THE INTEGUMENT OF CRYPTOCELLUS PELAEZI

(ARACHNIDA, RICINULEI)

by

VIRGINIA M. TIPTON, B.S.

A THESIS

IN

ZOOLOGY

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCE

Approved

Accepted

May, 1971

AEH-5932

AC
805
T3
1971
No.55
cop.2

ACKNOWLEDGMENTS

I am indebted to Dr. R. W. Mitchell for his direction of this thesis; Dr. R. W. Strandtmann, Dr. J. D. Berlin, Dr. G. Van Horn, and Dr. J. E. George for their helpful criticism; and Dr. P. R. Morey for his assistance in photography.

TABLE OF CONTENTS

ACKNOWLEDGMENTS. ii

LIST OF TABLES iv

LIST OF ILLUSTRATIONS. v

I. INTRODUCTION 1

II. MATERIALS AND METHODS. 4

III. RESULTS. 7

Sclerites 7

Intersegmental Membranes. 17

Arthrodial Membranes. 18

Pore Canals 19

Epidermis, Basement Membrane, Muscle Attachments. . . 20

Simple and Spike-like Setae 20

IV. DISCUSSION 22

ILLUSTRATIONS. 27

REFERENCES 42

LIST OF TABLES

Table		Page
1.	Results of staining and chemical tests on the cuticle of adult Cryptocellus pelaezi	8
2.	Results of staining and chemical tests on the cuticle of Cryptocellus pelaezi juveniles and third nymph exuviae	11

LIST OF ILLUSTRATIONS

Figure		Page
1.	Sclerite of adult opisthosoma, cross section. x18,000	29
2.	Intersegmental membrane of adult opisthosoma, cross section. x16,000	31
3.	Sclerite of adult opisthosoma, cross section. x1300	33
4.	Sclerite of adult opisthosoma, cross section. x16,000	35
5.	Epidermis of adult opisthosoma, cross section. x30,000	37
6.	Sclerite of adult opisthosoma, cross section. x14,000	39
7.	Seta, cross section. x16,000	41

CHAPTER I

INTRODUCTION

Although there have been many studies on the arthropod integument, the investigations into the arachnid integument have been few. More recent studies include the following. The acarine integument has been investigated with light microscopy by Hughes (1959) and Lees (1946, 1947); with light and electron microscopy by Brody (1969) and Gibbs and Morrison (1959); and with electron microscopy by Henneberry et al. (1965), Nathanson (1967, 1970), and Wharton et al. (1968). Browning (1943) did the first comprehensive study on the spider integument, followed by Sewell (1951, 1955); then with the electron microscope, Barth (1969, 1970) did two studies. Major investigations on the scorpion integument were done by Kennaugh (1959), Krishnan (1953, 1954), Krishnan et al. (1955), Lal and Shrivastava (1957), Malek (1963, 1964), and Shrivastava (1955). A brief study of the cuticle of Opiliones was done by Grainge and Pearson (1966). Kennaugh examined the ricinuleid cuticle in 1968.

The arthropod integument includes a non-chitinous epicuticle, a chitinous procuticle, and an epidermis. Confusion exists in the literature on terminology applied to the cuticle. The terminology pertaining to the epicuticle is the most unsatisfactory (see Lower, 1959). The only generally accepted point is that the epicuticle lacks chitin. The procuticle may be composed of three layers, exocuticle, mesocuticle, and endocuticle. Exocuticle is usually hardened, pig-

mented, refractory to staining, and interpreted chemically as the end product of progressive sclerotization. Schatz (1952) introduced the term "mesocuticle" to cover the intermediate step in the process of sclerotization in which a substance penetrates the cuticle prior to tanning (hardening and darkening). Mesocuticle characteristically stains red with Mallory triple. The endocuticle stains blue with Mallory triple, is neither impregnated with tanning agents nor tanned, and is resorbed at molting (Locke, 1964).

Cryptocellus pelaezi, the animal used in the present study, is an arachnid in the order Ricinulei. Ricinuleids are small, heavily sclerotized animals that are uncommonly found in nature because of their cryptic habits and limited distribution. They can be recognized by their tick-like appearance and cucullus, a movable hood articulating with the anterior edge of the prosoma. The adult C. pelaezi is 4-5 mm in length and dark reddish-brown in color. The four cream-colored juvenile stages are the six-legged larvae and eight-legged first, second, and third nymphs (Mitchell, 1970). Several pecularities of this unusual group of arthropods include the intricate copulatory apparatus on the distal portion of the male's third leg, the coupling device uniting the prosoma and opisthosoma which hides the pedicel, and a telescoping pygidium.

As recently as 1964, Savory remarked on the rarity of ricinuleids. Cooke (1967), however, believed these animals are rare only because of inadequate collecting techniques, since sizeable collections have been made. Few studies have been made because of the scarcity of specimens; and the biology of these animals remains relatively unknown.

Very little is known of the exoskeleton which is frequently referred to as being remarkably hard and thick (Millot, 1945; Savory, 1964; Kaestner, 1968) and very sensitive to desiccation (Kaestner, 1968; Cooke, 1967). A study of the integument of C. pelaezi was undertaken for these reasons. Electron microscopy was used to supplement the results obtained with light microscopy.

CHAPTER II

MATERIALS AND METHODS

Animals for this study were collected in La Cueva de la Florida, near the village of El Pachón, Tamaulipas, Mexico. Primarily, adults were studied, although some juveniles and exuviae were examined for comparison.

For light microscopy, the legs on one side of each animal were cut off, then the animals were fixed in tetra-Bouin, consisting of picric acid, dioxane, formalin, formic acid and chloroform (Rhode, 1964). Some were fixed in a mixture of formalin, isopropyl alcohol, and acetic acid (Williams, 1968).

Proper infiltration of the embedding medium was difficult to obtain due probably to the hardness of the exoskeleton. As a result, various embedding media were tried including paraffins with different melting points, double embedding in celloidin and paraffin (Kennedy, 1932), double embedding in N-butyl methacrylate and paraffin (Smith and Hynes, 1966), and methacrylate alone. The best results were obtained with 75:25 mixtures of paraffin and methacrylate polymerized in a 60°C oven. The block of paraffin and methacrylate containing the animal was transferred to pure paraffin in order to obtain serial sections easily.

Material was sectioned at 7 to 10μ on an American Optical rotary microtome and 0.5μ on LKB and Reichert ultratomes. The various stains and reagents used to distinguish the layers of the cuticle and

to determine its composition included Mallory triple (obtained from Carolina Biological Supply Co.), Masson trichrome (obtained from Carolina Biological Supply Co.), Richardson's, a combination of azure II and methylene blue (Richardson et al, 1960), Ehrlich's hematoxylin (obtained from Carolina Biological Supply Co.), periodic-acid Schiff (PAS) (Humason, 1967), Sudan III (obtained from Carolina Biological Supply Co.), Sudan IV (obtained from Carolina Biological Supply Co.), Sudan Black B (Lee, 1950) xanthoproteic (Lee, 1950), Millon (Lee, 1950), warm (42°C) concentrated HNO_3, and the chitosan preparation. The chitosan reaction bleached and softened the exoskeleton. To determine what chemical changes had occurred, some of the stains and chemical tests were run on these treated specimens. The procedure for converting chitin to chitosan was followed according to Jensen (1962) where material was placed in 23M KOH and autoclaved at 124°C at 20 p.s.i. for 45 minutes. All tests were run on sections prepared in paraffin for light microscopy. Sections were examined with an American Optical Phase Star microscope and Reichert Zetopan Research Scope using brightfield, phase contrast, and polarizing microscopy.

For electron microscopy, the legs were cut off and the body quartered for quicker infiltration. The two fixatives used were 1% glutaraldehyde in cacodylate buffer at pH 7.3 and 2.5% glutaraldehyde in phosphate buffer at pH 7.1. Animals remained overnight in fixative at 0°-4°C. Then the material was transferred for one hour to either Millonig's osium tetroxide (Millonig, 1961) or 2% osmium tetroxide in phosphate buffer at pH 7.1, also at 0°-4°C. Infiltration and adherence of the embedding media were a problem because of the

hardness of the cuticle; therefore, two kinds of embedding media were tried, Epon (Luft, 1961) and Spurr medium (Spurr, 1969). The Spurr medium has a lower viscosity and longer infiltration time, and it penetrated the exoskeleton better. Specimens were sectioned with glass knives on LKB and Reichert ultratomes, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) to enhance contrast, and examined with an Hitachi HS-8 transmission electron microscope.

