SENSE ORGANS ON THE HEAD OF LARVAE OF SOME ELATERIDAE (COLEOPTERA): THEIR DISTRIBUTION, STRUCTURE, INNERVATION, AND HISTOCHEMISTRY

A Thesis
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by
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Preface

The thesis consists of four interrelated manuscripts on sensory organs of wireworms. Each manuscript constitutes a separate Chapter. The manuscripts are currently in press or have been submitted for publication to the Journals indicated at the beginning of each Chapter. They are based solely on original research by the author, done at the Department of Zoology, University of Glasgow during 1959-61, and completed furth of Glasgow, at the University of Saskatchewan, during 1961-2. In addition to the acknowledgements given in each Chapter, I wish to express my appreciation for the constant interest and enthusiasm in this study shown by Mr. R. A. Crowson, Department of Zoology, University of Glasgow, and Professor J. G. Rempel, Department of Biology, University of Saskatchewan. The Biographic Unit, Research Branch, Canada Agriculture, Ottawa, assisted only in the preparation of the plates of photomicrographs presented in Chapter II. The work at Glasgow was done during a leave of absence with half pay from the Research Station, Research Branch, Canada Agriculture, Saskatoon, Sask.

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CHAPTER IV

Chapter I

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SOME NEW LARVAL CHARACTERS FOR THE CLASSIFICATION OF ELATERIDAE (COLEOPTERA) INTO MAJOR GROUPS

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In a comparative study of sense organs in elaterid larvae (Chapter II), some basic differences were observed among the species of the various groups that were examined. The most typical and consistent of these are: (1) the presence of certain setae and associated small sclerites in the post-gular region of the head and prothorax, and (2) the presence of certain setae on the first and second segments of the antennae and on the first segment of the labial palpi. These differences appear to be characteristic of the sub-families and some of the tribes of Hyslop's (1917) classification.

Living specimens were examined under a low power binocular microscope. It was necessary to extrude with force the heads of these in order to view the structures in the post-gular region. Preserved specimens were treated with KOH and mounted and cleared in a 70 per cent aqueous solution of Dimethyl Hydantoin Formaldehyde (Steedman, 1958), in a fully extended position, for examination under a high power microscope.

The characteristics of the species that were

examined are given in Table I. The following descriptions of these characteristics are based on examinations of at least five full-grown or nearly full-grown larvae of each species, with two exceptions. Only one specimen of Cardiophorus sp. and two of Oestodes puncticollis Horn were seen. The nomenclature is based on the current N. American usage.

Setae and Sclerites in the Post-gular Region

The setae and associated sclerites that were observed in the post-gular region of the head and prothorax of all the species that were examined are shown in the composite, diagrammatic illustration of figure 1. Those observed in representative species of the various groups are illustrated in figure 2.

With few exceptions, the setae usually occur in pairs (S, fig. 1), and are situated symmetrically, one on each side of the midline, as follows.

S1.- One pair; situated on the anterior margin of the prosternum; largest and most widely separated of the setae in this region; usually supported on a columnar sclerotic ring of the integument. So-called 'master' pair, present in all the representatives.

S2.- One pair; situated on the prosternum caudad

Table 1. Presence (P) or absence (-) of setae (S1 - S5) and plates (P1 - P5) in the post-gular region of the head and prothorax; of setae on the lateral walls of the first and second segments of the antennae and first segment of the labial palpi; and of a complete (D), partial (PD) or no (U) division along the midline of the prosternum in larvae of Elateridae.

Groups and Species	Setae in Post-gular Region					Plates in Post-gular Region					Antennal Setae		Labial Setae	Prosternum
	S1	S2	S3	S4	S5	P1	P2	P3	P4	P5	Seg 1	Seg 2		
CARDIOPHORINAE														
Cardiophorus sp.	P	-	-	-	-	-	-	-	-	-	-	-	-	U
OESTODINAE														
Oestodes puncticollis Horn	P	P	-	-	-	-	-	P	-	-	-	-	-	D
LEPTUROIDINI														
Ctenicera aena (L.)	P	P	P	-	-	-	-	P	-	-	-	-	-	D
C. cuprea (F.)	P	P	P	-	-	-	-	P	-	-	-	-	-	D
C. destructor (Brown)	P	P	P	-	-	-	-	P	-	-	-	-	-	D
Hypolithus bicolor Esch.	P	P	-[1]	-	-	-	-	-	-	-	-	-	-	PD
H. riparius (F.)	P	P	-	-	-	-	-	-	-	-	-	-	-	PD
Limonius minutus (L.)	P	P	P	-	-	-	-	P	-	-	-	-	-	U
Athous haemorrhoidalis (F.)	P	P	P	-	-	-	-	P	-	-	-	-	-	U
Lepturoides linearis (L.)	P	P	P	-	-	-	-	P	-	-	-	-	-	U
PYROPHORINI														
Adelocera murinus (L.)	P	P	-	P	-	P	P	-	P	P	P	P	P	U
ELATERINAE														
Procraerus tibialis (Lac.)	P	P[2]	-	P[2]	P	P[3]	-	-	-	-	P	-	P	U
Sericus brunneus (L.)	P	P	-	-	P	P	P	-	-	-	-	-	-	U
Dalopius marginatus (L.)	P	P	-	-	P	P[3]	P	-	-	-	P	-	P	U
Melanotus rufipes (Herbst)	P	P	-	-	P	P	P	-	-	-	P	-	P	U
Ampedus nigrinus Herbst	P	P	-	-	P	P	P	-	-	-	P	-	P	U
Agriotes obscurus (L.)	P	P	-	-	P	P	P	-	-	-	P	-	P	U
A. lineatus (L.)	P	P	-	-	P	P	P	-	-	-	P	-	P	U
A. sputator (L.)	P	P	-	-	P	P	P	-	-	-	P	-	P	U

[1] One pair present in one specimen of 11 examined.
[2] Tentatively interpreted as only one of the basic pair present.
[3] Basic pair of plates fused.

and slightly mesad to S1; occasionally supported on a columnar sclerotic ring of the integument. Present in all but Cardiophorus sp.; tentatively interpreted as only one of the pair present in Procraerus, left of the midline when viewed from the ventral aspect.

S3.- One to three pairs; situated on the prosternum caudad to S2, in a pattern resembling a V when viewed with setae S1 and S2, with the arms of the V directed cephalad. Present only in the representatives of Lepturoidini, but usually absent in Hypolithus.

S4.- One pair; situated on the prosternum, one directly caudad to each of the setae S2. (The setae S4 may be homologous with the anteriormost pair of setae S3, but are differentiated here by the slight but consistent difference in position). Present only in the representative of Pyrophorini; tentatively interpreted as only one of the pair present in Procraerus, right of the midline when viewed from the ventral aspect.

S5.- Four to six pairs; situated on the membrane between the gula and the prosternum, in a pattern approximating a V, the apex of which is directed cephalad. Present only in the representatives of Elaterinae, including those of the genera Sericus, Melanotus and Procraerus.

In a few apparently aberrant specimens, one of a

Fig. 1. Setae and small sclerites in the post-gular region of the head and prothorax of elaterid larvae;- a composite of the structures found in all the species examined. CM, connecting membrane; Epla, lateroepicranial plate; J, anterior prosternal fold; P, sclerotized plates; Prst, prosternum; S, setae.

Fig. 2. Setae and plates in the post-gular region of the head and prothorax of some elaterid larvae. (a) Cardiophorous sp.; (b) Hypolithus riparius (F.); (c) Athous haemorrhoidalis (F.); (d) Dalopius marginatus (L.); (e) Oestodes puncticollis Horn; (f) Ctenicera aena (L.); (g) Sericus brunneus (L.); (h) Agriotes lineatus (L.); (i) Adelocera murinus (L.); (j) Procraerus tibialis (Lac.); (k) Melanotus rufipes (Herbst); (l) Ampedus nigrinus Herbst.

Fig. 3. Antennae, lateral view. (a) Adelocera murinus; (b) Hypolithus riparius, anterior portion of second segment; (c) Ctenicera aena, ditto. AS1, setiform structures on the anterior margin of the second segment; AS2, setae on lateral walls of first and second segment.

Fig. 4. Labial palpus of Adelocera murinus. LS1, peg-like setiform structure on distal segment; LS2, setae on lateral walls of basal segment.

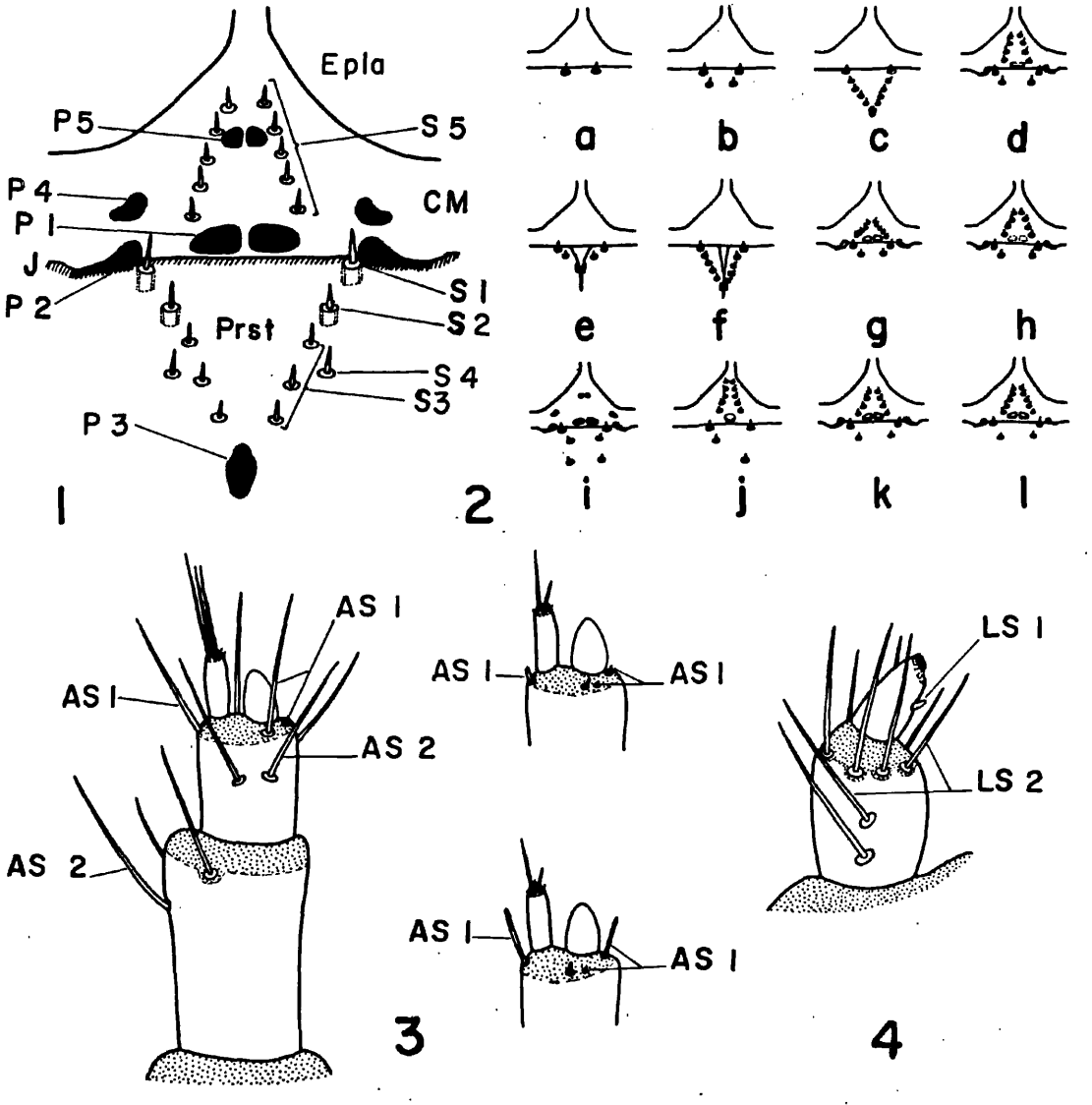
Epla
P5
S 5
P 4
CM
P 1
J
P 2
S 1
S 2
Prst
S 4
S3
P 3
1
2
a
b
c
d
e
f
g
h
i
j
k
l
AS 1
AS 1
AS 2
AS 2
3
AS 1
AS 1
AS 1
AS 1
LS 1
LS 2
4

Fig. 1

pair of setae S3 or S5 were missing, or were situated slightly asymmetrically. One of the 11 specimens of Hypolithus (Hypnoidus) bicolor Esch. that were examined had one pair of setae S3. These formed a V-shaped pattern with setae S1 and S2, typical of the other representatives of Lepturoidini that were seen.

Large campaniform organs or 'pores', in variable numbers and patterns, are often associated with some of the setae in this region. These could easily be confused with the sockets of the smaller setae.

The small plates in the post-gular region (P, fig. 1), are usually paired, heavily sclerotized, and characteristically shaped. Some are fused or lightly sclerotized in certain species. They are indistinct or absent in other species. The positions of these plates are as follows.

P1.- One pair; situated on the membrane, one on each side of the midline, just anterior to the prosternum. Distinct in the representatives of Elaterinae and Pyrophorini, but fused in Dalopius and Procraerus, and lightly sclerotized in Agriotes; absent or indistinct in the representatives of the other groups.

P2.- One pair; one plate situated just lateral to each setae S1. Present only in the representatives of Pyrophorini and Elaterinae, but absent in Procraerus.

P3.- Typically unpaired, but divided in some representatives with completely divided prosterna; situated on the midline of the prosternum, at the apex of the V formed by setae S1, S2, and S3, where the latter are present. Distinct only in the representatives of Oestodinae and Lepturoidini, but absent in Hypolithus; divided in Oestodes and some Ctenicera.

P4.- One pair; situated on the membrane, one anterior to each of the plates P2. Present in the representative of Pyrophorini only.

P5.- One pair; situated one on each side of the midline, on the membrane anterior to the plates P1. Present in the representative of Pyrophorini only.

Setae on Antennae and Labial Palpi

For taxonomic purposes, the setiform structures on the basal two segments of the antennae and on the labial palpi are differentiated into two types. Those of one type are usually short and peg-like, two to four in number, situated on the outer margin of the anterior membrane of the second segment of each antenna, surrounding the bases of the sensory appendix and the third segment (AS1, fig. 3c). They are present in the representatives of all the groups. One or two of them are long and hair-like in some of the representatives, as in Hypolithus (AS1, fig. 3b), and all but one are hair-like in the representative of Pyrophorini

(AS1, fig. 3a). One peg of a similar type was present on the lateral wall of the distal segment of each labial palpus in some of the larvae of Adelocera (LS1, fig. 4). The representatives of the groups other than Pyrophorini have no setiform structures on this segment. The setiform structures of the second type are typically long and hair-like. They are situated on the lateral walls of the basal two segments of the antennae (AS2, fig. 3a), and on the basal segment of the labial palpi (LS2, fig. 4).

Only the setae of the second type appear to be useful as taxonomic characters in the classification of Elateridae into major groups. They are present, in variable numbers and patterns, only in the representatives of Pyrophorini and Elaterinae, with one exception. None were observed in the specimens of Sericus that were examined. In the representatives of Elaterinae, unlike that of Pyrophorini, these setae are absent from the second segment of the antennae.

Division of the Prosternum

Three types of prosterna were observed in the representatives examined: (1) undivided; (2) partially divided along the midline, usually in the posterior region; and (3) completely divided along the midline. This character has been used previously by Glen (1950) in the lower classification of the tribe Lepturoidini. As suggested by

Table I, it may also be useful, in conjunction with the new characters described, in the higher classification of the family.

The above characterizations suggest that there may be at least five major groups of Elateridae, rather than only the four proposed by Hyslop (1917). That is, the tribes Lepturoidini and Pyrophorini, which he placed in the subfamily Pyrophorinae, probably should each be elevated to subfamily status. However, a revision of the major classification of the family, based on larval characters, must await a more adequate representation of species than is available at present.

I am indebted to Professor C.M. Yonge, C.B.E., F.R.S., for the use of the facilities of the Department of Zoology, University of Glasgow. Mr. R.A. Crowson of this Department, and Mr. A.R. Brooks, Research Station, Canada Agriculture, Saskatoon, Sask., provided helpful suggestions on the taxonomic aspects. This study was financed, in part, by the Scholarship and Research Foundation of the Agricultural Institute of Canada.

