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TABLE OF CONTENTS

ACKNOWLEDGEMENTS .................................................. ii
LIST OF ILLUSTRATIONS ........................................... iv

I. INTRODUCTION .................................................... 1

II. METHODS AND PROCEDURES .................................... 4

  Preparation of Protein and Electrophoresis ................. 4
  Mass Isolation of Salivary Glands ............................. 4
  Incubation of the Glands ....................................... 5
  Isolation of Nuclei ............................................. 5
  Nonhistone Chromosomal Protein Extraction .................. 6
  SDS Polyacrylamide Gel Electrophoresis ...................... 6
  Molecular Weight Determinations .............................. 7
  Differential Staining of the Glands ........................... 7

III. FINDINGS AND INTERPRETATIONS ............................ 9

  Isolation of polytene nuclei and staining of nuclei ....... 9
  Changes in Nonhistone Chromosomal Proteins in Polytene
    Nuclei in Response to Puffing ................................ 9
    Induction of Puff II-48C .................................... 9
    Ecdysone Induced Puffs ..................................... 21

IV. DISCUSSION .................................................... 27

  Fast Green Accumulation ..................................... 27
  Changes in Nonhistone Chromosomal Proteins in Polytene
    Nuclei in Response to Puffing .............................. 27

V. SUMMARY ......................................................... 36

LIST OF REFERENCES ............................................... 37
<table>
<thead>
<tr>
<th>Figure</th>
<th>Illustration Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kinetics of puff II-48C induction</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>Mass isolated salivary glands</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>Mass isolated polytene nucleus</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Feulgen stained polytene chromosomes counterstained with acidic fast green</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>Densitometric scans of nonhistone chromosomal proteins. Comparison of Control and Vitamin B₆ treated preparations</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>Standard molecular weight curve</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>Densitometric gel scans of nonhistone chromosomal proteins. Comparison of 6 and 8 day old larvae.</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>Densitometric gel scans of nonhistone chromosomal proteins. Comparison of Ecdysone, Vitamin B₆ and Control preparations.</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>Analysis of gel scans from Elgin and Boyd (1975)</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>Comparison of the gels of Helmsing and Berendes (1971) and the gels reported in this paper</td>
<td>32</td>
</tr>
</tbody>
</table>
**INTRODUCTION**

*Drosophila hydei* and other members of the Diptera offer a unique opportunity to approach questions of genetic activity in eucaryotes. Many of the tissues of these organisms have polytene chromosomes at various times during their development (Beerman, 1952). Both during normal development and as a result of experimental manipulation discrete puffs occur along the chromosomes (Beerman, 1952; Ritossa, 1964; Berendes, *et al.*, 1965; Leenders and Beckers, 1972; Leenders and Berendes, 1972). These puffs are morphological manifestations of intense gene activity.

A single large puff at region II-48C on the salivary gland chromosomes of *Drosophila hydei* can be induced *in vitro* by incubations of isolated salivary glands in \(1.5 \times 10^{-2}\) M Vitamin B\(_6\) (pyridoxine HCl). The ability to selectively induce this puff allows correlation of cytological and biochemical observations.

Puffing is accompanied by increased protein specific dye binding (Holt, 1970, 1971). This observation is consistent with previous studied which demonstrated an increase of UV absorbance (at 231 nm) at the puff sites (Rudkin, 1964). Gorovsky and Woodward (1966, 1967) have also noted that there is no increase in amino acid incorporation at the site of puffs. Goodman *et al.* (1976) confirm these reports and those of Holt (1971) that the puff induction occurs without new protein synthesis. This protein accumulation is one of the first events in puff formation (Berendes, 1968) and most intense staining is reached after 30 minutes of incubation.
There are two types of chromosomal proteins, the histones or basic proteins and the nonhistones or acidic proteins. The five classes of histones exhibit little variability among eucaryotic cells. These proteins are primarily thought to be involved in packaging of the DNA (Olins and Olins, 1973; Thomas and Kornberg, 1975). Due to their homogeneity among diverse cell types and their lack of variability during development, the histones have been dismissed as a source of regulation (other than being general repressors of gene activity) (Allfrey et al., 1963; Paul and Gilmour, 1966).