CHAPTER III

RESULTS

The results reported here were obtained using Cryptocellus pelaezi adults unless otherwise stated. Results of staining and cytochemical tests for adults and juveniles are summarized in Tables 1 and 2.

The three major division of exoskeleton that were examined were sclerites, intersegmental membranes, and arthrodial membranes. The sclerites are hardened plates located dorsally and ventrally on the main body and on the legs. The intersegmental membranes are tough and thick (about 72μ), but unsclerotized, articulating membranes between the sclerites on the opisthosoma. The arthrodial membranes are thin (about 5μ) and flexible articulating membranes found between leg segments, joining the prosoma to the opisthosoma, between the cucullus and carapace, and between the segments of the pygidium.

Sclerites

There are only two apparent layers in the sclerites, epicuticle and exocuticle. Although an epicuticle is present, it was difficult to distinguish at times since it is such a thin layer (less than oneμ). Generally, it lacked an affinity for stains, although it stained very lightly with Sudan Black B, a stain specific for lipids. The chitosan reaction was so weak that it was impossible to determine whether chitin is present or absent in the epicuticle. The xanthoproteic reaction, a general test for proteins (Lee, 1950), indicated the

Table 1. Results of staining and chemical tests on the cuticle of adult Cryptocellus pelaezi. A indicates a negative reaction; +'s indicate strength of positive reaction; 0 indicates no observable reactions.

	SCLERITES		INTERSEGMENTAL MEMBRANES		ARTHRODIAL MEMBRANES		SETAE[a]		
	Epicuticle	Exocuticle	Epicuticle	Mesocuticle	Epicuticle	Endocuticle	Outer Layer	Middle Layer	Inner Layer
Masson Trichrome	-	-	-	+ + + + (red)	+ + + + (red)	+ + + + (green)	-	-	+ + (red)
Mallory Triple	-	-	-	+ + + + (red)	+ + + + (red)	+ + + + (blue)	-	-	+ + (red)
Mallory Triple (after chitosan reaction)	0	+ + (blue or red)	-	+ + + + (red or blue)	+ + + (red or blue)	+ + + (red or blue)	-	-	+ + (red or blue)
Hematoxylin	-	-	0	+ to -	+ +	-	-	-	+
Sudan III	-	-	-	+ +	0	0	-	-	+ +
Sudan IV	-	-	0	+ +	+ + + +	+ +	0	0	0
Sudan Black B	+ to -	-	-	+	+ + + +	+	-	-	+

Table 1. (cont'd.)

	SCLERITES		INTERSEGMENTAL MEMBRANES		ARTHRODIAL MEMBRANES		SETAE[a]		
	Epicuticle	Exocuticle	Epicuticle	Mesocuticle	Epicuticle	Endocuticle	Outer Layer	Middle Layer	Inner Layer
Sudan Black B (after chitosan reaction)	0	+ + +	+	+ + +	+ + + +	+ +	+ + +	-	+ +
Richardson's (0.5μ thick)	0	+ + +	+ +	+ to -	+ + +	+ +	+ +	+ to -	+ + +
Richardson's (10.0μ thick)	-	+ to -	+	+ + + +	+ +	+ + +	+ +	+	+ + +
PAS	-	-	-	-	-	-	-	-	-
Chitosan ($I_2KI-H_2SO_4$)	0	+ to -	0	+ to -	0	0	+ +	+ + +	-
Xanthoproteic	+	+ + +	+	+ + +	0	0	0	+	+ to -
Xanthoproteic (after chitosan reaction)	-	-	-	-	0	0	-	-	-
Concentrated (42°C) HNO_3	Separates from rest	Starts to dissolve	Separates from rest	Starts to dissolve	0	0	-	-	-

Table 1. (cont'd.)

	SCLERITES		INTERSEGMENTAL MEMBRANES		ARTHRODIAL MEMBRANES		SETAE[a]		
	Epicuticle	Exocuticle	Epicuticle	Mesocuticle	Epicuticle	Endocuticle	Outer Layer	Middle Layer	Inner Layer
Millon	+ to -	+ to -	+	+ + +	-	-	-	-	-
Millon (after chitosan reaction)	-	-	-	-	0	0	-	-	-

[a]Includes juveniles.

Table 2. Results of staining and chemical tests on the cuticle of Cryptocellus pelaezi juveniles and third nymph exuviae.

	MALLORY TRIPLE	MASSON TRICHROME	HEMA-TOXYLIN	SUDAN III	SUDAN BLACK B	RICHARDSON'S (10μ)	MILLON
LARVAE							
SCLERITES							
epicuticle	-	-	+	-	+ to -	+ (light purple)	0
exocuticle	+ (red)	+ +	+ to -	-	-	+	0
mesocuticle	+ + + (red)	+ + (red)	+ to -	+ +	+ +	+	0
INTERSEGMENTAL MEMBRANES							
epicuticle	0	-	0	0	+ +	+ (light purple)	0
mesocuticle	+ (red)	+ + + (red)	+	0	+ +	+ + + +	0
ARTHORODIAL MEMBRANES							
epicuticle	0	0	+	0	0	0	0
endocuticle	0	0	-	0	0	0	0

(cont'd.)

Table 2. (cont'd.)

	MALLORY TRIPLE	MASSON TRICHROME	HEMA-TOXYLIN	SUDAN III	SUDAN BLACK B	RICHARDSON'S (10μ)	MILLON
FIRST NYMPH							
SCLERITES							
epicuticle	-	-	-	+ to -	-	+ (light purple)	+
exocuticle	-	-	+	-	+ +	+	+ +
mesocuticle	+ + (red)	+ + + (red)	+ to -	+ +	0	+	+ + +
INTERSEGMENTAL MEMBRANES							
epicuticle	0	0	0	0	0	+ (light purple)	0
mesocuticle	+ + + + (red)	+ + + + (red)	0	+ + +	+ + +	+ + + +	0
ARTHRODIAL MEMBRANES							
epicuticle	+ + + + (red)	+ + + (red)	0	+ to -	+	+ +	0
endocuticle	+ + + + (blue)	+ + + (green)	0	+ to -	+	+ + +	0

(cont'd.)

Table 2. (cont'd.)

	MALLORY TRIPLE	MASSON TRICHROME	HEMA-TOXYLIN	SUDAN III	SUDAN BLACK B	RICHARDSON'S (10μ)	MILLON
SECOND NYMPH							
SCLERITES							
epicuticle	-	-	-	-	+ to -	+ (light purple)	+ to -
exocuticle	-	-	-	-	-	+ + +	+ +
mesocuticle	+ + +	+ + + (red)	+ +	+ +	+ +	+ + +	+ + +
INTERSEGMENTAL MEMBRANES							
epicuticle	0	-	0	0	0	0	0
mesocuticle	+ + + (red)	+ + + + (red)	+ + +	+ + + +	+ + +	0	0
ARTHRODIAL MEMBRANES							
epicuticle	+ + + + (red)	+ + + (red)	+ + +	+	+ +	+	-
endocuticle	+ + + + (blue)	+ + + (green)	-	+	-	+ +	-

(cont'd.)

Table 2. (cont'd.)

	MALLORY TRIPLE	MASSON TRICHROME	HEMA-TOXYLIN	SUDAN III	SUDAN BLACK B	RICHARDSON'S (10μ)	MILLON
THIRD NYMPH							
SCLERITES							
epicuticle	-	-	+ to -	-	+ to -	+ (light purple)	0
exocuticle	-	-	-	-	-	-	+ +
mesocuticle	+ + + (red)	+ + (red)	+ to -	+ +	+ +	+ +	+ + +
INTERSEGMENTAL MEMBRANES							
epicuticle	-	-	-	0	0	-	+
mesocuticle	+ + + + (red)	+ + + (red)	+ +	+ +	+ + +	+ + + +	+ + +
ARTHRODIAL MEMBRANES							
epicuticle	0	+ + + + (red)	+ + + +	0	+ +	+	-
endocuticle	0	+ + + + (green)	-	0	+	+ + + +	-

(cont'd.)