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Chapter II

(J. Morph. Submitted to Editor Feb. 1, 1962)

SENSE ORGANS OF THE HEAD OF LARVAE OF SOME ELATERIDAE (COLEOPTERA): THEIR DISTRIBUTION, STRUCTURE AND INNERVATION[1]

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INTRODUCTION

Elaterid larvae live in diverse habitats and are often specific to certain types of these. Their feeding habits are also diverse: although most are omnivorous, many species are primarily phytophagous, some are carnivorous, and a few ingest fungi and decaying wood (Savely, 1939; Horion, 1953). They respond to substances in solution (Thorp et al., 1946; Crombie and Darrah, 1947), to differences in temperature (Campbell, 1937; Falconer, 1944; Stone and Foley, 1955) and moisture (Lees, 1943 a,b), and to gravity (Campbell 1937; Stone and Foley, 1955; Zacharuk, 1962 a). The responses to temperature and moisture often differ among species (Zacharuk, 1962 a).

The sensory mechanisms that are involved in the habits and responses of wireworms are virtually unknown. Lees (1943 a) observed five types of cephalic sensilla in Agriotes, but concluded that they were not involved in the

response to moisture. Working with the same species, Crombie and Darrah (1947) briefly described two types of these sensilla, which they believed to be contact chemoreceptors. Other sensilla appear to have been overlooked, some of which were later reported from Ctenicera by Glen (1950). No study was made of their detailed structure and innervation, a knowledge of which is basic to investigations of function by modern neurophysiological techniques.

This prompted the present study of the distribution, structure and innervation of all the sensilla that are present in the cuticle of the head of wireworms, with a view to their probable integrated as well as individual functions. It was also of interest to determine if there are any basic differences in the distribution and structure of sensilla among species that differ in habits and responses.

MATERIALS AND METHODS

The species studied, their major classification and their normal habitats are as follows: Lepturoidini - Athous haemorrhoidalis (F.), Ctenicera (Corymbites) aena (L.), C. destructor (Brown), Limonius minutus (L.) and Hypolithus (Hypnoidus) riparius (F.) from soil, and Lepturoides (Denticollis) linearis (L.) from decaying wood; Pyrophorini - Adelocera (Lacon) murinus (L.) from sand; Elaterinae - Melanotus rufipes (Herbst) and Ampedus (Elater) nigrinus

(Herbst) from decaying wood, and Dalopius (Dolopius) marginatus (L.) and mixed specimens of Agriotes obscurus and A. lineatus (L.) from soil. The descriptions are based on larvae that were nearly mature, and that were in the process of moulting, had just moulted, or had moulted several weeks previously.

The unstained whole mounts were treated with 10% aqueous KOH and cleared and mounted in 70% aqueous Dimethyl Hydantoin Formaldehyde (Steedman, 1958). Other specimens were fixed in aqueous Bouin's fluid or 10% Formol, embedded in Ester Wax (Steedman, 1947) or Paraffin Wax, sectioned serially at 3 to 10 μ, and stained with Heidenhein's iron haematoxylin or by Romane's (1950) silver method. Heavily sclerotized specimens were immersed overnight, after fixation, in 4% aqueous phenol or were incubated in an extract of mushrooms as outlined by Carlisle (1960). Some larvae were also stained intra-vitally with methylene blue, by a technique adapted from that of Hsü (1938).

The silver and methylene blue methods of staining gave variable results, as has been reported by other workers. With the silver method, nerve tissues were revealed more clearly and regularly in heavily sclerotized specimens than in those that had just moulted. With methylene blue, best results were obtained when larvae that were in the process of moulting were injected twice with the staining solution, at an interval of 45 to 60 min., and injected again 15 min.

later with a saturated aqueous solution of ammonium molybdate. For this, the hypodermic needle (glass hypodermic with a stainless steel needle) was inserted cephalad through the posterior connecting membrane of the prothorax, along the median dorsal line. The cephalic portions were fixed overnight in ammonium molybdate, washed well in running water, completely dehydrated and cleared, and mounted in DePeX polystyrene mountant (G.T. Gurr Ltd, London). Alternatively, cleared stained specimens were sectioned serially in paraffin at 15 μ and mounted similarly. Preparations that were completely dehydrated and infiltrated with xylene still retained the stain one year later, with no apparent loss in intensity or clarity.

Most of the following descriptions are based on at least five specimens of each species. They apply equally to all the species studied, except for the few minor differences that are indicated.

DISTRIBUTION AND STRUCTURE

Seven types of sensilla occur in fairly regular numbers and patterns in the cuticle of the head and its appendages. (1) Thick-walled hair organs; (2) campaniform organs; (3) mandibular pore canal organs; (4) scolopophorous organs; (5) peg or thin-walled hair organs; (6) plate organs; and (7) an antennal sensory appendix. Variations in the

number of sensilla within species were often as great as those among species. Both are included in the ranges given. Their number, distribution and structure are as follows.

Thick-walled hair organs

The thick-walled hair organs are differentiated here into long, short, and minute types on the basis of size, distribution, and probable differences in function. They are typical sensilla chaetica or thick-walled sensilla trichodea of Snodgrass' (1935) classification.

The long hair organs are present on most of the cephalic sclerites (fig. 1). They usually are situated on exposed surfaces, are directed perpendicularly or slightly forward from their point of attachment on the cuticle, and normally come into contact with external materials before the cuticle does. Typically there are: 4 on each side of the nasale, along the anterior margin, and 2 to 4 on each lateral aspect of the frontoclypeus; 6 to 10 on the lateral aspect and 3 to 4 along the medioventral margin of each epicranial plate; 1 on the dorsolateral ridge near the base and 1 near the middle of the lateral aspect of each mandible; 4 or 5 on the anterolateral aspect of each maxillary stipes; 1 to 4 on the first and third, 1 to 8 on the second, and 0 or 1 on the fourth segment of each maxillary palp; 4 to 12, often with broken or worn points, surrounding a minutely

lobate, thimble-shaped membranous structure at the tip of each galea; 3 near the middle and 1 near the tip of each lacinia, among the dense fine hairs that cover its mediodorsal aspect; 4 on the postmentum, 1 near each corner; 2 to 10 on the anterior part of the prementum, on the lateral aspect; 2 on the anterior protuberance of the ligula; 0 to 7 on the basal segment of each labial palp; and usually none on the third; 1 or 2 on the anterior margin of the second, and 0 to 3 on the basal segment of each antenna. Only A. murinus and the Elaterinae have hairs of this type on the first segments of the antennae and labial palps, and only the former has them, about 7 in number, on the lateral aspects of the second segment of the antenna. A. murinus usually has nearly twice as many of the long hair organs as are given above for the other species examined.

The short setae project slightly above the surface of the cuticle (fig. 6, B). Eight to 10 are situated in a row along the medioventral margin of each epicranial plate. The lateral edges of the ventral mouthparts touch or cover these when retracted. Three or 4 similar hairs are set in a row on the posterodorsal surface of each epicranial plate, one is on the connecting membrane posterior to each of these rows, and 4 to 14 are on the connecting membrane and the prosternum in the post-gular region. These are touched or covered by a sliding fold of the cuticle when the head is

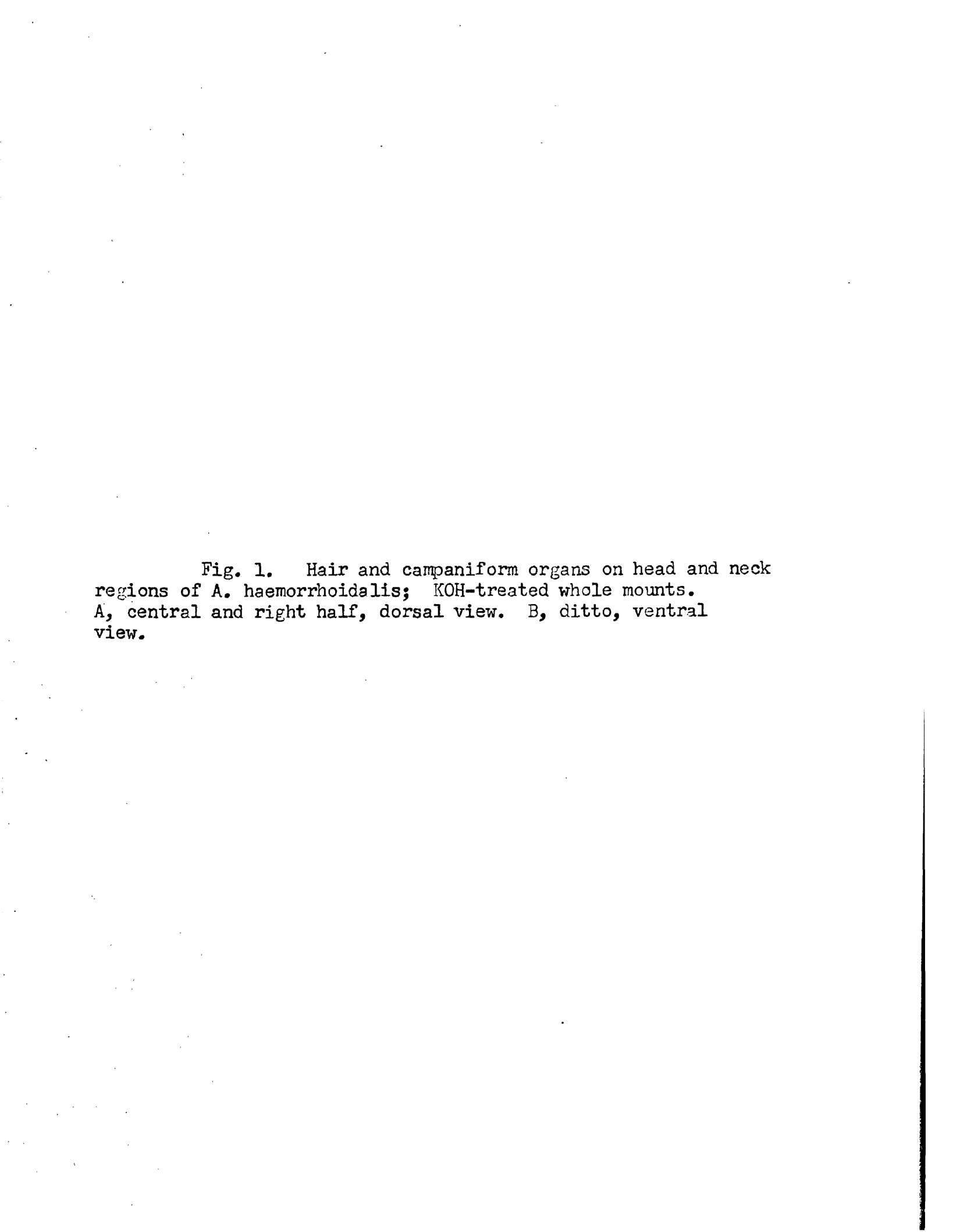
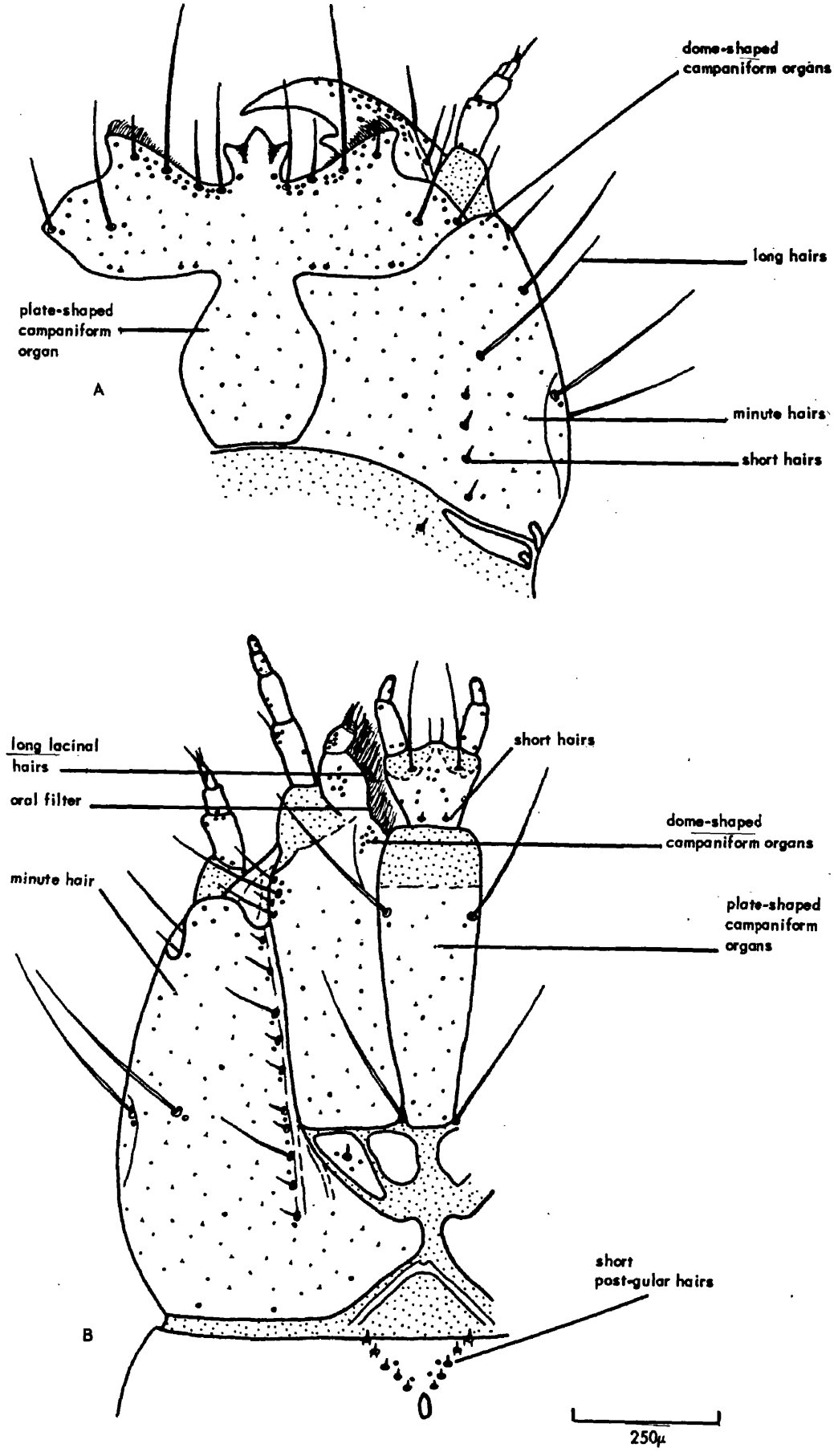


Fig. 1. Hair and campaniform organs on head and neck regions of A. haemorrhoidalis; KOH-treated whole mounts. A, central and right half, dorsal view. B, ditto, ventral view.


dome-shaped
campaniform organs
long hairs
plate-shaped
campaniform
organ
A
minute hairs
short hairs
long lacinal
hairs
oral filter
short hairs
dome-shaped
campaniform organs
minute hair
plate-shaped
campaniform
organs
short
post-gular hairs
B
250μ

raised or is partly retracted into the prothorax. Two others are situated at the base of the prementum, on the ventral aspect. They are touched or covered when the prementum is telescoped partly into the postmentum (fig. 1). Several other short hair organs are present on some of the other sclerites. They often are replaced by long ones in species or individuals with more than the typical number of long hair organs. Two apparently more specialized hair organs are set in deep sockets at the base of the nasale, on the ventral aspect. They are bent almost at a right-angle near their base, and are directed forward along the nasale. Although longer than a typical short seta, they project no further away from the cuticle (figs. 2,B; 6,D).

The minute hair organs are distributed more or less evenly over most of the surfaces of the frontoclypeus, epicranial plates, maxillary stipes and postmentum (fig. 1). Their tips are approximately level with the surface of the cuticle (fig. 6,C). Their number varies greatly among some of the species studied, but the exact extent of this variation was not determined.