The nonhistone chromosomal proteins are acidic and extremely heterogeneous. Both quantitative and qualitative variability of these proteins occurs between different tissues within one organism and at different developmental stages within the same tissue (Elgin et al., 1973; Gilmour and Paul, 1971; MacGillivray et al., 1972). The majority of the acidic proteins have enzymatic or structural functions common to all chromatin. However, Gilmour and Paul (1970), Kostraba and Wang (1973) and Kleinsmith et al. (1970) as well as many others present evidence which suggests that some nonhistone chromosomal protein fractions may play a role in gene activation. These proteins which have a specific regulatory function need be present in very small quantities (Gilmour, 1974).

The protein which accumulates at the puff during puff induction is an acidic protein on the basis of two lines of evidence. First, the staining procedure used by Holt (1970, 1971) is specific for acidic proteins (Alfert and Geschwund, 1953). Second, the histone:DNA ratio remains the same during puff development (Swift, 1964) leaving only the nonhistone chromosomal proteins to vary.
The recent work of Goodman et al. (1976) on Sciara coprophila indicates that the accumulation of acidic proteins at the puff site in response to ecdysterone is due to an influx of cytoplasmic proteins. Helmsing and Berendes (1971) and Helmsing (1972) examined the electrophoretic patterns of salivary gland nonhistone chromosomal proteins of Drosophila hydei under several conditions which induce puffs. Their work showed the accumulation of a 41,000 Dalton protein in response to ecdysterone treatment and a 23,000 D protein in response to heat shock or anaerobic shock. Both of these latter treatments produce the same series of puffs. Puff II-48C, which is selectively induced by Vitamin B₆ treatment, is one of the five puffs induced by heat shock or anaerobosis. Since the work of Helmsing and Berendes (1970) revealed a change in the nonhistone chromosomal protein pattern after induction of all five puffs, this work was undertaken to determine what type of changes (if any) in the nonhistone chromosomal proteins would be found when only puff II-48C was induced to form.
Mass Isolation of Salivary Glands

Gram quantities of salivary glands were isolated using a modification of the technique of Boyd et al. (1968). Mass cultures of *Drosophila hydei* were raised under standard conditions at 24°C. Larvae were synchronous in growth, coming from eggs laid over a two hour period. Sixty (60) grams of third instar larvae (either 136 or 184 hours old) were used in each experiment. Each experiment reported was performed at least three times. The larvae were spread on a siliconized glass plate and oriented with a strong light. The internal organs were extruded by rolling over the larvae from the rear with a steel rod. Tissues were washed from the rod and glass plate with cold (4°C) Ringers solution (Boyd et al., 1968). All subsequent operations were carried out at 4°C. The larvae were allowed to settle for five minutes. The fat bodies were poured off the top and the remaining suspension was then swirled and poured through a 1.1 mm mesh sieve to remove larval carcasses. The settling and sieving procedures were repeated four times. The suspension was then swirled and allowed to settle for 30 seconds. It was rapidly poured through a 0.5 mm mesh to remove large pieces of gut. Salivary glands preferentially adhered to the walls of the original beaker and were washed from the walls into a small beaker where contaminating tissues were removed with a pasteur pipette. This procedure was repeated six times and the salivary glands collected were pooled.

The salivary glands were pelleted at 60 x g for 5 minuted in a
refrigerated centrifuge and the supernatant was poured off.

**Incubation of the glands**

A variety of incubation conditions were employed to induce the different series of puffs. In each case the isolated glands were divided into two equal portions. One of the portions was suspended in Poels medium (Poels, 1972) and incubated at 24°C for either 30 or 90 minutes. This portion served as the control for each experiment. The other portion was treated to induce the specific puffs desired.

To induce the heat shock puffs the preparation was incubated in Poels medium (Poels, 1972) at 37°C for 30 minutes.

To chemically induce the heat shock puffs, salivary glands were incubated in Poels medium (Poels, 1972) with $1.5 \times 10^{-2}$ M pyridoxal 5' phosphate at 24°C for 30 minutes.

To induce puff II-48C the glands were incubated in $1.5 \times 10^{-2}$ M Vitamin B₆ (pyridoxine HCl) at 24°C for either 30 or 90 minutes.