Table 2. (cont'd.)

	MALLORY TRIPLE	MASSON TRICHROME	HEMA-TOXYLIN	SUDAN III	SUDAN BLACK B	RICHARDSON'S (10μ)	MILLON
THIRD NYMPH EXUVIAE							
SCLERITES							
epicuticle	-	-	0	0	+	0	+
exocuticle	+ + + +	irregularly red	0	0	+ to -	+ + + +	+ +
mesocuticle	+ + + +	irregularly red	0	0	+ to -	+ + + +	+
INTERSEGMENTAL MEMBRANE							
epicuticle	-	-	0	0	+	+	+

presence of a trace amount of tyrosine, a phenolic amino acid. However, after the chitosan reaction, both of the preceding tests were negative, indicating the absence of proteins and phenolic compounds. The epicuticle separated from the rest of the cuticle with warm (42°C) concentrated HNO_3.

The sclerite exocuticle also shows little affinity to stains. There was a fairly strong xanthoproteic reaction, and very weak Millon indicating the presence of proteins and very little tyrosine in these proteins. The chitosan reaction was very weak, indicating a small amount of chitin. However, after the chitosan reaction, both the acid fuchsin and aniline blue of Mallory triple and Sudan Black B stained the exocuticle, indicating the presence of lipid. The inner portion of the exocuticle began to dissolve in warm concentrated HNO_3 after four hours showing less resistance to the acid than the epicuticle which did not dissolve.

In the exocuticle, various layers were evident in light microscopy. In the dorsal portion of the body and the legs, there were two regions, an outer unstriated region and an inner horizontally striated one. The striated region measured one-third of the total thickness in the dorsal opisthosoma. Ventrally, extreme anterior and posterior regions resembled the dorsum and legs, but medially there was a dark pigmented area in the outer unstriated region. Neither striations nor coloration were observed in the 0.5μ sections. With electron microscopy, none of these subdivisions of the exocuticle are evident. The whole exocuticle is uniform, exhibiting fine light and dark striations (40mμ thick) parallel to the surface, and tra-

versed by pore canals (Figure 1).

The juvenile sclerites, composed of epicuticle, mesocuticle, and endocuticle, are also striated with the inner two-thirds to three-fourths showing striations parallel to the surface and relatively colorless in section. The outer unstriated one-eighth to one-fourth is yellowish. The mesocuticle coincides with the striated portion.

The third nymph exuviae (cast exoskeletons) showed a separation of the epicuticle from the rest of the cuticle. The setae were still in place, and striations could still be seen in the inner one-half to three-fourths. The cuticle of the exuvia is comparable in thickness to that of the third nymphs. Based on the presence of striations and thickness, regions corresponding to the meso- and exocuticle are still present, but there was no differential staining. The epicuticle stained with Sudan Black B indicating the presence of lipid. The exocuticle stained approximately as the mesocuticle did indicating the tanned exocuticle reverts to untanned mesocuticle at the time of molting. Only one reaction showed any differentiation; the exocuticle indicated more phenolic compounds with Millon than did the mesocuticle.

Intersegmental Membranes

As in the sclerites, there are two apparent layers in the intersegmental membrane. These, however, are epicuticle and mesocuticle. The epicuticle is unaffected by nearly all the chemical tests and stains. The mesocuticle, on the other hand, is more reactive. It is positive to all three Sudan stains indicating the presence of

lipid material. The chitin test was very weak indicating a very small amount of chitin. The xanthoproteic reaction was positive, indicating a large amount of protein. The Millon was also positive, indicating a large amount of tyrosine. The juvenile intersegmental membranes have the same two layers that react in practically an identical fashion as the adult. In the third nymph exuvia, the mesocuticle is absent; and all that remains is the epicuticle.

The whole adult mesocuticle appears striated in both light and electron microscopy. The laminae are thicker than the sclerite laminae and become thinner and closer together toward the outer surface. The inner surface of the intersegmental membrane is convoluted (Figure 2) as opposed to the straight inner region of the sclerite cuticle (Figure 1). The epicuticle (0.4μ thick) is present. It is recognizable by being less electron dense than the rest of the cuticle and homogeneous in texture.

Arthrodial Membranes

The two layers of the arthrodial membranes have been designated as the epicuticle and endocuticle. The epicuticle is more reactive to staining and chemical tests than the epicuticles of the sclerites and intersegmental membranes. It stains strongly red with Mallory triple and Masson trichrome, is positive to hematoxylin and Richardson's, and shows an affinity for two of the Sudan stains indicating the presence of a large amount of lipid. It is negative to Millon indicating no phenols to be present. Phenolic compounds are also absent from the endocuticle.

The endocuticle stains green with the light green of Masson trichrome and blue with the aniline blue of Mallory triple, is positive to Richardson's, and unreactive to hematoxylin. The presence of lipids is indicated with staining with Sudan IV and Sudan Black B.

The epicuticle is clearly demonstrated in the intersegmental and arthrodial membranes with electron microscopy. It measures from 0.4 to oneμ in thickness and is non-striated in the arthrodial membrane.

Pore Canals

Pore canals are very small ducts (oneμ or less in diameter) that pass vertically through the cuticle. Generally, they are twisted into a tight helix in most arthropods (Locke, 1961).

Pore canals are present in abundance in the sclerites, infrequently found in the intersegmental membranes, and are either absent or sparse in the arthrodial membranes. They can be seen in the 0.5μ sections for light microscopy as curved structures passing from the epidermis to the surface of the cuticle (Figure 3). However, they are too small to be seen well with the light microscope, the lumen being only 0.1μ in diameter. The laminations of the cuticle are not present immediately around the pore canals (Figure 1). This amorphous area measures from 0.5 to 1μ in diameter and enables the pore canals to be seen in light microscopy. Cytoplasm can be seen extending into the bases of the pore canals where they appear to be larger in diameter (Figure 4). Further from the epidermis, they are seen to be empty or filled with a granular substance. The pore canals have also been observed to branch.

Epidermis, Basement Membrane, Muscle Attachments

It was difficult to determine the exact limits of the epidermis. If there is basement membrane, its presence must be irregular. Some electron micrographs indicated that a basement membrane was present (Figure 5). The epidermis was limited to a single layer of cells beneath the intersegmental membranes and a complex multicellular layer below the sclerites. With light microscopy, the epidermis seemed to be syncitial, like that of the spider observed by Browning (1943), but with the electron microscope the presence of cell membranes was established. Often areas of microdigitations could be seen below the cuticle (Figure 2). The PAS reaction was positive in the epidermal cells showing the presence of glycogen. Lead staining also indicated the presence of glycogen in electron micrographs (Figure 1). Septate desmosomes have also been observed in the epidermis that are very similar to those seen by Wood (1959), Tsubo and Brandt (1962), and Locke (1965).

Myoepidermal junctions have been observed several times in electron micrographs of C. pelaezi. The tonofibrillae, which attach muscle to the cuticle, extend into the cuticle at which point they branch (Figure 6). The tonofibrillae seem to originate in sockets. Interdigitations of muscle with epidermis can also be seen (Figure 6).

Simple and Spike-like Setae

Simple setae (Pittard, 1970) are the most common sensory structures covering the surface of the body. They are connected to the epidermis by large ducts passing through the cuticle (Figure 3). The

setae are comprised of three layers. The inner layer is the most reactive; lipids, proteins, and chitin are indicated as being present. The outer and middle layers are unreactive in contrast. The middle layer is birefringent when observed with the polarizing microscope. With electron miscrosopy, the three layers can easily be distinguished, the middle one being rather irregular in density (Figure 7). At least two cells are seen extending into the setal ducts.

The surface of the cuticle is covered with numerous epicuticular spike-like setae (Pittard, 1970) measuring oneμ in diameter and on to fiveμ in height. These spike-like setae have been observed on other ricinuleids (Kennaugh, 1968).

