441.
- . 1970. The ultimobranchial body in *Rana pipiens*. XI. Response to increased dietary calcium-evidence of possible physiological function. *Endocrinology*, 87:1041-1050.
- . 1972. Influence of the parathyroids and ultimobranchial glands in the frog (*Rana pipiens*) during respiratory acidosis. *Gen. Comp. Endocrinol., suppl.* 3:421-429.
- . 1975a. The *in vitro* transport of calcium by the frog intestine and the effect of vitamin D₃. *Comp. Biochem. Physiol.*, 51A:705-710.
- . 1975b. Effects of the ultimobranchial and parathyroid glands and vitamins D₂, D₃, and dihydrotachysterol on blood calcium and intestinal calcium transport in the frog. *Endocrinology*, 96:934-940.
- . 1977. The annual pattern of plasma calcium in the frog and the seasonal effect of ultimobranchialectomy and parathyroidectomy. *Gen. Comp. Endocrinol.*, 33:336-343.
- ROBINSON, C. J., K. K. MAHAJAN, AND D. F. HORROBIN. 1975. Some effects of prolactin on calcium metabolism. *J. Endocrinol.*, 65:27P.
- ROOS, B. A., K. OKANO, AND L. J. DEFTOS. 1974. Biosynthesis and secretion of calcitonin *in vitro*. *Endocrinology*, 94(suppl.):206A.
- ROTH, S. I., AND A. L. SCHILLER. 1976. Comparative anatomy of the parathyroid glands. Pp. 281-312, in *Parathyroid gland* (G. D. Aurbach, vol. ed.), *Handbook of physiology*, section 7 (R. O. Greep and E. B. Astwood, section eds.), Amer. Physiol. Soc., Washington, D.C., 7:vii+1-480 pp.
- SAWYER, W. H. 1972. Lungfishes and amphibians: endocrine adaptation and transition from aquatic to terrestrial life. *Fed. Proc.*, 31:1609-1614.

- SAWYER, W. H., P. K. T. PANG, AND S. M. GALLI-GALLARDO. 1978. Environment and osmoregulation among lungfishes and amphibians. Pp. 210-216, in *Environmental endocrinology* (I. Assenmacher and D. S. Farner, eds.), Springer-Verlag, Berlin, xv+334 pp.
- SEDRANI, S., AND T. G. TAYLOR. 1977. Metabolism of 25-hydroxycholecalciferol in Japanese quail in relation to reproduction. *J. Endocrinol.*, 72:305-406.
- SIMKISS, K. 1961. The influence of large doses of estrogens upon the structure of the bone of some reptiles. *Nature*, 190:1217-1218.
- . 1967. Calcium in reproductive physiology. Reinhold Publ. Co., New York, xiv+264 pp.
- . 1974. Calcium and avian reproduction. *Symp. Zool. Soc. London*, 35:307-337.
- SO, Y. P., AND J. C. FENWICK. 1977. Relationship between net ⁴⁵calcium influx across a perfused isolated eel gill and the development of post-stannectomy hypercalcemia. *J. Exp. Zool.*, 200:259-264.
- SOKABE, H., H. NISHIMURA, M. OGAWA, AND M. OGURI. 1970. Determination of renin in the corpuscles of Stannius of the teleost. *Gen. Comp. Endocrinol.*, 14:510-516.
- SPANOS, E., K. W. COLSTON, I. M. A. EVANS, L. S. GALANTE, S. J. AULEY, AND I. MACINTYRE. 1976a. Effect of prolactin on vitamin D metabolism. *Mol. Cell. Endocrinol.*, 5:163-167.
- SPANOS, E., J. W. PIKE, M. R. HAUSSLER, K. W. COLSTON, I. M. A. EVANS, A. M. GOLDNER, T. A. MCCAIN, AND I. MACINTYRE. 1976b. Circulating 1,25-dihydroxyvitamin D in the chicken: enhancement by injection of prolactin and during egg laying. *Life Sci.*, 19:1751-1756.
- STANNIUS, H. 1839. Die Nebennieren bei Knochenfischen. *Arch. Anat. Physiol.*, 97:97-101.
- SUZUKI, H. K., AND R. L. PROSSER, III. 1968. The effects of estradiol valerate upon the serum and bone of the lizard, *Sceloporus cyanogenys*. *Proc. Soc. Exp. Biol. Med.*, 127:4-7.
- TASHJIAN, A. H., JR., B. G. HOWLAND, K. E. W. MELVIN, AND C. S. HILL, JR. 1970. Immunoassay of human calcitonin. *New England J. Med.*, 283:890-895.
- TANAKA, Y., L. CASTILLO, AND H. F. DELUCA. 1976. Control of renal vitamin D hydroxylases in birds by sex hormones. *Proc. Nat. Acad. Sci.*, 73:2701-2705.
- URIST, M. R. 1962. The bone body fluid continuum; calcium and phosphorus in the skeleton and blood of extinct and living vertebrates. *Perspect. Biol. Med.*, 6:75-115.
- . 1964. Further observations on the bone-body fluid continuum: composition of the skeleton and serums of cyclostomes, elasmobranchs, and bony vertebrates. Pp. 151-179, in *Bone biodynamics* (H. F. Frost, ed.), Little and Brown, Co., Boston, vi+327 pp.
- . 1967. Avian parathyroid physiology: including a special comment on calcitonin. *Amer. Zool.*, 7:883-895.
- URIST, M. R., AND O. A. SCHJEIDE. 1961. The partition of calcium and protein in the blood of oviparous vertebrates during estrus. *J. Gen. Physiol.*, 44:743-756.
- URIST, M. R., S. UYENO, E. KING, M. OKADA, AND S. APPELGATE. 1972. Calcium and phosphorus in the skeleton and blood of the lungfish *Lepidosiren paradoxa*, with comment on hormonal factors in calcium homeostasis in the osteichthyes. *Comp. Biochem. Physiol.*, 42A:393-408.
- VARGAS, F., F., AND J. CONCHA B. 1957. Histologia y tecnica de extirpacion de las glandulas adrenales en el teleosteo *Sicyases sanguineus*. *Invest. Zool. Chilensis*, 3:88-95.
- WAGGENER, R. A. 1930. An experimental study of the parathyroids in the Anura. *J. Exp. Zool.*, 57:13-55.
- WALLACE, R. A., AND E. W. BERGINK. 1974. Amphibian vitellogenin: properties, hormonal regulation of hepatic synthesis and ovarian uptake, and conversion to yolk proteins. *Amer. Zool.*, 14:1159-1176.
- WATTS, E. G., D. H. COPP, AND L. J. DEFTOS. 1972. Changes in plasma Ca and calcitonin in the spawning cycle of sockeye salmon. *Proc. Canadian Fed. Biol. Soc.*, 15:406.
- WENDELAAR BONGA, S. E., AND J. A. A. GREVEN. 1975. A second cell type in Stannius bodies of two euryhaline teleost species. *Cell Tissue Res.*, 159:287-290.

- WENDELAAR BONGA, S. E., J. A. A. GREVEN, AND M. VEENHIUS. 1977. Vascularization, innervation, and ultrastructure of the endocrine cell types of Stannius corpuscles in the teleost *Gasterosteus aculeatus*. J. Morphol., 153:225-244.
- WOODHEAD, P. M. J. 1969. Effects of oestradiol and thyroxine upon the plasma calcium content of a shark, *Scyliorhinus canicula*. Gen. Comp. Endocrinol., 13:310-312.
- YAMANE, S., AND J. YAMADA. 1977. Histological changes of the ultimobranchial gland through the life history of the masu salmon. Bull. Japanese Soc. Sci. Fish., 43: 385-386.
- ZIEGLER, R., M. TELIB, AND E. F. PFEIFER. 1969. The secretion of calcitonin by the perfused ultimobranchial glands of the hen. Hormone Metab. Res. 1:39-40.

PHYLOGENY OF VERTEBRATE ADRENAL CORTICOSTEROIDS

D. R. IDLER AND B. TRUSCOTT

Our goal is not to enumerate the vast number of steroids of adrenal origin that have been reported for vertebrates because such information is contained in several recent reviews (Idler and Truscott, 1972; Sandor, 1972; Butler, 1973; Idler, 1973; Sandor *et al.*, 1976). Most reviewers have emphasized the great similarity of adrenal steroids among animals and minimized the differences. This is understandable. Given an *in vitro* approach, sooner or later nearly every steroid enzyme now known to exist in any vertebrate probably will be found in all other vertebrates. *In vitro* techniques are valuable for establishing the existence of enzyme systems and in some instances even dominant enzyme systems, but such data must be interpreted with extreme caution. For example, the rat adrenal contains 17 α -hydroxylase (Young and Sweat, 1967) yet the rat is a corticosterone secretor. In this instance it is known that *in situ* the 17 α -hydroxylase activity is suppressed by the non-haem iron protein component of 11 β -hydroxylase. However, when the tissue is disrupted prior to and during incubation and depending on other experimental conditions the true situation *in situ* might or might not be revealed. Another problem concerns the choice of precursors for *in vitro* studies. *In vivo*, the tissue enzymes may have a choice of several substrates at the same time, or perhaps no substrates, whereas *in vitro* they are generally presented with pregnenolone or progesterone. A case in point is the demonstration that bovine medullary tissue can carry out many of the major steroid hydroxylations but it has not been shown that this tissue ever has the opportunity to do so *in vivo*. Medullary tissue apparently cannot transform cholesterol to the C-21 steroids on which these enzymes can then act (Carballeira *et al.*, 1965). The unique feature of adrenal cells is not their ability to transform steroids to other steroids but rather their ability to synthesize steroids from nonsteroid precursors, in this we would include cholesterol. Endogenous incubates in which no radioactive precursor is added probably give a more reliable reflection of *in situ* events; gland perfusion affords an even closer approximation. Unfortunately, the latter approach is generally difficult or impossible with many of the nonmammalian vertebrates.

In zoned adrenals an enzyme may occur in one or more than one zone and these may or may not interact *in situ*. Thus, 18-hydroxylase, which is involved in the synthesis of aldosterone in zona glomerulosa of the rat, also occurs in the inner zones where 18-hydroxy-11-deoxycorticosterone is the product (Lucis *et al.*, 1961).

The commonly used substrates, pregnenolone and progesterone, are known to permeate adrenal tissue at different rates *in vitro* (Matsumoto and

Samuels, 1969). Some enzymes leak into the media and reactions occur outside the cell structure *in vitro* (Carballeira and Durnhofer, 1968).

A potentially serious problem is the difference in lability of key steroid hydroxylases to temperature *in vitro* (Tsang and Stachenko, 1970). This raises also the question of how long one should incubate to attempt to reflect the *in vivo* situation. Then there is the importance of temperature optima of steroid hydroxylating enzymes and what to do about these in poikilotherms. The interrenal 1α -hydroxylase in elasmobranchs is temperature sensitive and glands subjected to freezing can give different end products than do fresh glands (Idler and Truscott, 1967; Simpson and Wright, 1970).

In 1957, Dr. A. S. Parks reminded the participants at a CIBA Colloquia of E. H. Sparling's original definition of a hormone as a substance produced in one organ of the body and carried by the circulating blood to another organ in which it evoked a response. Dr. Parks, faced with the same dilemma that we are today, urged a policy of back to blood. In a 1970 review, I urged that Parks' advice was as valid then as it had been in 1957 (Idler, 1971). Unfortunately for comparative endocrinology, few investigators have heeded these pleas.

The above observations are not meant to imply that there are no problems in the interpretation of plasmatic corticosteroid profiles. Rather, they are intended to point out that results can be more meaningful for the purposes of comparative endocrinology if suitable precautions are taken to avoid stress and to record data that might be relevant to such factors as diurnal rhythm and sexual maturity. In deciding whether an animal is a cortisol or corticosterone secretor it is important to consider stress. Chronic administration of ACTH to rabbits presumably induces stress and changes this corticosterone secretor to a cortisol secretor by preferential stimulation of 17α -hydroxylase (Kass *et al.*, 1954; Ganjam *et al.*, 1972). A similar increase in cortisol secretion with little effect on corticosterone was observed in a marsupial by Oddie *et al.* (1976). If corticosterone predominates in plasma it is probably safe to conclude that the animal is a corticosterone secretor, but if both cortisol and corticosterone are present and cortisol predominates one must be sure that the animal was not stressed.

Most of our further remarks will be confined to phylogenetic comparisons arising from studies on plasmatic steroids either known or believed to be of adrenal origin. New information on some species is presented.

Class AGNATHA

The surviving members of these ancient jawless vertebrates are the hagfishes and lampreys. Early reports of high plasma levels of 17 -hydroxycorticosteroids in a lamprey and of cortisol and corticosterone in hagfishes (for discussion see Idler *et al.*, 1971) have not been confirmed nor are they likely to be because methodology in use at the time was not sufficiently discriminatory.

We confirmed the production of adrenalcorticosteroids by hagfish in 1970 when cortisol, cortisone, and corticosterone were identified in peripheral

plasma by double isotope derivative assay (DIDA) (Idler *et al.*, 1971). Cortisol was the predominant steroid, but all three corticosteroids were present in nanogram amounts per 100 ml. of plasma. The metabolic clearance rate of steroid is not known for agnathans but the most likely explanation for the low plasma corticosteroid levels in relatively stressed hagfish is the dearth of adrenalcortical cells, which until recently has prevented their detection (Idler and Burton, 1976). Plasma corticosteroid levels in the hagfish were increased in a rather interesting manner by long term treatment with ACTH; 17 α -hydroxylase was stimulated as expected but 11-hydroxylation and 11-dehydrogenation were affected so that cortisone and 11-deoxycortisol increased substantially

Analysis by DIDA for cortisol, cortisone, and corticosterone in plasma of landlocked sea lamprey and migrating lamprey indicated even lower concentrations (Weisbart and Idler, 1970) than in hagfish. Weisbart and Idler were very cautious in their conclusions on lamprey steroids in 1970 because the definitive quantification of these three steroids in hagfish plasma was not possible until later. Reexamination of the published data leaves no doubt that the identity of cortisol was established for a large plasma sample from landlocked lamprey. The $^3\text{H}:$ ^{14}C ratio of cortisol acetate after purification by thin layer chromatography was 8.0; paper chromatography reduced the ratio first to 2.20 and then to 0.840; serial recrystallization of the radioactive material with authentic cortisol acetate gave consecutive ratios of 0.815 and 0.804 for the crystals and 0.810 for the final mother liquor. Inspection of the data shows that the $^3\text{H}:$ ^{14}C ratios were within the accepted limit of 5 per cent and, equally important, that the ratios in the mother liquor from the final crystallization were essentially identical to that of the final crop of crystals. In the same experiment, the final ratio for corticosterone crystals was only 0.484 compared to the theoretically lowest ratio based on the specific activity of the [^3H] acetic anhydride of 0.420. Cortisol was present at a concentration of only 5 ng/100 ml. Now that it is established that levels of the principal plasmatic steroids in lamprey are so low, quantification of the major corticosteroids should be repeated using high specific activity acetic anhydride.

In summary, the most characteristic feature of agnathans is the extremely low level of plasmatic corticosteroids and, like actinopterygians, they are normally cortisol secretors.

Class CHONDRICHTHYES

The two subclasses of Chondrichthyes are the Elasmobranchii and the Holocephali. Elasmobranchs generally are subdivided into the batoids (skates and rays) and the selachians (sharks).

Subclass ELASMOBRANCHII

The principal corticosteroid of elasmobranchs is 1 α -hydroxycorticosterone. It was first isolated from the blood and interrenal tissue of two species of *Raja* (Idler and Truscott, 1967). The synthesis of 1 α -hydroxycorticosterone

by the elasmobranch interrenal sets this subclass apart (Truscott and Idler, 1968) for to date 1α -hydroxylated corticosteroid as a normal product of the adrenal has not been described in any other animal class. The presence of a 1α -hydroxylase in elasmobranchs assumed wider significance with the discovery of 1 -hydroxylated pregnanes, probably of adrenal origin, in the urine of a hypertensive infant (Edwards and Trafford, 1968) and the identification of the active form of Vitamin D as a product of kidney 1α -hydroxylase on 25-hydroxycholecalciferol (Fraser and Kodicek, 1970).

There is very limited information on the plasmatic steroids of elasmobranchs. Quantitatively, 1α -hydroxycorticosterone was found to be the most important in both batoids and selachians: the concentration was generally low, less than $1\text{ }\mu\text{g}/100\text{ ml.}$, in four species of batoids, but higher in two of three species of selachians, for example, $5\text{ }\mu\text{g}/100\text{ ml.}$ in the Mako shark, *Isurus oxyrinchus* (Idler and Truscott, 1969; Truscott and Idler, 1972). Corticosterone and 11 -deoxycorticosterone, intermediates in the synthesis of 1α -hydroxycorticosterone, and 11 -dehydrocorticosterone, a metabolite of corticosterone, was detected in the plasma of both groups. Corticosterone occurred in submicrogram amounts except in the spiny dogfish *Squalus acanthias* where its concentration was equal to that of 1α -hydroxycorticosterone. In the batoids, 11 -deoxycorticosterone was, quantitatively, the second most important corticosteroid. In some samples the concentration of 11 -dehydrocorticosterone exceeded that of corticosterone; this situation is reminiscent of cortisol and cortisone in the blood of teleosts where cortisol is considered to be the secretory product of the interrenal and cortisone a peripheral metabolite in dynamic equilibrium with cortisol (Leloup-Hatey and De Lignieres, 1974). Korotaev *et al.*, (1973) measured the total 11 -oxygenated corticosteroids by a fluorometric method in plasma from several species of pelagic sharks and found concentrations ranging between 2 and $4\text{ }\mu\text{g}/100\text{ ml.}$ These authors pointed out that the values for the sharks were much lower than for the tunas and marlins (26 and $18\text{ }\mu\text{g}/100\text{ ml.}$), which inhabit the same waters. Although there are several reports of cortisol and cortisone in elasmobranchs in the earlier literature, 17 -hydroxylated corticosteroids have not been identified definitively in the plasma or as products of the interrenal *in vitro*. However, there is always the possibility that 17 -hydroxylated steroids can be produced by the elasmobranch interrenal, either under certain conditions, or in nanogram amounts. To our knowledge the methods necessary to measure such quantities have not been applied to elasmobranch plasma.