To induce ecdysone puffs the glands were incubated in Poels medium (Poels, 1972) with 1 mg/ml ecdysone for 90 minutes at 24°C.

At the end of the incubation period the incubation medium was pipetted off and discarded.

**Isolation of Nuclei**

Nuclei of the salivary glands were isolated and purified according to the procedure of Elgin and Boyd (1975). The glands were transferred to a homogenizer and broken in three ml of nuclear isolation buffer (0.11 M NaCl, 0.002 M KCl, 0.0025 M MgCl₂ and 0.01 M Tris-HCl at pH 7.2) which contained 0.001% spermidine and 0.2% Triton-X.
Fragments were allowed to settle and the supernatant was removed. The homogenization was repeated with fresh buffer. The supernatants were combined and brought to 0.5% Triton-X. The nuclear suspension was filtered through a 53 micron mesh and washed with 100 ml nuclear isolation buffer. This collected suspension was washed and centrifuged at 40 x g for five minutes to pellet the nuclei. Nuclei were resuspended in 10 ml of nuclear isolation buffer containing 1.67 M sucrose and layered on two ml buffer with 2.3 M sucrose in a polyallomer tube. The tubes were centrifuged at 40,000 rpm for 42 minutes in a Beckman 42 preparative ultracentrifuge to form a nuclear pellet.

Nonhistone Chromosomal Protein Extraction

The nonhistone chromosomal proteins were extracted using the method of Konings et al. (1970). The nuclear pellet was suspended in 1 ml of Ringers (Leenders and Berender, 1972) solution with 0.16 mM EDTA and 1% SDS. Three ml of phenol saturated with 0.25 M Tris-HCl (pH 7.8) was added and the suspension vortexed for 10 minutes. The solution was centrifuged for 10 minutes at 16,000 x g. The phenol layer was removed and reserved. The procedure was repeated for the water and interphase layers. Both phenol layers were combined with two volumes of ice cold acetone which contained 0.1 M acetic acid. The solution was kept at 0°C for at least two hours. The precipitate that formed was pelleted and washed with 1:1 ethanol: ether, then with ether and dried.

SDS Polyacrylamide Gel Electrophoresis

The dried precipitate was mixed with 20 microliters of 0.01 M
sodium phosphate buffer (pH 7.6) containing 0.1% SDS, 0.14 M B mercaptoethanol and 4 M urea and incubated for two hours at 37°C. The protein was heated to 100°C for 1.5 minutes. One drop of glycerol was added and the preparation was applied to the top of SDS-acrylamide disc gels (Weber and Osborn, 1969) which had been modified to carry a 3% acrylamide, 0.1% SDS, phosphate buffered stacking gel. The gels were run at 8 mA per gel for four hours. The gels were then removed from the tubes and stained in 0.25% Coomassie Brilliant Blue B dissolved in 45.5% methanol and 9% acetic acid. The gels were stained overnight, rinsed in 7.5% acetic acid and 5% methanol. A Beckman 24 spectrophotometer was used to scan the gels at 550 nm. The scans of the gels were examined for changes in the protein pattern. Gels were stored in 10% acetic acid.

**Molecular Weight Determinations**

Apparent molecular weights were determined by the method of Weber and Osborn (1969). The following molecular weight marker proteins were electrophoresed; Cytochrome C (12,400 Daltons), Myoglobin (17,500 D.), Chymotrypsinogen (24,000 D.), Ovalbumin (45,000 D. and Bovine serum albumin (68,000 D.). The relative mobility of each protein in the gel was calculated and plotted against the log of the molecular weight to construct a standard molecular weight curve (fig. 6). The relative mobility of any protein in a gel may be calculated and then by referring to the standard molecular weight curve the apparent molecular weight may be determined.