CHAPTER IV

DISCUSSION

Examination of the integument of Cryptocellus pelaezi reveals a cuticle conforming to the basic arthropod pattern, consisting of epicuticle and procuticle. There are, however, some pecularities. C. pelaezi does not have the sclerite endocuticle found in most other arthropods including scorpions (Kennaugh, 1959; Krishnan, 1953; Malek, 1964; Shrivastava, 1955), spiders (Barth, 1969; Browning, 1943; Sewell, 1955), opiliones (Grainge and Pearson, 1966) and mites (Brody, 1969). The means of expansion with feeding is also different. Most articulating membranes of arthropods are either folded or pleated. During engorgement, these membranes straighten. In C. pelaezi, no folds or pleats are present in the intersegmental membranes. The mechanism of expansion may be due to stretching of these membranes.

The mesocuticle of insects is digested at the time of molting (Richards, 1967). The mesocuticle of the intersegmental membranes of the third nymph exuviae is absent, but the mesocuticle of the sclerites is still present. So there appears to be a difference between the two although I have called them both mesocuticle. An examination of the juveniles revealed that the mesocuticle of the intersegmental membranes is generally more reactive to staining and chemical tests than that of sclerites. Taylor and Richards (1965) also noticed that mesocuticle in different portions of the same

insect will react differently to Sudan Black B. They also observed that mesocuticle reverted to endocuticle (based on Mallory stain) before digestion of the cuticle. The exocuticle of C. pelaezi reverted to mesocuticle although it was not digested.

Treatment with warm (42°C) concentrated HNO_3 left the epicuticle still intact while the exocuticle and mesocuticle started to dissolve and lose their definition. One of the characteristics of the epicuticle of arachnids and other arthropods is its resistance to concentrated mineral acids (Lees, 1947; Sewell, 1955). Kennaugh (1968) could only distinguish one layer of the epicuticle which he referred to as the cuticulin layer. Undoubtedly, he was referring to the "cuticulin" of Dennell and Malek (1953), the "inner epicuticle" which is thick enough to see with the light microscope. This layer in C. pelaezi is composed of lipid and protein. It may be tanned or untanned in scorpions (Krishnan, 1954) and in insects (Dennell and Malek, 1955). The epicuticle of the sclerites and intersegmental membranes of C. pelaezi is tanned, whereas the epicuticle of the arthrodial membranes is impregnated but untanned. No other layers of epicuticle could be distinguished in this study.

The distribution of tyrosine, one of the tanning agents, is difficult to explain. It is absent in the arthrodial membranes, present in very small quantities in sclerites, and present in abundance in the intersegmental membranes. In the process of tanning, the impregnating substance contains tyrosine (Blower, 1951; Dennell, 1958). This would explain its presence in mesocuticle which is impregnated but untanned in C. pelaezi. However, the epicuticle of the

arthrodial membranes is also impregnated and untanned, but contains no tyrosine. Perhaps the tyrosine has already been converted to a quinone, which is the next step in the tanning process (Dennell, 1958). Tyrosine is also found in tanned exocuticle and tanned epicuticle. It seems that tyrosine should be absent here, being utilized in the tanning process.

According to Taylor and Richards (1965), tanned cuticle of insects is PAS-negative. But there are certain regions that are PAS-positive such as flexible membranes, endocuticles of some insects, and the basement membrane. The subcuticle discovered by Schmidt (1956) is also PAS-positive. In this study, no subcuticle was identified with PAS or electron microscopy. Nor was a basement membrane identified with PAS even though one may be irregularly present. This should not be surprising; for according to Richards (1951), only insect basement membranes are well-defined structures. In scorpions and spiders, the basement membrane is imperfectly formed (Richards, 1951). However, it has been observed in some mites (Henneberry et al, 1965) and not in others (Gibbs and Morrison, 1959).

Kennaugh (1968) refers to two helical patterns of the pore canals, a tight helix that conforms to that of other arthropods and a spiral with a large radius. The large spiral has been observed in C. pelaezi; but, the tight helix is not present. Neville and Luke (1969) describe two patterns of cuticle formation and resulting pore canal shapes. The two-system model suggests a helicoidal structure of cuticular laminae with resulting twisted, ribbon-shaped pore canals, and cuticle of laminae oriented in one direction with straight pore

canals. Cryptocellus pelaezi seems to follow the more uncommon preferred orientation with untwisted pore canals. Cytoplasm of the epidermal cells can be seen extending into the pore canals in C. pelaezi. Other studies with electron microscopy also show these cytoplasmic extensions in acarines (Nathanson, 1967, 1970; Wharton et al., 1968). Only Neville and Luke (1969) have seen cytoplasmic membranes bordering the inside of the pore canals. In this study, a membrane seems to be present inside the pore canals (Figure 1).

Locke (1964) observed in insects a microvillate plasma membrane beneath the cuticle during cuticle formation, and he suggested a connection between the two. Barbier (1968) also suggested cuticle deposition by microdigitations of the membrane beneath the cuticle of Galleria mellonella. Microdigitations have also been observed in C. pelaezi beneath the cuticle, although not to as great an extent. Others have observed the same in adult arthropods (Nathanson, 1967, 1970; Wharton et al, 1968; Locke, 1961). This seems unusual if the microdigitations are involved in cuticle formation, for the cuticle is already complete in adults.

The two cells seen extending into the setal ducts are probably the tormogen and trichogen cells that form the sockets and the setae respectively (Wigglesworth, 1933). The inner layer of the setae reacts like mesocuticle. The outer and middle layers react like sclerite exocuticle and epicuticle. According to Richards (1952) and Barth (1969), setae are always sclerotized and are composed of exocuticle. On the other hand, Slifer et al. (1959) say the walls of sensory pegs of insects are more than likely epicuticle. Electron

microscopy revealed an outer layer that was more dense than the middle layer in C. pelaezi. This outer layer corresponds to epicuticle in location, but contains chitin. Since it contains chitin, then according to definition, it is not epicuticle. Therefore, C. pelaezi setae consist of epicuticle.

The sockets and tonofibrillae in the myoepidermal junctions appear to be the same as those seen in insects by Lai-Fook (1967) and Caveney (1969).

It would be interesting to compare the ricinuleid integument with that of other arachnids based on structure and composition of the integument. Since there have been such a limited number of studies on other arachnid cuticles, there is inadequate information for such a comparison at the present time.

ILLUSTRATIONS

Figure 1. The sclerite of the adult opisthosoma in cross section shows a laminated exocuticle (Exo) traversed with pore canals (PC), some of which are lined with a membrane (MB). The epidermis (Epi) contains an abundance of glycogen (arrows). x18,000.