It would be appropriate to consider here two observations concerning corticosteroids, or more correctly C_{21} steroids, in elasmobranchs because they aptly illustrate the danger of generalizations in endocrinological studies. First, 11 -deoxycorticosterone was isolated in "surprisingly high concentrations (*ca.* $500\text{ }\mu\text{g}/100\text{ g.}$)" from semen of the dogfish *Squalus acanthias* (Simpson *et al.*, 1963*a*), yet it was undetectable in the semen of four other species of elasmobranchs (Simpson *et al.*, 1963*b*). Second, with regard to elasmobranch body fluids (Maren, 1967), perivisceral fluids vary in volume

from one species to another and within a species may vary from a few to over 100 ml. In the thorny skate, *Raja radiata*, 1α -hydroxycorticosterone occurs at concentrations approximately 10 times higher in the perivisceral and pericardial fluids than in the plasma (Idler and Truscott, 1968) and can be transferred to maintain low levels of the steroid in plasma of interrenalec-tomized animals (Idler, 1971). Other Atlantic species, including three batoids and one selachian, showed no evidence of 1α -hydroxycorticosterone concentrated in the perivisceral fluids.

To conclude our discussion of corticosteroids in elasmobranchs we would like to point out that the biological role of 1α -hydroxycorticosterone has not been firmly established. In mammalian tests, it has been shown to possess mineralocorticoid as well as glucocorticoid activity (see Idler, 1971). In the skate, *R. ocellata*, 1α -hydroxycorticosterone exhibited mineralocorticoid activity; interrenalectomy and steroid replacement therapy controlled the fluid output of the rectal gland (Holt and Idler, 1975). At the same time, interrenalectomy of another species, *R. radiata*, had minimal effects on plasmatic electrolytes (Idler and Szeplaki, 1968), a result in agreement with much earlier studies on *R. erinacea* (Hartman *et al.*, 1944). Whether or not 1α -hydroxycorticosterone acts as a glucocorticoid in the skate remains unanswered.

Subclass HOLOCEPHALI

To our knowledge there are no studies reported on the plasma corticoste-roids of a holocephalan. Bern and his co-workers gave evidence for cortisol in adrenal incubates of the Pacific ratfish and were unable to detect corticos-terone (Bern *et al.*, 1962). Inasmuch as 1α -hydroxycorticosterone was not known when Bern's work was done, we looked specifically for 1α -hydroxycorticosterone from [^{14}C] progesterone and [^{14}C] corticosterone; there was no 1α -hydroxylase in ratfish adrenals. It was of interest that corticos-terone was converted to cortisol in 32 per cent yield, which would seem to preclude the possibility of this holocephalan accumulating any significant amount of 17-deoxycorticosteroids. When [4- ^{14}C] progesterone was incubated with ratfish interrenal, the major products were cortisol (48 per cent) and 11-deoxycortisol (37 per cent), with a lesser amount of 17α -hydroxy-progesterone (6.8 per cent), and only 0.1 per cent corticosterone (Idler *et al.*, 1969). The absence of 1α -hydroxylase in the elasmobranchs' nearest living relative supports Patterson's conclusion that "holocephalans and selachians have evolved in parallel throughout a large part of their history" (Patterson, 1965).

Class OSTEICHTHYES

Most higher bony fishes have ray fins and are referred to as actinopterygi-ans. The fleshy finned subclass is referred to as sarcopterygians; the three lungfishes and the coelacanth (*Latimeria*) are the only living species known.

Subclass ACTINOPTERYGII

Infraclass CHONDROSTEI

Three genera of the *Chondrostei* survive: the sturgeon, paddlefish and polypterines. The sturgeons and paddlefish have similar features, that is, little ganoid scale cover a mainly cartilaginous skelton but *Polypterus* has fleshy paired fins, lungs, and lives in an environment similar to that of the lungfishes in Central Africa.

A sample of plasma from an Atlantic sturgeon, *Acipenser oxyrhynchus*, held in captivity for several months contained extremely low levels of cortisol, cortisone, and corticosterone; the concentrations were 0.18, 0.016, and 0.007 $\mu\text{g}/100\text{ ml.}$, respectively (Sangalang *et al.*, 1971). To our knowledge, there are no other published data on plasmatic steroids of the chondrosteans. However, we have acquired some data, as yet unpublished, on the other two genera.

A sample of whole blood from the paddlefish *Polyodon spathula* was obtained through the courtesy of Dr. Norman Benson, N.C. Reservoir Investigations, Yankton, South Dakota. Analysis of 40 ml. for corticosteroids by DIDA indicated that in the paddlefish, as in the sturgeon, 17 α -hydroxylated steroids were predominant. The concentration of cortisol, cortisone, and corticosterone in this particular sample were 5.2, 1.0 and 0.02 $\mu\text{g}/100\text{ ml.}$ respectively. These values are much higher than those reported for the sturgeon but may have resulted from the stress of capture immediately before the blood sample was taken. (The fish was bled in the field, so to speak, and returned to its native waters). Aldosterone could not be detected and under the conditions of this assay a concentration of 0.002 $\mu\text{g}/100\text{ ml.}$ should have been apparent.

Live specimens of two species of *Polypterus* were purchased from Paramount Aquarium Inc., Ardsley, New York. Only small samples of plasma, 2.3 ml. from five fish and 0.3 ml. from two fish, were obtained and in both cases, only corticosterone could be identified. The concentration of corticosterone in the two samples was 0.4 and 1.0 $\mu\text{g}/100\text{ ml.}$ Inasmuch as there were no technical problems with the assay, it would appear that in *Polypterus* 17-deoxycorticosteroids are quantitatively important. Analysis of a 30 minute incubate of presumptive interrenal tissue from these fish also failed to detect cortisol or cortisone, but corticosterone was isolated and its identity confirmed by DIDA in both species.

Thus, *Polypterus*, a fish that must cope with prolonged periods of drought, can be classified as a 17-deoxycorticosteroid secretor whereas its relatives the sturgeon and paddlefish, which are not faced with a shortage of water, can be included in the group of animals considered to be cortisol secretors. This pattern will be repeated when we discuss other animals faced with similar environmental pressures.

Infraclass HOLOSTEI

Holosteans succeeded the chondrosteans as the dominant fishes in the middle Mesozoic (Romer, 1956). Since that time, the oceanic forms have disappeared and only two freshwater genera survive, the garpikes (*Lepidosteus* sp.) and bowfin (*Amia* sp.).

We have analyzed a combined pool of plasma from four relatively immature bowfin by DIDA (Idler *et al.*, 1971) and identified cortisol and lesser amounts of corticosterone. The plasmatic steroids of garpikes have not been investigated before, and in the face of limited data it would be premature to label the holosteans as cortisol secretors.

Infraclass TELEOSTEI

Teleosts replaced holosteans as the most prevalent aquatic vertebrates before the end of the Cretaceous. Today the number of recognized teleost species ranks in the thousands, making them the most abundant of all vertebrates.

Teleosts examined to date are predominantly cortisol secretors, based on analyses of plasmatic corticosteroids. Nothing reported since this subject was reviewed in 1972 would appear to make it necessary to qualify this conclusion (Idler and Truscott, 1972). However, there appear to be circumstances (such as stress) when large amounts of 17-deoxycorticosteroids can accumulate *in vivo* in some species.

There seems no doubt that teleosts investigated so far by *in vitro* methods have potent 17 α -hydroxylation systems and that some are able to convert even 21-hydroxylated precursors to 17-hydroxycorticosteroids. However, work by Colombo *et al.* (1972) on *Tilapia* established that 11-deoxycorticosterone was not converted to cortisol by head kidney. This is in contrast to findings with eel, herring, Atlantic salmon, and probably with cod and haddock (for references see Colombo *et al.*, 1972). However, it might be worth recalling that while 17 α -hydroxylation of 21-hydroxylated steroids occurred in the more ancient teleosts (Sangalang *et al.*, 1972), the conversion was an order of magnitude less than with the even more ancient holocephalan, the ratfish (Idler *et al.*, 1969; Idler, 1969). It is possible that the more evolved teleost, *Tilapia*, has lost the ability to produce 17 α -hydroxylate 21-hydroxycorticosteroids; more species should be examined.

In many teleosts, aldosterone occurs in the plasma at very low concentrations, if at all. When we last reviewed this subject in 1972, the 18-hydroxylating enzyme necessary to synthesize aldosterone had been demonstrated *in vitro* in several species but had been identified in the plasma of one species only, *Clupea harengus* (Idler and Truscott, 1972). Since that time, aldosterone has been identified and measured (0.11 μ g/100 ml.) in the goldfish *Carassius auratus* (Chavin and Singley, 1972) and in the freshwater powan of Loch Lomond, *Coregonus clupeoides* (Fuller *et al.*, 1976).

Subclass SARCOPTERYGII

Order DIPNOI

Living lungfishes are the Australian (*Neoceratodus*), the South American (*Lepidosiren*), and four quite closely related African species (*Protopterus*). There are certain criteria that established a closer relationship between *Lepidosiren* and *Protopterus* than that between either of these and *Neoceratodus*. These criteria include, but are not restricted to, Chavin's (1976) studies on the thyroid and a very recent investigation of the bile salts (Amos *et al.*, 1977). Romer (1956) points out that lungfishes are found only in regions of the world where drought occurs, such as is believed to have existed in the Devonian.

We reported on the plasmatic steroids of South American lungfish (*Lepidosiren*) previously (Idler *et al.*, 1972). Plasma levels of aldosterone were comparable to those found by others in the bullfrog and toad. The concentration of cortisol was equal to that of aldosterone and comparable to resting levels in bony fishes. Two corticosteroids found in lesser amounts, corticosterone and 11-deoxycortisol, also could be considered typical of the amphibian and actinopterygian, respectively. The absence of cortisone in *Lepidosiren* plasma is of interest inasmuch as it is generally present in actinopterygians but not in amphibians.

We know of no detailed published studies relating to the identity of corticosteroids in *Protopterus* although Janssens and associates (1965) reported preliminary evidence for the conversion of radioactive progesterone to corticosterone by presumptive adrenal tissue of the African lungfish, *Protopterus* sp. Lungfish plasma was obtained by us through the courtesy of Dr. M. Hyder and a 50 ml. sample has now been analyzed by DIDA. The only steroids positively identified to date were 11-deoxycorticosterone (60 ng./100 ml.) and testosterone (0.6 μ /100 ml.). No cortisol, cortisone, corticosterone, 11-deoxycortisol, or aldosterone were detected within the assay limit of 20 ng./100 ml. The compound 1 α -hydroxycorticosterone was not detected within the limit of 10 ng./100 ml., and neither 18-hydroxy-11-deoxycorticosterone nor 18-hydroxycorticosterone were detected within a sensitivity limit of 100 ng./100 ml. The sample thawed on route but there was no evidence of spoilage, and thawing of the plasma was not suspected to be a problem.

Recently, Blair-West *et al.* (1977) assayed blood samples of several specimens of *Neoceratodus* for cortisol, corticosterone, 11-deoxycorticosterone, and aldosterone. Neither cortisol nor corticosterone was present in detectable amounts. The principal steroid found was 11-deoxycorticosterone; in females the concentration was 32 ng./100 ml. and in males, 4.4 ng./100 ml. Aldosterone also was present in measurable amounts (5.0 ng./100 ml. in females and 1.2 ng./100 ml. in males).

A freeze-dried plasma sample from *Neoceratodus* was kindly supplied by Dr. Janssens but the analyses are not yet completed. Aldosterone assayed at only 0.6 ng./100 ml. by radioimmunoassay and in the same assay no aldosterone was detected in the plasma from *Protopterus*.

Much remains to be done before a meaningful comparison of corticosteroids can be made among the lungfishes but the African and Australian lungfishes would appear to be similar and quite different from the South American species.

Order CROSSOPTERYGII

Through the courtesy of Dr. Grace Pickford and Dr. R. W. Griffith we received 1.4 ml. coelacanth plasma for corticosteroid analysis. In order to establish identity as well as quantity, a double isotope derivative assay was used. Neither cortisol nor cortisone were detected at the limit of sensitivity ($0.25 \mu\text{g}/100 \text{ ml.}$) in the coelacanth plasma. For the assay of aldosterone and corticosterone a minimum amount of [^{14}C] tracer was added to improve the sensitivity of the method. Under these conditions, there was no evidence of aldosterone; if present, the concentration was less than $0.06 \mu\text{g}/100 \text{ ml.}$ However, the results for corticosterone were more positive. Coelacanth plasmatic corticosterone was not separable from the added [^{14}C] tracer by chromatography. A minimal amount of the [^{14}C] tracer remained after chromatography but upon recrystallization with radioinert corticosterone, the isotope ratio ($^3\text{H}:$ ^{14}C) was constant at 5.01 ± 1 per cent through four consecutive crystallizations and the ratio for the final supernatant was 5.32. Because authentic [^{14}C] corticosterone under the same conditions gave a final ratio upon recrystallization of 3.62, the coelacanth plasma was calculated as containing $0.09 \mu\text{g}/100 \text{ ml.}$ These results should serve as a guide for future work. Consideration of our results with the African lungfish prompts us to suggest that 11-deoxycorticosterone be included in any future assays for coelacanth corticosteroids. This sample was analyzed some time ago, but we had hoped to complete some *in vitro* incubation studies with presumptive interrenal tissue to support the above conclusions. It is now apparent that this work will not be finished soon.

Class AMPHIBIA

Based on limited information, almost entirely confined to Anura, the amphibians are 17-deoxycorticosteroid secretors; corticosterone predominates and 18-oxygenated steroids occur in significant quantities. It is of interest that 18-hydroxycorticosterone, a precursor of aldosterone, was discovered first in the bullfrog (Ulick and Kusch, 1960). Early reports of significant quantities of cortisol in blood have not been confirmed.

Class REPTILIA

Corticosterone has been quantified in the blood of the grass snake, iguanid, agamid, lizard, and the freshwater turtle (see review by Gist and Kaplan, 1976). Many *in vitro* studies leave no doubt that the reptiles investigated to date are 17-deoxycorticosteroid secretors and corticosterone, 18-hydroxycorticosterone, and aldosterone have been isolated. There may be a very weak 17α -hydroxylase activity but further study is required.

Class AVES

Corticosterone has been identified as the principle steroid in the peripheral plasma of the domestic duck (Donaldson and Holmes, 1965), along with mammalian levels of aldosterone (Sandor, 1972). The chicken also has corticosterone as the major plasmatic corticosteroid and aldosterone is present (Taylor *et al.*, 1970). Several *in vitro* studies confirm the chicken, duck, western gull, pigeon, and goose as 17-deoxycorticosteroid secretors (Sandor, 1972).

Cortisol has been identified in plasma (65 ng./100 ml.) of the embryonic chick by DIDA (Idler *et al.*, 1976). Like reptiles and amphibians, birds appear to produce only minor quantities of cortisol.

In summary, amphibians, reptiles, and birds are 17-deoxycorticosteroid secretors. It would be interesting to see how aldosterone levels compare in the three classes; *in vitro* and limited *in vivo* studies suggest that the order may be amphibians > reptiles > birds. In any event, this is an hypothesis to be confirmed or rejected.

Class MAMMALIA

The two major groups of living mammals are the Prototheria (egg-laying monotremes) and the Theria (Metatheria and Eutheria, the marsupial and placental mammals, respectively).

Subclass PROTOTHERIA

There are two extant families of egg-laying mammals, each adapted to a different environment. The duck-billed platypus is aquatic in habit whereas spiny anteater lives in the sandy and rocky parts of Australia. Both have characteristics in common with reptiles and mammals. The adrenal cortex of the platypus resembles that of some elasmobranchs and Amphibia; the spiny anteater, on the other hand, has adrenal features similar to those of birds and reptiles (see Chester Jones, 1957).

The aquatic platypus is a predominantly cortisol secretor, but the terrestrial anteater secretes predominantly corticosterone (Weiss, 1973). *In vitro*, adrenals of the anteater produced small quantities of corticosteroids, when compared to the aquatic platypus, and a weak 11-hydroxylase system resulted in the accumulation of 11-deoxycorticosterone and 11-deoxycortisol. The possibility may still exist that the potent mineralocorticoid 11-deoxycorticosterone is a significant secretory product of the anteater *in vivo* because it appears that this steroid was not measured in a recent analysis of plasma corticosteroids (Oddie *et al.*, 1976). It would seem that the terrestrial anteater adapted to its environment by producing 17-deoxycorticosteroids, the more potent mineralocorticoids, and water conservation may be one goal. It is reported that the spiny anteater can retain urine for long periods (Weiss and McDonald, 1965). On the other hand, the platypus adapted to the aquatic environment as most fish had done before them; it has high

plasma cortisol levels and this steroid could conceivably function as a mineralocorticoid in addition to its other functions.

Subclass THERIA

Infraclass METATHERIA

Cortisol is the principal steroid secreted by several marsupials investigated by Weiss and her collaborators and recently studied also by Scoggins and Coghlan's laboratory (for example, Weiss and Richards, 1971; Oddie *et al.*, 1976). An interesting feature of the marsupials is the secretion of 11-deoxycortisol (Reichstein's substance S) in amounts comparable to corticosterone, which is the other major adrenalcorticosteroid. It has been suggested that the adrenal production of 21-deoxycortisol in some marsupials in amounts in excess of 11-deoxycortisol (the immediate precursor of cortisol in eutherians) indicates a possible different preferential sequence of hydroxylations (Weiss, 1968). In a recent study of nine marsupials, killed instantly to avoid stress, seven were cortisol secretors, one secreted predominantly 11-deoxycortisol, and one primarily corticosterone (Oddie *et al.*, 1976). It might be worth noting that in the seven species where cortisol was most abundant the quantities far exceeded those of the two species where corticosterone or 11-deoxycortisol predominated. These low plasma levels in marsupials, which secrete 11-deoxycorticosteroids, are similar to the situation in the corticosterone secreting monotreme, the spiny anteater.