**Differential Staining of the Glands**

Glands were fixed in 3:1 (ethanol: acetic acid) at times 0, 30,
90 minutes after incubation of isolated salivary glands in $1.5 \times 10^{-2}$M Vitamin B$_6$ in Poels medium (Poels, 1972). The glands were stained with Feulgen (Lillie, 1954) and squashed. The coverslips were removed by freezing the slides on dry ice and popping them off with a razor blade. The preparations were post fixed with 3:1 (ethanol: acetic acid), dipped in 95% ethanol and air dried. The slides were then counterstained with acidic (pH 2.4) fast green (Alfert and Geschwund, 1953), dehydrated through an alcohol series, cleared with xylol and made permanent with permount.
FINDINGS AND INTERPRETATIONS

Isolation of polytene nuclei and staining of the nuclei

The mass isolation techniques used yield salivary glands free of other larval tissues, except for a small strip of fat adhering to the isolated salivary glands (Fig. 2). The isolated polytene nuclei are free of cytoplasmic contamination (Fig. 3).

A large puff is selectively induced at region II-48C of the polytene chromosomes after incubation of isolated salivary glands in Vitamin B6. The kinetics of puff formation was followed (Fig. 1) and these results indicate that puffing begins within 15 minutes. The puff size doubles in two hours.

Fast green stain (pH 2.4) is specific for acidic proteins (Alfert and Geschwund, 1953). Feulgen stained polytene chromosomes counterstained with acidic fast green show intense stain accumulation when puff II-48C is induced (Fig. 4). This accumulation, one of the first events of puff formation, does not occur in the noninduced control nuclei (Fig. 4a). Dense stain is present after 30 minutes in Vitamin B6 (Fig. 4b) whereas the measurements of the puff indicate that the puff is just beginning to form (Fig. 1). The intense staining in the puff persists for the longest time tested, 90 minutes (Fig. 4c).

Changes in Nonhistone Chromosomal Proteins in Polytene Nuclei in Response to Puffing

Induction of Puff II-48C

A number of diverse treatments of isolated salivary glands from Drosophila hydei result in the formation of a large puff at band II-48C.
Fig. 1.  Kinetics of puff II-48C induction by $1.5 \times 10^{-2}$ M Vitamin B$_6$ (pyridoxine HCl). Puff measurements were made relative to band II-47B.
Fig. 2. Mass isolated salivary glands. The glands are free from contamination by other larval tissues, except for a small fat body adhering to some of the salivary glands (arrow). 10X

Fig. 3. Mass isolated polytene nucleus, free of cytoplasmic contamination.
Fig. 4. Feulgen stained polytene chromosomes which have been counterstained with fast green (pH 2.4).

(a) Band II-48C after 90 minutes in Poels medium lacking Vitamin B₆ (pyridoxine HCl).

(b) Puff II-48C after 30 minutes in $1.5 \times 10^{-2}$ M Vitamin B₆ in Poels medium.

(c) Puff II-48C after 90 minutes in $1.5 \times 10^{-2}$ M Vitamin B₆ in Poels medium.
In vitro incubations in Vitamin B₆ (pyridoxine HCl) induces only puff II-48C, whereas heat shock, anaerobic shock and pyridoxal 5' phosphate result in a series of six puffs (II-32A, II-36A, II-48C, III-51B, III-58B and IV-81B) one of which is II-48C. Helmsing and Berendes (1971) and Helmsing (1972) report the appearance of a 23,000 Dalton protein in the nonhistone chromosomal protein fraction after induction of the heat shock puffs by incubation of the larvae at 37°C prior to isolation of the salivary glands.

Three treatments were employed in this study to determine what effect if any, the induction of puff II-48C had on the pattern of nonhistone chromosomal proteins in the salivary gland nuclei. Figure 5 shows densitometric gel scans of nonhistone chromosomal proteins prepared from nuclei with no induced puffs and from nuclei with puffs induced by one of the following treatments; 1.5 x 10⁻² M Vitamin B₆ (pyridoxine HCl), 1.5 x 10⁻² M pyridoxal 5' phosphate or heat shock at 37°C. It is of interest to note that all of the three treatments result in the appearance of a 46,000 D. protein after 30 minute incubations. This protein is never observed in control glands incubated in Poels medium (Poels, 1972) at 25°C.

The apparent molecular weight of the unknown protein was determined by referring to the standard molecular weight curve (Fig. 6). This standard curve plots the relative mobility of a protein in the gel against the molecular weight. The relative mobility of each unknown protein has been calculated by the method of Weber and Osborn (1969).