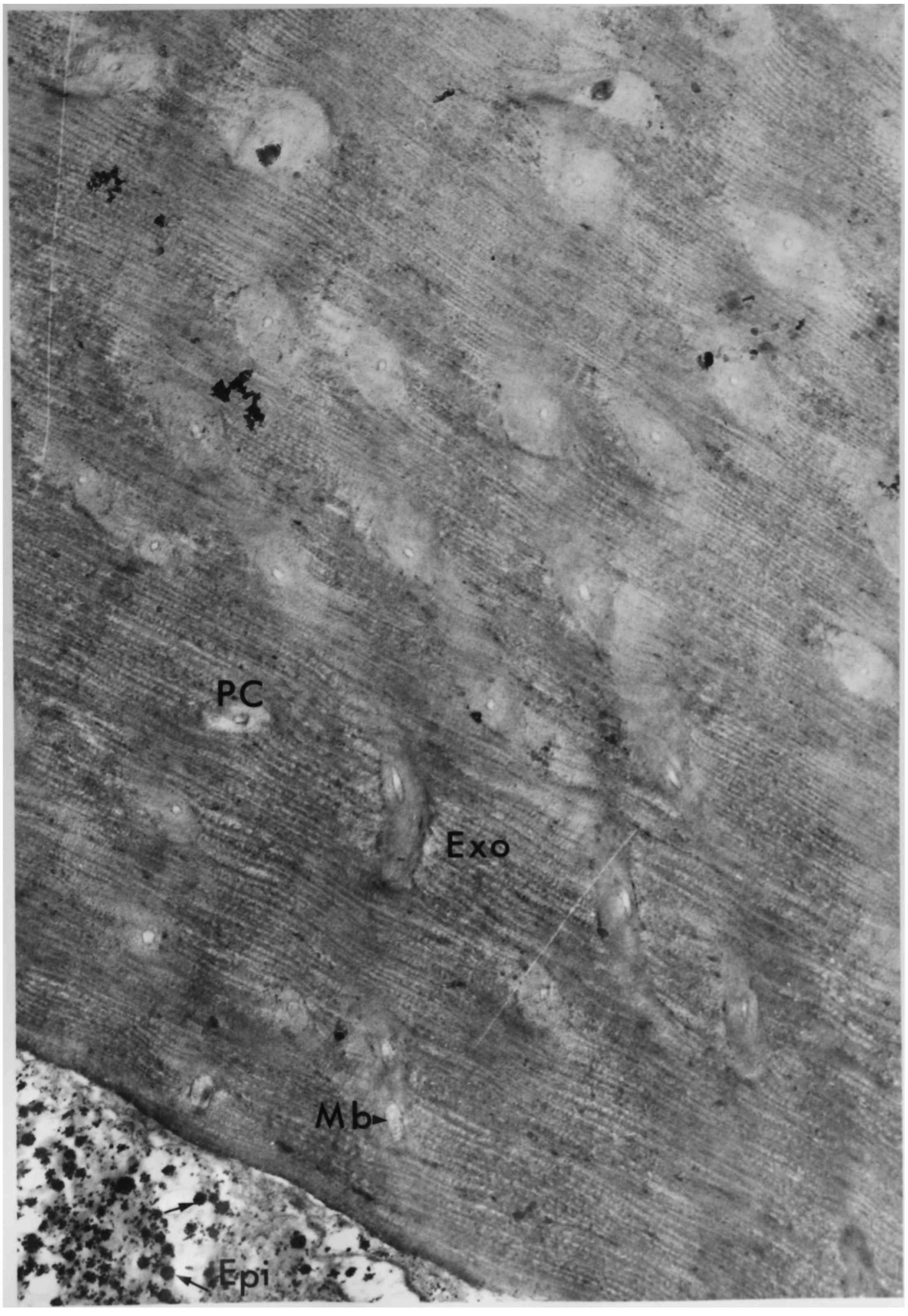

PC
Exo
Mb
Epi

Figure 2. The adult intersegmental membrane in cross section can be seen to be convoluted next to the epidermis (Epi). Microdigitations (Md) can be seen at the interface of the cell and mesocuticle (Mes). x16,000.

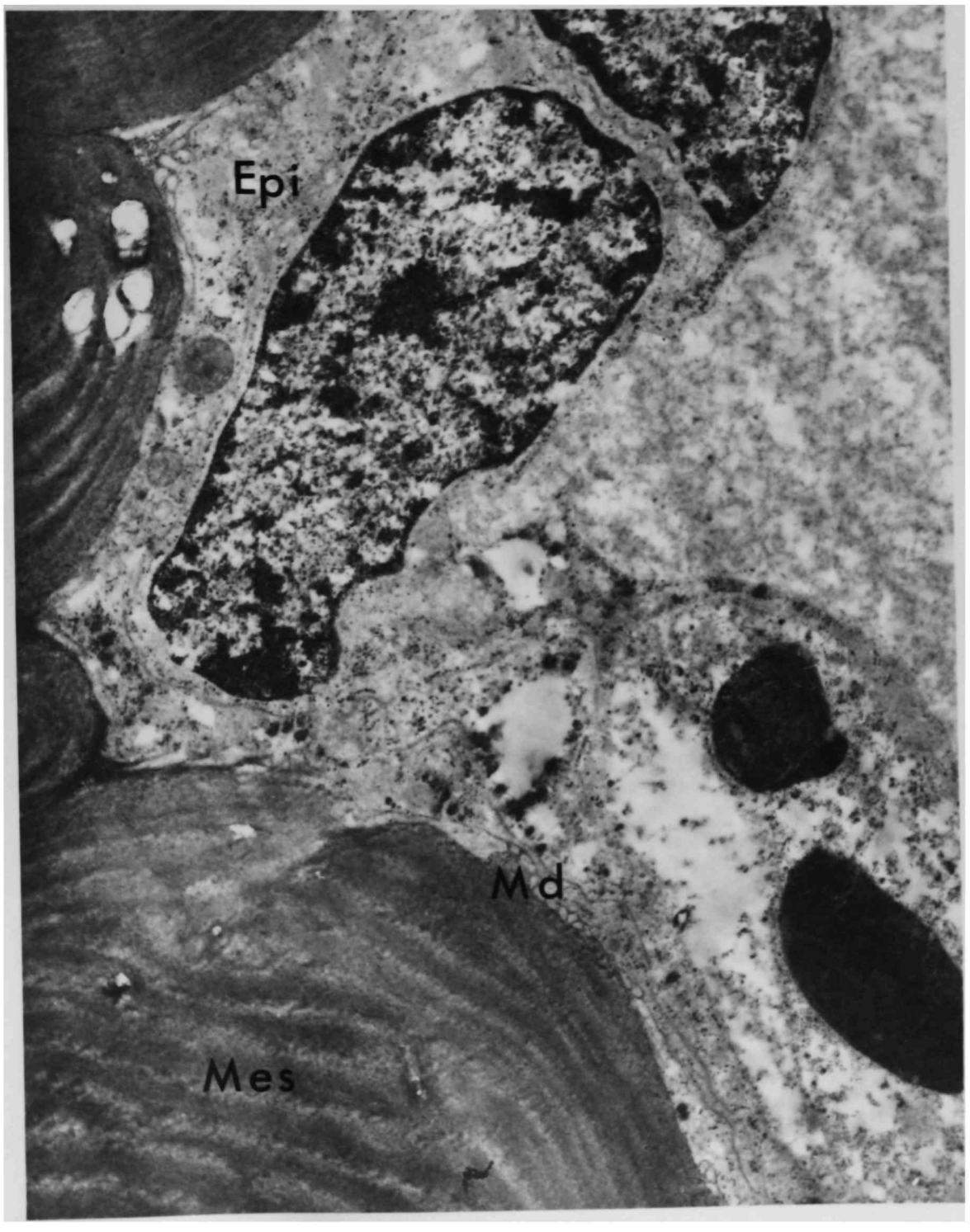

Epi
Md
Mes

Figure 3. The sclerite of the adult opisthosoma seen in cross section shows a multicellular epidermis (Epi) and thick exoskeleton traversed by pore canals (arrows). Simple setae (not shown) are connected to the epidermis through setal ducts (SD). Spike-like setae (Sk), on the other hand, are strictly epicuticular structures with no cytoplasmic connections. x1300.