Infraclass EUTHERIA

Rabbits, rats, and mice generally are referred to as corticosterone secretors, but this is an oversimplification because several species are not. Some of the apparent discrepancies may be attributed to methodology but in other instances this is not the case. It is well documented that different strains of laboratory rodents show major differences in corticosteroid hydroxylating enzyme systems (for review see Shire, 1974).

Progress was made in sorting out the cortisol secreting voles and mice from the corticosterone secretors by the work of Ogunsua *et al.* (1971), but more species need to be investigated before generalizations can be made.

Among the cortisol secretors are guinea pigs and golden hamsters. Gerbils, otherwise known as sand rats, inhabit the dry parts of Asia and Africa and have an excellent capacity to conserve water. It has been established that the production of a highly concentrated urine by the Mongolian gerbil is dependent upon an intact adrenal (Burns, 1956). An analysis of adrenal vein blood from the gerbil resulted in the conclusion that cortisol and 19-hydroxy-11-deoxycortisol were the principal corticosteroids (Oliver and Peron, 1964). However, neither of these components changed in response to salt or water loading of salt, and 19-hydroxy-11-deoxycortisol had no effect on salt retention in the rat assay. The situation was complicated when other researchers were unable to confirm the principal steroid following *in vitro*

incubation of gerbil adrenals with ^{14}C -progesterone (Francois *et al.*, 1966). In this latter study, 11-deoxycorticosterone and 18-hydroxy-11-deoxycorticosterone were identified as the principal products. This problem is worthy of careful reexamination because both steroids found in the latter study are mineralocorticoids and have an antidiuretic action in the rat (Birmingham *et al.*, 1968).

A recent study of six desert rodents found that plasma corticosterone levels correlated with the degree of habitat aridity (Vanjonack *et al.*, 1975). It was suggested that the lower corticosterone levels in those species inhabiting the more arid regions might be related to water conservation.

Marine mammals appear to secrete both 17-deoxy and 17-hydroxycorticosteroids. A report by Sangalang and Freeman (1976), using a DIDA assay, established that cortisol, cortisone, and corticosterone were present in peripheral plasma of the gray seal. In two seals, cortisol predominated whereas nearly equal quantities of cortisol and corticosterone were found in a third animal; aldosterone was detected in all. Cortisol and corticosterone were reported in nearly equal quantities by Seal and Doe (1965) in plasma of a dolphin and a whale.

The ratio of cortisol to corticosterone, at least in eutherian mammals, probably has little significance inasmuch as it can vary considerably over relatively short periods of time (Zolovick *et al.*, 1966). Nevertheless, cortisol predominates, and, with the exceptions noted above, eutherians appear to be predominantly cortisol secretors. Even among eutherians, there have been relatively few definitive studies of blood compared to the many hundreds of published reports on synthesis of steroids by the adrenal *in vitro*.

We are convinced that adaptation to environment is a major factor in determining the nature of the circulating adrenal steroids. However, it must be remembered that adrenal steroids control many processes other than those involved with water and salt metabolism. There is insufficient information concerning the action of 17-hydroxycorticosteroids and 17-deoxycorticosteroids on nonmammalian vertebrates to warrant drawing further conclusions at this time. For example, it has been established that cortisol, which is a glucocorticoid in mammals, can be a mineralocorticoid in some fishes. The proteins that bind steroids in fish also are quite different from those in mammals (Idler and Truscott, 1972). Thus there is evidence that the nature of the plasma transport proteins and the protein receptor of the target tissue may be as important in evolution as the structure of the corticosteroids.

At this point it seems appropriate to refer to an observation that N. H. Horowitz (1965) attributed to G. W. Beadle when the former was reviewing the evolution of biochemical synthesis: "It's hard to make a good theory—a theory has to be reasonable but a fact doesn't." Certainly we need more facts to support theories of the evolution of steroid hormones. In conclusion, we would reiterate our plea for more emphasis on corticosteroids in blood.

ACKNOWLEDGMENT

This paper is contribution number 275 of the Marine Sciences Research Laboratory, Memorial University of Newfoundland.

LITERATURE CITED

- AMOS, B., I. G. ANDERSON, G. A. D. HASLEWOOD, AND L. TOKES. 1977. Bile salts of the lungfishes *Lepidosiren*, *Neoceratodus* and *Protopterus* and those of the coelacanth *Latimeria chalumnae* Smith. *Biochem. J.*, 161:201-204.
- BERN, H. A., C. C. DE ROOS, AND E. G. BIGLIERI. 1962. Aldosterone and other corticosteroids from Chondrichthyan interrenal glands. *Gen. Comp. Endocrinol.*, 2:490-494.
- BIRMINGHAM, M. K., M. L. MACDONALD, AND J. G. ROCHEFORT. 1968. Adrenal function in normal rats and in rats bearing regenerated adrenal glands. Pp. 647-689, in *Functions of the adrenal cortex* (K. W. McKerns, ed.), Appleton-Century-Crofts, New York, 2:xiv+647-1176 pp.
- BLAIR-WEST, J. R., J. P. COGHLAN, D. A. DENTON, A. P. GIBSON, C. J. ODDIE, W. H. SAWYER, AND B. A. SCOGGINS. 1977. Plasma renin activity and blood corticosteroids in the Australian lungfish *Neoceratodus forsteri*. *J. Endocrinol.*, 74:137-142.
- BURNS, T. W. 1956. Endocrine factors in water metabolism of the desert mammals, *G. gerbilus*. *Endocrinology*, 58:243-254.
- BUTLER, D. G. 1973. Structure and function of the adrenal gland of fishes. *Amer. Zool.*, 13:839-879.
- CARBALLEIRA, A., AND I. DURNHOFFER. 1968. Side-chain scission of cholesterol-4-¹⁴C by adrenocortical preincubation media (PIM) and tissue sections. *Steroids*, 11:513-526.
- CARBALLEIRA, A., A. MEHDI, AND E. H. VENNING. 1965. Metabolism of labelled steroid precursors by normal bovine adrenal medulla *in vitro*. *Proc. Soc. Exp. Biol. Med.*, 119:751-756.
- CHAVIN, W. 1976. The thyroid of the sarcopterygian fishes (Dipnoi and Crossopterygii) and the origin of the tetrapod thyroid. *Gen. Comp. Endocrinol.*, 30:142-155.
- CHAVIN, W., AND J. A. SINGLEY. 1972. Adrenocorticoids of the goldfish *Carassius auratus* L. *Comp. Biochem. Physiol.*, 42B:547-562.
- CHESTER JONES, I. 1957. The adrenal cortex. Cambridge Univ. Press, x+316 pp.
- COLOMBO, L., H. A. BERN, J. PIEPRZYK, AND D. W. JOHNSON. 1972. Corticosteroidogenesis *in vitro* by the head kidney of *Tilapia mossambica* (Cichlidae, Teleostei). *Endocrinology*, 91:450-462.
- DONALDSON, W. M., AND W. N. HOLMES. 1965. Corticosteroidogenesis in the freshwater and saline maintained duck (*Anas platyrhynchos*). *J. Endocrinol.*, 32:329-336.
- EDWARDS, R. W., AND D. J. TRAFFORD. 1968. The partial characterization of 1-oxygenated steroids from urine of a hypertensive newborn child. *Biochem. J.*, 108:185-193.
- FRANCOIS, D., D. F. JOHNSON, AND H. Y. WONG. 1966. *In vitro* metabolism of progesterone-4-¹⁴C by the adrenal gland of the Mongolian gerbil. *Steroids*, 7:297-307.
- FRASER, D. R., AND E. KODICEK. 1970. Unique biosynthesis by kidney of a biologically active Vitamin D metabolite. *Nature*, 228:764-766.
- FULLER, J. D., P. A. MASON, AND R. FRASER. 1976. Gas-liquid chromatography of corticosteroids in plasma of Salmonidae. *J. Endocrinol.*, 71:163-164.
- GANJAM, V. K., A. L. CAMPBELL, AND B. E. P. MURPHY. 1972. Changing patterns of circulating corticosteroids in rabbits following prolonged treatment with ACTH. *Endocrinology*, 91:607-611.
- GIST, D. H., AND M. L. KAPLAN. 1976. Effects of stress and ACTH on plasma corticosterone levels in the caiman, *C. crocodilus*. *Gen. Comp. Endocrinol.*, 28:413-419.
- HARTMAN, F. A., L. A. LEWIS, K. A. BROWNELL, C. A. ANGERER, AND F. F. SHELDON. 1944. Effect of interrenalectomy on some blood constituents in the skate. *Physiol. Zool.*, 17:228-238.

- HOLT, W. F., AND D. R. IDLER. 1975. Influence of the interrenal gland on the rectal gland of the skate. *Comp. Biochem. Physiol.*, 50C:111-119.
- HOROWITZ, N. H. 1965. The evolution of biochemical syntheses-retrospect and prospect. Pp. 15-23, in *Evolving genes and proteins* (V. Bryson and V. J. Vogel, eds.), Academic Press, New York, xxi+629 pp.
- IDLER, D. R. 1969. Steroidogenesis in fish. Pp. 121-133, in *Fish in research* (O. W. Neuhaus and J. E. Halver, eds.), Academic Press, New York, xii+311 pp.
- . 1971. Some comparative aspects of corticosteroid metabolism. Pp. 14-28, in *Hormonal steroids*, Proc. 3rd. Int. Congr. Hormonal Steroids, 1970, Hamburg, Germany (V. H. T. James and L. Martini, eds.), Int. Congr. Ser. 219, Excerpta Medica, Amsterdam, xvi+1063.
- . 1973. Comments on "Structure and function of the adrenal gland of fishes" by David Gordon Butler. *Amer. Zool.*, 13:881-884.
- IDLER, D. R., AND M. P. M. BURTON. 1976. The pronephroi as the site of presumptive interrenal cells in the hagfish *Myxine glutinosa* L. *Comp. Biochem. Physiol.*, 53A:73-77.
- IDLER, D. R., AND B. J. SZEPLAKI. 1968. Interrenalectomy and stress in relation to some blood components of an elasmobranch (*Raja radiata*). *J. Fish. Res. Board Canada* 25:2549-2560.
- IDLER, D. R., AND B. TRUSCOTT. 1967. 1α -Hydroxycorticosterone: synthesis *in vitro* and properties of an interrenal steroid in the blood of cartilaginous fish (Genus *Raja*). *Steroids*, 9:457-477.
- . 1968. 1α -Hydroxycorticosterone and testosterone in body fluids of a cartilaginous fish (*Raja radiata*). *J. Endocrinol.*, 42:165-166.
- . 1969. Production of 1α -hydroxycorticosterone *in vivo* and *in vitro* by elasmobranchs. *Gen. Comp. Endocrinol.*, suppl. 2:325-330.
- . 1972. Corticosteroids in fish. Pp. 126-252, in *Steroids in nonmammalian vertebrates*. (D. R. Idler, ed.), Academic Press, New York, xii+504 pp.
- IDLER, D. R., B. TRUSCOTT, AND H. C. STEWART. 1969. Some distinctive aspects of steroidogenesis in fish. Pp. 724-729, in *Progress in endocrinology*, Proc. 3rd. Int. Congr. Endocrinol., 1968, Mexico City, Mexico. (C. Gual and F. J. G. Ebling, eds.), Int. Congr. Ser. 184, Excerpta Medica, Amsterdam, xii+1276 pp.
- IDLER, D. R., G. B. SANGALANG, AND M. WEISBART. 1971. Are corticosteroids present in the blood of all fish? Pp. 983-989, in *Hormonal steroids*, Proc. 3rd. Int. Congr. hormonal steroids, 1970, Hamburg, Germany (V. H. T. James and L. Martini, eds.), Int. Congr. Ser. 219, Excerpta Medica, Amsterdam, xvi+1063 pp.
- IDLER, D. R., G. B. SANGALANG, AND B. TRUSCOTT. 1972. Corticosteroids in the South American lungfish. *Gen. Comp. Endocrinol.*, suppl. 3:238-244.
- IDLER, D. R., J. M. WALSH, R. KALLIECHARAN, AND B. K. HALL. 1976. Identification of cortisol in plasma of the embryonic chick. *Gen. Comp. Endocrinol.*, 30:539-540.
- JANSSENS, P. A., G. P. VINSON, I. CHESTER JONES, AND W. MOSLEY. 1965. Amphibian characteristics of the adrenal cortex of the African lungfish (*Protopterus* sp.). *J. Endocrinol.*, 32:373-382.
- KASS, E. H., O. HECHTER, I. A. MACCHI, AND T. W. MOU. 1954. Changes in patterns of secretion of corticosteroids in rabbits after prolonged treatment with ACTH. *Proc. Soc. Exp. Biol. Med.*, 85:583-587.
- KOROTAEV, G. K., A. A. ARDASHEV, AND E. F. BROVENKO. 1973. The 11α -oxycorticosteroid level in the blood plasma of *Plagiostomi* and *Teleostei*. *J. Evol. Biochem. Physiol.*, 9:455-456.
- LELOUP-HATEY, J., AND A. DE LIGNIERES. 1974. Origine de la cortisone du plasma chez *Anguilla anguilla*. *Gen. Comp. Endocrinol.*, 22:398.
- LUCIS, O. J., I. DYRENFURTH, AND E. H. VENNING. 1961. Effect of various preparations of pituitary and diencephalon on the *in vitro* secretion of aldosterone and corticosterone by the rat adrenal gland. *Canadian J. Biochem.*, 39:901-903.

- MAREN, T. H. 1967. Special body fluids of the elasmobranch. Pp. 287-292, in *Sharks, skates and rays* (P. W. Gilbert, R. F. Mathewson, and D. P. Rall, eds.), Johns Hopkins Press, Baltimore, xv+624 pp.
- MATSUMOTO, K., AND L. T. SAMUELS. 1969. Influence of steroid distribution between microsomes and soluble fraction on steroid metabolism by microsomal enzymes. *Endocrinology*, 85:402-409.
- ODDIE, C. J., E. H. BLAINE, S. D. BRADSHAW, J. P. COGHLAN, D. A. DENTON, J. F. NELSON, AND B. A. SCOGGINS. 1976. Blood corticosteroids in Australian marsupial and placental mammals and one monotreme. *J. Endocrinol.*, 69:341-348.
- OGUNSUA, A. O., A. F. DENICOLA, H. TRAIKOV, M. K. BIRMINGHAM, AND S. LEVINE. 1971. Adrenal steroid biosynthesis by different species of mouse-like rodents. *Gen. Comp. Endocrinol.*, 16:192-199.
- OLIVER, J. T., AND F. G. PERON. 1964. 19-Hydroxy-11-deoxycortisol, a major steroid secreted by the adrenal gland of the Mongolian gerbil. *Steroids*, 4:351-363.
- PATTERSON, C. 1965. The phylogeny of the chimaeroids. *Phil. Trans. Roy. Soc., ser. B.*, 249:101-219.
- ROMER, A. S. 1956. The vertebrate body. 2nd ed., W. B. Saunders Co., viii+644 pp.
- SANDOR, T. 1972. Corticosteroids in Amphibia, Reptilia and Aves. Pp. 253-327, in *Steroids in nonmammalian vertebrates* (D. R. Idler, ed.), Academic Press, New York, xii+ 504 pp.
- SANDOR, T., A. G. FAZEKAS, AND B. H. ROBINSON. 1976. The biosynthesis of corticosteroids throughout the vertebrates. Pp. 25-142, in *General, comparative and clinical endocrinology of the adrenal cortex* (I. Chester Jones and I. W. Henderson, eds.), Academic Press, New York, 1:xv+1-461 pp.
- SANGALANG, G. B., AND H. C. FREEMAN. 1976. Steroids in the plasma of the gray seal, *Halichoerus grypus*. *Gen. Comp. Endocrinol.*, 29:419-422.
- SANGALANG, G. B., M. WEISBART, AND D. R. IDLER. 1971. Steroids of a chondrosteian: corticosteroids and testosterone in the plasma of the American Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill. *J. Endocrinol.*, 50:413-421.
- SANGALANG, G. B., B. TRUSCOTT, AND D. R. IDLER. 1972. A comparison of steroidogenesis *in vitro* in two teleosts, the marine herring, *Clupea* and the freshwater Atlantic salmon, *Salmo*. *J. Endocrinol.*, 53:433-446.
- SEAL, U. S., AND R. P. DOE. 1965. Vertebrate distribution of corticosteroid-binding globulin and some endocrine effects on concentration. *Steroids*, 5:827-841.
- SHIRE, J. G. M. 1974. Review: endocrine genetics of the adrenal gland. *J. Endocrinol.*, 62:173-207.
- SIMPSON, T. H., AND R. S. WRIGHT. 1970. Synthesis of corticosteroids by the interrenal gland of selachian elasmobranch fish. *J. Endocrinol.*, 46:261-268.
- SIMPSON, T. H., R. S. WRIGHT, AND H. GOTTFRIED. 1963a. Steroids in the semen of dogfish (*Squalus acanthias*). *J. Endocrinol.*, 26:489-498.
- SIMPSON, T. H., R. S. WRIGHT, AND S. V. HUNT. 1963b. Steroids of the semen of elasmobranch fish. *J. Endocrinol.*, 27:131-132.
- TAYLOR, A. A., J. O. DAVIS, R. P. BREITENBACH, AND P. M. HARTROFT. 1970. Adrenal steroid secretion and a renal pressor system in the chicken (*Gallus domesticus*). *Gen. Comp. Endocrinol.*, 14:321-333.
- TRUSCOTT, B., AND D. R. IDLER. 1968. The widespread occurrence of a corticosteroid 1 α -hydroxylase in the interrenals of Elasmobranchii. *J. Endocrinol.*, 40:515-526.
- . 1972. Corticosteroids in the plasma of elasmobranchs. *Comp. Biochem. Physiol.*, 42A:41-50.
- TSANG, P. W. C., AND J. STACHENKO. 1970. The effect of temperature and of cofactor concentrations on the conversion of 4-¹⁴C progesterone to corticosterone by rat adrenal preparations. *Canadian J. Biochem.*, 48:740-745.
- ULICK, S., AND K. J. KUSCH. 1960. A new C-18 oxygenated corticosteroid from bullfrog adrenal. *J. Amer. Chem. Soc.*, 82:6421-6422.