Late third instar larvae (8 days old) had been used because of the large size of the glands and the relative ease of isolation and handling.
Fig. 5. Densitometric scans of nonhistone chromosomal proteins which were run on polyacrylamide- SDS gels. The gels were scanned at 550 nm. The arrows correspond to the location of protein bands of molecular weights, 40,000 Daltons and 46,000 D.

The incubation media used to induce the puffs are indicated to the right of the scan lines.

All incubations were done for 30 minutes at 24°C.
Vitamin B<sub>6</sub>

Pyridoxal 5'P<sub>O</sub><sub>4</sub>

Heat Shock

Control

A<sub>280</sub>

MW 46,000
Fig. 6. Standard molecular weight graph.

The calculated relative mobility is plotted against the log of the molecular weight for each known molecular marker protein run.

The arrows show the relative mobility of the 46,000 Dalton and the 40,000 D. proteins found in the nonhistone chromosomal protein preparations.
However, the polytene nuclei from 8 day old larvae have a series of puffs arising as a part of normal development in these insects. These complex series of puffs are induced by ecdysone and do not include puff II-48C or any of the other heat shock puffs. To determine whether the 46,000 D. protein found in the polytene nuclei is specific to the induction of puff II-48C or whether it is a protein found in conjunction with the puffing process in general, salivary glands lacking natural puffs were needed. Six day old larvae (136 hours) raised in our laboratory under standard conditions lacked puffs, but were of a size which allowed isolation of the salivary glands and polytene nuclei. Incubation of salivary glands from these 6 day old larvae in Vitamin B₆ induces the formation of puff II-48C.

Analysis of Figure 7 reveals that the 46,000 D protein appears in the nucleus after induction of puff II-48C by Vitamin B₆, but not in control glands. This accumulation is notable in both the 6 and 8 day old larvae. In the 6 day old larvae, the appearance of a 40,000 D protein in the Vitamin B₆ treated glands is also noted, but never found in the control (uninduced) preparations. The 8 day old larvae control preparations show the presence of a 40,000 D protein, but not the 46,000 D protein. Neither of these proteins is found in the 6 day old control larvae, which were incubated under similar conditions except that the culture media lacked the Vitamin B₆.

Ecdysone Induced Puffs

To determine if the proteins which were found to accumulate in the nucleus following incubation in Vitamin B₆ were associated specifically with the induction of puff II-48C (or the other heat shock puffs) or were associated with the general phenomenon of puff induction,
Fig. 7.  Densitometric scans of nonhistone chromosomal proteins from salivary gland nuclei. Scans were run at 550 nm.

Incubations of the salivary glands were done at 24°C for 30 minutes in either Poels media or Poels media with $1.5 \times 10^{-2}$ M Vitamin $B_6$. 
23 DAY OLD LARVAE
VITAMIN B₆
CONTROL

8 DAY OLD LARVAE
VITAMIN B₆

CONTROL

6 DAY OLD LARVAE
VITAMIN B₆
CONTROL
the next series of experiments were undertaken. Salivary glands were 24 mass isolated from 6 day old larvae. These larvae do not yet have the complex puff series associated with the normal pattern of development in the Diptera. These puffs result from ecdysone secretions in the larvae. Isolated salivary glands were incubated in Poles media (Poels, 1972) to serve as a control or in Poels media with 1 mg/ml ecdysone. The incubations were conducted for 90 minutes. The latter treatment induced the appearance of the ecdysone puffs.

Examination of figure 8 reveals the accumulation of a 40,000 D protein in the nonhistone chromosomal proteins of the glands incubated in the ecdysone which is not found in the nonhistone chromosomal proteins from the control glands. This protein is present in the gel pattern of nonhistone chromosomal proteins from 8 day old control larvae preparations. These 8 day old larvae have the normal developmentally induced ecdysone puffs in their salivary gland polytene chromosomes. Thus the same molecular weight protein is found in the nucleus of both glands with naturally occurring and experimentally induced ecdysone puffs, but never in salivary glands that lack any puffs.
Fig. 8. Densitometric scans of nonhistone chromosomal proteins run on SDS-acrylamide gels. Scans were run at 550 nm.

Incubations were done for 90 minutes in all cases.