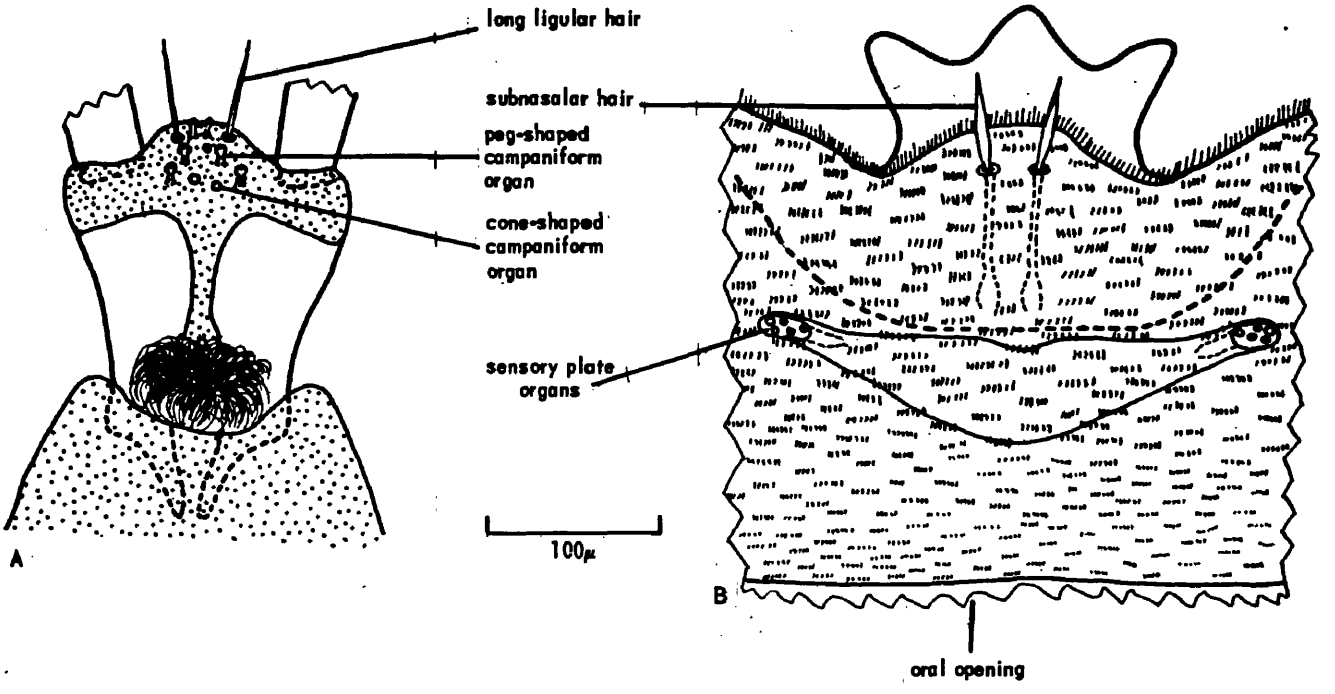
The structure of the three types of thick-walled hair organs is basically similar to that generalized for similar organs by Snodgrass (1935). The long and short hairs

are hollow; the narrow internal cavities usually are expanded slightly at the base. The minute hairs appear to be solid. All the hairs are set in membranous sockets (fig. 6,H), and appear to be movable. The sockets of hairs situated on membranous areas of the cuticle often are supported on annular sclerites that encircle the pore canal. The four cells that are associated with each hair (trichogen, tormogen, sensory and neurilemmal) are grouped in the hypodermis at the base of the pore canal (fig. 6,E). The distal process from the bipolar sense cell (fig. 6,F) terminates near the base of the hair in a spear-shaped, darkly stained tip (fig. 6,A,C,H). It is attached to the inner wall of the hair, within the cavity at its base, by a very delicate cuticular strand (fig. 3,C,D). This strand usually stains by the haematoxylin but rarely by the silver and methylene blue methods. The neurilemma cell is situated near the axonal end of the sensory cell. The axon usually traverses the basement membrane of the hypodermis near the four-cell cluster. Under the larger sclerites of the head, it joins axons from neighbouring thick-walled hair and campaniform organs to form progressively thicker nerve branches. These enter the central nervous system through one of the cephalic nerve trunks. This system of branches has the appearance of a subhypodermal nerve net (fig. 7,C,D). The axons from the hair organs on the cephalic appendages usually enter directly into the nerve trunk that

serves the appendage concerned.

The structure and distribution of the above thick-walled hair organs suggest that they probably are primarily tactile, in accordance with the generally accepted view for similar organs present in all the major groups of Arthropoda. However, the specific functions of the three types of hair organs undoubtedly differ. Most of the long hairs appear to be extero-receptors that serve as protective mechanisms for the head generally, or for other more specialized sensilla, such as those on the galeae and ligula. They may also serve as static organs in the orientation and movement of the larvae in the dense medium of their habitats. Most of the short setae are usually touched by other parts of the body during the normal movements of the head and its appendages, and are probably tactile proprioceptors. Those in the neck region may be stimulated by certain positions or movements of the head, particularly during feeding, in a manner similar to that reported by Haskell (1959) for Locusta and by Popham (1960) for Forficula. The pair of short subnasaler hairs perhaps also function as tactile proprioceptors during feeding. The minute hairs, if primarily tactile, may serve as static organs. However, because they are poorly exposed to stimulation by touch, they may be primarily involved in some other capacity, perhaps in the response to temperature or moisture.

Fig. 2. Unstained KOH-treated whole mounts of A. haemorrhoidalis. A, prementum and ligula of labium, dorsal view. B, nasale and dorsal lining of the pre-oral cavity, ventral view.


long ligular hair
subnasalar hair
peg-shaped campaniform organ
cone-shaped campaniform organ
sensory plate organs
100μ
A
B
oral opening

2

The fine long hairs that form the pre-oral filter (fig. 7,B), discussed by Eidt (1959), and the spicules on the dorsal lining of the pre-oral cavity (fig. 6,D), termed "sensory" by Glen (1950), are not innervated.

Campaniform organs

Four types of sensilla campaniformia were observed. They are designated here as Types A,B,C and D, on the basis of the appearance of their cuticular parts. In surface view Types A and C appear as circular or oval pores (figs. 2,A; 6,G), Type D as knob-like pegs (fig. 2,A), and Type B as small, round, deep-seated plates (fig. 6,G).

Campaniform organs of Type A are present in most of the cephalic sclerites (fig. 1). Usually at least one is situated near a short or long seta in the frontoclypeus, epicranial plates, maxillary stipes, and post- and prementum. A few are widely scattered on the other parts of these sclerites They are most numerous around the long setae that are situated along the anterior margin of the frontoclypeus. The largest are a pair at the base of the nasale on the dorsal aspect, and another on the prementum, one in each lateral wall. Those near the lateroepicranial setae have heavily sclerotized domes, which makes them appear more darkly pigmented than

are the others. On the other sclerites there are: numerous such organs in the mandibles, particularly in the lateral walls, with 1 or 2 larger ones in the dorsal wall; 3 to 7 in the basal, 1 or 2 in the second, usually near the anterior and posterior margins, and sometimes 1 in the third segment of each antenna; 1 to 8 in the basal, 1 to 6 in the second, 2 to 4 in the third, and usually 1 or 2 in the fourth segment of the maxillary palps; 0 to 7 in the basal and 2 to 7 in the distal segment of the galeae, in the ventral and lateral walls; 2 to 12 at the base of each lacinia; and 2 to 7 in the basal and 1 in the distal segment of the labial palps.

The campaniform organs of Type B are also numerous but they are confined to the fronto⌒clypeus, epicranial plates, maxillary stipes, and postmentum (fig. 1). Most of them are in or near areas where muscles are attached to the cuticle (fig. 6,J).

The campaniform organs of Types C and D are present only in the membranous ligula, on the anterodorsal aspect (fig. 2,A). Five to 9 of the latter type are situated in two rows between and posterior to the pair of long setae, and are usually on slight ridges of the ligular wall (fig. 7,A). Two to 5 of the former type are situated between and posterior to these rows, usually in folds between the ridges (fig. 6,L).

In the organs of Types A, B and C the pore canal opens into a spherical chamber in the cuticle. This chamber is covered dorsally and lined laterally by a dome-like sclerite in the first and a cone-like sclerite in the third, and covered only by a plate-like sclerite in the second of these types. The conical sclerite consists of a heavily sclerotized base and apex connected by an almost membranous central region. The apex projects almost to the level of the surface of the cuticle through a large pore (fig. 6,L). The dome-like sclerite is thickest at the apex. It is partly or entirely in the exocuticular layers of the cuticle, usually near the surface, and is partly exposed to the exterior by a small pore (fig. 6,H,I). The plate-like sclerite is entirely in the endocuticular layer, often in the hypodermal half. A very fine pore connects the minute space over this sclerotic cap with the exterior (fig. 6,J,K). The main axis of the organs of Type A and C is perpendicular and that of Type B is at an oblique angle to the surface of the cuticle.

The structure of the cuticular part of an organ of Type D differs considerably from that of the other three types. There are two distinct sclerites. The basal supporting sclerite is annular, tapered distally, and is embedded entirely in the endocuticle. The innervated distal sclerite is spherical and appears to be hollow. It is set in a semicircular cavity

at the top of a papilla-like projection of the cuticle. The pore canal extends through the basal sclerite and is continuous with the cavity in the distal sclerite through an opening in its base. A thin membrane encircles this opening. It connects the distal sclerite to the wall of the papillar cavity (fig. 7,A).

The cells associated with the four types of campaniform organs and the innervation of these organs are basically similar to those of a minute thick-walled hair organ. In the organs of Types A and B, the spear-shaped terminal part of the distal nerve process, which projects into the spherical chamber above the pore canal, is usually curved or sickle-shaped (fig. 6,K). A delicate cuticular strand, similar to that in a thick-walled hair organ, connects the tip of the distal nerve process to the apex of the covering sclerite. In the organs of Types C and D the distal nerve process is expanded only slightly near the base of the sclerotized part of the organ. The fibre that connects the tip of the nerve process to the apex of the sclerite is only slightly thinner than the rest of the nerve process, and often stains similarly to it by the silver method (figs. 6,L; 7,A).

The axon from the bipolar neurone that innervates each organ traverses the basement membrane of the hypodermis

near the sensorial cell cluster. It enters the subhypodermal nerve net or one of the appendicular sensory nerve branches in a manner similar to that of the axons from the thick-walled hair organs.

Pringle (1938 a,b) has shown that the campaniform organs in the legs and maxillary palps of Periplaneta are mechanical proprioceptors, which respond to stresses created in the cuticle by external pressure or by the contraction of muscles attached to the cuticle. The campaniform organs of Types A and B in wireworms appear to be structurally adapted to function similarly. The suggestion is that the organs of Type A respond primarily to stresses created in the exocuticular layers by direct external contact, as in the mandibles, palps, galeae and antennae, or indirectly through the bending of the long or short thick-walled hairs. The organs of Type B are perhaps stimulated primarily by stresses created in the endocuticular layers of the larger sclerites by the contraction of muscles attached to them.

The structural mechanisms for stimulation in the organs of Types C and D appear to be basically similar to those in the first two types. However, their probable function as proprioceptive stress organs appears to be a more specialized and delicate one. They are confined only to the thin membrane that covers the ligula, where there are no

muscle attachments and where the stresses created in the membrane when the pair of protective long hairs are touched would be very limited. From their positions, the cones in folds of the membrane and the pegs on ridges between the folds, one would infer that they perhaps respond to stresses created in the membrane through changes in the pressure of the body fluids within the labium.

Mandibular pore canal organ

This type of sensillum was revealed successfully only by the methylene blue method, in whole mounts from larvae that were in the process of moulting or that had just moulted. Because the mandibles become very heavily sclerotized and darkly pigmented soon after a moult, satisfactory sections could not be obtained for staining by the silver and haematoxylin methods.

There are six pore canals in each mandible. Two are at the apex of the main tooth (fig. 7,F), two are in the lateral wall and one is in the medial wall of this tooth, near the apex, and one is at the apex of the retinaculum. They traverse the endo- and exocuticular layers from the mandibular cavity to the epicuticular layer, under which they end blindly. There are no external cuticular structures associated with these canals.

Six pairs of bipolar sense cells lie in the central

area of the mandibular cavity (fig. 7,E). Each cell is about twice as large as that of the campaniform organs of Type A that are situated along the lateral wall nearer the base of the mandible. The paired neurones lie one behind the other in a compact fusiform unit. The paired distal processes from five of the units are directed towards the bases of the pore canals in the main tooth, two centrally, two along the lateral, and one along the medial walls of the mandibular cavity. Those from the sixth pair of cells are directed towards the base of the pore canal in the retinaculum. The two processes from each cell unit appear to unite near the canal they innervate, and the so-formed terminal fibre enters the base of the canal (fig. 7,G). The manner in which the terminal fibre ends within the canal was not determined, because the staining was always incomplete in this region. The other cells that are undoubtedly also associated with these organs also were not demonstrated. However, argyrophil inclusions were observed in certain large cells within the mandibular cavity, near its base, in a few specimens. These inclusions, discussed in a later section, suggest the presence of trichogen cells. The paired proximal processes from the six cell units join each other near the base of the mandible. They form one of the two sensory nerve branches that innervate each mandible, as described in a later section.

As far as is known, sensilla of this type have not been reported previously from insects. They resemble sensilla placodea in some respects, but they lack the external cuticular manifestations and are innervated by only half the number of neurones present in the plate organs of wireworms. The absence of specialized external structures, even in newly moulted larvae, would seem to be an adaptation to the erosion or wearing of the surfaces of the teeth that normally occurs during each larval stadium. Thus, only the thin epiculticle that caps the pore canals, and which is continuous with that covering the rest of the mandible, would need to be replaced as it became worn.

The inference from their position and structure is that the pore canal organs are probably contact chemoreceptors, which respond primarily to substances in solution, and which are probably concerned more with activities involved in gustation than with orientation.

Scolopophorous organs of the palps

Sensilla scolopophora were observed only in the maxillary and labial palps. There are usually six in each. The terminal scolopales are thick, tubular, slightly curved structures. They lie in or along the inner surface of the lateral wall in the distal segment. These sensory tubes are often fused with each other, and their distal ends are fused

with the cuticular wall near the tip of the segment. In KOH-treated whole mounts they have the same appearance as does the exocuticle. They are shed with the exuviae at each moult. There are no pore canals or external cuticular structures associated with these organs (fig. 7,H,I).

Each organ is innervated by a single, large, bipolar sense cell (fig. 7,J). The distal nerve process is finely granular from the cyton to the base of the scolopale, but appears as a uniformly dark, thick rod within the scolopaler cavity, which it traverses to the closed, rounded tip (figs. 7,J; 8,A). In methylene blue preparations this terminal nerve rod is surrounded by a granular matrix. The proximal processes from the sense cells join the palpal nerve trunks behind the more central neurones that are associated with the terminal peg organs. The epithelial cells that may be associated with the scolopophorous organs were not demonstrated clearly.

Snodgrass (1935) suggests that in general scolopophorous organs are probably receptors of vibratory stimuli . The inference from the morphology of the palpal scolopophorous organs of wireworms is that they respond to some form of mechanical stimuli, probably also vibratory.

Peg or thin-walled hair organs

Some of the cephalic peg or thin-walled hair organs of wireworms are similar to the sensilla basiconica, and

others to the thin-walled sensilla trichodea of Snodgrass' (1935) classification. Nine types are differentiated here, primarily on the basis of the appearance of their cuticular processes. Two types occur at the tips of the galeae, two at the tips of the maxillary and labial palps, four at the tip of the third segment of the antennae, two of which are also present on the anterior margin of the second segment of this appendage, and one is situated on the annular sclerites of the maxillary and labial palps.

Of the three innervated processes usually present on the lobate membranous structure at the tip of each galea, two are short, broadly lanceolate and setiform (fig. 8,B) and one is bladder-shaped (fig. 8,C). Both types are thin-walled, the latter more so than the former. The lanceolate processes are set in membranous sockets similar to those of the thick-walled hairs, and are above the surface of the cuticle. The bladder-shaped one completely fills a deep socket, and its flattened apex is level with the surface of the cuticle. The sockets of all three processes are supported by heavy cylindrical sclerites embedded in the length of the lobate terminal structure. A protective ring of thick-walled hair organs encircles these peg organs, as mentioned previously.

One to 3 large and 10 to 40 small peg-like processes are situated on the membrane at the tip of each maxillary and labial palp (fig. 8,D). They are set in individual small

membranous sockets, each of which is supported by a short annular sclerite. The large pegs are about three times as large as the small ones, and have gently rounded tips. The small pegs usually have truncate tips. All are thin-walled, and are above the surface of the cuticle.

Six or seven peg-like or hair-like processes are situated on the terminal membrane of the third segment of each antenna. One is large, broadly lanceolate, with a pointed tip and very thin walls (fig. 8,E); one is small, usually about three times as long as broad, with thin, almost membranous walls and a truncate tip (fig. 8,F); one is very small, usually as long as broad, with heavily sclerotized walls and a truncate tip (fig. 8,H); and three or four are long and slender and, except for their rounded tips and slightly thinner walls, are not unlike thick-walled hairs in their external appearance (fig. 8,E,G). One or two of each of the third and fourth of the above types are present also on the lateral margin of the anterior membrane of the second antennal segment (fig. 8,G,H). Each process is set in a membranous socket that is supported by a short, annular basal sclerite. The basal sclerite of the shortest of the antennal "pegs" is the largest and heaviest, and encloses the basal half or more of the process (fig. 8,H). The other three types of processes are above the surface of the cuticle.