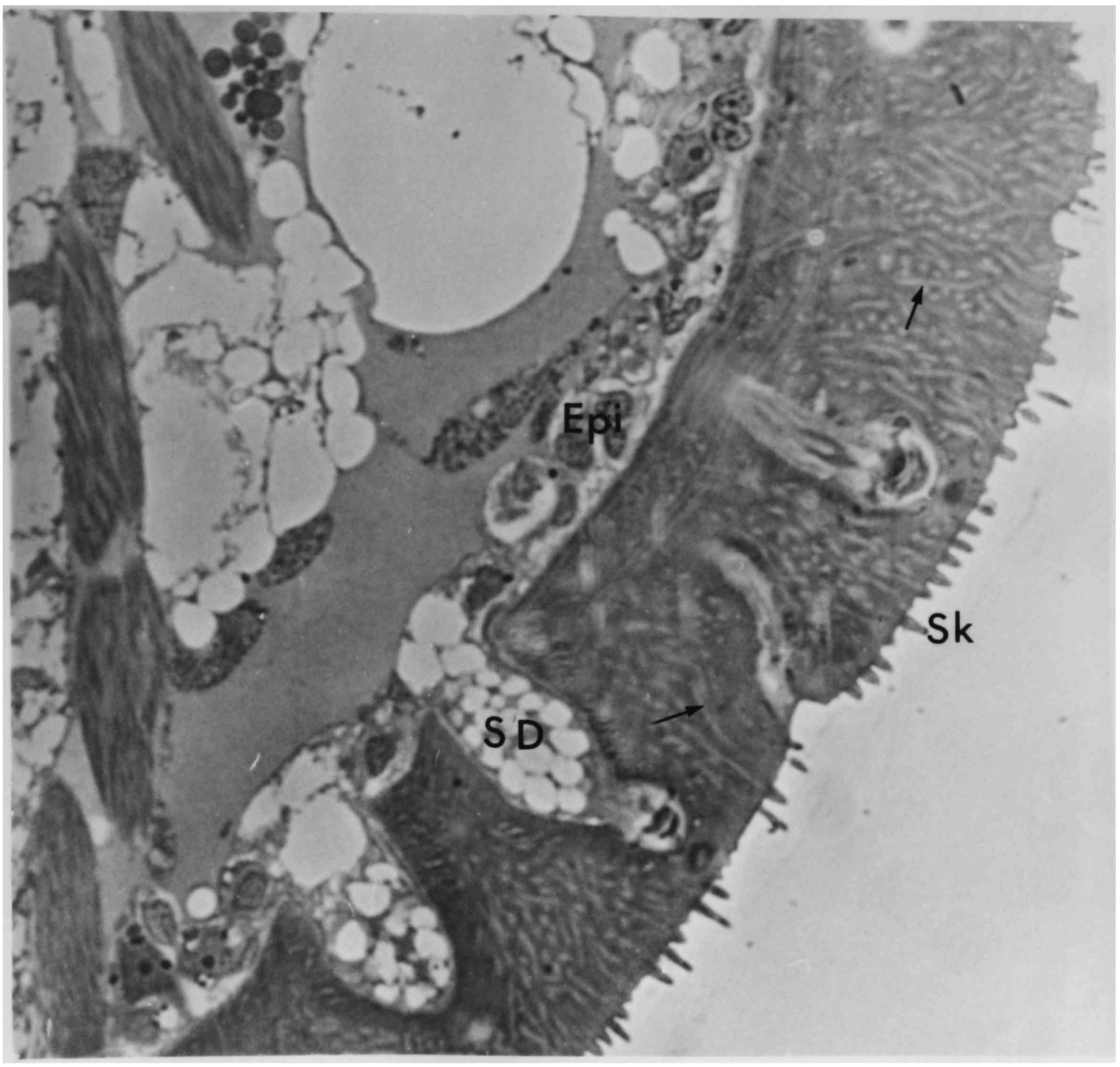

Epi
Sk
SD

Figure 4. Cytoplasmic extensions (CE) of the epidermis (Epi) into the pore canals (PC) can frequently be seen in cross section of sclerite exocuticle (Exo). x16,000.

Exo
PC
CE
Epi

Figure 5. In this cross section of epidermis, a basement membrane (BM) is present. The direction of the arrow is toward the cuticle. x30,000.

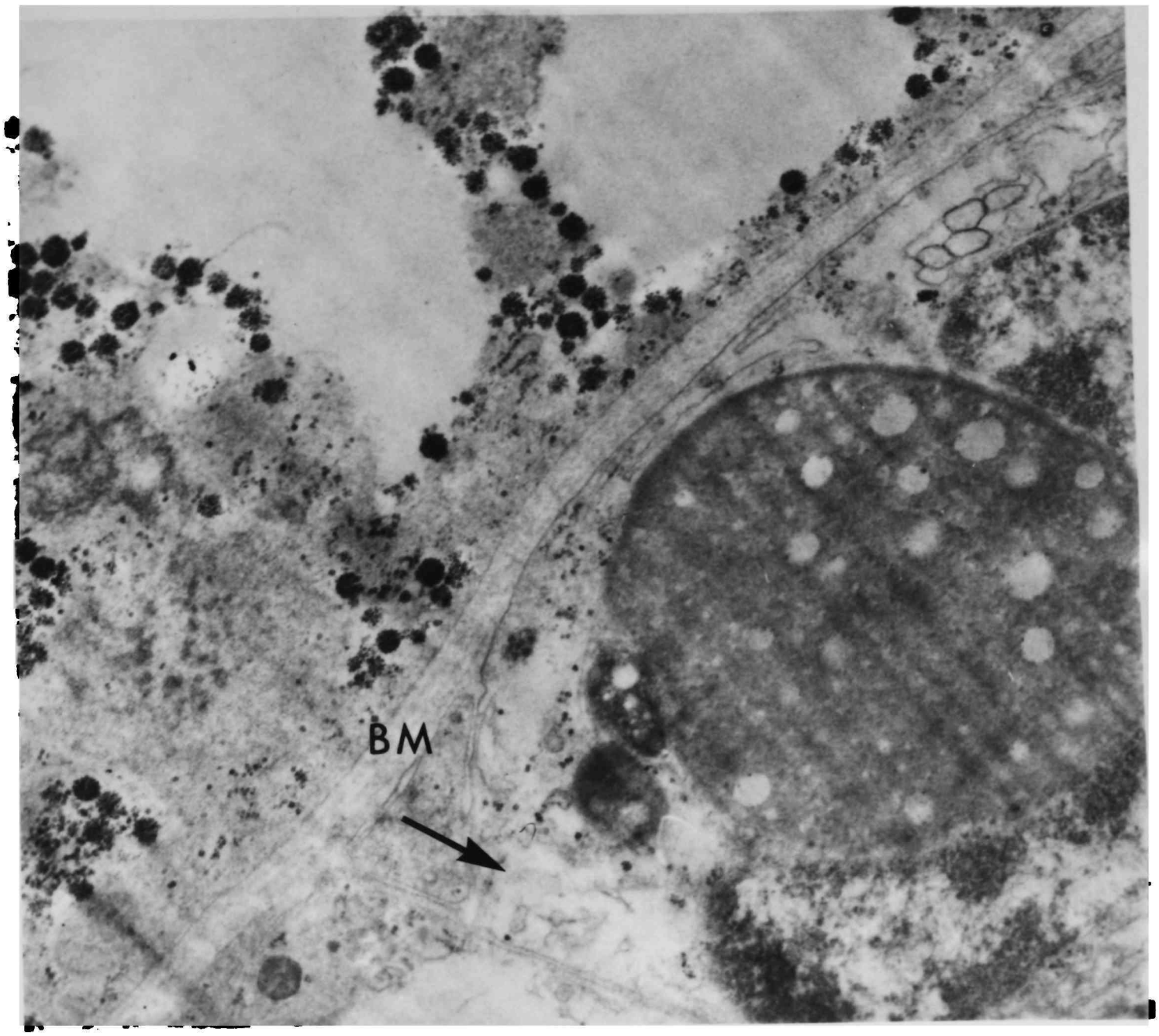

BM

Figure 6. Tonofibrillae (T), originating in sockets (S), are seen extending into the exocuticle (Exo) and branching. Myoepidermal junctions are also evident (arrows). x14,000.