Incubation media used to induce puffs is indicated to the right of the scan lines. Controls indicate preparations incubated in Poels medium under the same conditions.
6 Day Old Larvae

CONTROL

VITAMIN B₆

ECDYSONE

8 Day Old Larvae

CONTROL
DISCUSSION

Fast Green Accumulation

Acidic fast green stain accumulation in puffs has been reported for many years (Holt, 1970; Holt, 1971). This accumulation has intrigued investigators especially in light of the mounting evidence suggesting that nonhistone chromosomal proteins may function as regulatory molecules in the transcription of eucaryotic DNA (Gilmour and Paul, 1970; Kostraba and Wang, 1973; Kleinsmith et al., 1970). These nonhistone chromosomal proteins probably have a number of diverse functions in the nucleus (Swift, 1964). The increase in acidic fast green stainability demonstrates that the puff is accumulating acidic protein, but gives no indication of the diversity, number, size or function of these proteins.

Changes in Nonhistone Chromosomal Proteins in Polytene Nuclei in Response to Puffing

The polytene chromosomes of the salivary gland nuclei of Drosophila allow us the opportunity to correlate this accumulation of acidic protein at the puff site with biochemical changes in the total nonhistone chromosomal protein fraction. This approach has been taken with respect to the puffs formed by heat shock, anaerobic conditions and ecdysone treatments with confusing and contradictory results. Helmsing and Berendes (1971) reported the appearance of a 23,000 D. protein after a 30 minute heat shock and a 40,000 D. protein after treatment of isolated salivary glands in ecdysone. Efforts by Elgin and Boyd (1975) to repeat these experiments failed to verify these results. Examination
of the densitometric gel scans presented by Elgin and Boyd (1975) do
indeed fail to show any accumulation of a 23,000 D. protein in nuclei
subjected to heat shock (Fig. 9). Their attempts to induce ecdysone
puffs in mass isolated salivary glands were reportedly unsuccessful
(Elgin and Blyd, 1975). The discrepancies arising between the data
reported in this paper and the earlier reports of Helmsing and Berendes
(1971) and Elgin and Boyd (1975) are explained by a reexamination of
the data of these earlier investigators. Figure 10 is a photograph
of the gels of Helmsing and Berendes (1971) along with control and
Vitamin B6 gels reported here. The location on the gel of the newly
accumulated protein in the work of Helmsing and Berendes (1971) with
heat shock and anaerobic shock puffs and that reported here with puffs
arising from Vitamin B6, heat shock, and pyridoxal 5' phosphate is
identical. The disparity arises in the assignment of a molecular weight
to this protein. Comparison of the position of this protein in the
gel with the positions of known molecular weight marker proteins indi­
cated the molecular weight to be 46,000 D. not 23,000D.

This requires a reexamination of the scans presented by Elgin and
Boyd (1975). They discount the claim by Helmsing and Berendes (1971)
that a protein of 23,000 D. accumulated in the nuclei of heat shocked
salivary glands and their gel scans bear up this disclaimer. However,
if one examines the Elgin and Boyd (1975) scans (Fig. 9) in the area
between 40,000 and 50,000 D. a very distinct shoulder is present in the
nuclear proteins from heat shocked larvae that is not present in the con­
trols. Excellent reproducibility is found in the scans of Elgin and Boyd
(1975) except in this particular region. Therefore we are led to the con­
clusion that when the six heat shock puffs are induced in Drosophila
Fig. 9. Densitometric scans of nonhistone chromosomal proteins from salivary gland nuclei. Solid line is the scan from heat shocked salivary glands. Dotted line is scan of proteins from control nuclei. Electrophoresis was done on SDS-Tris-glycine polyacrylamide disc gels.

Fig. 10. SDS-polyacrylamide gel separation of phenol soluble nuclear proteins from larval salivary glands.

(a) Proteins from control nuclei

(b) Proteins from larvae which have been subjected to heat shock at 37°C for 30 minutes prior to isolation of the salivary glands.

(c) Proteins from larvae which have been subjected to CO2 treatment 30 minutes prior to isolation of the salivary glands.

(From Helmsing, P. J. and M. D. Berendes. Induced accumulation of nonhistone proteins in polytene nuclei of *Drosophila hydei*. J. Cell Biol. 50: 893 (1971).)