Most of the eight types of cuticular processes described above have large internal cavities, which appear to be filled with fluid. This fluid, seen best in the larger processes, is usually vacuolate in newly moulted larvae and is often finely granular or reticulate in the heavily sclerotized individuals, fixed several weeks after a moult (fig. 8,E). In the bladder-shaped process of the galea, however, the fluid always stains darkly, and there is no evidence of vacuoles or granules in it.

Each peg is innervated by a unit of four bipolar neurones (fig. 10,A). The four distal processes from this cell unit are closely united, appearing as a single fibre in some preparations. They appear to unite near the base of the peg into a single terminal fibre, which traverses the cavity of the peg and terminates at its apex (fig. 3,A). In some of the preparations stained by the silver and haematoxylin methods, this fibre appears to end bluntly under or in a very lightly sclerotized "cap" at the apex of some types of pegs (fig. 8,D,F,H). In others, it traverses this cap by means of a fine pore (fig. 8,B) and terminates in a small, darkly stained apical body (fig. 8,G), similar to that observed by Dethier and Wolbarsht (1956) in certain chemosensory hairs of Phormia and by Slifer et al. (1957) in some basiconic sensilla of grasshoppers. In successful methylene blue preparations such apical bodies were observed at the

tips of most of the peg-like processes, but no connection was evident between the terminal nerve fibre within the cavity and the external apical body. It is probable that the variable results obtained by the three staining methods are partly due to the small size of these structures, and that such apical bodies are present and are connected with the terminal nerve fibres in most of these sensilla (fig. 3,A). In the bladder-shaped process of the galea the terminal nerve fibre ends in an expanded brush-like body in the fluid within the cavity, rather than in an external apical body (fig. 8,C). The proximal processes from the sensory cells of these organs constitute the major part of the nerve that serves the appendage concerned (fig. 8,J,K).

Two specialized epithelial cells, one with a very large, elongate nucleus and the other with a slightly smaller, oval one, are associated with each peg-like sensillum. These are believed to be the formative cells (tormogen and trichogen, respectively) of the membranous socket, of the peg, and probably also of the cuticular sheath of the distal nerve process. In heavily sclerotized specimens the cytoplasmic connection between the epithelial cell with the smaller nucleus and the external process is most distinct in the bladder-shaped organ of the galea. It ends in an expanded, dense cytoplasmic body within the base of the process (fig. 8,C). Four neurilemma cells, with very small, oval, darkly

stained nuclei, are present near the axonal ends of the sensory cells.

Snodgrass (1935) suggests that sensilla of this type, each innervated by more than one neurone and with the distal nerve process ending at the apex of the peg, are primarily chemosensory. The histological evidence suggests a similar function for the cephalic peg-like sensilla of wireworms. The receptive site for stimuli seems to be at the apex of the peg, as was determined physiologically for certain chemosensory hairs of Phormia by Dethier and Wolbarsht (1956), in all but the bladder-shaped organ of the galea. In this, the entire surface of the external process may be chemosensitive. The inference is that the peg-like sensilla situated on the exposed tips of the bilaterally symmetrical antennae and maxillary and labial palps are contact chemoreceptors concerned primarily with orientation to substances in solution. Those on the galeae, protected from external contact by the surrounding thick-walled hair organs and, in the case of the bladder-shaped organ, by its sunken position in the cuticle, may be olfactory organs concerned with orientation and/or gustation. However, because they are at the lateral margins of the pre-oral filter and would normally be bathed by the digestive fluids and dissolved food substances during the processes of feeding described by Eidt (1959), an alternative oradditional function

may be contact chemoreception, concerned primarily with gustation. Some of the peg organs, particularly those at the tips of the antennae and maxillary and labial palps where no typically tactile hair organs are present, may also respond to touch. Such a dual response was demonstrated in some of the chemosensory hairs of Phormia by Wolbarsht and Dethier (1958).

The ninth type of peg organ differs significantly from those described above. In heavily sclerotized larvae the external cuticular process is usually very minute, knob-shaped, thick-walled, and is set in a small membranous socket. In newly moulted larvae these pegs are similar in appearance to those described and figured by Crombie and Darrah(1947). Several of these organs are situated usually at the basal margins of the annular sclerites of the second and third maxillary and the second labial segments. The pegs project just above the surface of the cuticle, and are touched by the intersegmental fold during even the slightest telescoping of one segment into another (fig. 8,I). The cells associated with these pegs and their innervation are similar to those of a minute thick-walled hair organ.

The structure of this type of peg organ is related more closely to that of a thick-walled hair organ than of a thin-walled peg organ. It seems to be primarily tactile, and perhaps is stimulated when the segments of the palps

are extended or telescoped through changes in pressure of body fluids within the ventral mouthparts.

Plate organs

One sensillum placodeum is situated in the ventral wall of the annular sclerite of the third segment of each antenna, near the terminal sensory pegs (fig. 8,L). Five others are supported by each of two oval sclerites situated in the membranous dorsal lining of the pre-oral cavity, anterior to and one on each side of the opening to the pharynx (figs. 2,B; 9,A). The pair of supporting sclerites are bilaterally symmetrical in position, and are joined medially by a heavy bow-shaped sclerite in some species.

The pore canal of each plate organ traverses the endo- and exocuticular layers of the cuticle, and is covered exteriorly by a very thin, convex plate (fig. 9,B). This plate appears to be continuous with the epicuticular layer of the cuticle. Each organ is innervated by four bipolar neurones. The distal processes from, and the epithelial cells associated with, each tetrad of neurones appear to be similar to those of the thin-walled peg organs. The terminal fibre, formed by the union of the four distal processes of the sense cell unit, usually ends in the central region of the covering plate (figs. 8,L; 9,A,B), in a darkly stained matrix that coats the inner surface of each plate (fig. 9,B).

The axons from the sensory cells of the antennal plate organ enter the antennal nerve trunk along with those from the antennal peg organs. Those from the oral plate organs, the sensory cells of which are grouped in the distal part of a subhypodermal nerve cell bundle (fig. 9,C), enter the labral nerve trunk. These axons also are involved in a complex system of peripheral nerve connections, as outlined in a later section.

According to Snodgrass (1935), placoid sensilla are particularly numerous on the antennae of several groups of insects. He suggests that they are probably olfactory organs. The sensory plates on the antennae of wireworms are suitably positioned to function similarly for orientation to chemical stimuli. However, as only one such organ is present on each antenna, it would require a very low threshold of response, or a very high sensitivity to stimulation by a low concentration of molecules, to function effectively in this capacity. The oral plate organs are undoubtedly concerned primarily with gustation. If olfactory, they may respond to stimuli that perhaps initiate the extra-oral digestive processes. It is more probable that they are organs of taste, which are stimulated by substances dissolved in the fluids present within the pre-oral cavity during feeding.

Antennal sensory appendix

The antennal sensory appendix is the largest of the cephalic sensilla in wireworms, and is structurally the most variable among species. In the species examined, one is present on each antenna. The cuticular part of each sensilla is situated on the anterior membrane of the second segment, ventral to the third segment. However, Glen (1950) observed more than one on each antenna, in the same position, in several other wireworm species.

The cuticular external portion of the sensory appendix is usually cone-shaped and about half the size of the third antennal segment (fig. 8,J) with two exceptions. It is similar in shape but larger than the third segment in Ampedus nigrinus (fig. 9,D), and is convex or lens-shaped in Melanotus rufipes (fig. 9,E). It consists of a lightly sclerotized sensory distal part, which projects above the surface of the cuticle, and a supporting heavily sclerotized, annular basal part, which is embedded in the membrane of the segment (fig. 9,I). The distal sensory part consists of a darkly stained cuticular inner layer and a very thin, lightly stained or unstained epicuticular-like outer layer (fig. 9,F). The inner layer is preforated by numerous small canals, and has the appearance of a honey-comb in surface view (fig. 9,G).

The cells associated with this sensilla extend in a bundle, alongside the bundle of cells from the sensillae on the third segment, to near the base of the antenna. The number of cells is greatest in A. nigrinus and M. rufipes. In the former, they are concentrated in a bulbous mass at the base of the antenna (fig. 9,D). They form a fusiform bundle within the basal segment of the antenna in the other eleven species (fig. 9,E).

Several of each of four types of cells (sensory, neurilemma, and two types of epithelial cells) are present in the cell bundle. The bipolar sensory cells, with large, round or slightly oval nuclei, are situated medially and basally. Their number varies among species from at least 8 to more than 30. The distal processes of the neurones extend into the external cuticular structure in a loose bundle (fig. 9,D,E,H-J). Epithelial cells of one type, with slightly smaller, oval nuclei, surround the sensory cells and their processes. Thick cytoplasmic processes extend from these cells also into the cuticular structure, where each ends in a bulbous cytoplasmic mass. These masses contain numerous vacuoles and are closely applied to the perforate layer of the sensory cuticle in newly moulted larvae (fig. 9,J), but stain uniformly and are separated from the cuticle by a fibrous matrix of the distal nerve processes in heavily sclerotized specimens (fig. 9,I). Epithelial cells of a second type,

with very large, elongate nuclei, are situated laterally and distally to those of the first type. Their cytoplasmic processes terminate near the basal part of the external cuticular structure. These two types of cells appear to be the formative cells for the sensory and the supporting parts of the cuticular structure, respectively. Neurilemma cells with small, oval, darkly stained nuclei, occur near the axonal ends of the sensory cells. A few also extend into the antennal nerve. Other neurilemma cells, with elongate, flattened, very darkly stained nuclei, ensheath the basal part of the sensory cell cluster.

The distal processes from the sensory cells extend anteriorly in groups of four in A. nigrinus and singly in the other species. In moulting and newly moulted larvae stained by the methylene blue method, each distal nerve process ends in a darkly stained terminal fibre within the cuticular structure. These terminal fibres taper distally and curve towards the inner surface of the sensory cuticle, near which they end in small, rounded tips (fig. 9,H). Several very fine fibrils extend towards the cuticle from these tips. In newly moulted and heavily sclerotized specimens stained by the haematoxylin and silver methods, the distal nerve processes branch profusely near the base of the cuticular structure, forming a mass of very fine fibrils. These fibrils usually pass ventrally among the bulbous endings of the formative

epithelial cells, and spread out along the entire surface of the sensory cuticle (fig. 9,I). Fine strands from this fibrillar matrix appear to enter the canicular perforations in the inner layer of the sensory cuticle, but this could not be demonstrated with certainty by the methods used. The axons of the sensory cells form a short, thick nerve branch, which unites near the base of the antenna with the nerve comprised of axons from the other antennal sensilla to form the major part of the antennal nerve trunk.

The antennal sensory appendix of Agriotes larvae was classified as a multiple-celled sensilla basiconica by Crombie and Darrah (1947). That of Rhyzopertha larvae was referred to similarly by Crombie (1944). Roth and Willis (1951) described apparently homologous sensilla in larvae of Tenebrio and Dermestes as sensilla placodea. On the basis of the histological evidence, however, the antennal sensory appendix of wireworms cannot be related closely to any of the typical sensilla described and classified by Snodgrass (1926, 1935). Histologically, it is an additional type not included in previous classifications of sense organs of Arthropoda.

This sensillum exposes a large, apparently sensitive surface to stimulation, which suggests that it could be an olfactory organ. However, if the findings of Thorpe et al. (1946) that Agriotes larvae do not respond to air-borne odors is valid for this and other wireworms, it would indicate that

this sensillum is primarily a contact chemoreceptor. According to Crombie and Darrah (1947), it is a chemoreceptor concerned with orientation only.

INNERVATION

All the peripheral cephalic sensilla of wireworms are innervated by bipolar neurones similar to those classified by Zawarzin (1912 a) as sensory cells of Type I. The sensilla that are believed to respond primarily to mechanical stimuli are innervated by individual neurones situated in the hypodermis (figs. 6,F; 7,J). Those that are believed to be chemoreceptive are innervated by two, four, or more than four neurones, situated subhypodermally. In these the neurones are usually grouped into a unit, so that cell boundaries and individual processes often cannot be distinguished (figs. 9, K; 10,A).

Distal nerve processes

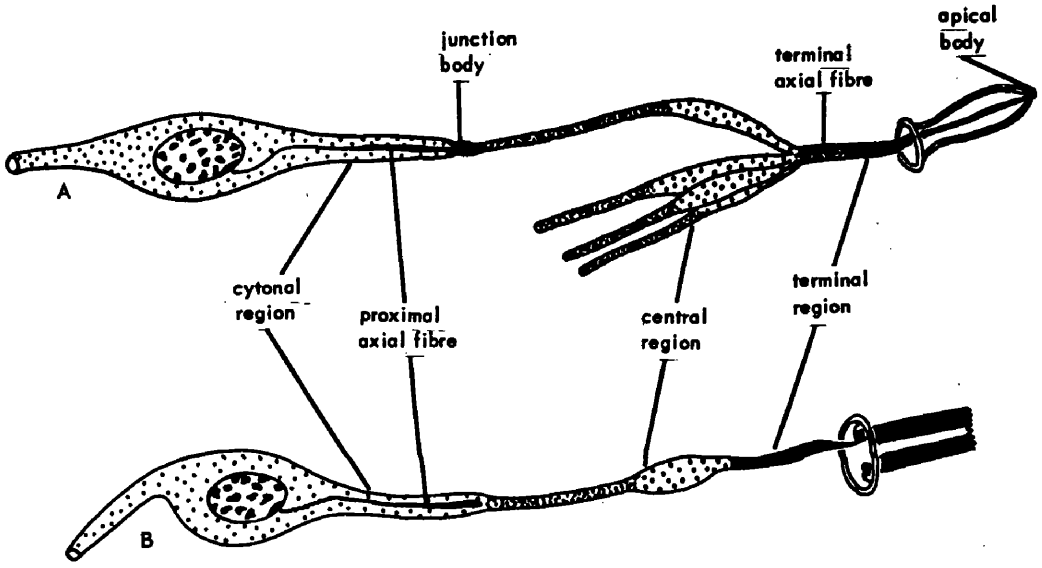
The distal nerve processes in most of the sensilla described above are typically unbranched (fig. 6,K). However, a slight terminal branching into barely distinguishable fibrils is apparent in the bladder-shaped organ of the galea. This type of branching is more developed in the antennal sensory appendix, where the numerous terminal fibrils from the nerve processes extend through the length of the external cuticular structure (fig. 9,I). Apart from the terminal modifications,

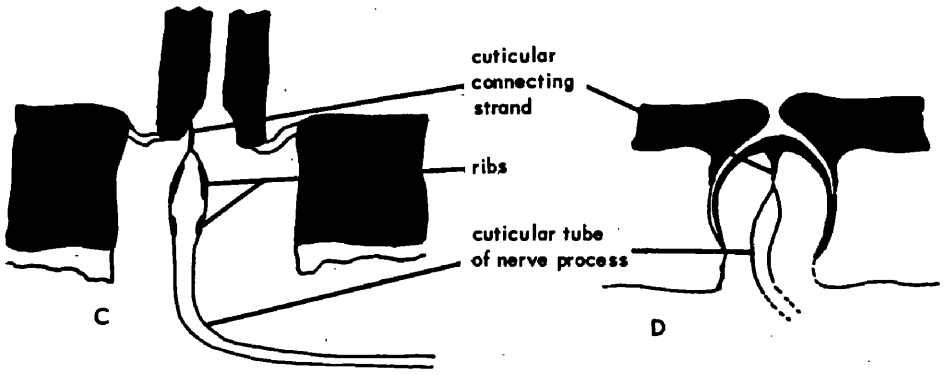
Fig. 3. Reconstructions. A, typical neurone and ending of the distal nerve process in a thin-walled peg organ. (From whole mounts stained intra-vitally with Methylene blue, and from sections stained by the Silver and Haematoxylin methods. The distal processes are separated for clarity, although they actually are grouped dightly into a single unit).

B, ditto of a typical thick-walled hair organ.

C, terminal cuticular sheath of the distal nerve process and the cuticular strand that connects it to a long thick-walled seta, as it appears when shed at ecdysis. (Stained intra-vitally with Methylene blue just before the cuticle was shed).

D, ditto of a Type A campaniform organ in the new cuticle early in the moulting process. (Stained with Methylene blue after treatment with KOH).


apical body
junction body
terminal axial fibre
A
cytonal region
proximal axial fibre
central region
terminal region
B


cuticular connecting strand
ribs
cuticular tube of nerve process
C
D

the histology of the distal nerve processes in all the sensilla seems to be basically the same.