EVOLUTION OF THE RENIN-ANGIOTENSIN SYSTEM

HIROKO NISHIMURA

There have been remarkable advances during the past ten years in our understanding of the chemistry and functions of the renin-angiotensin system in mammals. The investigators and the literature involved in the comparative aspects of this hormonal system also are increasing. Sokabe and Ogawa (1974) reviewed the literature extensively and summarized the concepts in comparative studies of the juxtaglomerular apparatus. The aim of this chapter is to review the morphological, chemical, and physiological aspects of the renin-angiotensin system in nonmammalian vertebrates, with emphasis on more recent studies. I will also speculate on evolutionary trends. Function of the renin-angiotensin system in fishes, phylogeny of renal effects of angiotensin, and function of the system in various vertebrates in relation to hydromineral regulation were reviewed previously by Nishimura and Ogawa (1973), Sokabe (1974), and Taylor (1977), respectively.

RENIN-ANGIOTENSIN SYSTEM IN MAMMALS

Components

In 1898, Tigerstedt and Bergman (1898) observed that saline extract of the rabbit kidney increased blood pressure of another rabbit when injected intravenously. They named this pressor substance "renin". Renin (Skeggs *et al.*, 1977) works on the renin substrate, angiotensinogen (Skeggs *et al.*, 1974), which is located largely in the plasma albumin and alpha-2-globulin to produce angiotensin I. Angiotensin I (decapeptide) is converted subsequently to angiotensin II by the action of a converting enzyme(s) (Bakhle, 1974; Vane, 1974; Erdős, 1977) that splits two amino acids from the carboxyl end of angiotensin I. This biologically active octapeptide, angiotensin II (Bumpus and Smeby, 1968), exerts actions at various receptor sites of the body (Khairallah, 1971). Angiotensin II is metabolized to an inactive form by angiotensinases (Ryan, 1974). This whole system diagrammed in Fig. 1, is called the "renin-angiotensin system" (for review, see Peart, 1965; Bumpus and Smeby, 1968).

More recently, an alternative degradative pathway has been proposed (Blair-West *et al.*, 1971; Bumpus, 1977). Aspartic acid (first position) of the decapeptide angiotensin I loses the amino group by the action of aminopeptidase. This [des-1-aspartic acid] angiotensin I is then converted to [des-1-aspartic acid] angiotensin II by converting enzyme(s). This C-terminal heptapeptide is approximately as potent as angiotensin II at various action sites, except in vasopressor action, and is perhaps an important mediator of the renin-angiotensin system (for review, see Freeman *et al.*, 1977).

Renin exists in the granules of the epithelioid cells (juxtaglomerular cells) of the media of the afferent arteriole of the kidney. The renal distal tubule returns to its own glomerulus and attaches to the afferent arteriole at the vascular pole. There, tubular epithelial cells are deformed, becoming narrow and tall macula densa cells. The afferent arteriole, including the juxtaglomerular cells, the efferent arteriole, the macula densa, and a dense aggregation of cells in the triangular space between afferent and efferent arterioles and the distal tubule (extraglomerular mesangium, Polkissen), compose the juxtaglomerular apparatus outlined in Fig. 2 (Barajas and Latta, 1967; Barajas, 1970). Adrenergic nerve fibers are innervated along the juxtaglomerular cells.

Function

It has been shown in several mammalian species that angiotensin stimulates aldosterone secretion from the adrenal cortex (Davis, 1971, 1974). A role of the renin-angiotensin-aldosterone system in control of blood pressure has been suggested because angiotensin II exerts a potent vasopressor action by constricting peripheral arterioles, the renin and angiotensin levels are high in some forms of hypertension induced experimentally or by diseases in man (Wilson *et al.*, 1971; Laragh *et al.*, 1972; Laragh and Sealey, 1973), and because aldosterone is an important regulator for blood volume and sodium balance. However, the participation of the renin-angiotensin system in maintaining physiological levels of blood pressure is uncertain.

Angiotensin may regulate renal blood flow and glomerular filtration rate (Navar and Langford, 1974) by intrarenal feedback mechanisms (Thurau, 1974). Under certain circumstances, angiotensin produces natriuresis (Peters and Bonjour, 1971), although it is not clear whether angiotensin inhibits Na reabsorption by directly affecting tubular Na transport mechanisms, or whether the natriuresis is secondary to hemodynamic changes produced by angiotensin.

Angiotensin acts on the central nervous system (for review, see Severs and Daniels-Severs, 1973). In rabbits, dogs, and men, angiotensin produces a greater rise of blood pressure when infused into the vertebral arteries than when given intravenously (Dickinson and Ferrario, 1974). The possible site of action is the area of postrema in the distal medulla, where the blood-brain barrier is deficient. Intracranial injection of angiotensin stimulates water intake (dipsogenic effect) in mammals (Fitzsimons, 1972; Severs and Summy-Long, 1975). Dipsogenic sites appear to be the medial preoptic areas, third ventricle, and anterior hypothalamus. A recent study shows that angiotensin injected directly into the subfornical organ in a dose as small as 0.1 ng. elicited drinking behavior in water-sated rats (Simpson and Routtenberg, 1973). It has been suggested that the anterior-ventral wall of the third ventricle is important both for vasopressor and drinking responses to angiotensin (Phillips and Hoffman, 1976; Anderson and Olsson, 1977). A lesion or an injection of cold cream in this area causes adipsia or loss of vasopressor and dipsogenic responses to angiotensin.

THE MAMMALIAN RENIN-ANGIOTENSIN SYSTEM

FIG. 1.—The mammalian renin-angiotensin system. JG, juxtaglomerular.

Interactions between the renin-angiotensin system and other vasoactive systems have been demonstrated. Introduction of angiotensin into the central nervous system appears to release antidiuretic hormone from the neurohypophysis (for review, see Severs and Daniels-Severs, 1973). Infusions of angiotensin into the carotid artery of conscious dogs or angiotensin perfusion through the ventriculo-cisternal system in the anesthetized dog increased plasma ADH (Mouw *et al.*, 1971). It has been shown that the brain contains all the components of the renin-angiotensin system: renin substrate, renin-like enzyme, converting enzyme, and angiotensin I and II (for review, see Severs and Daniels-Severs, 1973; Ganten *et al.*, 1976; Ganong, 1977). However, the biochemical nature of the renin-angiotensin system in the brain and physiological links among the vasopressor, dipsogenic, and ADH releasing actions of angiotensin and the brain renin-angiotensin system are not yet clear.

It has been suggested that the renal kallikrein-kinin system protects the renal vasculature against the constricting action of angiotensin. Although urinary kallikrein excretion is reduced during renal arterial constriction and in some hypertensive diseases (Margolius *et al.*, 1976), it is not clear whether the renal kallikrein-kinin system participates in the etiology of hypertension

JUXTAGLOMERULAR APPARATUS

FIG. 2.—Schematic illustration of the juxtaglomerular apparatus in the mammalian kidney.

independently or in relation to the renin-angiotensin system. Both angiotensin level and urinary kallikrein excretion are high in sodium-depleted subjects (Mills *et al.*, 1976).

Infusion of angiotensin II into the dog kidney is accompanied by dose-dependent increases of prostaglandins in the venous effluent and blunting of the vasoconstrictor-antidiuretic action of angiotensin II (McGiff *et al.*, 1970). The renal prostaglandins and renin seem to stimulate each other's formation or release, but oppose each other's actions (Ånggård *et al.*, 1976).

Release Mechanisms

This subject was thoroughly reviewed recently by Davis and Freeman (1976). It is generally recognized that three groups of mechanisms are involved in the control of renin release in mammals: two intrarenal receptors, the renal vascular receptor and the macula densa; the renal sympathetic nerves, catecholamines, and an adrenergic receptor; and several humoral agents, including sodium, potassium, and calcium ions, angiotensin II, vasopressin (antidiuretic hormone), prostaglandins, kinins, and some steroid hormones.

The renal vascular receptor, which is presumably located in the juxtaglomerular cells of the afferent arteriole, responds to decreased renal perfusion pressure by increasing renin secretion (Tobian *et al.*, 1959; Davis and Freeman, 1976). The macula densa, a renal tubular receptor, appears to

detect changes in either the load or concentration of sodium or chloride at the macula densa site, and transfers the information to the juxtaglomerular cells (Vander, 1967; Schnermann *et al.*, 1976).

Sympathetic nerve fibers containing adrenergic vesicles are innervated to the region of the juxtaglomerular cells, according to Barajas (1964). Stimulation of renal nerves (Johnson *et al.*, 1971) or the central nervous system (midbrain and medulla oblongata) causes renin release (Ueda *et al.*, 1967; Passo *et al.*, 1971), whereas renal renin content decreased after denervation (Taquini *et al.*, 1964). It appears that beta adrenergic receptors, which are presumably located in the juxtaglomerular cells, mediate renin release (Ganong, 1972; Johnson *et al.*, 1976).

Recent studies indicate that prostaglandins might be a mediator for renin release. Treatment with indomethacin, a prostaglandin synthetase inhibitor, inhibited renin release by acute hemorrhage (Romero *et al.*, 1976) and constriction of the renal artery (Romero and Strong, 1977), and inhibited renin release from kidney slices (Weber *et al.*, 1976). We cannot exclude the possibility, however, that this blockage of renin release might be due to the effect of indomethacin itself, rather than inhibition of prostaglandin synthesis.

RENIN-ANGIOTENSIN SYSTEM AMONG VERTEBRATES

Renal Renin Activity and Granulated Cells

The current concept of the presence and absence of the renin-angiotensin system among vertebrates is summarized in Fig. 3. Renal renin activity (determined biochemically), juxtaglomerular cells, and the macula densa have not been found in cyclostomes and elasmobranchs (Nishimura *et al.*, 1970). Renal renin (renin-like activity) has been detected in primitive bony fishes (Nishimura *et al.*, 1973) and in representative species of teleosts, lungfishes, coelacanths, amphibians, reptiles, birds, and mammals (Sokabe *et al.*, 1969; Nolly and Fasciolo, 1972; Nishimura *et al.*, 1973). Thus, the renin-angiotensin system probably evolved during the early evolution of the bony fishes.

Juxtaglomerular granules cannot be stained in the primitive bony fishes (chondrosteans and holosteans), with the exception of a longnose gar, *Lepisosteus osseus*, although all primitive bony fish examined possess renal renin activity. This suggests that their renin has differing histochemical properties (Nishimura *et al.*, 1973). The kidney from the ratfish, a holocephalian, appears to possess renal renin, but the level is low. In teleost fishes and lungfishes (Fig. 4), granulated cells are distributed along the small arteries and arterioles of the kidney (Krishnamurthy and Bern, 1969; Sokabe *et al.*, 1969; Nishimura *et al.*, 1973). Distribution of the granulated cells changes along the phylogenetic scale. Primitive animals have a diffuse distribution of granulated cells along the arterioles and small arteries, whereas more advanced animals show a tendency to localize these cells near the glomeruli (Krishnamurthy and Bern, 1969; Sokabe and Ogawa, 1974).

PRESENCE AND ABSENCE OF RENIN AND JUXTAGLOMERULAR APPARATUS

FIG. 3.—A diagram of the vertebrate phylogenetic tree and the presence and absence of the renin-angiotensin system among various vertebrates classes. Studies from several groups are summarized. A single asterisk indicates renin activity was noted, but no granules were stained by Bowie's method; a double asterisk, an atypical transitional form of macula densa cells.

Saline extracts of corpuscles of Stannius from the European eel showed substantial amounts of pressor activity resembling renin (Chester Jones *et al.*, 1966). Extract of corpuscles of Stannius from the carp, goldfish, and Japanese goosefish, *Lophius litulon*, formed angiotensin-like substances when incubated with homologous plasmas (Sokabe *et al.*, 1970). The total content of renin in the corpuscles of Stannius, however, was less than 1 per cent of that of the kidneys in these fishes. The role of renin and angiotensin-like substances in the corpuscles of Stannius is unknown. Stannius corpuscle homogenate produced hypocalcemia in the killifish, *Fundulus heteroclitus*, adapted to a low-calcium medium (hypocalcin; Pang *et al.*, 1974). This hypocalcemic response was not elicited by angiotensin II-amide. Details of the occurrence of the renin-angiotensin system among fishes and nonmammalian tetrapods have been reviewed by Nishimura and Ogawa (1973), Sokabe and Ogawa (1974), and Taylor (1977).

Macula Densa Cells

In most primitive animals, there is no topographical relationship between epithelioid granulated cells and renal tubules, and thus they lack a macula densa (Sokabe *et al.*, 1969). In holocephalians, elasmobranchs, and perhaps some amphibians (Edwards, 1940), distal tubules do return to their own glomeruli and attach to the afferent arterioles, but no cells resembling mammalian macula densa cells were found; birds have some transitional

Dipnoi African Lungfish, *Protopterus aethiopicus*

Glomerulus spherical, $180 \times 180 \mu$

FIG. 4.—Schematic illustration of juxtaglomerular area of African lungfish, *Protopterus aethiopicus*. Granules are observed in the media of arterioles distant from the glomeruli. JGG, juxtaglomerular granules; NS, neck segment. (Reproduced from Nishimura *et al.*, 1973).

formation of the cell type (Sokabe *et al.*, 1969; Ogawa and Sokabe, 1971). Only mammals have all the components of the juxtaglomerular apparatus. Therefore, the vascular component of the juxtaglomerular apparatus, renin and granulated cells, evolved in an earlier stage of vertebrate phylogeny than the tubular macula densa. This discrepancy in emergence of the granulated cells and macula densa is important when we consider the physiological role of the renin-angiotensin system in primitive animals.

Converting Enzyme

Partially purified natural eel angiotensin (presumably angiotensin I) and [5-valine, 9-serine]-angiotensin I (fowl angiotensin I; Nakayama *et al.*, 1973) increased the dorsal aortic pressure of freshwater-adapted eels (Nishimura *et al.*, 1976, 1978). Vasopressor action in eels is exerted also by [1-asparagine, 5-valine]-angiotensin II (angiotensin II-amide). Pretreatment of eels with SQ 20,881 (a nonapeptide from snake venom; Ondetti *et al.*, 1971), which inhibits converting enzyme in mammals, abolished the pressor responses to eel and fowl angiotensin I in eels, but not the response to angiotensin II-amide. This finding suggests that an additional enzymatic conversion is necessary to exert the vasopressor action of angiotensin I, whereas angiotensin II may increase blood pressure by working directly on the blood vessels. A converting enzyme (or converting enzyme-like substance) might exist in eels (Nishimura *et al.*, 1978).

Opdyke and Holcombe (1976) found that the vasopressor effect of human angiotensin I was inhibited by SQ 20,881, but the effect of angiotensin II was not. This observation is particularly interesting when we consider that neither renal renin activity nor granulated cells have been found in elasmobranchs (Nishimura *et al.*, 1970). The emergence of a converting enzyme and angiotensin receptors in blood vessels may precede the evolution of renin. However, the angiotensin I converting enzyme has a dual function in mammals: it converts angiotensin I to angiotensin II, and it inactivates bradykinin (for review see Erdős, 1977). There is a possibility, therefore, that the enzyme that was blocked by SQ 20,881 is physiologically bradykininase in elasmobranchs.

Angiotensinases

Angiotensinases have been found in the plasma, kidney, and other tissues from representative species in vertebrates (Nishimura and Sokabe, 1968). Aminopeptidases, carboxypeptidases, and endopeptidases have been shown to act as angiotensinases in mammals, but the chemical properties of angiotensinases in nonmammalian vertebrates are not known. *In vitro* inactivation of angiotensin by angiotensinases from nonmammalian vertebrates is inhibited efficiently by acid or alkaline treatment, EDTA, diisopropyl fluorophosphate (DFP), phenylmethylsulfonylfluoride (PMSF), adsorption of angiotensin onto Dowex-resin, or a combination of these (Mizogami *et al.*, 1968; Nishimura and Sokabe, 1968; Nishimura *et al.*, 1970, 1977).

Summary

Renal renin evolved during the early evolution of bony fishes. A holocephalian appears to have a similar substance.

Primitive bony fishes, holocephalians, teleosts, dipnoans, coelacanths, amphibians, and reptiles possess granulated epithelioid cells in the small arteries and arterioles of the kidney, but they lack a macula densa. Birds

TABLE 1.—*Amino acid sequence of angiotensin I. Data for fowl are from Nakayama et al. (1973); those for the Japanese goosefish are from Hayashi et al. (1978).*

Organism	Amino acid sequence
Equine, Hog, Rat, Dog	Asp ¹ -Arg ² -Val ³ -Tyr ⁴ -Ile ⁵ -His ⁶ -Pro ⁷ -Phe ⁸ -His ⁹ -Leu ¹⁰
Bovine	Asp ¹ -Arg ² -Val ³ -Tyr ⁴ -Ile ⁵ -His ⁶ -Pro ⁷ -Phe ⁸ -His ⁹ -Leu ¹⁰
Fowl	Asp ¹ -Arg ² -Val ³ -Tyr ⁴ -Ile ⁵ -His ⁶ -Pro ⁷ -Phe ⁸ -Ser ⁹ -Leu ¹⁰
Japanese goosefish <i>Lophius litulon</i>	Asn ¹ -Arg ² -Val ³ -Tyr ⁴ -Ile ⁵ -His ⁶ -Pro ⁷ -Phe ⁸ -His ⁹ -Leu ¹⁰

have a transitional structure of macula densa cells. Thus, the vascular component of the juxtaglomerular apparatus could have evolved earlier than the tubular component (macula densa) during vertebrate phylogeny.

A converting enzyme (or converting enzyme-like substance) might exist in elasmobranchs and teleosts. The chemical properties of converting enzyme in nonmammalian vertebrates remain to be determined.

Angiotensinases in the plasma and kidney have been found in all classes, including cyclostomes and elasmobranchs. They are probably nonspecific peptidases.

ANGIOTENSIN CHEMISTRY IN NONMAMMALIAN VERTEBRATES

Amino Acid Sequence

Substances produced by incubating kidney extracts from primitive animals with homologous plasma biologically resemble mammalian angiotensins. The substances evoke vasopressor responses in the rat as well as in homologous species. The substances are dialyzable, heat-stable, and susceptible to digestion by alpha chymotrypsin (Mizogami *et al.*, 1968; Nishimura *et al.*, 1973). Vasopressor responses in the rat are not blocked by alpha adrenergic blocking drugs.