(d) Proteins from control salivary glands which were incubated in Boels medium.

(e) Proteins from salivary glands incubated in $1.5 \times 10^{-2}$ M Vitamin B$_6$ in Poels medium at 24°C.

The closed circles indicate the position of the accumulating protein of Helmsing and Berendes (1971). The arrows indicate the position of the protein in the gels reported here. The band is evident in the glands incubated in Vitamin B$_6$ but not in the control glands.
salivary gland polytene chromosomes or more interestingly when a single one of these puffs, II-48C, is induced the observable accumulation of a single protein band may be demonstrated.

The appearance of this 46,000 D. protein in the nucleus after treatment of salivary glands with either Vitamin B₆ or heat shock, but not after ecdysone treatment, indicates that this protein is specifically involved with the appearance of a puff at II-48C. These data do not eliminate the possibility that it is associated with the heat shock puffs, but do eliminate its involvement in the general puffing phenomenon.

Although the data presented here make no attempt to determine the nature of this protein several possible functions for the acidic proteins which associate with the chromatin in the eucaryotic cells have been postulated (Swift, 1964). First, these proteins could be involved in the transcription of mRNA. For example they could be molecules like RNA polymerase. Second, they might be structural proteins. Third, one could envision such proteins used to transport or package newly formed RNA. Fourth, the proteins may be of a regulatory nature, either involved in the transport of the regulatory message or they could act as the regulatory message itself.

It seems unlikely that the 46,000 D. protein is such a common entity as a RNA polymerase since it is found only in conjunction with the formation of puff II-48C (and the other heat shock puffs) but not found in ecdysone puffed nuclei.

The possibility does exist that this is a structural protein involved with puff formation. If so it would necessary to propose that it is specific in nature since it is not seen in the nonhistone
chromosomal proteins of ecdysone puffed nuclei.

The third possibility can not be so easily dismissed in light of the peculiarity of puff II-48C. This puff unlike others that have been looked at in this species (Leenders et al., 1973) is known to build up large aggregations of RNP (ribonuclear protein) particles as the puff forms (Berendes et al., 1973). Since this build up is restricted to the puff II-48C and the 46,000 D. protein reported here seems to be found in conjunction with the formation of this puff, it is possible that this protein is involved in the packaging phenomenon associated with this puff.

The possibility that these proteins are of a regulatory nature is of course very attractive. The evidence points to some specificity of the 46,000 D. protein since it is not found in all cases that puffs are formed. Analysis of the gel scans shows that this protein band makes up only a small fraction of the overall nonhistone chromosomal proteins in the nucleus (Fig. 5). Both of these points are necessary if one is to consider these molecules as candidates for a regulatory function in the nucleus.

The appearance of a 42,000 D. protein was reported by Helmsing and Berendes (1971) when salivary glands were treated in vitro with ecdysone. Reconsideration of the molecular weight of this protein is necessary in light of the evidence reported earlier in this paper in regard to their molecular weight determinations. Reevaluation leads to a molecular weight of 80,000D. The data reported here (Fig. 8) indicates that a protein of about 40,000 D. accumulates in the nucleus when heat shock or ecdysone puffs are induced. The reason for the discrepancy is not known.
The appearance of the 40,000 D. protein is noted in each case where puffing is found in the nucleus, but it is not found in 6 day old controls which have no puffs. This protein is likely to be one of a general nature and function, perhaps a protein associated with the general formation of puffs.
SUMMARY

Two nonhistone chromosomal proteins are found in the nucleus of *Drosophila hydei* salivary glands in response to puff formation.

A 40,000 D. protein is found to accumulate in response to both induction of ecdysone puffs and puff II-48C. This protein is likely one associated with the general phenomenon of puff formation or a protein involved in the general transcription of eucaryotic DNA.

A 46,000 D. protein is found to accumulate in the salivary glands when a specific puff, II-48 C is induced to form, but not when the ecdysone puffs are formed. This protein may have one of several functions in the nucleus of polytene chromosome bearing cells. The most interesting of these possibilities is that the protein may function by regulating the DNA transcription in these cells.
LIST OF REFERENCES