Each process, or unit of processes where the component fibres are not distinguishable, consists of three histologically different regions (figs. 3,A,B; 9,K; 10,A,B,E). The finely granular basal region is tapered distally, and appears as an undifferentiated elongation of the cytoplasmic portion of the cyton (fig. 3,A,B). An axial fibre, most apparent in preparations stained by the silver method, traverses the length of this region. It ends proximally in or near a darkly stained area of the nucleus (fig. 10,E). Distally, it is connected to a junction body in chemoreceptive neurones (fig. 3,A), and ends blindly in an homologous part of the process in mechanoreceptive neurones (figs. 3,B; 10,E).

In individual processes stained by the silver method, the junction body is small, oval and solid in the peg-like organs (fig. 10,C), and is similar in shape but has a lightly stained centre in the antennal sensory appendix (fig. 10,D). In methylene blue preparations, its position in an individual process or in a unit of processes often is denoted by a small, darkly stained area (fig. 10,B). This body, or the homologous part of the process in a mechanoreceptive neurone, demarcates the basal from the central region of the process.

The central region of the distal nerve process appears as a thin, darkly stained fibre composed of very dense fine granules in preparations of heavily sclerotized specimens stained by the silver method (figs. 6,K; 9,I; 10,C,D). It is shorter, thicker and lightly stained in those of newly moulted larvae. In preparations of moulting and newly moulted larvae stained by the methylene blue method, this region consists of two sections. The basal section is darkly stained and is composed of dense, fine granules; the distal section is expanded, lightly stained, and contains a few large, widely scattered granules (figs. 3,A,B; 10,A,B). The latter section is still distinct but much shorter in similar preparations of heavily sclerotized specimens. Perhaps the nerve process lengthens primarily in this region, possibly in the distal section, as the layers of the cuticle thicken during and after a moult. No axial fibre was evident in this region.

The third or terminal region is almost entirely within the pore canal and the external cuticular structure of the sensilla. As already indicated, it is structurally the most variable of the three regions of the nerve process among the different types of sensilla. Basic similarities are evident, however, especially in preparations of moulting and newly moulted specimens stained by the methylene blue method. It stains darkly and uniformly, but appears to be

more solid distally than proximally. A delicate axial fibre traverses the length of the proximal part (figs. 3,A,B; 9,H; 10,A). Distal regions stained by the silver method are uniformly dark throughout (figs. 8,B; 9,B), or the distal part is uniformly dark and the basal part granular (fig. 6,K). In the antennal sensory appendix, the differences in the appearance of the terminal region between the methylene blue preparations of moulting and newly moulted larvae (fig. 9,H) and the silver preparations of heavily sclerotized larvae (fig. 9,I) are perhaps partly developmental and partly due to the differences in the staining properties of the two methods.

A tubular, thin-walled sheath is shed with the old cuticle at each larval moult by the distal processes of the sense cells of the cutaneous sensilla. Such tubes are shown clearly in whole mounts of exuviae that were removed from moulting larvae stained intra-vitally with methylene blue, but only in specimens or regions of the head where the terminal fibres of the underlying newly formed sensilla also stained successfully.

In the exuviae that split easily along the ecdysial line during removal, indicating that the moulting process and histolysis were nearly complete, the lengths of these nerve sheaths varied among sensilla. It appeared to be directly

proportional to the original length (before ecdysis) of the pore canal that the process traversed. This corresponds closely to the length of the terminal region of the nerve process that shed it. The shape and diameter of these tubes conform closely to the surface contours of the terminal nerve fibres of similar types of sensilla before ecdysis (figs. 3,C; 10,F,G). Several thickened longitudinal ribs are evident in the walls of the expanded apical part, at two levels, particularly in the thick-walled hair organs (figs. 3,C; 10,F). In these and in the campaniform organs, the closed, thickened, pointed apex is attached to the cuticular structure of a sensillum by a very fine, solid strand (fig. 3,C). The internal cavities of all the cast-off tubes examined were empty.

The apical portions of partly formed terminal nerve sheaths were observed in a few campaniform sensilla in the new cuticle of a moulting larva. These stained the same as the surrounding cuticle when placed in 0.1% methylene blue after the non-cuticular tissues were largely removed with warm 10% KOH and the closely adhering old cuticle was peeled off. The connection between the apex of the tube and the apical connecting strand was not distinct in this preparation (fig. 3,D).

These cuticular sensory nerve sheaths appear to be homologous with the sense rods or scolopales described from

similar types of sensilla of other insects by Snodgrass (1926). That they are shed with the exuviae at ecdysis has been reported previously by Sihler (1924) for tactile hairs of the Acridian, Gomphocerus, by Richard (1952) for trichoid sense organs of termites, and more recently by other workers for other insects. The nature of these sheaths, their homologies among the various types of sensilla, and the shedding of more delicate sheaths by the axons at ecdysis, will be considered in a later paper.

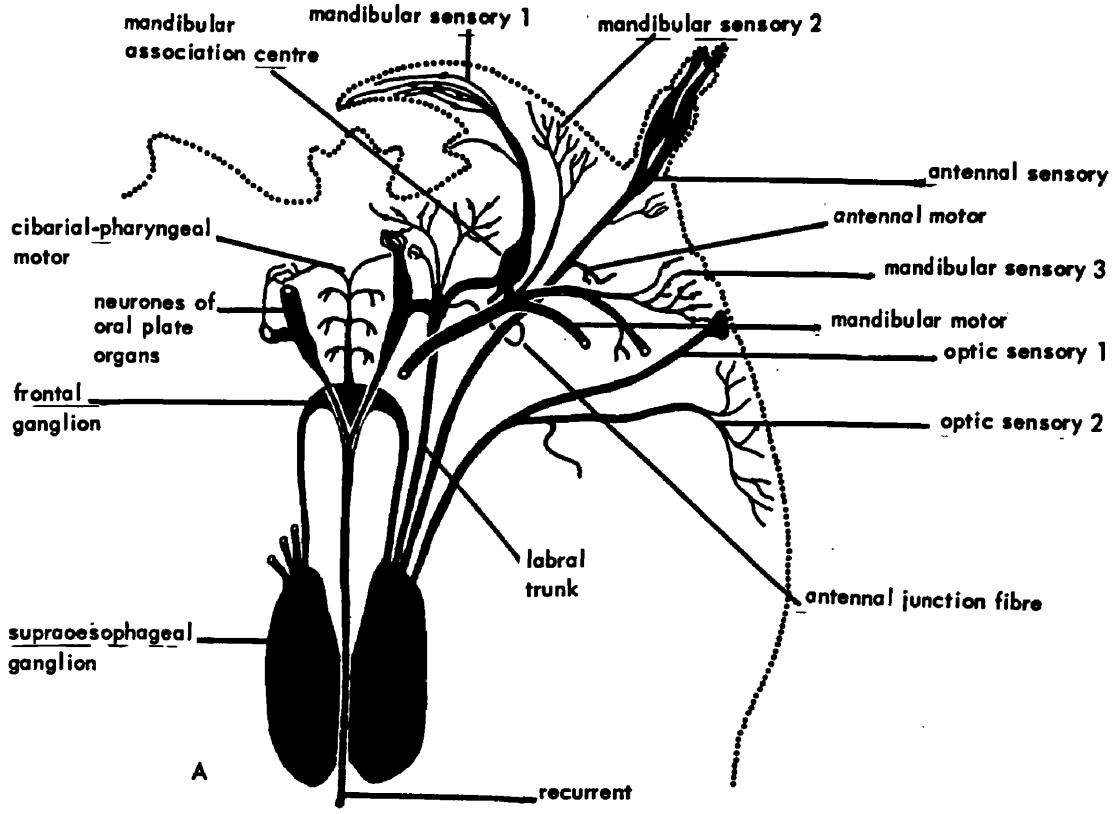
Cephalic nerves

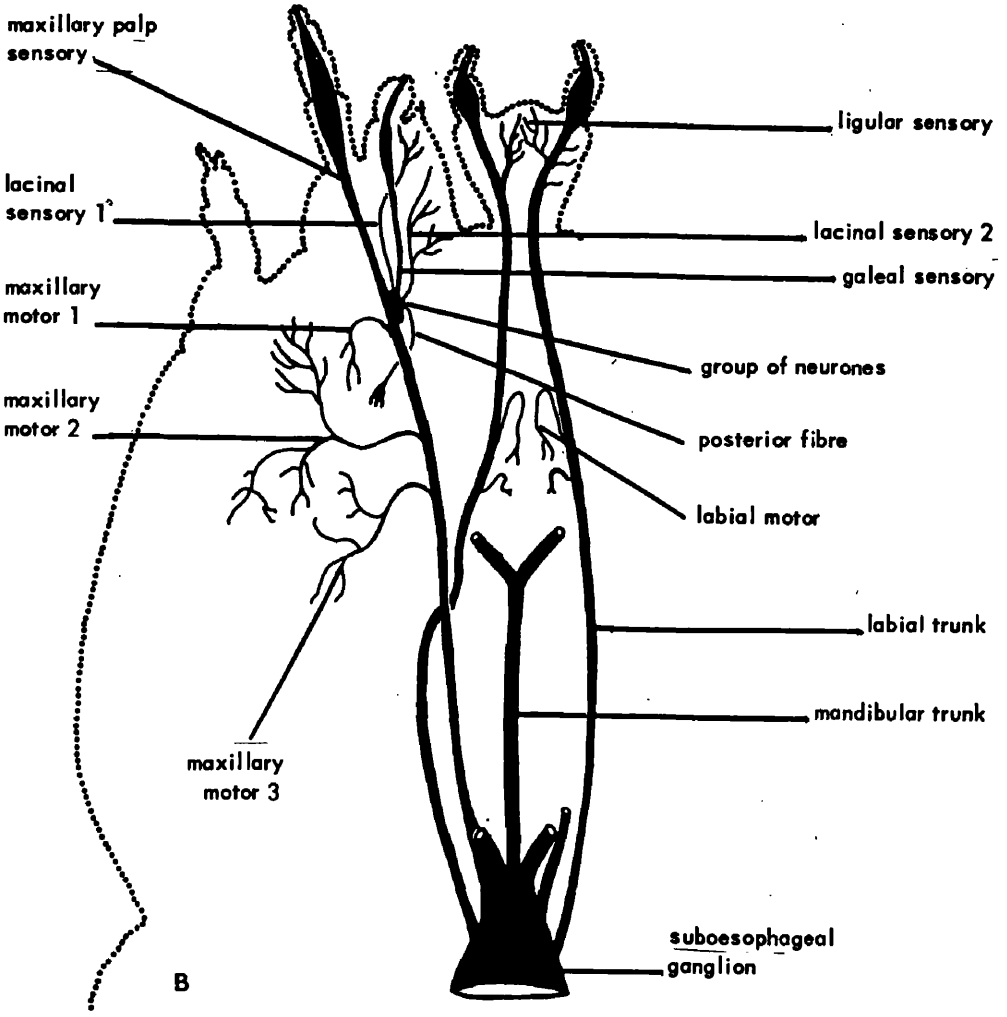
The pathways of the main cephalic nerve trunks of the wireworms examined correspond in most instances to those outlined by Eidt (1958) for C. destructor. The paired antennal, labral and optic nerves and the connectives of the frontal ganglion enter the supraoesophageal ganglion anteriorly, one on each side. The unpaired recurrent nerve extends posteriorly from the frontal ganglion along the dorsal surface of the digestive tube, beneath the supraoesophageal ganglion (fig. 4,A). The paired maxillary and labial nerves enter the suboesophageal ganglion also anteriorly and one on each side. The paired main mandibular nerve branches enter this ganglion medially through a common nerve trunk (fig. 4,B). The pathways of the nerve fibres within the ganglia were not investigated. Their peripheral ramifications, however, are more complex than those of any other insect for which descriptions are

Fig. 4. Innervation of the sensilla in the head of wireworms; reconstructed from Methylene blue whole mounts.

A, central and left half of the dorsal region of the head, ventral view.

B, ditto of ventral region of the head, dorsal view.


mandibular association centre
mandibular sensory 1
mandibular sensory 2
antennal sensory
antennal motor
cibarial-pharyngeal motor
mandibular sensory 3
neurones of oral plate organs
mandibular motor
optic sensory 1
frontal ganglion
optic sensory 2
labral trunk
antennal junction fibre
supraoesophageal ganglion
A
recurrent


maxillary palp sensory
ligular sensory
lacinal sensory 1
lacinal sensory 2
galeal sensory
maxillary motor 1
group of neurones
maxillary motor 2
posterior fibre
labial motor
labial trunk
mandibular trunk
maxillary motor 3
suboesophageal ganglion
B

available.

According to Eidt (1958), the paired optic nerves branch shortly after they leave the supraoesophageal ganglion. However, a closer study reveals that the two so-called branches of each optic nerve are distinct nerve trunks, which are separated distally but are closely united proximally (fig. 4,A). They enter the supraoesophageal ganglion on the anterolateral aspect, alongside one another. The anterior nerve (optic sensory 1) extends directly to the ocellus; it is the true optic nerve. The posterior nerve (optic sensory 2) branches peripherally into numerous smaller nerves and individual axons, which terminate in the sense cells of the campaniform and thick-walled hair organs that surround the ocellus in the lateroepicranial plate. It also gives off a small branch near its base, directed caudad, the destination of which was not determined.

The main part of the antennal nerve extends directly to the base of the antenna. It branches here into two short, thick nerves. One consists of axons from the antennal sensory appendix and the other, of axons from the other sensilla on the antenna. A small motor nerve leaves the trunk proximal to the base of the antenna; it innervates the antennal muscles. (Motor nerve endings on these muscles can be seen at the

extreme right in the lower half of fig. 10,H). Another small nerve leaves the trunk between the motor nerve branch and the terminal fork. It branches near the apex of the hypopharyngeal rod into individual axons, which innervate some of the long setae and campaniform organs on the lateroepicranial plate at the base of the antenna (fig. 5). A third small nerve, the antennal junction fibre, leaves the trunk proximal to the motor nerve branch and enters the labral plexus (fig. 4,A). It seems to consist of a single nerve fibre.

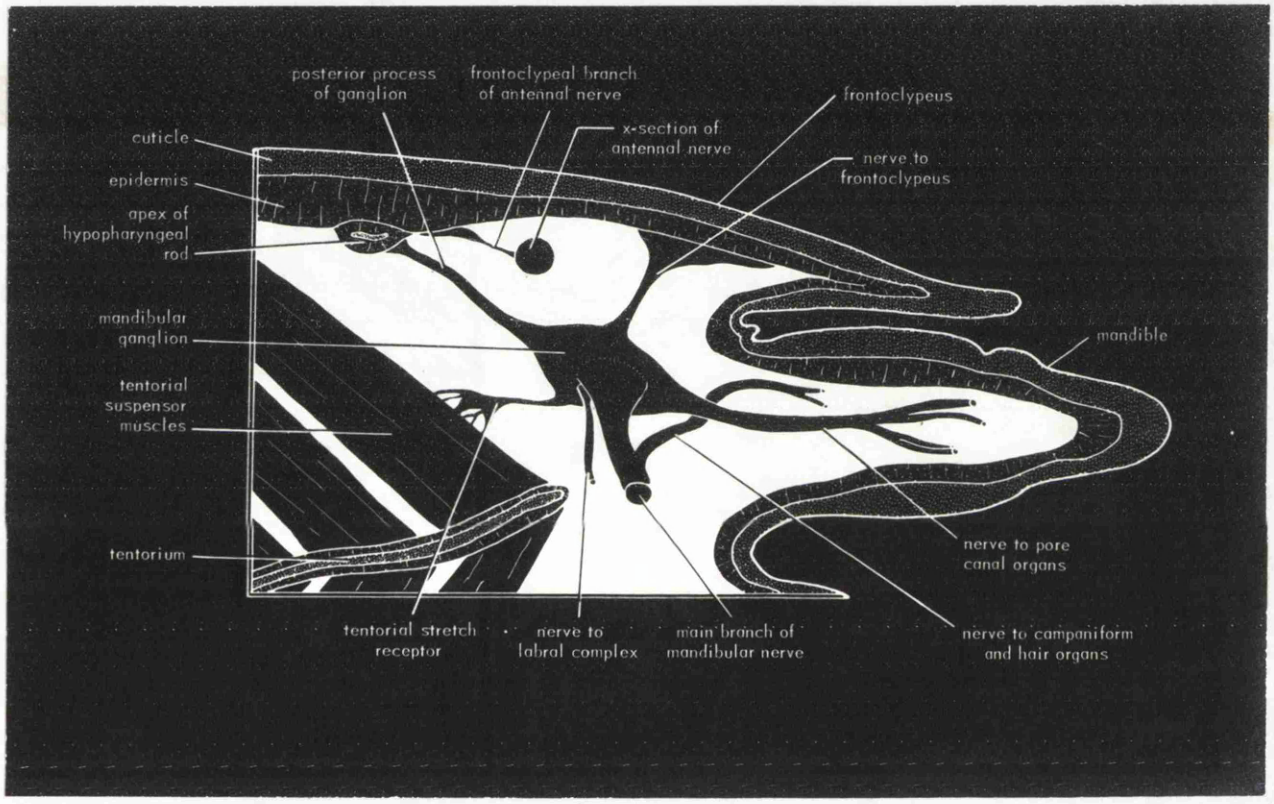
The labral plexus is situated laterally to the cibarial and pharyngeal muscles, approximately dorsolaterally to the oral opening to the pharynx. The labral nerve trunk leaves this plexus posteriorly and extends unbranched to the brain (fig. 4,A). Axons from the pre-oral sensory plate organs and processes from the four bipolar stomodaeal sensory neurones, situated basal to the neurones of the sensory plates in the same bundle, enter the plexus medially. The processes from the opposite poles of the stomodaeal receptive neurones enter the recurrent nerve just posterior to the frontal ganglion (figs. 4,A; 9,C; 10,H). Nerve processes from the subnasaler and anterofrontoclypeal hair and associated campaniform organs enter the plexus anteriorly (fig. 4,A). Two or three nerve fibres extend anteriorly and medially from the plexus, and seem to be associated with the lateral extensions of the anteriormost branches of the cibarial-pharyngeal motor nerve

through fine, varicose, synaptic-like fibrils (figs. 4,A; 10,H). These form a cibarial bridge between the predominantly afferent labral plexus and the efferent cibarial-pharyngeal nerve branches. The latter originate as an unpaired nerve that leaves the frontal ganglion anteriorly (figs. 4,A; 10,H; 11,A). One large, unipolar neurone, not unlike a typical large ganglion cell, is situated in each labral plexus. The two processes from a bipolar neurone (partly out of focus just above the unipolar neurone in the same figures) are also associated with this plexus. A short lateral nerve connects the labral plexus with the mandibular association centre (figs. 4,A; 11,A).