The chemical and biochemical properties of the renin-angiotensin system in nonmammalian vertebrates have been little known. The amino acid sequences of angiotensin I from fowl and Japanese goosefish, *Lophius litulon*, have been determined by Nakayama *et al.* (1973) and Hayashi *et al.* (1978), respectively. Fowl angiotensin I has serine in the ninth position, and native angiotensin I from the goosefish has asparagine instead of aspartic acid in position 1 (Table 1). The corresponding angiotensin II of this goosefish angiotensin I is the same as [1-asparagine, 5-valine]-angiotensin II, which has been synthesized and used as standard angiotensin for various experimental and clinical studies in man and other mammals. Angiotensin I from a snake possesses tyrosine at the ninth position (Nakayama *et al.*, 1977), although it is not clear yet whether the amino acid in the first position is aspartic acid or asparagine.

Nakajima *et al.* (1971) attempted to characterize angiotensin-like substances in representative species from teleost fishes, amphibians, reptiles, birds, and mammals by fractionating them with SE-Sephadex column chromatography. Products derived from teleost fishes were eluted by a pH and ionic

strength gradient in two peaks, neither of which exactly coincides with those of known mammalian angiotensins I or II.

Immunological Properties

Nishimura *et al.* (1977) determined the immunological reaction between fish angiotensin and human angiotensin I antibody. Partially purified angiotensin (presumably angiotensin I) from the toadfish, *Opsanus tau*, and African lungfish, *Protopterus aethiopicus*, was bound by a human angiotensin I antibody, whereas angiotensins from the American eel, *Anguilla rostrata*, channel catfish, *Ictalurus punctatus*, and South American lungfish, *Lepidosiren paradoxa*, were not (Nishimura *et al.*, 1977, and unpublished data). The activity of toadfish angiotensin, measured by the vasopressor bioassay in the rat with [1-asparagine, 5-valine]-angiotensin II (angiotensin II-amide) as a standard, and that determined by the radioimmunoassay method with human angiotensin I (Table 1) standard were nearly equal in the range of 5-70 ng (angiotensin equivalent)/ml. Plasma renin activity (rate of angiotensin I generation) and plasma angiotensinogen levels (amount of angiotensin produced from 1 milliliter of plasma at maximum) determined by the radioimmunoassay system are well correlated with those measured by the bioassay system, although the levels are higher in the radioimmunoassay (Nishimura *et al.*, 1977). African lungfish angiotensin I was only partially bound to the antibody, and activity determined by the vasopressor bioassay was much higher than that measured by radioimmunoassay.

Summary

The amino acid sequences of angiotensin I from the fowl and Japanese goosefish are determined as [5-valine, 9-serine]-angiotensin I and [1-asparagine, 5-valine]-angiotensin I, respectively.

It appears that there are at least two types of native angiotensin I among bony fishes: one is bound by human angiotensin I antibody, and the other is not.

EXOGENOUSLY ADMINISTERED ANGIOTENSIN IN NONMAMMALIAN VERTEBRATES

Vasopressor Action

Synthetic angiotensins I and II, natural homologous angiotensin (product of the incubation of kidney extract with homologous plasma), or kidney extract exert vasopressor action in the dogfish, teleosts, lungfishes, amphibians, reptiles, birds, and mammals (Table 2). Angiotensin I produces a pressor effect by being converted to angiotensin II, presumably by a converting enzyme in the test animals (Opdyke and Holcombe, 1976; Nishimura *et al.*, 1978). The injected kidney extract (renin) works on angiotensinogen in the test animals to form angiotensin I and subsequently angiotensin II. Therefore, injections of homologous kidney extracts into eels (Chester Jones *et al.*, 1966) or the bullfrog, *Rana catesbeiana* (Johnston *et al.*, 1967), produce a

TABLE 2.—*Vasopressor action of angiotensin in nonmammalian vertebrates. Abbreviations are: AI, angiotensin I; AII-amide, [1-asparagine, 5-valine]-angiotensin II.*

Species	Experimental conditions	Injected substance	Blood pressure (route)	Reference
Elasmobranchs				
Spiny dogfish <i>Squalus acanthias</i>	Unanesthetized (seawater)	[Asp ¹ , Ile ⁶]-AI (human AI) or AII-amide	Increased (dorsal aorta)	Opdyke and Holcombe, 1976
Teleosts				
European eel <i>Anguilla anguilla</i>	Unanesthetized (freshwater)	AII-amide or extract from corpuscles of Stannius	Increased (pneumogastric artery)	Chester Jones <i>et al.</i> , 1966
American eel <i>Anguilla rostrata</i>	Unanesthetized (freshwater)	Natural eel angiotensin*	Increased (dorsal aorta)	Nishimura and Sawyer, 1976
Toadfish <i>Opsanus tau</i>	Unanesthetized (50% seawater)	Natural toadfish angiotensin* or AII-amide	Increased (celiac artery)	Zucker and Nishimura, (unpublished)
Lungfishes				
African lungfish <i>Protopterus aethiopicus</i>	Unanesthetized (freshwater)	AII-amide	Increased (caudal artery or celiac artery)	Sawyer, 1970
Australian lungfish <i>Neoceratodus forsteri</i>	Unanesthetized (freshwater)	AII-amide	Increased (dorsal aorta)	Sawyer <i>et al.</i> , 1976
Amphibians				
Frog <i>Rana esculenta</i>	Anesthetized (urethane)	AII-amide or natural amphibian angiotensin*	Slight or no increase	Grill <i>et al.</i> , 1972
Frog <i>Rana catesbeiana</i>	Pithed or hypophysectomized and anesthetized (tricaine methane-sulphonate)	Homologous kidney extract (renin preparation) or AII-amide	Prolonged increase (sciatic artery)	Johnston <i>et al.</i> , 1967
Frog <i>Rana catesbeiana</i>	Hypophysectomized and anesthetized (tricaine methane-sulphonate)	Kidney extract from carp, <i>Cyprinus carpio</i> , natural carp angiotensin** or AII-amide	Increased (sciatic artery)	Taylor and Davis, 1971
Reptiles				
Freshwater turtle <i>Pseudemys sueanniensis</i>	Anesthetized (sodium pentobarbital) and given dexamethasone	Homologous kidney extract or natural turtle angiotensin**	Increased (carotid artery)	Nothstine <i>et al.</i> , 1971
Crocodile <i>Caiman sclerops</i>	Anesthetized (sodium pentobarbital) and given dexamethasone	Homologous kidney extract	Increased (sciatic artery), but no property resembling renin	Nothstine <i>et al.</i> , 1971
Birds				
Chicken <i>Gallus domesticus</i>	Hypophysectomized and anesthetized (sodium pentobarbital)	Homologous kidney extract	Increased (sciatic artery)	Taylor <i>et al.</i> , 1970
Chicken <i>Gallus domesticus</i>	Anesthetized (sodium pentobarbital)	Homologous kidney extract, natural chicken angiotensin** or AII-amide	Increased (sciatic artery)	Taylor <i>et al.</i> , 1970

*Kidney extract was incubated with homologous plasma under inhibition of angiotensinases. Produced pressor substance was extracted with a modification of the method of Boucher *et al.* (1967). **Kidney extract was incubated with homologous plasma under inhibition of angiotensinases. The supernatant of the incubation mixture was injected.

prolonged pressor effect, whereas injection of angiotensin produces quicker and shorter vasopressor responses. No study has been done on the pressor effect of angiotensin or kidney extract in the cyclostomes.

Although the vasopressor action of angiotensins seems to be phylogenetically old, the properties of angiotensin receptors of the vascular smooth muscles appear to differ in primitive animals from those in mammals. The pharmacological properties of the angiotensin receptors and the structure-activity relationship in synthetic angiotensin analogs have been extensively studied in mammals (Khosla *et al.*, 1974; Regoli *et al.*, 1974). Angiotensin analogs in which the phenylalanine in position 8 of the peptide chain was replaced by aliphatic residues (leucine, valine, isoleucine) act as specific antagonists against the vasopressor action of angiotensin (Türker *et al.*, 1974), as well as against myotropic action *in vitro*. Antagonistic properties of some 8-substituted angiotensin II analogs are enhanced when aspartic acid in position 1 is replaced with sarcosine (Hall *et al.*, 1974).

[1-sarcosine, 8-threonine]-angiotensin II (10 $\mu\text{g/kg}$. per min.), which possesses a polar group on the aliphatic side chain of position 8, did not inhibit in eels the pressure rises produced either by angiotensin II-amide or natural eel angiotensin, although the responses to both angiotensin II-amide and eel angiotensin were blocked in the rat after 1 $\mu\text{g/kg}$. per minute of this antagonist (Nishimura *et al.*, 1978). Infusion of 1 $\mu\text{g/kg}$. per minute of [1-sarcosine, 8-isoleucine]-angiotensin II, the dose which effectively inhibits angiotensin's pressor action in mammals, did not decrease pressor responses in eels. These findings appear to suggest that the properties of the angiotensin receptors of the blood vessels differ in eels from those in mammals.

On the other hand, [8-thienylalanine]-angiotensin II, which has the aromatic group in position 8, evoked considerable agonist pressor action in eels. There were no differences between rats and eels in agonistic pressor potency of either angiotensin II-amide or [8-thienylalanine]-angiotensin II when responses were expressed as percentages of increases in blood pressure from preinjection levels (Nishimura *et al.*, 1978).

In mammals, the structure of position 8 of angiotensin II is important in determining the affinity of angiotensin to receptors, as well as acting as a part of the molecule with transcribed information to evoke intrinsic activity, presumably through the secondary mechanisms in the receptor (Türker, 1974; Bumpus, 1977). In eels, the aromatic group in position 8 of the octapeptide may be necessary both for inducing intrinsic pressor action and for binding to the receptors on the vascular smooth muscles. The analogs substituted in position 8 by the aliphatic group may not be bound, or may be bound only weakly, to the receptors. It may be possible also that the local environment, such as pH or ionic composition of the interstitial fluid (Marshall, 1976), differs in eels and thus alters the responsiveness to angiotensin derivatives.

Release of adrenal catecholamines by angiotensins has been shown in both *in vivo* and *in vitro* isolated adrenal preparations in various mammalian species (Peach, 1974; Peach and Ackerly, 1976). Alpha adrenergic blocking drugs, phentolamine and phenoxybenzamine, reduced the vasopressor action of angiotensin II-amide in eels approximately 30 per cent (Nishimura *et al.*, 1978). Repeated injection of reserpine in eels also reduced the pressor

response to angiotensin II-amide. These findings suggest that a part of the vasopressor action of angiotensin may be ascribed to release of catecholamines either from the chromaffin tissue cells or the adrenergic nerve ends.

Opdyke and Holcombe (1976) found that pressor responses to both angiotensin II and norepinephrine were blocked completely by phentolamine in the dogfish, *Squalus acanthias*. These authors suggest that the pressor response of angiotensin may be entirely ascribed to release of catecholamines, or that angiotensin and norepinephrine may act through a common receptor in this species.

Increase in Steroid Secretion

Angiotensin or homologous kidney extract (renin preparation) increased aldosterone and/or corticosterone secretion in several nonmammalian animals (Table 3). Intravenous administration of the homologous kidney extract increased aldosterone (Johnston *et al.*, 1967) or aldosterone and corticosterone (Taylor *et al.*, 1972) secretion into the postcaval vein in the hypophysectomized and anesthetized frog, and increased both aldosterone and corticosterone secretion in the pithed frog (Johnston *et al.*, 1967). Corticosterone secretion increased in the anesthetized and dexamethazone treated turtle, *Pseudemys sueanniensis*, after infusion of homologous kidney extract, but no increase in steroid secretion occurred in a crocodile, *Caiman sclerops* (Nothstine *et al.*, 1971), or cockerel, *Gallus domesticus* (Taylor *et al.*, 1970). In these experiments, however, arterial blood pressure, and often postcaval plasma flow also, increased after infusion of the kidney extract.

The adrenal (interrenal) tissue of teleosts does not compose a coherent organ, and it is thus difficult to collect venous effluent from the interrenal tissues to determine angiotensin's effect on steroid secretion. Taylor and Davis (1971) noted that an injection of natural carp angiotensin (product upon incubation of the kidney extract with homologous plasma) into hypophysectomized and anesthetized frogs increased aldosterone and corticosterone secretion. On the other hand, carp kidney extract increased blood pressure but had no effect on steroid secretion. Injection of eel kidney extract or angiotensin II-amide into intact, hypophysectomized, or Stanniectomized (remove corpuscles of Stannius) eels increased the cortisol level in the arterial blood (Henderson *et al.*, 1976). Inasmuch as blood pressure increased concomitantly, the possibility cannot be excluded that the increased cortisol secretion may be accounted for by the increased blood flow that perfuses the interrenal cells.

Angiotensin II-amide, added to the incubation medium, failed to increase corticoid production of chicken adrenal tissue *in vitro* (DeRoos and DeRoos, 1963).

Renal Action

Administration of angiotensin usually produced dual effects in normotensive man and other mammals: antidiuresis and antinatriuresis at relatively

TABLE 3.—Stimulation of adrenocorticosteroid secretion by angiotensins in nonmammalian vertebrates. Abbreviations and symbols as in Table 2.

Species	Experimental conditions	Injected substance	Steroid Secretion		Reference
			Source	Steroids	
Teleosts					
European eel <i>Anguilla anguilla</i>	Intact or hypophysectomized (freshwater)	Eel kidney extract	<i>Pneumogastric artery</i>	Henderson <i>et al.</i> , 1976 plasma level of cortisol	<i>al.</i> , 1976
European eel <i>Anguilla anguilla</i>	Hypophysectomized or Stannius corpuscles removed (freshwater)	All-amide	<i>Pneumogastric artery</i>	Increased plasma level of cortisol	Henderson <i>et al.</i> , 1976
Amphibians					
Frog <i>Rana catesbeiana</i>	Hypophysectomized and anesthetized (tricaine methanesulphonate)	Homologous kidney extract (renin preparation)	<i>Postcaval vein</i>	Increased aldosterone secretion	Johnston <i>et al.</i> , 1967
Frog <i>Rana catesbeiana</i>	Hypophysectomized and anesthetized (tricaine methanesulphonate)	Homologous kidney extract (renin preparation)	<i>Postcaval vein</i>	Increased aldosterone and corticosterone secretion	Taylor <i>et al.</i> , 1972
Frog <i>Rana catesbeiana</i>	Pithed	Homologous kidney extract (renin preparation)	<i>Postcaval vein</i>	Increased aldosterone and corticosterone secretion	Johnston <i>et al.</i> , 1967
Frog <i>Rana catesbeiana</i>	Hypophysectomized and anesthetized (tricaine methanesulphonate)	Carp kidney extract (renin preparation) or All-amide	<i>Postcaval vein</i>	No increase in steroid secretion	Taylor and Davis, 1971
Frog <i>Rana catesbeiana</i>	Hypophysectomized and anesthetized (tricaine methanesulphonate)	Natural carp angiotensin**	<i>Postcaval vein</i>	Increased aldosterone and corticosterone secretion	Taylor and Davis, 1971
Reptiles					
Freshwater turtle <i>Pseudemys sueanniensis</i>	Anesthetized (sodium pentobarbital) and dexamethasone treated	Homologous kidney extract	<i>Postcaval vein</i>	Increased corticosterone secretion	Nothstine <i>et al.</i> , 1971
Crocodile <i>Caiman sclerops</i>	Anesthetized (sodium pentobarbital) and dexamethasone treated	Homologous kidney extract	<i>Postcaval vein</i>	No increase in steroid secretion	Nothstine <i>et al.</i> , 1971
Birds					
Cockerel <i>Gallus domesticus</i>	Hypophysectomized and anesthetized (sodium pentobarbital)	Homologous kidney extract	<i>Adrenal vein</i>	No increase in aldosterone or corticosterone secretion	Taylor <i>et al.</i> , 1970
Cockerel <i>Gallus domesticus</i>	<i>In vitro</i> incubation	All-amide	<i>Adrenal gland</i>	No increase in corticoid production	DeRoos and DeRoos, 1963

small doses, and natriuresis at higher doses (Peters and Bonjour, 1971). Renal effects of angiotensin have been studied in several nonmammalian species (Table 4). Synthetic angiotensin II (Chester Jones *et al.*, 1969; Nishimura and Sawyer, 1976) and homologous natural eel angiotensin (Nishimura and Sawyer, 1976) caused natriuresis and diuresis with concomitant increases in glomerular filtration rate and dorsal aortic pressure (Fig. 5). Diuresis occurred only after clear vasopressor doses of angiotensins sug-

TABLE 4.—Renal action of angiotensins in primitive vertebrates. Symbols are: V, urine flow; C_{IN} , inulin clearance; Na, urinary Na excretion; AII, angiotensin II; GFR, glomerular filtration rate. Asterisks denote same references as in Table 1.