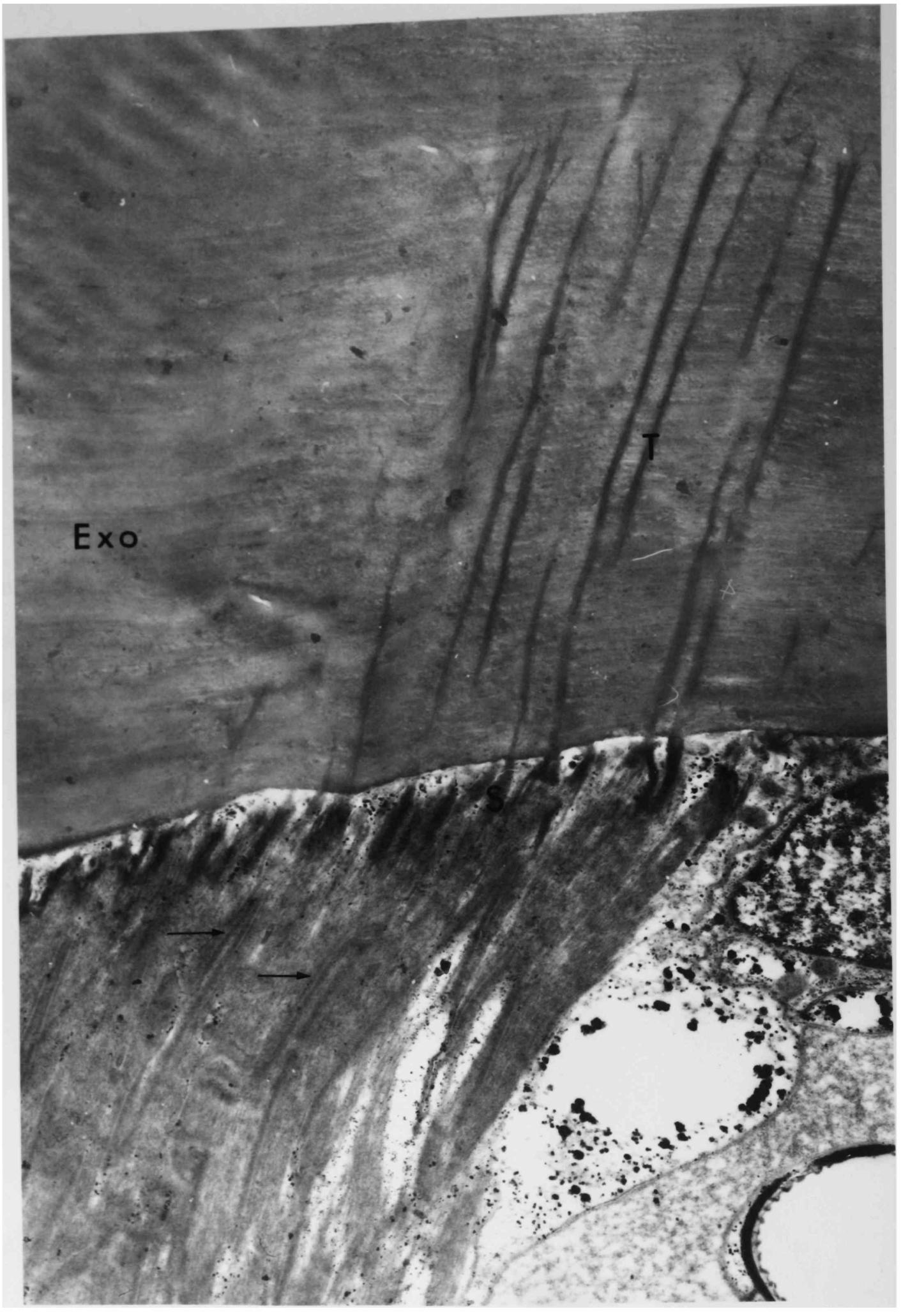
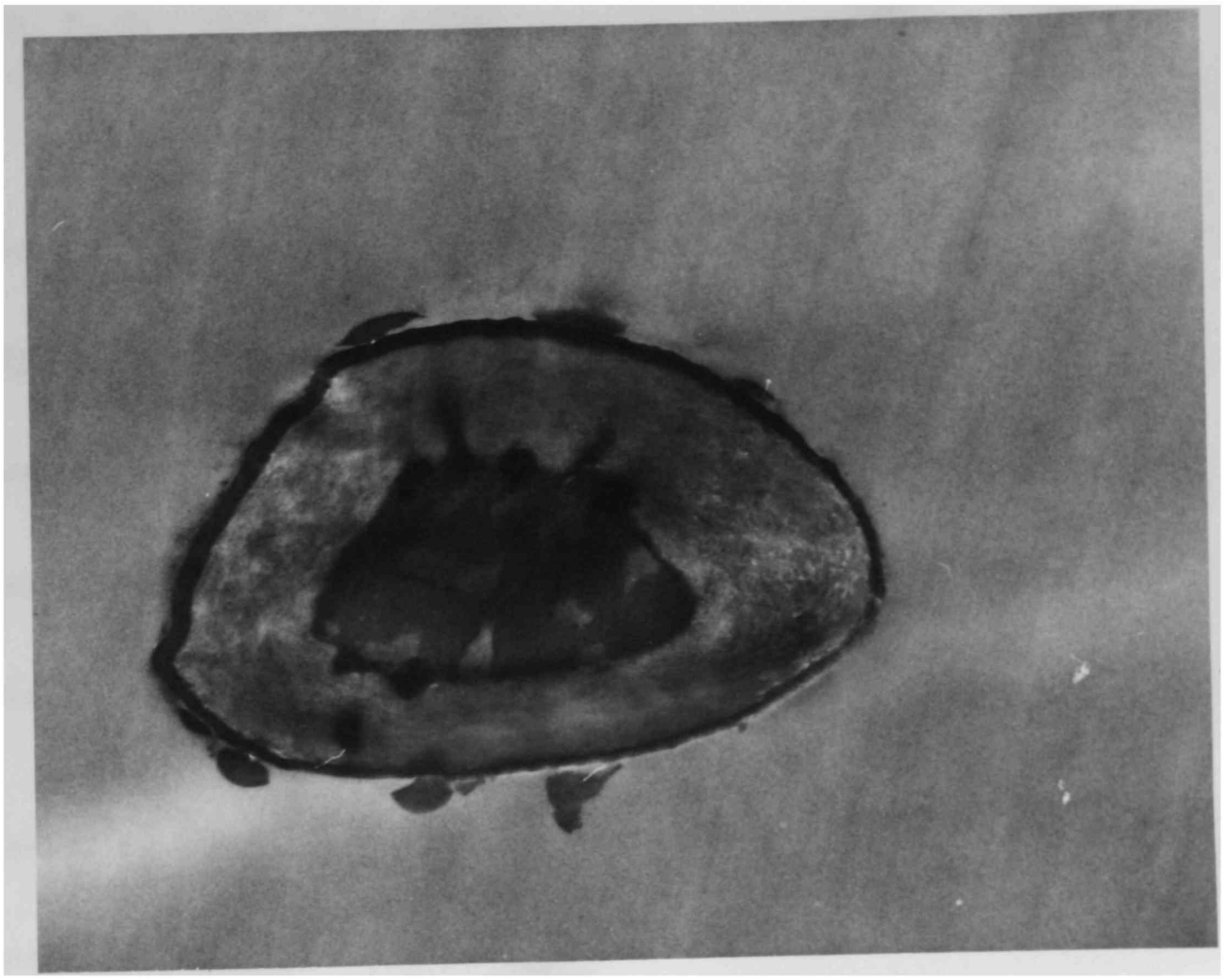

Exo
T
S

Figure 7. Three layers are evident in cross sections of setae. x16,000.



REFERENCES

Barbier, M. R. 1968. Etude expérimentale et observations, au microscope életronique du dépôt de l'endocuticle et de l'exocuticle chez la larve de Galleria mellonella L.(Lépidoptère Pyralidae). C. R. Acad. Sci. (Paris) 266:1613-1615.

Barth, F. G. 1969. Die Feinstruktur des Spinnenintegu ments. I. Die Cuticula des Laufbeins adulter häutungsferner Tiere(Cupiennius salei Keys). Zeitsch. Zellforsch. 97:137-159.

Barth, F. G. 1970. Die Fainstruktur des Spinnenintegu ments. II. Die räumliche Anordnung der Mikrofasern in der lamellierten Cuticula und ihre Beziehung zur Gestalt der Porenkanäle(Cupiennius salei Kaysl, adult, häutungsfern, Tarsus). Zeitsch. Zellforsch. 104:87-106.

Blower, G. 1951. A comparative study of the chilopod and diplopod cuticle. Quart. J. Microscop. Sci. 92:141-161.

Brody, A. R. 1969. Comparative fine structure of the acarine integument. J. New York Entomol. Soc. 77:105-116.

Browning, H. C. 1943. The integument and moult cycle of Tegenaria atrica(Araneae). Proc. Roy. Soc. Lond.(Ser. B)131:65-86.

Caveney, S. 1969, Muscle attachment related to cuticle architecture in apterygota, J. Cell Sci. 4:541-559.

Cooke, J. A. L. 1967. Observations on the biology of Ricinulei (Arachnida) with descriptions of two new species of Cryptocellus. J. Zool. Lond. 151:31-42.

Dennell, R. 1958. The hardening of insect cuticles, Biol. Rev. 33:178-196.

Dennell, R. and S. R. A. Malek. 1953. Homology of the layers of the epicuticle of insects. Nature 171:298-299.

Dennell, R. and S. R. A. Malek. 1955. The cuticle of the cockroach Periplaneta americana. II. The epicuticle. Proc. Roy. Soc. (Ser. B) 143:239-257.