The mandibular association centre or mandibular ganglion (figs. 4,A; 5; 10,H; 11,A) is situated laterally and slightly anteriorly to the labral plexus. It contains a group of at least six large, unipolar cells, which superficially resemble the ganglion cells of the central nervous system. The ganglion is ensheathed by a membrane, which is continuous with the neurilemma of the nerves connected to it. The bundle of axons from the mandibular pore canal organs extends directly into the ganglion anteriorly (mandibular sensory 1). Posteriorly, a tubular ligament-like process, which has thick walls and contains one or two nerve fibres, connects the ganglion to the apex of the hypopharyngeal rod. This rod, described and figured by Glen (1950), extends

medially and ventrally to the lateral margin of the oral opening. This posterior innervated process may be a type of stretch receptor that is involved in the operation of the gustatory mechanisms. A small bundle of axons from a few laterofrontoclypeal setae and associated campaniform organs enters the ganglion dorsally. A fine ventral nerve from the ganglion terminates in a fan-shaped mass of processes, which are attached to the sarcolemma of the anterior suspensor muscle fibre of the tentorium. This structure appears to be a stretch receptor for the anterior arm of the tentorium. It resembles some of the stretch receptors described by Finalyson and Lowenstein (1958) from other groups of insects. A short lateral nerve leaves the ganglion alongside the nerve to the labral plexus, and enters the main mandibular nerve branch. Just distal to this junction the main mandibular nerve branch divides into three. One branch consists of axons from the two hair and the numerous campaniform organs in the mandible (mandibular sensory 2). The other two branches extend laterally and posteriorly. One innervates the mandibular muscles, and also sends a branch (mandibular sensory 3) to the setae on the anterolateral aspect of the epicranial plate. The other innervates the skeletal muscles of the head. Although primarily motor, nerves from most of the subhypodermal nerve net of the head also enter one or the other of these two branches.

Fig. 5. Longitudinal section through the left mandible and frontoclypeal region; diagrammatic, medial view; reconstructed from all the preparations to show the mandibular ganglion and the nerves connected to it.


posterior process of ganglion
frontoclypeal branch of antennal nerve
frontoclypeus
x-section of antennal nerve
cuticle
nerve to frontoclypeus
epidermis
apex of hypopharyngeal rod
mandibular ganglion
mandible
tentorial suspensor muscles
tentorium
nerve to pore canal organs
tentorial stretch receptor
nerve to labral complex
main branch of mandibular nerve
nerve to campaniform and hair organs

The major part of each labial nerve trunk consists of axons from the sensilla on the labial palp of the corresponding side. Axons from the sensilla on the ligula and prementum join one or the other of the labial nerves near the base of the prementum. Two small motor nerves branch off each trunk within the postmentum. These innervate the labial muscles (fig. 4,B).

Each maxillary nerve trunk gives off three branches in the region of the stipes. The two basal branches are primarily motor, but also receive afferent nerve fibres from the subhypodermal nerve net of the stipes and postmentum. The distal branch contains motor fibres only (fig. 4,B). Just anterior to this branch is a cluster of four maxillary accessory neurones, which resemble typical bipolar receptive neurones. This cluster is situated within the nerve, alongside the bundle of axons that extends from the sensilla on the maxillary palp to the brain (fig. 11,B). The nerves from the galea, the four thick-walled hairs on the lacinia, and the campaniform organs at the base of the lacinia, join the maxillary nerve trunk through this cluster of neurones. A process from the most medial of the four neurones extends posteriorly, and terminates in a fibrillar mass at the peripheral end of one of the maxillary muscle fibres, in

the region where the myofibrillae meet the tonofibrillae that attach the muscle to the integument. This appears to be a third type of wireworm stretch receptor (fig. 11,C). The processes of the other three accessory neurones did not stain completely, and their destinations could not be determined. No peripheral connections were evident between the maxillary and labial nerves, or between these and the more dorsal cephalic nerves described previously.

A discussion of the significance of these peripheral complexes, that is, of the mandibular association centre, the labral plexus, the cibarial bridge, and the maxillary accessory neurones, must await a more thorough mapping of the pathways and associations of the individual nerve cell processes involved. This is beyond the scope of the present study. However, the evidence strongly suggests the presence of peripheral synaptic or association centres between the afferent and efferent systems, involving particularly those receptors believed to be organs of taste. Thus, Pflugfelder's (1936) term "Bukkalganglions" for the peripheral labral nerve cell complex of the aphid, Pemphagus, in which he also includes the neurones that innervate the "pharyngeale Sinnesorgan" (homologous with the pre-oral plate organ described here), may be an appropriate one. His description of this so-called ganglion lacks sufficient detail to permit comparison with the labral complex of wireworms, which superficially does not

have the appearance of a ganglion.

Subhypodermal nerve net

The subhypodermal nerve net was demonstrated in preparations stained by the silver and methylene blue methods, but its fine structure was apparent only in the latter. It ramifies through connective tissue along the medial surface of the basement membrane of the hypodermis. Nerve branches extend medially from it at intervals, join similar neighbouring branches, and enter one of the nerve trunks to the central ganglia.

A primary open system of coarse fibres consists of individual or groups of axons from the sense cells of Type I that innervate the campaniform and thick-walled hair organs on the larger sclerites, as described above. In lightly stained preparations, it resembles the branching of a tree: a thick nerve extends to the central nervous system, and branches from it are reduced in size peripherally to fine terminal twiglets, each of which ends in a sensillum through a sense cell. This is similar to the subcutaneous ramifications of the nerve fibres in larvae of Aeschna (Zawarzin, 1912 a) and Rhodnius (Wigglesworth, 1953), and in the crustacean, Squilla (Tonner, 1936).

A secondary closed system of very fine fibrils is evident in preparations that have stained more deeply

(fig. 7,D). It originates as fine branches from the coarser fibres of the primary system. These fine fibrils branch profusely, and form interconnections between the coarser fibres as well as between one another. The significance of these secondary fibrils and their proximal connections within the primary system are not known. Perhaps they are the processes of the triangular cells that are situated at some of the junctions of the fibres of the primary net. (One such cell is present in the triangular junction at the extreme left of fig. 7,D). Such cells are proximal to the points where the secondary fibrils leave the primary. They seem to be homologous with the web-like cells of Rhodnius, which Wigglesworth (1953) considers to be neurilemma cells. However, in the lightly stained methylene blue preparations of wireworms, these cells stain almost as deeply as do the neurones of Type I, but the more typical neurilemma cells fail to stain or only their nuclei stain a very pale violet. Apart from these cells, neurones of Type II, similar to those that form the bulk of the subhypodermal nerve net in Melolontha (Zawarzin, 1912 b), other caterpillars, and most of the crustaceans (Tonner, 1936), were not evident in the subhypodermal nerve net of wireworms.

ARGYROPHIL GRANULES

Intra-cellular silver-staining inclusions are

characteristic of those sensilla of wireworms that are innervated by more than one neurone. They appear to be confined to the trichogen cells, the cytoplasm of which often extends to the base of the sensillar cell bundle where the granules usually are concentrated (fig. 11,D). In the antennal sensory appendix such inclusions occur in the homologous cells that form the sensitive cuticle of the external process, and which ensheath the sense cells and their distal processes (fig. 11,E). These inclusions vary in size and are either globular or granular in shape. They stain brownish-black or black by Romane's silver (ammoniacal) method, and usually occur in dense aggregations throughout the main body of the cell (fig. 11,F). They are very abundant and are most distinct after a moult, and are again abundant and distinct in the more heavily sclerotized larvae.

In only one instance were such inclusions observed in those sensilla that are innervated by single neurones. One to four such granules were present in each trichogen cell of some of the long thick-walled hair organs of a larva that had just moulted. Similar inclusions were not evident in the neurones, nor in cells of any of the other tissues in the head.

Slifer et al (1957) observed similar inclusions in the trichogen cells of the chemosensory pegs on the antennae of grasshoppers, which they term "secretion droplets". The

argyrophil globules in the sensilla of wireworms also have the appearance of secretion droplets. They are most numerous around the nuclei of the trichogen cells, where they perhaps originate. These inclusions may be involved in the formation and maintenance of the "sensitized" cuticular structures (the external process and the cuticular nerve sheath) of the sensilla. Their nature and significance are under further investigation.

GENERAL DISCUSSION AND CONCLUSIONS

There are only a few slight differences in the number, distribution and structure of the cephalic sensilla among species. The organs believed to be chemosensory differ least. The only difference that may be of functional significance is the presence of more neurones in the antennal sensory appendices of the wood-inhabiting larvae of Ampedus and Melanotus than in those of the other species examined. The organs believed to be mechanoreceptors vary primarily in number. In some instances this variation may be developmental, and is perhaps related to the size of the individuals within and among species. In others, such as the greater number of tactile hairs in the very active sand-inhabiting and largely predacious larva of Adelocera than in those of the other species studied, it may be related to differences in habits. A few of the differences in number and distribution of the

tactile hairs among species are of taxonomic significance (Zacharuk, 1962 b).

On the basis of structure and distribution, as mentioned previously, the antennal sensory appendix and the terminal, thin-walled, multi-celled peg organs on the antennae, galeae, and maxillary and labial palps are believed to be chemosensory. When various combinations of the cephalic appendages are removed (Crombie and Darrah, 1947), larvae of Agriotes continue to orientate to solutions of glucose or asparagine when only the antennae, the galeae, or the labial palps and ligula are present. They fail to do so when these and the maxillary palps are removed. A solution of glucose elicits a biting response when only the galeae or the labial palps and ligula are present, but not when these and the maxillary palps are removed and only the antennae are left. On the basis of these responses, the sensory appendix and/or the terminal multi-celled peg organs of the antennae are chemoreceptors concerned only with orientation. The thin-walled peg organs on the tips of the galeae and of the maxillary and labial palps are chemoreceptors concerned with both orientation and the biting response. The mandibular pore canals and the oral plate organs, which are also believed to be chemosensory, apparently are not involved in either response. Perhaps they are involved in the extra-oral digestive processes that take place during feeding.

Crombie and Darrah (1947) concluded that the peg organs ".... found on the labial and maxillary palps and galeae, mostly on the ventral surfaces" and the "cup-shaped structure" on the antennae (termed antennal sensory appendix here) seem to be the chemoreceptors involved in the orientating and biting responses. The "... minute projections on the tips of the labial and maxillary palps are not necessary for ..." either response. However, the position and structure of the pegs that are found mostly on the ventral walls of the maxillary and labial palps suggest that they are tactile. It is more probable, as concluded above, that the pegs at the tips of these appendages are the chemosensory organs involved. Crombie and Darrah's results show that there are no significant changes in response when other cephalic appendages are removed and only the galeae or only the labial palps and ligula are left. Because the ligula has no chemoreceptor-like organs, it would seem that, contrary to their conclusions, the "minute projections" at the tips of the labial palps (and presumably also the similar terminal pegs of the maxillary palps) are as necessary for the orientating and biting responses of Agriotes as are the pegs at the tips of the galeae.

The number of neurones and the manner in which their peripheral nerve processes terminate differ considerably among most of the seven types of sensillae described. This is to be expected in organs that respond to stimuli as different

as touch, stress, vibrations, and molecules of various chemical substances. However, the histological differentiation of the peripheral nerve processes into three distinct regions is consistently similar in all the sensilla when stained intra-vitally. Perhaps the mechanisms that create the action potential in one or more of the regions of the nerve processes are basically the same, even though the sites of stimulation and the types of stimuli that elicit a response may differ considerably.

The histological and anatomical evidence suggests that groups of neurones in certain sensilla are integrated to function as a unit, as in some of the chemoreceptors. Similarly, individual neurones of neighbouring sensilla are perhaps also integrated to function as a unit. This may take place through the subhypodermal nerve net in the case of the campaniform and thick-walled hair organs on the larger sclerites of the head. The complexes of the maxillary accessory neurones, the mandibular association centre and the labral plexus indicate other peripheral associations within the afferent system and between this and the efferent system. Further histological and neurophysiological studies are required to determine the exact nature and significance of these.

SUMMARY

There are no major differences in the distribution, structure and innervation and usually only few variations in the number of the cutaneous cephalic sensilla among twelve species of wireworms from three major taxonomic groups and from three different habitats.

Seven types of sensilla are described. (1) Long thick-walled setae occur on most of the cephalic sclerites; short ones are usually on parts that are covered by folds of the cuticle during certain movements; minute setae occur only on the large, flat skeletal plates. (2) Dome-shaped campaniform organs (Type A) are associated with the exocuticle and are usually near the longer setae; plate-shaped organs (Type B) are entirely in the endocuticle, mostly near muscle attachments; cone-shaped (Type C) and peg-shaped (Type D) campaniform organs are confined to the membranous ligula. (3) Pore canal organs are in the tips of the mandibles; they have no external cuticular manifestations, and have not been recorded previously from insects. (4) Scolopophorous organs are attached to the cuticle in the distal segment of the maxillary and labial palps. (5) Nine varieties of peg or thin-walled hair organs are primarily at the tips of the antennae, galeae, and the labial and maxillary palps. (6) Sensory plate organs occur at the tips of the antennae and

in the dorsal lining of the pre-oral cavity. (7) A large sensory appendix is situated on the second segment of each antennae. The first three types are innervated by 1, the fourth by 2, the fifth and sixth by 4, and the last by more than four neurones. The probable functions of these sensilla are considered on the basis of their structure and location.

The peripheral processes of the sensory neurones consist of a basal, a central, and a distal region. Axial fibres are evident only in the basal and distal regions. The basal axial fibre is closely associated with the nucleus in the cyton. A tubular cuticular sheath is shed with the exuviae from the distal region at each larval moult.

The subhypodermal nerve net under the larger sclerites of the head is comprised of uniting axons from the companiform and thick-walled hair organs and of fine inter-connecting fibrils.

Most of the cephalic sensory nerve fibres are connected directly to the supra- or suboesophageal ganglion. In addition, there are peripheral connections with the stomodaeal nervous system, and between the labral, mandibular, and antennal nerves through a labral plexus. A peripheral mandibular association centre, not previously reported from insects, receives nerve fibres from the mandibular pore canal organs, the main mandibular nerve branch, the labral plexus, sensilla on the frontoclypeus, and the apex of the

hypopharyngeal rod. A cluster of four accessory neurones is associated with the maxillary nerve trunk at the point where the axons from the sensilla on the lacinia and galea join it.

Globular inclusions usually are present in the cytoplasm of the trichogen cells of sensilla of both the newly moulted and the heavily sclerotized larvae. They are most numerous in those multi-celled sensilla that are believed to be chemoreceptors.

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Fig. 6 (Plate). A, base of long hair on maxillary stipes of heavily sclerotized A. haemorrhoidalis. (Silver).

B, short hairs on prosternal fold of newly moulted C. aena. (Silver).