Species	Experimental conditions	Injected substance and route	Renal responses	Reference
Teleosts				
European eel <i>Anguilla anguilla</i>	Unanesthetized, Freshwater	AII-amide, pressor dose (pneumatic duct vein)	Increased V, C_{IN} , and Na	Chester Jones <i>et al.</i> , 1969
American eel <i>Anguilla rostrata</i>	Unanesthetized, Freshwater	AII-amide, pressor dose (dorsal aorta) or natural eel angiotensin*, pressor dose (dorsal aorta)	Increased V, C_{IN} , and Na	Nishimura and Sawyer, 1976
Lungfishes				
African lungfish <i>Protopterus aethiopicus</i>	Unanesthetized, Freshwater	AII-amide, high dose (caudal vein or ventral intestinal vein)	Mild diuresis and natriuresis	Sawyer, 1970
Australian lungfish <i>Neoceratodus forsteri</i>	Unanesthetized, Freshwater	AII-amide, prolonged infusion of high dose (pulmonary or hepatic vein)	Moderate diuresis and natriuresis	Sawyer <i>et al.</i> , 1976
Amphibians				
<i>Xenopus laevis</i>	Unanesthetized, distilled water	AII-amide, high dose (abdominal vein)	Increased V and Na; no change in GFR	Henderson and Edwards, 1969
<i>Xenopus laevis</i>	Unanesthetized and 0.65% NaCl infused	AII-amide (abdominal vein)	Decreased V and C_{IN}	Henderson and Edwards, 1969
Toad <i>Bufo paracnemis</i>	Isolated kidney perfused with Ringer's solution	AII-amide (renal portal vein)	Antidiuresis and antinatriuresis	Coviello, 1969
Toad <i>Bufo paracnemis</i>	Isolated urinary bladder	AII	Increased water permeability	Coviello, 1972
Birds				
Chicken <i>Gallus domesticus</i>	Anesthetized (sodium pentobarbital)	AII-amide, high dose (leg vein)	Increased V and Na; Na; decreased C_{IN}	Langford and Fallis, 1966

gesting that angiotensin diuresis may largely be a consequence of increased renal perfusion pressure. Norepinephrine increased dorsal aortic pressure of eels, as did the angiotensin, but urine flow and filtration rate decreased (Nishimura and Sawyer, 1976). Mild to moderate diuresis and natriuresis was produced by [1-asparagine, 5-valine]-angiotensin II in the African lungfish, *Protopterus aethiopicus* (Sawyer, 1970), and in the Australian lungfish, *Neoceratodus forsteri* (Sawyer *et al.*, 1976).

The angiotensin effect on amphibian kidneys varies (Table 4). Angiotensin II-amide increased urine flow and Na excretion without change in filtration rate in *Xenopus laevis* that were maintained in distilled water and had a high glomerular filtration rate (GFR) (Henderson and Edwards, 1969). This suggests that angiotensin may have inhibited Na reabsorption in the renal tubules. However, in *Xenopus* infused with isotonic saline and which showed high urinary Na excretion, angiotensin seems to have caused glomerular antidiuresis. Angiotensin II-amide injected into the renal portal vein of an isolated toad kidney, *Bufo paracnemis*, so that angiotensin reached the renal tubules without influencing glomerular circulation, caused antidiuresis and antinatriuresis (Coviello, 1969). This observation

FIG. 5.—Typical renal responses of a freshwater-adapted and saline-loaded eel, *Anguilla rostrata* (FW, 2989.), to intra-arterial infusions of small and large doses of semipurified natural eel angiotensin (presumably angiotensin I). C_{IN} , insulin clearance; V, urine flow; BP, mean dorsal aortic pressure; $U_{Na}V$ and U_KV , urinary sodium and potassium excretion; U_{Na} and U_K , urinary sodium and potassium concentration. The doses of eel angiotensin are expressed as nanograms of synthetic angiotensin II-amide with equivalent rat vasopressor activities. (Reproduced from Nishimura and Sawyer, 1976.)

agrees with the finding that angiotensin II increased water permeability of the urinary bladder in *Bufo paracnemis* (Coviello, 1972). The variability of findings in amphibians may largely be ascribed to the status of fluid and mineral balance of test animals, endogenous level of mineralocorticoids and angiotensin levels, and to other hemodynamic or humoral mechanisms. Responses in isolated kidneys and in intact animals may also differ.

Angiotensin II-amide infused into the leg vein of the chicken eventually perfuses the renal tubules independently of glomerular circulation (Langford and Fallis, 1966). Diuresis and natriuresis in spite of decreased glomerular filtration rate were more evident at the angiotensin's infusion site. Thus, angiotensin may inhibit tubular sodium reabsorption in birds.

Stimulation of Drinking by Angiotensin

Intracranial injection of angiotensin II-amide induced drinking in the white crowned sparrow, *Zonotrichia leucophrys gambelii* (Wada *et al.*, 1975), domestic fowl (Snapir *et al.*, 1976; Schwob and Johnson, 1977), and in the Japanese quail, *Coturnix coturnix japonica* (Takei, 1977). Sensitive sites to angiotensin are the preoptic area, lateral and anterior hypothalamus, and third ventricle. The minimum effective dose was 5 ng. in the quail (Takei, 1977), where water intake increased in a dose-dependent manner, but a higher dose was necessary to stimulate drinking behavior in the white crowned sparrow and fowl. The effective dosage and sensitive region in the quail are similar to those of the rat.

Summary

The vasopressor action of angiotensins has been demonstrated in representative species of vertebrate classes from elasmobranchs to mammals. Properties of the angiotensin receptors in primitive animals, however, may differ from those in mammals.

Angiotensin or homologous kidney extract (renin preparation) increases mineralocorticoid secretion in a teleost, amphibian, and reptile. We cannot exclude the possibility, however, that increased mineralocorticoid secretion is due to increased blood flow to adrenocortical tissues.

Angiotensin appears to produce glomerular diuresis in the teleost, and perhaps in the lungfish, presumably due to increased renal perfusion pressure. Angiotensin's effects in amphibian kidneys are conflicting.

Angiotensin may release catecholamines in elasmobranchs and teleosts.

Intracranial injection of angiotensin stimulates drinking in birds.

FACTORS REGULATING ENDOGENOUS RENIN ACTIVITY IN NONMAMMALIAN VERTEBRATES

Hemodynamic Factors and the Sympathetic Nervous System

Kidneys from primitive vertebrates provide a pertinent model for elucidating a possible participation of the vascular receptor, which presumably

exists in the granulated cells, and the role of hemodynamic factors in renin release. Inasmuch as angiotensin from the aglomerular toadfish, *Opsanus tau*, is bound to human angiotensin I antibody (Nishimura *et al.*, 1977), the radioimmunoassay method is usable for determining plasma renin activity (rate of angiotensin I generation). The sensitive radioimmunoassay makes it possible to determine changes of plasma renin activity in a given animal by repeatedly drawing blood samples of small amounts.

Toadfish kidneys have neither functioning glomeruli nor a macula densa, but possess granulated epithelioid cells similar to mammalian juxtaglomerular cells in the small arteries and arterioles (Oguri *et al.*, 1972). Plasma renin activity increased markedly in unanesthetized toadfish after a cumulative hemorrhage, a single massive hemorrhage (Fig. 6), or after injection of a vasodilator, papaverine (Nishimura *et al.*, 1979). These results suggest that renin was released in toadfish, despite an absence of a macula densa, in response to a decrease in blood pressure and/or blood volume.

Blaine *et al.* (1970) developed a nonfiltering kidney model in a dog by ligating and sectioning the ureters with a 2-hour period of bilateral clamping of renal arteries so that the macula densa was made nonfunctional. Hemorrhage increased renin secretion from the nonfiltering and denervated kidney in the absence of a change in plasma renin substrate, presumably by stimulating the afferent arteriolar receptor of the juxtaglomerular apparatus (for review, see Davis and Freeman, 1976). Sodium delivery to the renal tubule is therefore not essential for renin release after hemorrhage in either the dog or the toadfish.

Although the presence of intact renal nerves is not essential for renin release after hemorrhage, renal nerves participate in increased renin secretion, because renin release occurs in the innervated nonfiltering kidney of the dog in which the function of the arteriolar receptor was blocked by intrarenal infusion of papaverine (Witty *et al.*, 1971). Thus, it has to be clarified in the toadfish whether a reduction in renal perfusion pressure due to decreased systemic pressure may stimulate a possible vascular receptor in the granulated cells, or whether decreased blood pressure or blood volume may affect the activity of granulated cells reflexly by signals transmitted by the renal nerves.

Beta adrenergic receptors, supposedly located in the juxtaglomerular cells, appear to mediate renin release (Johnson *et al.*, 1976). Intrarenal infusion of isoproterenol, norepinephrine, and electrical stimulation of renal nerves cause renin release in the dog, which is blocked by beta adrenergic blocking drugs (for review, see Davis and Freeman, 1976). Systemic injection of isoproterenol into the aglomerular toadfish increased plasma renin activity with a concomitant decrease of blood pressure (Nishimura *et al.*, and unpublished data). It remains to be determined, however, whether the increase in renin activity may be ascribed to direct stimulation of the granulated cells by isoproterenol or whether decreased blood pressure caused renin release. A ganglionic blocking drug, pentolinium tartrate (Ansolsen), did

PLASMA RENIN ACTIVITY AND BLOOD PRESSURE AFTER A SINGLE MASSIVE HEMORRHAGE

FIG. 6.—Renin and blood pressure responses to a single massive hemorrhage (60 per cent of estimated blood volume) in an unanesthetized toadfish. In the time control study, blood samples were taken periodically without a massive hemorrhage. Dextran-saline solution was replaced after each blood sample collection (350 μ l), both in time control and hemorrhage experiments.

not inhibit the increase in plasma renin activity in the toadfish after papaverine.

Decreased renal and hepatic clearances could contribute to the increase in plasma renin activity during hypotension in the toadfish. The contribution is probably minor, because the acute and drastic increase in plasma renin

after hemorrhage is far beyond the increase that can be accounted for by decreased renin clearance.

Chan and Holmes (1971) found that acute hemorrhage increased plasma renin activity in intact and hypophysectomized pigeons proportionately to the amount of blood withdrawn. Hypophysectomized pigeons showed low blood pressure and high plasma renin levels, when compared to intact pigeons.

It has been shown in mammals that cardiopulmonary receptors are involved in renin release (Zehr *et al.*, 1976). An increase in left atrial pressure, which reflects a change of blood volume, reflexly reduces the rates of renin secretion via vagal afferent and renal sympathetic efferent pathways. Isosmotic volume expansion produced in the Australian lungfish, *Neoceratodus forsteri*, by injecting isosmotic saline decreased plasma renin activity (Blair-West *et al.*, 1977). The receptor and pathway through which the stimulus is transmitted, however, remain to be determined.

Hemorrhage and hypotension appear to be potent stimuli for increasing plasma renin activity in an aglomerular teleost, a bird, and mammals. The observation by Johnston *et al.* (1967) that aldosterone increases markedly after hemorrhage may indicate that renin is released, and thus aldosterone increased, in response to hemorrhage in this species. When we consider these physiological observations and the anatomical evidence that the evolution of the juxtaglomerular cells precedes that of the macula densa, together with the fact that vasopressor actions of angiotensin have been noted extensively among various classes of vertebrates (Fig. 7), one might speculate that the renin-angiotensin system evolved as a humoral regulator of blood pressure and/or blood volume homeostasis. It is necessary to determine renin responses to hemodynamic factors in glomerular teleosts, amphibians, and reptiles.

Sodium Balance

Because the renin-angiotensin system stimulates aldosterone secretion, and plasma renin activity increased in response to sodium depletion in man and other mammals, evidence for homologous function has been sought in primitive animals (Table 5). There is no clear indication, however, that the renin-angiotensin system helps to conserve sodium in teleosts or amphibians exposed to hyposmotic media. There is no significant difference in plasma renin activity between *Tilapia* adapted to seawater and those transferred from seawater to freshwater (Malvin and Vander, 1967). Plasma renin levels decreased in American eels (Nishimura *et al.*, 1976) and in European eels (Henderson *et al.*, 1976), but showed no change in aglomerular toadfish adapted to hyposmotic media (Nishimura *et al.*, 1976). Plasma levels of cortisol, a major mineralocorticoid in teleosts, remained relatively constant both in eels and toadfish after transfer to hyposmotic environments, whereas plasma sodium concentrations decreased in most animals. There was no difference in plasma renin activity between toads adapted to distilled water and

FIG. 7.—Current summary of function of the renin-angiotensin system in various vertebrate classes.

those adapted to saline, although glomerular filtration rate and the secretion rate of adrenocorticosteroids are much higher in toads in distilled water (Garland and Henderson, 1975). Contrarily, plasma renin levels and plasma renin and cortisol levels showed a transient increase after transfer in the opposite direction, from freshwater to seawater (Sokabe *et al.*, 1973; Henderson *et al.*, 1976) (Table 6).

The histological appearance of juxtaglomerular cells in fishes from media of various salinities seems to be compatible with the observed changes in plasma renin activity. Granular epithelioid cells showed histological involutional changes in *Cymatogaster* moved from bay water (68 per cent seawater) to hypotonic media (Lagios, 1968). Krishnamurthy and Bern (1973) noted that juxtaglomerular cells of *Tilapia mossambica* increased in number and size following transfer from freshwater to seawater (Table 6). Their number reached a maximum at 12 days and returned toward the original freshwater level by 30 days. Juxtaglomerular cells of the three-spined stickleback, *Gasterosteus aculeatus*, were more prominent in fish from seawater than in those adapted to freshwater (Wendelaar Bonga, 1973).

Previously available reports on the relation of external salinity to renal renin are, however, conflicting. The renin content of the kidney decreased

TABLE 5.—Activities of the renin-angiotensin system in sodium-depleted animals. Abbreviations are SW, seawater; FW, freshwater.

Species	Sodium depletion	Activity	Reference
<i>Plasma Renin Activity</i>			
Teleosts			
<i>Tilapia mossambica</i>	SW to FW	No change	Malvin and Vander, 1967
American eel	SW to FW	Decreased	Nishimura <i>et al.</i> , 1976
<i>Anguilla rostrata</i>			
Agglomerular toadfish	50% SW to 5% SW	No change	Nishimura <i>et al.</i> , 1976
<i>Opsanus tau</i>			
European eel	SW to FW	Decreased	Henderson <i>et al.</i> , 1976
<i>Anguilla anguilla</i>			
Amphibians			
<i>Bufo marinus</i>	Distilled water	No change	Garland and Henderson, 1975
Primitive Mammals			
Echidna	Furosemide injection	Increased	Reid, 1971
<i>Tachyglossus aculeatus</i>			
<i>Renal Renin Activity</i>			
Teleosts			
Agglomerular toadfish	2.8% NaCl to	Increased	Capelli <i>et al.</i> , 1970
<i>Opsanus beta</i>	0.009% NaCl		
<i>Alosa sapidissima</i>	2.7% NaCl to	Increased	Capelli <i>et al.</i> , 1970
	0.004% NaCl		
Amphibians			
Frog	Distilled water,	No change	Nolly and Fasciolo, 1971
<i>Bufo arenarum</i>	furosemide injection		
	or peritoneal dialysis		
Frog	Deionized water	Increased	Capelli <i>et al.</i> , 1970
<i>Rana pipiens</i>			
Birds			
Cockerel	Low Na diet plus	Increased	Taylor <i>et al.</i> , 1970
<i>Gallus domesticus</i>	mercurial diuretic		
	drug		
Primitive mammals			
Echidna	Furosemide injection	Increased	Reid, 1971
<i>Tachyglossus aculeatus</i>			
<i>Juxtaglomerular Cells</i>			
Teleosts			
<i>Cymatogaster aggregata</i>	68% seawater to	Involuntal	Lagios, 1968
	hyposmotic media	change	
Birds			
Cockerel	Low Na diet plus	Increased	Taylor <i>et al.</i> , 1970
<i>Gallus domesticus</i>	mercurial diuretic	granularity	
	drug		

when Japanese eels or *Tilapia* from freshwater were adapted to seawater for over three weeks (Sokabe *et al.*, 1968, 1973). Kidney renin activity also was found to be higher in freshwater stenohaline teleosts or euryhaline teleosts adapted to hyposmotic media than in marine species (Mizogami *et al.*, 1968; Capelli *et al.*, 1970). Nolly and Fasciolo (1972), however, did not find any

TABLE 6.—Activities of the renin-angiotensin system in sodium-loaded animals. Symbols are: SW, seawater; FW, freshwater.

Species	Sodium load	Activity	Reference
<i>Plasma Renin Activity</i>			
Teleosts			
Japanese eel <i>Anguilla japonica</i>	FW to SW	Transiently increased	Sokabe <i>et al.</i> , 1973
European eel <i>Anguilla anguilla</i>	FW to SW	Increased	Henderson <i>et al.</i> , 1976
Amphibians			
Toad <i>Bufo marinus</i>	Hyperosmotic NaCl	No change	Garland and Henderson, 1975
<i>Renal Renin Activity</i>			
Teleosts			
Japanese eel <i>Anguilla japonica</i>	FW to SW	Decreased	Sokabe <i>et al.</i> , 1968
<i>Tilapia mossambica</i>	FW to SW	Decreased	Sokabe <i>et al.</i> , 1968
Amphibians			
Toad <i>Bufo arenarum</i>	NaCl solution	No change	Nolly and Fasciolo, 1971
<i>Juxtaglomerular Cells</i>			
Teleosts			
<i>Tilapia mossambica</i>	FW to SW	Increased in number and size	Krishnamurthy and Bern, 1973

significant difference among five species of teleosts from seawater or freshwater. There seems to be some discrepancy between plasma renin and renal renin levels, and between renal renin levels and the histological activity of juxtaglomerular cells. Renal renin activity is usually 100 to 1000 times higher than plasma renin activity in fishes. Decreases in plasma renin activity, which presumably reflect decreased renin secretion or increased renin metabolism, or both, may not necessarily be associated with measurable changes in renal renin. It is thus necessary to determine plasma renin activity, or both plasma renin activity and renal renin, to see the changes in renin-angiotensin system activity during the short periods of adaptation.

The relationships between salinity and renal renin activity are conflicting also in amphibians. There was no difference in renal renin activity between the toad, *Bufo arenarum*, loaded with sodium by immersion in saline and the toad depleted in sodium by keeping in distilled water, or treated with natriuretic drugs, or peritoneal dialysis (Nolly and Fasciolo, 1971). However, Capelli *et al.* (1970) reported that renal renin activity was higher in the frog, *Rana pipiens*, in distilled water than in those in 0.02 per cent NaCl water.

Renal renin activity and granularity of the juxtaglomerular cells increased in the cockerel, *Gallus domesticus*, sodium-depleted by maintenance on a low Na diet plus a mercurial diuretic drug (Taylor *et al.*, 1970). A monotreme, the spiny echidna, *Tachyglossus aculeatus*, responded to sodium depletion by increasing plasma renin activity and renal renin concentration (Reid, 1971). This primitive mammalian species exhibits juxtaglomerular cells and a macula densa that resemble those of more advanced mammals.