Gibbs, K. E. and F. O. Morrison. 1959. The cuticle of the two-spotted spider mite, Tetranychus telarius(Linnaeus)(Acarina: Tetranychidae). Can. J. Zool. 7:633-637.

Grainge, C. A. and R. G. Pearson. 1966. Cuticular structure in the Phalangida. Nature 211:866.

Henneberry, T. J., J. R. Adams, and G. E. Cantwell. 1965. Fine structure of the integument of the two-spotted spider mite, Tetranychus telarius(Acarine:Tetranychidae). Ann. Entomol. Soc. Amer. 58:532-535.

Hughes, T. E. 1959. The cuticle of Acarus siro L. (Tyroglyphus farinae). J. Exp. Biol. 36:363-368.

Humason, G. L. 1967. Animal tissue techniques. 2nd ed. W. H. Freeman and Co., San Francisco. 569 p.

Jensen, W. A. 1962. Botanical histochemistry. W. H. Freeman and Co., San Francisco. p. 203.

Kaestner, A. 1968. Invertebrate zoology. Vol. II. Interscience Publishers, New York. 472 p.

Kennaugh, J. 1959. An examination of the cuticles of two scorpions, Pandinus imperator and Scorpioos hardwickii. Quart. J. Microscop. Sci. 100:41-50.

Kennaugh, J. H. 1968. An examination of the cuticle of three species of Ricinulei(Arachnida). J. Zool. Lond. 156:393-404.

Kennedy, C. H. 1932. Methods for the study of the internal anatomy of insects. By the author, Ohio State Univ., Columbus. 103 p.

Krishnan, G. 1953. On the cuticle of the scorpion Palamneus swammerdami. Quart. J. Microscop. Sci. 94:11-21.

Krishnan, G. 1954. The epicuticle of an arachnid, Palamneus swammerdami. Quart. J. Microscop. Sci. 95:371-381.

Krishnan, G., G. N. Ramachandran, M. S. Santanam. 1955. Occurrence of chitin in the epicuticle of an arachnid Palamneus swammerdami. Nature 176:557-558.

Lai-Fook, J. 1967. The structure of developing muscle insertions in insects. J. Morphol. 123:503-527.

Lal, M. B. and S. C. Shrivastava. 1957. Absence of chitin in epicuticle of some Indian scorpions. Science 126:508-509.

Lee, B. 1950. The microtomist's vade-mecum. 11th ed. J. B. Gatenby and H. W. Beams (eds). The Blakinston Co., Philadelphia. 753 p.

Lees, A. D. 1946. The water balance in Ixodes ricinus L. and certain other species of ticks. Parasitology 37:1-20.

Lees, A. D. 1947. Transpiration and the structure of the epicuticle in ticks. J. Exp. Biol. 23:379-410.

Locke, M. 1961. Pore canals and related structures in insect cuticle. J. Biophys. Biochem. Cytol. 10:589-618.

Locke, M. 1964. The structure and formation of the integument in insects. In M. Rockstein (ed.) The physiology of insects. Vol. III. Academic Press, New York. p. 380-470.

Locke, M. 1965. The structure of septate desmosomes. J. Cell Biol. 25:166-169.

Lower, H. F. 1959. The insect epicuticle and its terminology. Ann. Entomol. Soc. Amer. 52:381-385.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:409-414.

Malek, S. R. A. Chitin in the hyaline exocuticle of the scorpion. Nature 198:301-302.

Malek, S. R. A. 1964. A study of a scorpion cuticle. I. The structure and staining reactions of the fully formed cuticle of Buthus guinquestriatus (H.&E.). Proc. Linn. Soc. Lond. 175:101-116.

Millonig, G. 1961. Advantages of a phosphate buffer for OsO_4 solutions in fixation. J. Appl. Phys. 32:1637.

Millot, J. 1945. L'anatomie interne des Ricinulei(Arachnides). Ann. Sci. Natur. Zool. Ser. 11, 7:1-29.

Mitchell, R. W. 1970. Population size and dispersion and species associations of a Mexican cavernicole Ricinuleid(Arachnida). Ciencia 27:63-74.

Nathanson, M. E. 1967. Comparative fine structure of sclerotized and unsclerotized integument of the rabbit tick, Haemaphysalis leporispalustris(Acari:Ixodides:Ixodidae). Ann. Entomol. Soc. Amer. 60:1125-1135.

Nathanson, M. E. 1970. Changes in the fine structure of the integument of the rabbit tick, Haemaphysalis leporispalustris(Acari: Ixodides:Ixodidae), which occur during feeding. Ann. Entomol. Soc. Amer. 63:1768-1774.

Neville, A. C. and B. M. Luke. 1969. Molecular architecture of adult locust cuticle at the electron microscope level. Tissue & Cell 1:355-366.

Pittard, S. K. 1970. Comparative external morphology of the life stages of Cryptocellus pelaezi(Arachnida,Ricinulei). M.S. thesis, Texas Tech Univ. 122 p.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208-212.

Rhode, C. J., Jr. 1964. Some techniques in the preparation of stained whole mounts and serial sections of mite embryos and adults. Acarologia, fasc. h.s. 1964:208-214.

Richards, A. G. 1951. The integument of arthropods. Univ. Minnesota Press, Minneapolis. 411 p.

Richards, A. G. 1952. Studies on arthropod cuticle. VIII. The antennal cuticle of honeybees, with particular reference to the sense plates. Biol. Bull. 103:201-225.

Richards, A. G. 1967. Sclerotization and the localization of brown and black colors in insects. Zool. Jahrb. Anat. 84:25-62.

Richardson, K. C., L. Jarett, and E. H. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. 35:313-325.

Savory, T. 1964. Arachnida. Academic Press, New York. 291 p.

Schatz, L. 1952. The development and differentiation of arthropod procuticle:staining. Ann. Entomol. Soc. Amer. 45:678-686.

Schmidt, E. L. 1956. Observations on the subcuticular layer in the insect integument. J. Morphol. 99:211-226.

Sewell, M. T. 1951. Pore canals in a spider cuticle. Nature 167:857-858.

Sewell, M. T. 1955. The histology and histochemistry of the cuticle of a spider, Teoenaria domestica(L.). Ann. Entomol. Soc. Amer. 48:107-118.

Shrivastava, S. C. 1955. Some peculiarities of the structure of the cuticle of some Indian scorpions. Curr. Sci. 24:24-25.

Slifer, E. H., J. J. Prestage, and H. W. Beams. 1959. The chemoreceptors and other sense organs on the antennal flagellum of the grasshopper(Orthoptera;Acrididae). J. Morphol. 105:145-191.

Smith, H. G. and C. D. Hynes. 1966. Modifications of the N-butyl methacrylate imbedding method as applied to insect larval head capsules. Ann. Entomol. Soc. Amer. 59:230.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Res. 26:31-43.

Taylor, R. L. and A. G. Richards. 1965. Integumentary changes during moulting of arthropods with special reference to the subcuticle and ecdysial membrane. J. Morphol. 116:1-22.

Tsubo, I. and P. W. Brandt. 1962. An electron microscopic study of the Malpighian tubules of the grasshopper, Dissosteira carolina. J. Ultrastructure Res. 6:28-35.

Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. 4:475-478.

Wharton, G. W., W. Parrish, and D. E. Johnston. 1968. Observations on the fine structure of the cuticle of the spiny rat mite, Laelaps echidnina(Acari-Mesostigmata). Acarologia 10:206-214.

Wigglesworth, V. B. 1933. The physiology of the cuticle and of ecdysis in Rhodnius prolixus(Triatomidae, Hemiptera); with special reference to the function of the oenocytes and of the dermal glands. Quart. J. Microscop. Sci. 76:269-318.

Wigglesworth, V. B. 1945. Transpiration through the cuticle of insects. J. Exp. Biol. 21:97-114.

Williams, S. C. 1968. Scorpion preservation for taxonomic and morphological studies. Wasmann J. Biol. 26:133-136.

Wood, R. L. 1959. Intercellular attachment in the epithelium of Hydra as revealed by electron microscopy. J. Biophys. Biochem. Cytol. 6:343-352.