C, minute hair on frontoclypeus of heavily sclerotized C. aena. (Silver).

D, subnasaler hair (arrow) and non-innervated spicules (left) on dorsal lining of pre-oral cavity of same.

E, group of four cells associated with minute hair organ of same.

F, individual bipolar neurones of the four long hairs on lacinia of C. destructor. (Methylene blue; whole mount).

G, surface view of campaniform organs of Type A (left) and Type B (right), and of minute hair organ (centre) on the epicranial plate of a heavily sclerotized Agriotes. (KOH-treated whole mount; unstained).

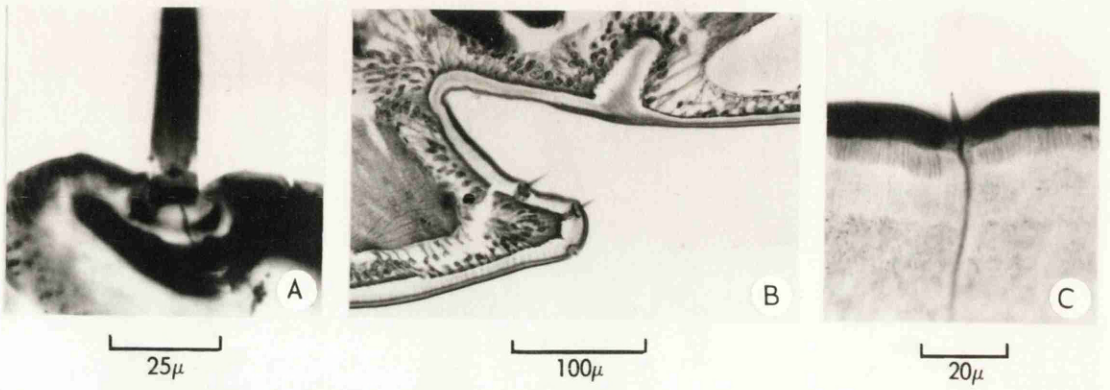
H, Type A campaniform organ (left) and minute hair or an on frontoclypeus of a heavily sclerotized C. destructor. (Haematoxylin).

I, Type A campaniform organs in maxillary palp of heavily sclerotized A. haemorrhoidalis. (Silver).

J, Type B campaniform organs near a muscle attachment on the epicranial plate of a newly moulted C. aena. (Silver).

K, ditto in heavily sclerotized specimen of same.

L, Type C campaniform organs in the ligula of a heavily sclerotized M. rufipes. (Silver).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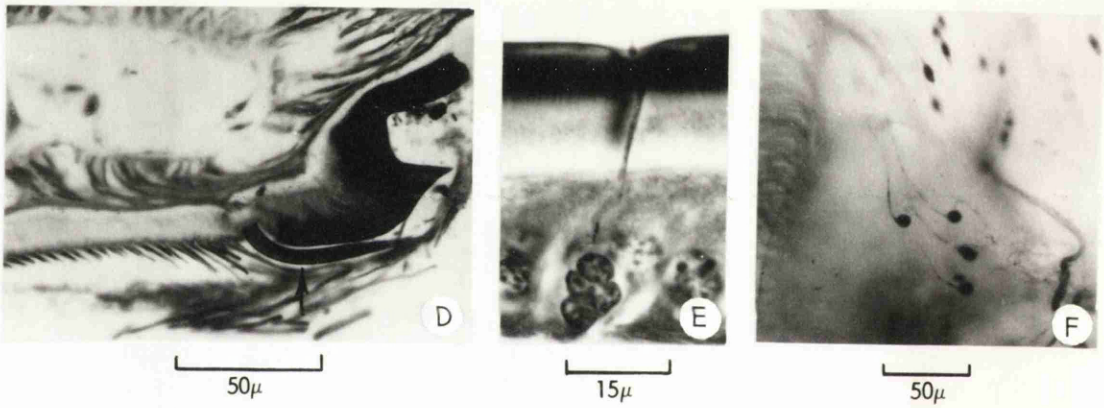
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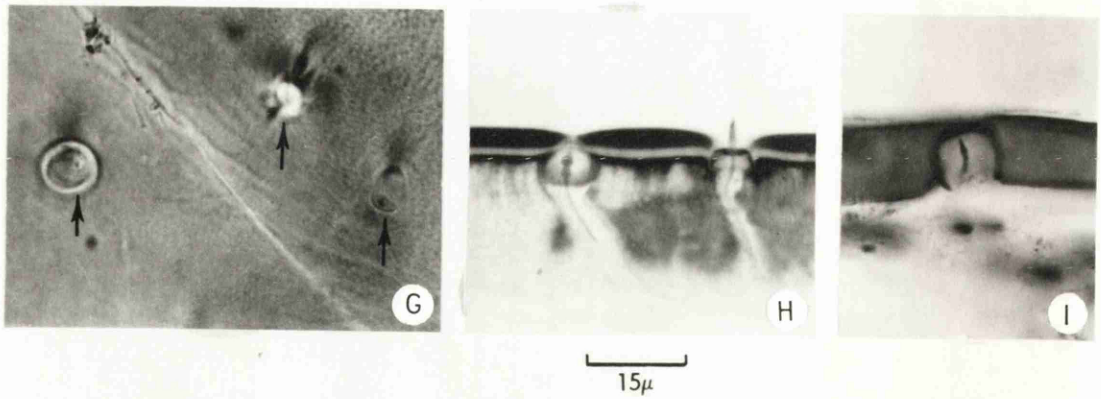
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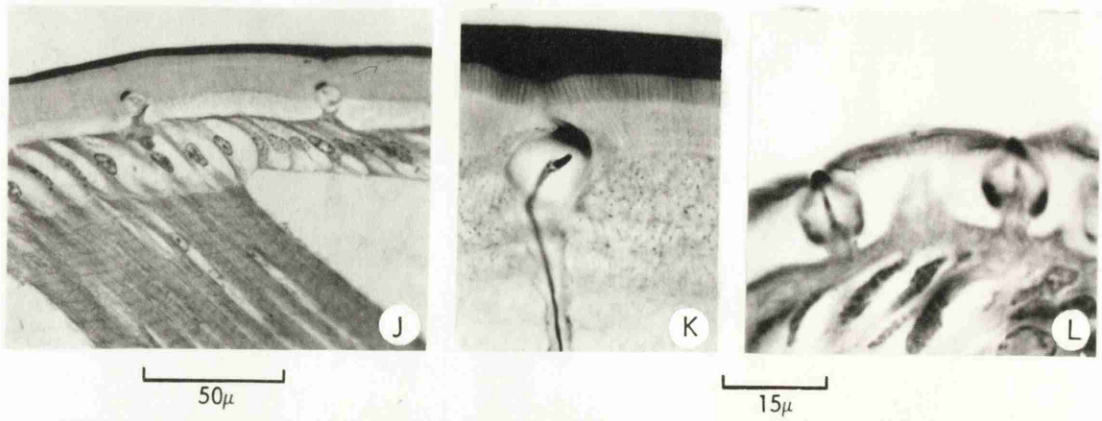
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Fig. 7 (Plate). A, Type D campaniform organs on ligula of a newly moulted and a heavily sclerotized (inset) H. riparius. (Silver).

B, non-innervated hairs of the pre-oral filter of a heavily sclerotized A. haemorrhoidalis. (Silver).

C, medial view of the subhypodermal 'nerve net' under the epicranial plate of a heavily sclerotized C. destructor. (Methylene blue).

D, ditto, showing the delicate net of interconnecting fibrils.

E, neurones of the campaniform organs (near base) and of the pore canal organs (central and distal) in the mandible of a moulting C. destructor. (Methylene blue; whole mount).

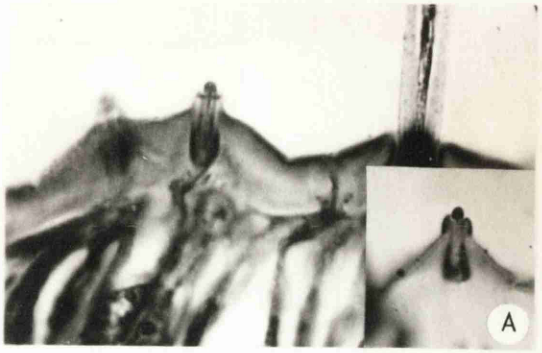
F, apical pore canals in mandible of heavily sclerotized L. linearis. (Unstained; KOH-treated whole mount).

G, terminal nerve processes at the base of the apical pore canals in mandible of moulting C. destructor. (Methylene blue; whole mount).

H, lateral view of scolopale of a scolopophorous organ in a maxillary palp of a heavily sclerotized C. destructor. (Unstained; KOH-treated whole mount).

I, ditto, medial view, showing cavity within scolopale.

J, individual bipolar sense cells and distal nerve process of scolopophorous organs in labial palp of a moulting C. destructor. (Methylene blue; whole mount).



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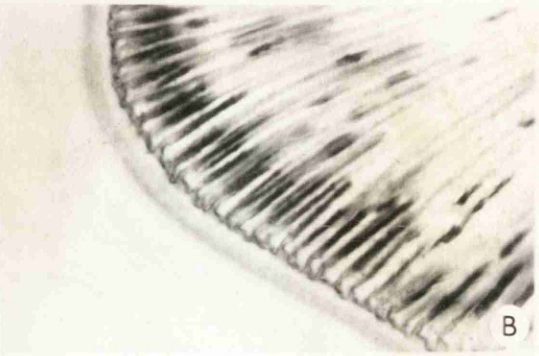

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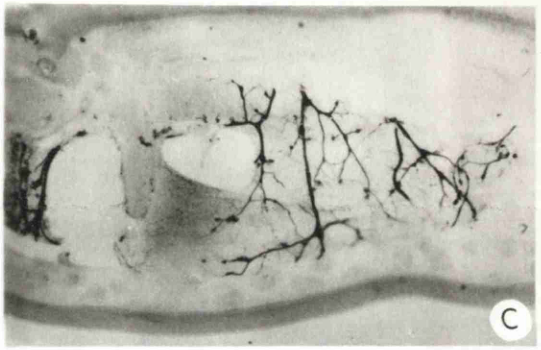

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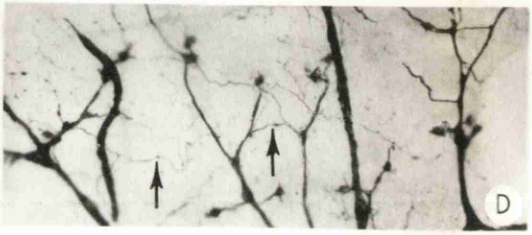

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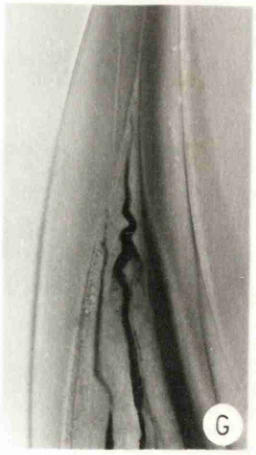

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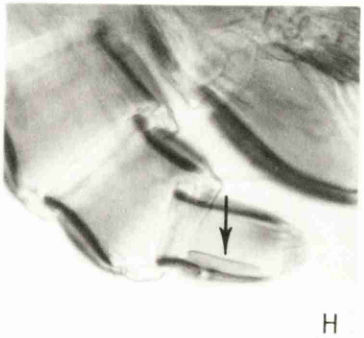

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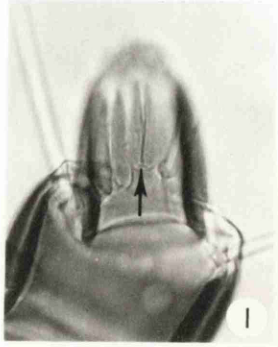

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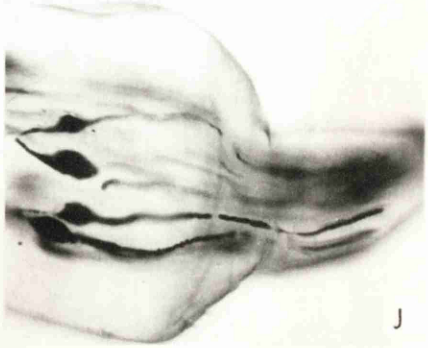

40μ

Fig. 8 (Plate). A, rod-like terminal region of the distal nerve process within the scolopale of a scolopophorous organ in the labial palp of a moulting (top, Methylene blue whole mount) and a heavily sclerotized (Haematoxylin) C. destructor.

B, lance-shaped peg at the tip of the galea of heavily sclerotized Agriotes (right, Silver) and C. destructor (Haematoxylin).

C, bladder-shaped peg on the galea of a heavily sclerotized H. riparius. (Silver).

D, large and small pegs on a maxillary palp of a newly moulted M. rufipes, and the nerve fibre terminating in a small peg (inset) on same of heavily sclerotized H. riparius. (Silver).

E, large lanceolate pegs (arrows) and setiform peg on third segment of the antenna of a heavily sclerotized D. marginatus (left) and L. minutus. (Silver).

F, small truncate peg (arrow) on the antenna of a heavily sclerotized D. marginatus. (Silver).

G, setiform peg on the second segment of the antenna of a heavily sclerotized D. marginatus, and (inset) nerve ending at tip of same on the third segment of the antenna of a heavily sclerotized A. haemorrhoidalis. (Silver).

H, small sunken pegs on the second segment of the antenna of a newly moulted H. riparius (left) and D. marginatus. (Silver).

I, minute thick-walled peg on the ventral wall of the second segment of the maxillary palp of a newly moulted Agriotes. (Silver).

J, bundles of neurones and distal nerve process of the sensilla on the third segment of the antenna (lower) and of the sensory appendix of a moulting C. destructor. (Methylene blue whole mount; antenna inverted).

K, innervation of the sensilla on the appendages of the ventral mouthparts of same. (Dorsal view).

L, placoid sensilla (arrows) on the third segment of the antenna (inverted) of a heavily sclerotized A. haemorrhoidalis (upper, Silver) and of a moulting C. destructor (Methylene blue whole mount).

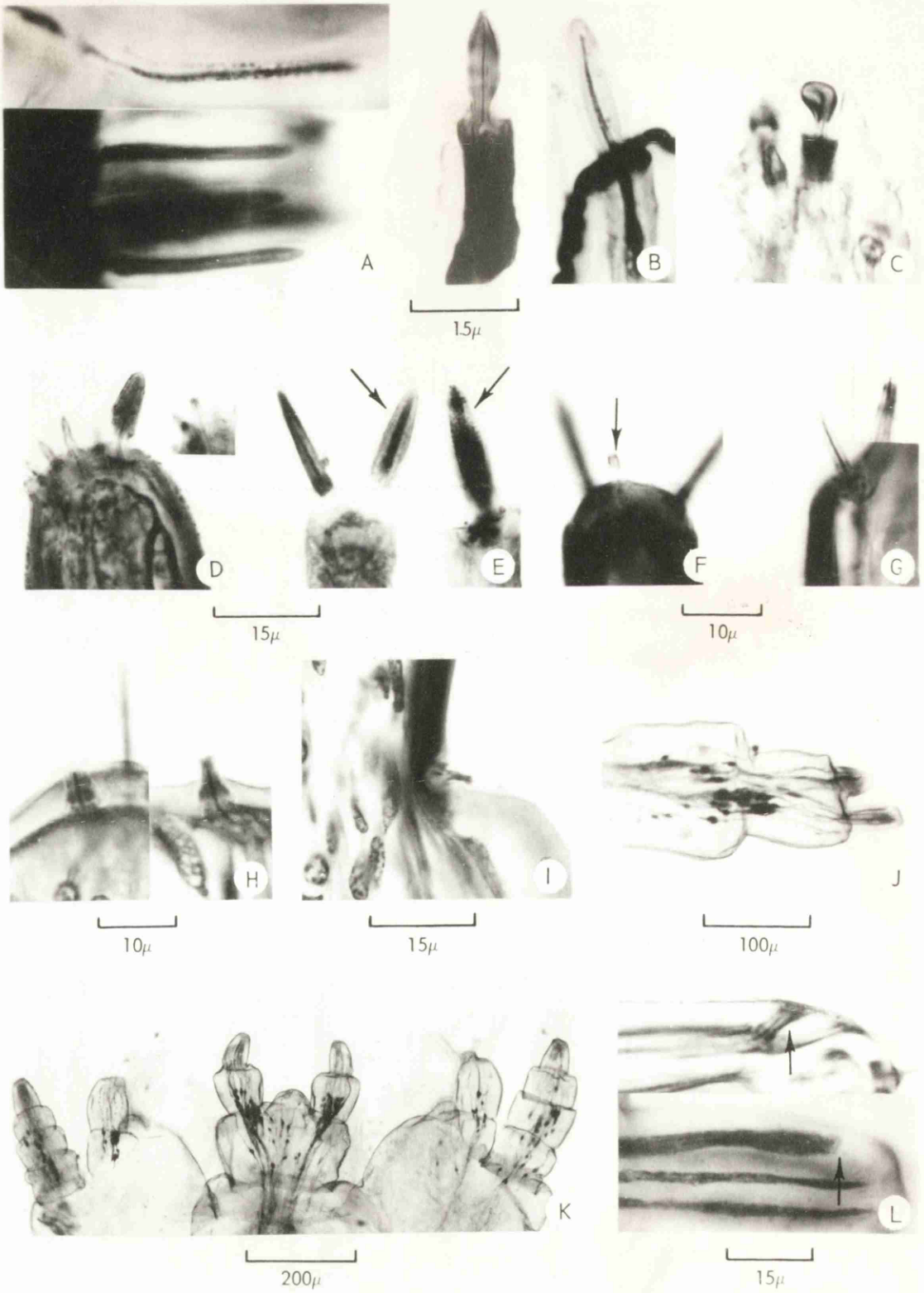
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Fig. 9. (Plate). A, surface view of the terminal nerve fibres in the five placoid sensilla on a supporting sclerite in the dorsal lining of the pre-oral cavity, in a heavily sclerotized A. haemorrhoidalis. (Silver).