The question arises as to whether the renin-angiotensin-mineralocorticoid system exists in nonmammalian vertebrates. In mammals, plasma renin activity usually increases in response to sodium depletion, but plasma renin activity showed either a decrease or no change in sodium-depleted teleosts. On the other hand, plasma renin activity increased transiently during seawater adaptation. These apparently contradictory findings may be feasible if we consider that angiotensin may stimulate cortisol secretion in teleosts. Cortisol plays an important role in seawater osmoregulation in teleosts by promoting the extrusion of sodium across the gill and increasing water permeability of the gut. Both plasma renin activity (Sokabe *et al.*, 1973; Henderson *et al.*, 1976) and plasma cortisol (Ball *et al.*, 1971; Hirano, 1969; Hirano and Utida, 1971) increased during seawater adaptation of freshwater eels.

Although exogenous administration of homologous kidney extract or angiotensin increased secretion of aldosterone and corticosterone in the frog (Table 3), elevated endogenous mineralocorticoid levels seen in the toad adapted in distilled water do not accompany the increase in plasma renin activity (Garland and Henderson, 1975). Because plasma renin levels were determined by the rat vasopressor bioassay, which requires a considerable amount of blood samples, the collection of blood samples itself may have produced renin release and masked the small changes of plasma renin levels. It is interesting to note that sodium depletion appears to stimulate the renin-angiotensin system in birds and a monotreme, which possess a macula densa.

Renal Function

Functional interrelationships among the macula densa, juxtaglomerular cells, and the glomerulus have been suggested (Guyton *et al.*, 1964). A possible intrarenal feedback mechanism (the macula densa senses some changes in composition or flow of renal tubular fluid and influences the rate of glomerular filtration in the same nephron) has been tested by means of the micropuncture method (Davis and Freeman, 1976). In this feedback mechanism, juxtaglomerular cells and the renin-angiotensin system may be involved in regulating single-nephron filtration rate. Thureau and coworkers (Thureau *et al.*, 1972; Thureau, 1974) demonstrated that retrograde perfusion of the macula densa with isotonic or hypertonic sodium chloride solutions increased the renin activity in a single juxtaglomerular apparatus. They proposed that the renin-angiotensin system regulates GFR by constricting the afferent arteriole upon the signals transmitted from the macula densa. More recent study indicates that feedback responses depend critically upon the rate of chloride transport across the macula densa (Schnermann *et al.*, 1976).

Sokabe proposed the hypothesis that the renin-angiotensin system regulates GFR by constricting the efferent arterioles (Sokabe, 1974; Sokabe and

Ogawa, 1974). Plasma renin activity decreased by dehydration with a decrease in GFR, but both GFR and plasma renin activity increased after infusion of isotonic saline or glucose solution (Sokabe *et al.*, 1972). Constriction of the efferent arteriole was observed microscopically under direct observation of the glomerular circulation.

In teleost fishes, however, increase in plasma renin activity is not correlated with increases in filtration rate. When euryhaline teleosts, which can survive either in seawater or freshwater by maintaining internal osmolality relatively constant, are introduced from hyper to hyposmotic media, the kidney plays a role in adaptation in a biphasic manner (Hickman and Trump, 1969). Glomerular filtration rate increases immediately (primary adjustment), and renal tubules become less permeable to water within a few days (secondary adjustment), which results in producing a large amount of dilute urine in order to excrete excess water invading along the osmotic gradient. However, plasma renin activity decreased in eels during freshwater adaptation (Henderson *et al.*, 1976; Nishimura *et al.*, 1976), and it increased during seawater adaptation with a concomitant decrease of GFR (Sokabe *et al.*, 1973; Henderson *et al.*, 1976). Exogenous administrations of angiotensins, however, are accompanied by increased filtration rate (Chester Jones *et al.*, 1969; Nishimura and Sawyer, 1976). The functions of endogenous and exogenous angiotensin may not be the same. It has to be considered, also, that measurement of plasma renin activity by the bioassay method may not be adequate to detect small changes (Nishimura *et al.*, 1977).

Summary

Hemorrhage and acute hypotension appear to be potent stimuli for causing renin release in the aglomerular teleost, which lacks both macula densa and glomeruli. A reduction in blood pressure and/or blood volume may stimulate a possible vascular receptor of the granulated cells either directly or indirectly through the extrarenal mechanisms. Hemorrhage increased plasma renin activity in a bird.

There is no evidence that the renin-angiotensin system is stimulated in sodium-depleted teleosts or amphibians. Both the plasma renin activity and plasma cortisol level increased during seawater adaptation in teleosts. The renin-angiotensin-mineralocorticoid system may exist in primitive animals.

An intrarenal feedback mechanism for regulating glomerular filtration rate and close interrelationships among juxtaglomerular cells, GFR, and macula densa has been suggested in mammals. The renin-angiotensin system may participate in the regulation of GFR in amphibians by working on the efferent arterioles of the glomeruli, although the actions of endogenous and exogenous angiotensins appear to differ.

It is necessary to determine the sympathetic innervation of the juxtaglomerular cells and whether adrenergic receptors are involved in the mechanisms of renin release in nonmammalian vertebrates.

ACKNOWLEDGMENTS

Studies leading to the unpublished observations mentioned in the text were supported by Research Grants from the National Science Foundation, PCM 75-20645, a Grant-in-Aid from the American Heart Association (74-680), and by Research Grant AM 17824 from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

LITERATURE CITED

- ANDERSON, B., AND K. OLSSON. 1977. Evidence for periventricular sodium-sensitive receptors of importance in the regulation of ADH secretion. Pp. 118-127, in *Neurohypophysis; International conference on the neurohypophysis* (A. M. Moses, and L. Share, eds.), Karger, Basel, x+253 pp.
- ÄNGGÅRD, E., C. LARSSON, AND P. WEBER. 1976. Interactions between the renal prostaglandins and the renin-angiotensin system. Pp. 587-594, in *Advances in prostaglandin and thromboxane research* (B. Samuelsson and R. Paoletti, eds.), Raven Press, New York, 2:xvi+507-1028 pp.
- BAKHLE, Y. S. 1974. Converting enzyme *in vitro* measurement and properties. Pp. 41-80, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- BALL, J. N., I. CHESTER JONES, M. E. FORSTER, G. HARGREAVES, E. F. HAWKINS, AND K. P. MILNE. 1971. Measurement of plasma cortisol levels in the eel, *Anguilla anguilla*, in relation to osmotic adjustments. *J. Endocrinol.*, 50:75-96.
- BARAJAS, L. 1964. The innervation of the juxtaglomerular apparatus. An electron microscopic study of the innervation of the glomerular arterioles. *Lab. Invest.*, 13:916-929.
- . 1970. The ultrastructure of the juxtaglomerular apparatus as disclosed by three-dimensional reconstructions from serial sections. The anatomical relationship between the tubular and vascular components. *J. Ultrastruct. Res.*, 33:116-147.
- BARAJAS, L., AND H. LATTA. 1967. Structure of the juxtaglomerular apparatus. *Circ. Res.*, 20/21(suppl. II):II-15-II-28.
- BLAINE, E. H., J. O. DAVIS, AND R. T. WITTY. 1970. Renin release after hemorrhage and after suprarenal aortic constriction in dogs without sodium delivery to the macula densa. *Circ. Res.*, 27:1081-1089.
- BLAIR-WEST, J. R., J. P. COGLAN, D. A. DENTON, J. W. FUNDER, B. A. SCOGGINS, AND R. D. WRIGHT. 1971. The effect of the heptapeptide (2-8) and hexapeptide (3-8) fragments of angiotensin II on aldosterone secretion. *J. Clin. Endocrinol. Metab.*, 32:575-578.
- BLAIR-WEST, J. R., J. P. COGLAN, D. A. DENTON, A. P. GIBSON, C. J. ODDIE, W. H. SAWYER, AND B. A. SCOGGINS. 1977. Plasma renin activity and blood corticosteroids in the Australian lungfish, *Neoceratodus forsteri*. *J. Endocrinol.*, 74:137-142.
- BOUCHER, R., J. MENARD, AND J. GENEST. 1967. A micromethod for measurement of renin in the plasma and kidney of rats. *Canadian J. Physiol. Pharmacol.*, 45:882-890.
- BUMPUS, F. M. 1977. Mechanisms and sites of action of newer angiotensin agonists and antagonists in terms of activity and receptor. *Fed. Proc.*, 36:2128-2132.
- BUMPUS, F. M., AND R. R. SMEBY. 1968. Angiotensin. Pp. 62-98, in *Renal hypertension* (I. H. Page and J. W. McCubbin, eds.), Year Book Medical Publisher, Chicago, 493 pp.
- CAPELLI, J. P., L. G. WESSON, JR., AND G. E. APONTE. 1970. A phylogenetic study of the renin-angiotensin system. *Amer. J. Physiol.*, 218:1171-1178.
- CHAN, M. Y., AND W. N. HOLMES. 1971. Studies on a 'Renin-Angiotensin' system in the normal and hypophysectomized pigeon (*Columba livia*). *Gen. Comp. Endocrinol.*, 16:304-311.
- CHESTER JONES, I., I. W. HENDERSON, D. K. O. CHAN, J. C. RANKIN, W. MOSELY, J. J. BROWN, A. F. LEVER, J. I. S. ROBERTSON, AND M. TREE. 1966. Pressor activity in extracts of the corpuscles of Stannius from the European eel (*Anguilla anguilla* L.). *J. Endocrinol.*, 34:393-408.

- CHESTER JONES, I., D. K. O. CHAN, AND J. C. RANKIN. 1969. Renal function in the European eel (*Anguilla anguilla* L.): effects of the caudal neurosecretory system, corpuscles of Stannius, neurohypophysial peptides and vasoactive substances. *J. Endocrinol.*, 43:21-31.
- COVIELLO, A. 1969. Tubular effect of angiotensin II on the toad kidney. *Acta Physiol. Latinoamerica*, 19:73-82.
- . 1972. Hydrosmotic effect of angiotensin II: isolated toad bladder. *Acta Physiol. Latinoamerica*, 22:218-226.
- DAVIS, J. O. 1971. The renin-angiotensin system in the control of aldosterone secretion. Pp. 173-205, in *Kidney hormones*, (J. W. Fisher, ed.), Academic Press, New York, xviii+665 pp.
- . 1974. The renin-angiotensin system in the control of aldosterone secretion. Pp. 322-336, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- DAVIS, J. O., AND R. H. FREEMAN. 1976. Mechanisms regulating renin release. *Physiol. Rev.*, 56:1-56.
- DEROOS, R., AND C. C. DEROOS. 1963. Angiotensin II: its effects on corticoid production by chicken adrenals *in vitro*. *Science*, 141:1284.
- DICKINSON, C. J., AND C. M. FERRARIO. 1974. Central neurogenic effects of angiotensin. Pp. 408-416, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- EDWARDS, J. G. 1940. The vascular pole of the glomerulus in the kidney of vertebrates. *Anat. Rec.*, 76:381-389.
- ERDŐS, E. G. 1977. The angiotensin I converting enzyme. *Fed. Proc.*, 36:1760-1765.
- FITZSIMONS, J. T. 1972. Thirst. *Physiol. Rev.*, 52:468-561.
- FREEMAN, R. H., J. O. DAVIS, T. E. LOHMEIER, AND W. S. SPIELMAN. 1977. Des-Asp¹ angiotensin II: mediator of the renin-angiotensin system? *Fed. Proc.*, 36:1766-1770.
- GANONG, W. F. 1972. Sympathetic effects on renin secretion: mechanism and physiological role. Pp. 17-32, in *Advances in experimental medicine and biology: control of renin secretion*, (T. A. Assaykeen, ed.), Plenum Press, New York, 17:xii+1-290 pp.
- . 1977. The renin-angiotensin system and the central nervous system. *Fed. Proc.*, 36:1771-1775.
- GANTEN, D., J. S. HUTCHINSON, P. SCHELLING, U. GANTEN, AND H. FISCHER. 1976. The iso-renin angiotensin systems in extrarenal tissue. *Clin. Exp. Pharmacol. Physiol.*, 3:103-126.
- GARLAND, H. O., AND I. W. HENDERSON. 1975. Influence of environmental salinity on renal and adrenocortical function in the toad, *Bufo marinus*. *Gen. Comp. Endocrinol.*, 27:136-143.
- GRILL, G., P. GRANGER, AND K. THURAU. 1972. The renin angiotensin system of amphibians. I. Determination of the renin content of amphibian kidneys. *Pflugers Arch. European J. Physiol.*, 331:1-12.
- GUYTON, A. C., J. B. LANGSTON, AND G. NAVAR. 1964. Theory for renal autoregulation by feedback at the juxtaglomerular apparatus. *Circ. Res.*, 15(Suppl. I):I-187-I-196.
- HALL, M. M., M. C. KHOSLA, P. A. KHAIRALLAH, AND F. M. BUMPUS. 1974. Angiotensin analogs: the influence of sarcosine substituted in position 1. *J. Pharmacol. Exp. Ther.*, 188:222-228.
- HAYASHI, T., T. NAKAJIMA, AND H. SOKABE. 1978. Comparative studies on angiotensins. V. Structure of angiotensin formed by the kidney of Japanese goosefish and its identification by dansyl method. *Chem. Pharm. Bull.*, 26:215-219.
- HENDERSON, I. W., AND B. R. EDWARDS. 1969. Effect of angiotensin-II-amide on renal function in the clawed toad, *Xenopus laevis* Daudin. *J. Endocrinol.*, 44:iii-iv.
- HENDERSON, I. W., V. JOTISANKASA, W. MOSLEY, AND M. OGURI. 1976. Endocrine and environmental influences upon plasma cortisol concentrations and plasma renin activity of the eel, *Anguilla anguilla* L. *J. Endocrinol.*, 70:81-95.

- HICKMAN, C. P., AND B. F. TRUMP. 1969. The kidney. Pp. 91-239, in *Fish physiology* (W. S. Hoar and D. J. Randall, eds.), Academic Press, New York, 1:xiv+1-465 pp.
- HIRANO, T. 1969. Effects of hypophysectomy and salinity change on plasma cortisol concentration in the Japanese eel, *Anguilla japonica*. *Endocrinol. Japonica*, 16:557-560.
- HIRANO, T., AND S. UTIDA. 1971. Plasma cortisol concentration and the rate of intestinal water absorption in the eel, *Anguilla japonica*. *Endocrinol. Japonica*, 18:47-52.
- JOHNSON, J. A., J. O. DAVIS, AND R. T. WITTY. 1971. Effects of catecholamines and renal nerve stimulation on renin release in the nonfiltering kidney. *Circ. Res.*, 29:646-653.
- JOHNSON, J. A., J. O. DAVIS, R. W. GOTSHALL, T. E. LOHMEIER, J. L. DAVIS, B. BRAVERMAN, AND G. E. TEMPEL. 1976. Evidence for an intrarenal beta receptor in control of renin release. *Amer. J. Physiol.*, 230:410-418.
- JOHNSTON, C. I., J. O. DAVIS, F. S. WRIGHT, AND S. S. HOWARDS. 1967. Effects of renin and ACTH on adrenal steroid secretion in the American bullfrog. *Amer. J. Physiol.*, 213:393-399.
- KHAIRALLAH, P. A. 1971. Pharmacology of angiotensin. Pp. 129-171, in *Kidney hormones* (J. W. Fisher, ed.), Academic Press, New York, xviii+665 pp.
- KHOSLA, M. C., R. R. SNEY, AND F. M. BUMPUS. 1974. Structure-activity relationship in angiotensin II analogs. Pp. 126-161, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- KRISHNAMURTHY, V. G., AND H. A. BERN. 1969. Correlative and histologic study of the corpuscles of Stannius and the juxtaglomerular cells of teleost fishes. *Gen. Comp. Endocrinol.*, 13:313-335.
- . 1973. Juxtaglomerular cell changes in the euryhaline freshwater fish, *Tilapia mosambica*, during adaptation to sea water. *Acta Zool. (Stockholm)*, 54:9-14.
- LAGIOS, M. D. 1968. Granular epithelioid cell involution in the renal arteries of a euryhaline fish, *Cymatogaster*, adapted to hypotonic salinities. *Gen. Comp. Endocrinol.*, 11:248-250.
- LANGFORD, H. G., AND N. FALLIS. 1966. Diuretic effect of angiotensin in the chicken. *Proc. Soc. Exp. Biol. Med.*, 123:317-321.
- LARAGH, J. H., AND J. E. SEALEY. 1973. The renin-angiotensin-aldosterone hormonal system and regulation of sodium, potassium, and blood pressure homeostasis. Pp. 831-908, in *Handbook of physiology, Section 8, Renal physiology* (J. Orloff and R. W. Berliner, section eds.), Amer. Physiol. Soc., Washington, D.C., xiii+1082.
- LARAGH, J. H., L. BAER, H. R. BRUNNER, F. R. BÜHLER, J. E. SEALEY, AND E. D. VAUGHAN, JR. 1972. Renin, angiotensin, and aldosterone system in pathogenesis and management of hypertensive vascular disease. *Amer. J. Med.*, 52:633-652.
- MALVIN, R. L., AND A. J. VANDER. 1967. Plasma renin activity in marine teleosts and cetacea. *Amer. J. Physiol.*, 213:1582-1584.
- MARGOLIUS, H. S., D. HORWITZ, J. J. PISANO, AND H. R. KEISER. 1976. Relationships among urinary kallikrein, mineralocorticoids and human hypertensive disease. *Fed. Proc.*, 35:203-206.
- MARSHALL, G. R. 1976. Structure-activity relations of antagonists of the renin-angiotensin system. *Fed. Proc.*, 35:2494-2501.
- MCGIFF, J. C., K. CROWSHAW, N. A. TERRAGNO, AND A. J. LONIGRO. 1970. Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. *Circ. Res.*, 26/27(suppl. I):I-121-I-130.
- MILLS, I. H., N. A. A. MACFARLANE, P. E. WARD, AND L. F. O. OBIKA. 1976. The renal kallikrein-kinin system and the regulation of salt and water excretion. *Fed. Proc.*, 35:181-188.
- MIZOGAMI, S., M. OGURI, H. SOKABE, AND H. NISHIMURA. 1968. Presence of renin in the glomerular and aglomerular kidney of marine teleosts. *Amer. J. Physiol.*, 215:991-994.
- MOUW, D., J. P. BONJOUR, R. L. MALVIN, AND A. VANDER. 1971. Central action of angiotensin in stimulating ADH release. *Amer. J. Physiol.*, 220:239-242.

- NAKAJIMA, T., T. NAKAYAMA, AND H. SOKABE. 1971. Examination of angiotensin-like substances from renal and extrarenal sources in mammalian and nonmammalian species. *Gen. Comp. Endocrinol.*, 17:458-466.
- NAKAYAMA, T., T. NAKAJIMA, AND H. SOKABE. 1973. Comparative studies on angiotensins. III. Structure of fowl angiotensin and its identification by DNS-method. *Chem. Pharmacol. Bull.*, 21:2085-2087.
- . 1977. Comparative studies on angiotensins. IV. Structure of snake (*Elaphe climacophora*) angiotensin. *Chem. Pharm. Bull.*, 25:3255-3260.
- NAVAR, L. G., AND H. G. LANGFORD. 1974. Effects of angiotensin on the renal circulation. Pp. 455-474, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- NISHIMURA, H., AND M. OGAWA. 1973. The renin-angiotensin system in fishes. *Amer. Zool.*, 13:823-838.
- NISHIMURA, H., AND W. H. SAWYER. 1976. Vasopressin, diuretic, and natriuretic responses to angiotensins by the American eel, *Anguilla rostrata*. *Gen. Comp. Endocrinol.*, 29:337-348.
- NISHIMURA, H., AND H. SOKABE. 1968. The role of angiotensinases in the renin-angiotensin system. *Proc. Symp. Chem. Physiol. Pathol.*, 8:116-120.
- NISHIMURA, H., M. OGURI, M. OGAWA, H. SOKABE, AND M. IMAI. 1970. Absence of renin in kidneys of elasmobranchs and cyclostomes. *Amer. J. Physiol.*, 218:911-915.
- NISHIMURA, H., M. OGAWA, AND W. H. SAWYER. 1973. Renin-angiotensin system in primitive bony fishes and a holoccephalian. *Amer. J. Physiol.*, 224:950-956.
- NISHIMURA, H., W. H. SAWYER, AND R. F. NIGRELLI. 1976. Renin, cortisol and plasma volume in marine teleost fishes adapted to dilute media. *J. Endocrinol.*, 70:47-59.
- NISHIMURA, H., J. T. CROFTON, V. M. NORTON, AND L. SHARE. 1977. Angiotensin generation in teleost fish determined by radioimmunoassay and bioassay. *Gen. Comp. Endocrinol.*, 32:236-247.
- NISHIMURA, H., L. G. LUNDE, AND A. ZUCKER. 1979. Renin response to hemorrhage and hypotension in the aglomerular toadfish, *Opsanus tau*. *Amer. J. Physiol.*, 237: H105-H111.
- NISHIMURA, H., V. M. NORTON, AND F. M. BUMPUS. 1978. Lack of specific inhibition of angiotensin II in eels by angiotensin antagonists. *Amer. J. Physiol.*, 235:H95-H103.
- NOLLY, H. L., AND J. C. FASCILOLO. 1971. Renin-angiotensin system and sodium homeostasis in *Bufo arenarum*. *Comp. Biochem. Physiol.*, 39A:833-841.
- . 1972. The renin-angiotensin system through the phylogenetic scale. *Comp. Biochem. Physiol.*, 41A:249-254.
- NOTHSTINE, S. A., J. O. DAVIS, AND R. M. DEROOS. 1971. Kidney extracts and ACTH on adrenal steroid secretion in a turtle and a crocodilian. *Amer. J. Physiol.*, 221:726-732.
- OGAWA, M., AND H. SOKABE. 1971. The macula densa site of avian kidney. *Z. Zellforsch. Mikroskop. Anat.*, 120:29-36.
- OGURI, M., M. OGAWA, AND H. SOKABE. 1972. Juxtaglomerular cells in aglomerular teleosts. *Bull. Japanese Soc. Sci. Fish.*, 38:195-200.
- ONDETTI, M. A., N. J. WILLIAMS, E. F. SABO, J. PLUSCEC, E. R. WEAVER, AND O. KOCY. 1971. Angiotensin-converting enzyme inhibitors from the venom of *Bothrops jararaca*. Isolation, elucidation of structure, and synthesis. *Biochemistry*, 19:4033-4039.
- OPDYKE, D. F., AND R. HOLCOMBE. 1976. Response to angiotensins I and II and to AI-converting enzyme inhibitor in a shark. *Amer. J. Physiol.*, 231:1750-1753.
- PANG, P. K. T., R. K. PANG, AND W. H. SAWYER. 1974. Environmental calcium and the sensitivity of killifish (*Fundulus heteroclitus*) in bioassays for the hypocalcemic response to Stannius corpuscles from killifish and cod (*Gadus morhua*). *Endocrinology*, 94:548-555.
- PASSO, S. S., T. A. ASSAYKEEN, A. GOLDFIEN, AND W. F. GANONG. 1971. Effect of α - and β -adrenergic blocking agents on the increase in renin secretion produced by stimulation of the medulla oblongata in dogs. *Neuroendocrinology*, 7:97-104.

- PEACH, M. J. 1974. Adrenal medulla. Pp. 400-407, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- PEACH, M. J., AND J. A. ACKERLY. 1976. Angiotensin antagonists and the adrenal cortex and medulla. *Fed. Proc.*, 35:2502-2507.
- PEART, W. S. 1965. The renin-angiotensin system. *Pharmacol. Rev.*, 17:143-182.
- PETERS, G., AND J. P. BONJOUR. 1971. Renal effects of renin and angiotensin. Pp. 81-154, in *The kidney: morphology, biochemistry, physiology* (C. Rouiller and A. F. Muller, eds.), Academic Press, New York, 4:xiv+1-430 pp.
- PHILLIPS, M. I., AND W. E. HOFFMAN. 1976. Sensitive sites in the brain for the blood pressure and drinking responses to angiotensin II. Pp. 82-93, in *International symposium on the central actions of angiotensin and related hormones* (J. P. Buckley and C. Ferrario, eds.), Pergamon Press, New York, vii+352 pp.
- REGOLI, D., W. K. PARK, AND F. RIOUX. 1974. Pharmacology of angiotensin. *Pharmacol. Rev.*, 26:69-123.
- REID, I. A. 1971. Renin secretion in a monotreme (*Tachyglossus aculeatus*). *Comp. Biochem. Physiol.*, 40A:249-255.
- ROMERO, J. C., AND C. G. STRONG. 1977. The effect of indomethacin blockade of prostaglandin synthesis on blood pressure of normal rabbits and rabbits with renovascular hypertension. *Circ. Res.*, 40:35-41.
- ROMERO, J. C., C. L. DUNLAP, AND C. G. STRONG. 1976. The effect of indomethacin and other anti-inflammatory drugs on the renin-angiotensin system. *J. Clin. Invest.*, 58:282-288.
- RYAN, J. W. 1974. The fate of angiotensin. II. Pp. 81-110, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- SAWYER, W. H. 1970. Vasopressor, diuretic, and natriuretic responses by lungfish to arginine vasotocin. *Amer. J. Physiol.*, 218:1789-1794.
- SAWYER, W. H., J. R. BLAIR-WEST, P. A. SIMPSON, AND M. K. SAWYER. 1976. Renal responses of Australian lungfish to vasotocin, angiotensin II, and NaCl infusion. *Amer. J. Physiol.*, 231:593-602.
- SCHNERMANN, J., D. W. PLOTH, AND M. HERMLE. 1976. Activation of tubulo-glomerular feedback by chloride transport. *Pflügers Arch. European J. Physiol.*, 362:229-240.
- SCHWOB, J. E., AND A. K. JOHNSON. 1977. Angiotensin-induced dipsogenesis in domestic fowl (*Gallus gallus*). *J. Comp. Physiol. Psychol.*, 91:182-188.
- SEVERS, W. B., AND A. E. DANIELS-SEVERS. 1973. Effects of angiotensin on the central nervous system. *Pharmacol. Rev.*, 25:415-449.
- SEVERS, W. B., AND J. SUMMY-LONG. 1975. The role of angiotensin in thirst. *Life Sci.*, 17:1513-1526.
- SIMPSON, J. B., AND A. ROUTTENBERG. 1973. Subfornical organ: site of drinking elicitation by angiotensin II. *Science*, 181:1172-1175.
- SKEGGS, L. T., F. E. DORER, J. R. KAHN, K. E. LENTZ, AND M. LEVINE. 1974. The biological production of angiotensin. Pp. 1-16, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- SKEGGS, L. T., M. LEVINE, K. E. LENTZ, J. R. KAHN, AND F. E. DORER. 1977. New developments in our knowledge of the chemistry of renin. *Fed. Proc.*, 36:1755-1759.
- SNAPIR, N., B. ROBINSON, AND M. GODSCHALK. 1976. The drinking response of the chicken to peripheral and central administration of angiotensin II. *Pharmacol. Biochem. Behav.*, 5:5-10.
- SOKABE, H. 1974. Phylogeny of the renal effects of angiotensin. *Kidney Int.*, 6:263-271.
- SOKABE, H., AND M. OGAWA. 1974. Comparative studies of the juxtaglomerular apparatus. *Int. Rev. Cytol.*, 37:271-327.
- SOKABE, H., S. MIZOGAMI, AND A. SATO. 1968. Role of renin in adaptation to seawater in euryhaline fishes. *Japanese J. Pharmacol.*, 18:332-343.

- SOKABE, H., M. OGAWA, M. OGURI, AND H. NISHIMURA. 1969. Evolution of the juxtaglomerular apparatus in the vertebrate kidneys. *Tex. Rep. Biol. Med.*, 27:867-885.
- SOKABE, H., H. NISHIMURA, M. OGAWA, AND M. OGURI. 1970. Determination of renin in the corpuscles of Stannius of the teleost. *Gen. Comp. Endocrinol.*, 14:510-516.
- SOKABE, H., H. NISHIMURA, K. KAWABE, S. TENMOKU, AND T. ARAI. 1972. Plasma renin activity in varying hydrated states in the bullfrog. *Amer. J. Physiol.*, 222:142-146.
- SOKABE, H., H. OIDE, M. OGAWA, AND S. UTIDA. 1973. Plasma renin activity in Japanese eels (*Anguilla japonica*) adapted to seawater or in dehydration. *Gen. Comp. Endocrinol.*, 21:160-167.
- TAKEI, Y. 1977. Angiotensin and water intake in the Japanese quail (*Coturnix coturnix japonica*). *Gen. Comp. Endocrinol.*, 31:364-372.
- TAQUINI, A. C., P. BLAQUIER, AND A. C. TANQUINI, JR. 1964. On the production and role of renin. *Canadian Med. Assoc. J.*, 90:210-213.
- TAYLOR, A. A. 1977. Comparative physiology of the renin-angiotensin system. *Fed. Proc.*, 36:1776-1780.
- TAYLOR, A. A., AND J. O. DAVIS. 1971. Effects of carp kidney extracts and angiotensin II on adrenal steroid secretion. *Amer. J. Physiol.*, 221:652-657.
- TAYLOR, A. A., J. O. DAVIS, R. P. BREITENBACH, AND P. M. HARTROFT. 1970. Adrenal steroid secretion and a renal-pressor system in the chicken (*Gallus domesticus*). *Gen. Comp. Endocrinol.*, 14:321-333.
- TAYLOR, A. A., J. O. DAVIS, AND B. BRAVERMAN. 1972. Deoxycorticosterone secretion in the bullfrog: effects of ACTH, hypophysectomy and renin. *Amer. J. Physiol.*, 223:858-863.
- THURAU, K. 1974. Intrarenal action of angiotensin. Pp. 475-489, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- THURAU, K., H. DAHLHEIM, A. GRÜNER, J. MASON, AND P. GRANGER. 1972. Activation of renin in the single juxtaglomerular apparatus by sodium chloride in the tubular fluid at the macula densa. *Circ. Res.*, 30-31(suppl. II):II-182-II-186.
- TIGERSTEDT, R., AND P. G. BERGMAN. 1898. Niere und Kreislauf. *Scandinavian Arch. Physiol.*, 8:223-271.
- TOBIAN, L., A. TOMBOULIAN, AND J. JANACEK. 1959. The effect of high perfusion pressure on the granulation of juxtaglomerular cells in an isolated kidney. *J. Clin. Invest.*, 38:605-610.
- TURKER, R. K., I. H. PAGE, AND F. M. BUMPUS. 1974. Antagonists of angiotensin II. Pp. 162-169, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591 pp.
- UEDA, H., H. YASUDA, Y. TAKABATAKE, M. IIZUKA, T. IIZUKA, M. IHORI, M. YAMAMOTO, AND Y. SAKAMOTO. 1967. Increased renin release evoked by mesencephalic stimulation in the dog. *Japanese Heart J.*, 8:498-506.
- VANDER, A. J. 1967. Control of renin release. *Physiol. Rev.*, 47:359-382.
- VANE, J. R. 1974. The fate of angiotensin I. Pp. 17-40, in *Handbook of experimental pharmacology: angiotensin* (I. H. Page and F. M. Bumpus, eds.), Springer, Heidelberg, 37:xix+1-591.
- WADA, M., H. KOBAYASHI, AND D. S. FARNER. 1975. Induction of drinking in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*, by intracranial injection of angiotensin II. *Gen. Comp. Endocrinol.*, 26:192-197.
- WEBER, P. C., C. LARSSON, E. ÅNGGÅRD, M. HAMBERG, E. J. COREY, K. C. NICOLAOU, AND B. SAMUELSSON. 1976. Stimulation of renin release from rabbit renal cortex by arachidonic acid and prostaglandin endoperoxides. *Cir. Res.*, 39:868-874.
- WENDELAAR BONGA, S. E. 1973. Morphometrical analysis with the light and electron microscope of the kidney of the anadromous three-spined stickleback, *Gasterosteus aculeatus*, form *trachurus*, from freshwater and from seawater. *Z. Zellforsch. Microskop. Anat.*, 137:563-588.

- WILSON, C., J. M. LEDINGHAM, AND M. A. FLOYER. 1971. Experimental renal and renoprival hypertension. Pp. 155-247, *in* The kidney: morphology, biochemistry, physiology (C. Rouiller and A. F. Muller, eds.), Academic Press, New York, 4:xiv+1-430 pp.
- WITTY, R. T., J. O. DAVIS, J. A. JOHNSON, AND R. L. PREWITT. 1971. Effects of papaverine and hemorrhage on renin secretion in the nonfiltering kidney. *Amer. J. Physiol.*, 221: 1666-1671.
- ZEHR, J. E., J. A. HASBARGEN, AND K. D. KURZ. 1976. Reflex suppression of renin secretion during distension of cardiopulmonary receptors in dogs. *Circ. Res.*, 38:232-239.

Copies of the following numbers of Graduate Studies may be purchased from the Texas Tech Press Sales Office, Texas Tech University Library, Lubbock, Texas 79409, or obtained on an exchange basis through the Exchange Librarian at Texas Tech University.

- No. 1 Pittard, K., and R. W. Mitchell. 1972. Comparative Morphology of the Life Stages of *Cryptocellus pelaezi* (Arachnida, Ricinulei), 77 pp., 130 figs. \$2.00
- No. 2 Shoppee, C. W., ed. 1973. Excited States of Matter, 174 pp. \$5.00
- No. 3 Levinsky, R. 1973. Nathalie Sarraute and Fedor Dostoevsky: Their Philosophy, Psychology, and Literary Techniques, 44 pp. \$2.00
- No. 4 Ketner, K. L. 1973. An Emendation of R. G. Collingwood's Doctrine of Absolute Presuppositions, 41 pp. \$1.00
- No. 5 Wirth, W. W., and W. R. Atchley. 1973. A Review of the North American Lepto-conops (Diptera: Ceratopogonidae), 57 pp. \$1.00
- No. 6 Gillis, E. 1974. *The Waste Land* as Grail Romance: Eliot's Use of the Medieval Grail Legends, 26 pp. \$1.00
- No. 7 Garner, H. W. 1974. Population Dynamics, Reproduction, and Activities of the Kangaroo Rat, *Dipodomys ordii*, in Western Texas, 28 pp. \$1.00
- No. 8 Stratton, L. H. 1974. Emilio Rabasa: Life and Works, 101 pp. \$2.00
- No. 9 Baker, T. L. 1975. The Early History of Panna Maria, Texas, 69 pp. \$2.00
- No. 10 Ketner, K. L., and J. E. Cook, comps. 1975. Charles Sanders Peirce: Contributions to *The Nation*. Part One: 1869-1893, 208 pp. \$6.00
- No. 11 Campbell, R. G. 1976. The Panhandle Aspect of the Chaquagua Plateau, 118 pp., 7 figs. \$3.00
- No. 12 Vail, D. 1976. Robert Frost's Imagery and the Poetic Consciousness, 83 pp. \$3.00
- No. 13 Tarwater, J. D., J. T. White, and J. D. Miller, eds. 1976. Men and Institutions in American Mathematics, 136 pp. \$5.00
- No. 14 Baird, F. L., ed. 1977. Mexican Americans: Political Power, Influence, or Resource, 108 pp. \$5.00
- No. 15 Morris, R. J. 1977. The Contemporary Peruvian Theatre, 98 pp. \$6.00
- No. 16 Ketner, K. L., and J. E. Cook, comps. 1978. Charles Sanders Peirce: Contributions to *The Nation*. Part Two: 1894-1900, 281 pp. \$14.00
- No. 17 Organ, D. M. 1979. Tennyson's Dramas: A Critical Study, 125 pp. \$7.00
- No. 18 Tijerina, A. A. 1979. History of Mexican Americans in Lubbock County, Texas, 73 pp. \$5.00
- No. 19 Ketner, K. L., and J. E. Cook, comps. 1979. Charles Sanders Peirce: Contributions to *The Nation*. Part Three: 1901-1908, 306 pp. \$16.00
- No. 20 Kennedy, S. M. 1979. The Monroe Doctrine Clause of The League of Nations Covenant, 60 pp. \$5.00
- No. 21 Pang, P. K. T., and A. Eppele, eds. 1980. Evolution of Vertebrate Endocrine Systems, 404 pp. (cloth, \$35.00) \$25.00