B, lateral view of same in a newly moulted H. riparius, with the terminal nerve fibre and epicuticular covering plate in a heavily sclerotized A. haemorrhoidalis (upper left) and the darkly stained matrix beneath the covering plate in a newly moulted D. marginatus (upper right). (Silver).

C, paired bundles of bipolar neurones of the oral sensory plate organs (upper arrows) and of the stomodaeal nervous system in a moulting C. destructor. (Methylene blue whole mount).

D, sensory appendix on the antenna (inverted) of a heavily sclerotized A. nigrinus. (Silver).

E, ditto of M. rufipes.

F, thin outer and perforated inner layers of the sensitized cuticle of same.

G, surface view of the canicular perforations in the inner layer of same of A. nigrinus. (Silver).

H, distal nerve processes extending into the cuticular structure of the antennal sensory appendix of a moulting C. destructor. (Methylene blue whole mount).

I, nerve (n) and epithelial cytoplasmic (c) processes in same of a heavily sclerotized Agriotes. (Silver).

J, ditto of newly moulted C. destructor.

K, paired proximal processes and the three regions (arrows) in the distal processes of the paired sense cell units of pore canal organs in the mandible of a moulting C. destructor. (Methylene blue whole mount; composite of five focal levels).

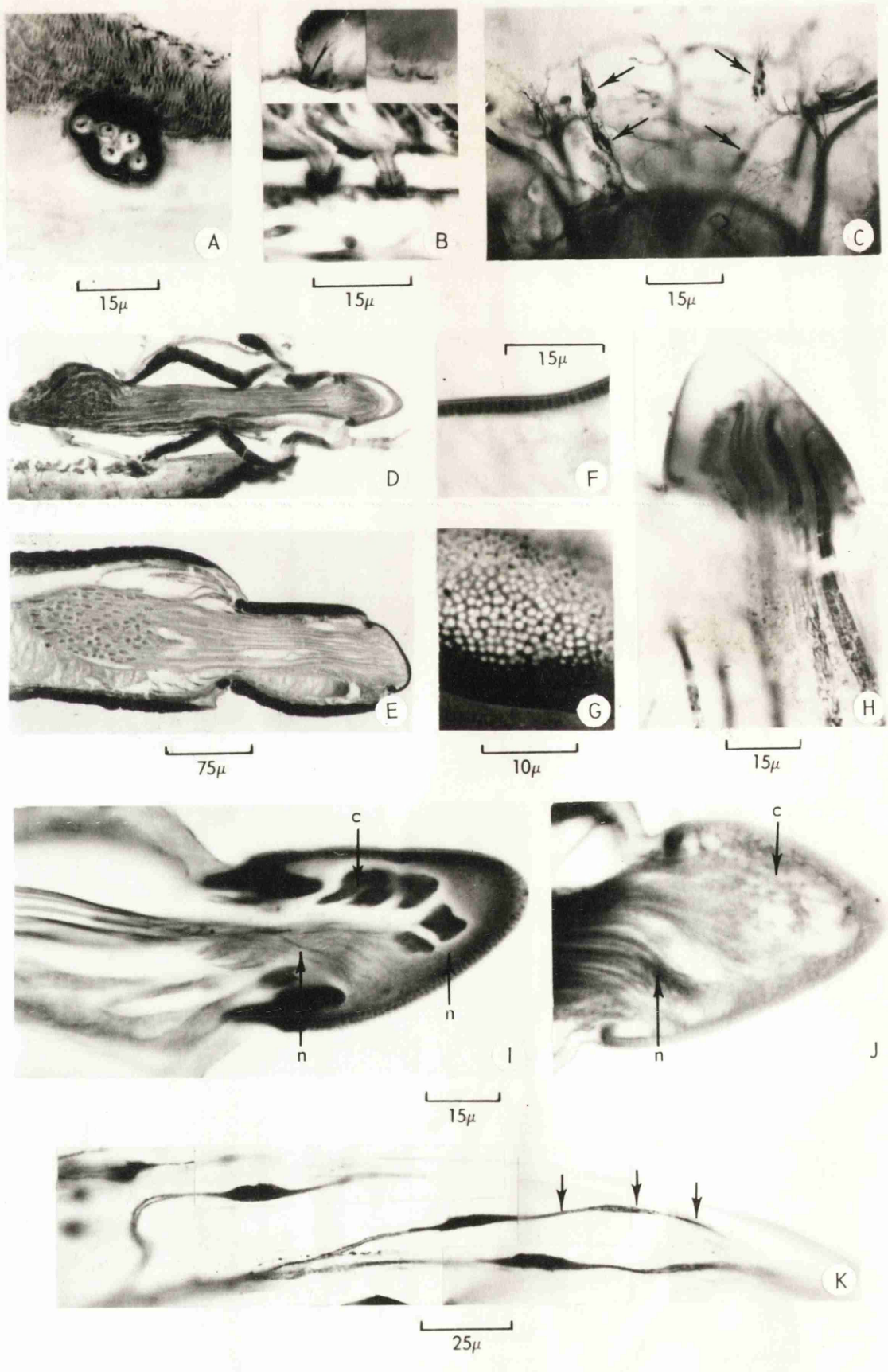
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Fig. 10 (Plate). A, unit of four sense cells and the three regions in their distal processes (arrows) of a setiform peg organ on the third segment of the antenna of a moulting C. destructor. (Methylene blue whole mount; composite of six focal levels).

B, the three regions and the junction body (arrow) in the unit of four distal nerve processes of the large lanceolate peg of same. (Composite of three focal levels).

C, junction bodies (arrows) in the distal nerve processes of the peg organs on the maxillary palp of a heavily sclerotized M. rufipes. (Silver).

D, ditto in the antennal sensory appendix of a heavily sclerotized C. destructor. (Silver).

E, three regions in a distal nerve process of a Type B campaniform organ on the frontoclypeus of a heavily sclerotized A. haemorrhoidalis; the axial fibre of the proximal region either enters the nucleus (upper; composite of three focal levels) or ends near it (lower; composite of two focal levels). (Silver).

F, tubular sheath of the distal nerve process of a long hair organ on the epicranial plate of the old cuticle, just before it was shed by a moulting C. destructor; arrow points to longitudinal ribs near the apex. (Whole mount stained intra-vitally with Methylene blue; surface view of the inner aspect).

G, ditto of a Type B campaniform organ in same; focal level at inner surface of cuticle (right) and near the apex of the sheath.

H, ventral view of the central and left regions of the nerve complex in the dorsal part of the head, anterior to the brain, of a moulting C. destructor; the neurones of the oral sense plate organs (sn) enter into a complex of nerve connections with the sympathetic nervous system through the bipolar neurones (r) that connect with the recurrent nerve behind the frontal ganglion (f), with the cibarial-pharyngeal motor system (c) through a varicose region (s), with the peripheral mandibular association centre (a), and with the central nervous system through the labral nerve (l). (Methylene blue whole mount; two focal levels).

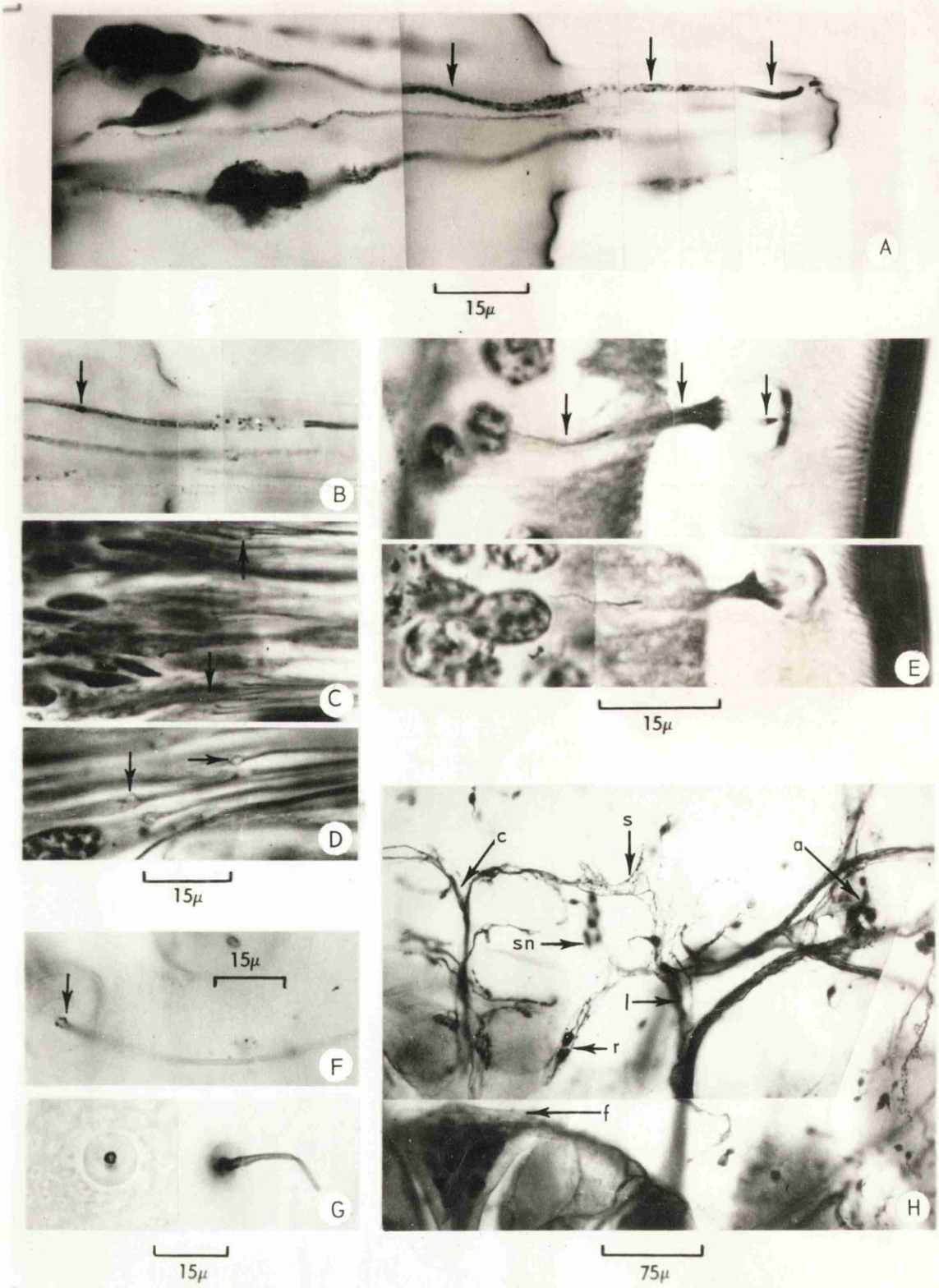
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Fig. 11 (Plate). A-C were stained by the Methylene blue and D-F by the Silver method.

A, ventral view of left labral, mandibular and antennal nerve connections and the mandibular association centre in a moulting C. destructor. (Whole mount).

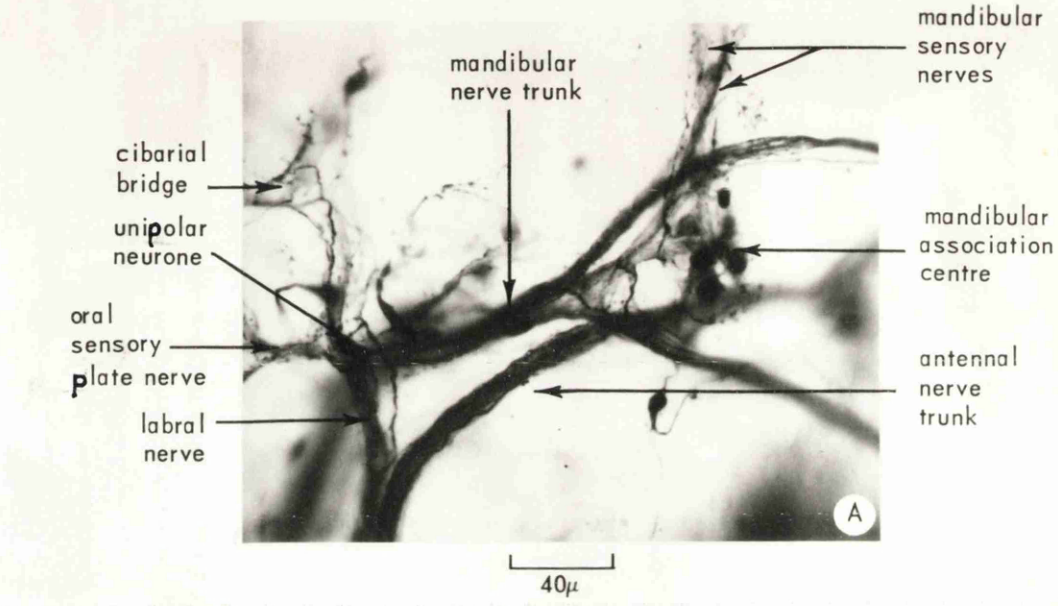
B, cluster of neurones (n) at the junction of the sensory nerve fibres from the maxillary palps (ms), galea (g), and lacinia (l), which is just anterior to the distal-most maxillary motor nerve (mm); a fibre from the cluster of neurones is directed posteriorly (p) towards the insertions of the muscles of the maxillae. (Whole mount of same).

C, posterior fibre (p) from the maxillary cluster of neurones, with its termination (t) in the insertion of a muscle fibre. (Whole mount of same).

D, argyrophil inclusions in the trichogen cells of the terminal maxillary peg organs of a newly moulted A. murinus. (Longitudinal section).

E, ditto surrounding the distal nerve processes in the antennal sensory appendix of a heavily sclerotized A. nigrinus. (Oblique section).

F, inclusions surrounding the nucleus in a trichogen cell of a peg organ in the galea of a newly moulted C. destructor. (Longitudinal section).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cibarial bridge
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oral sensory plate nerve
antennal nerve trunk
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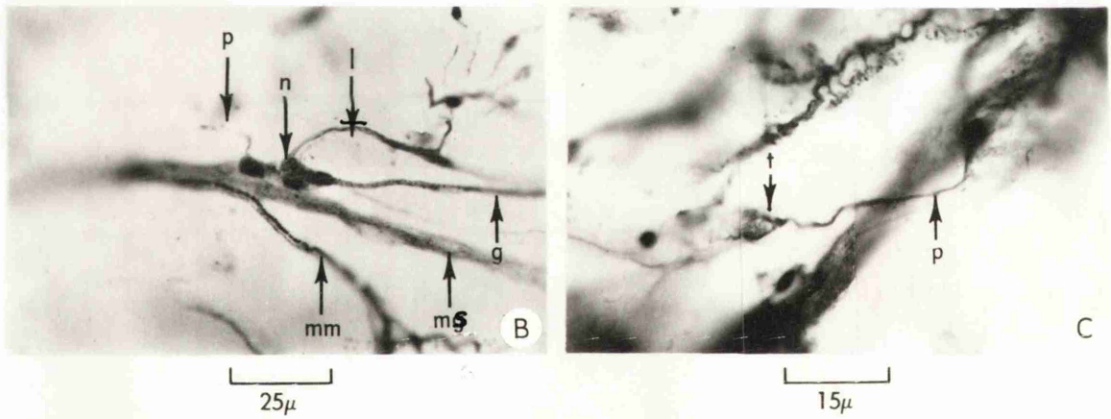
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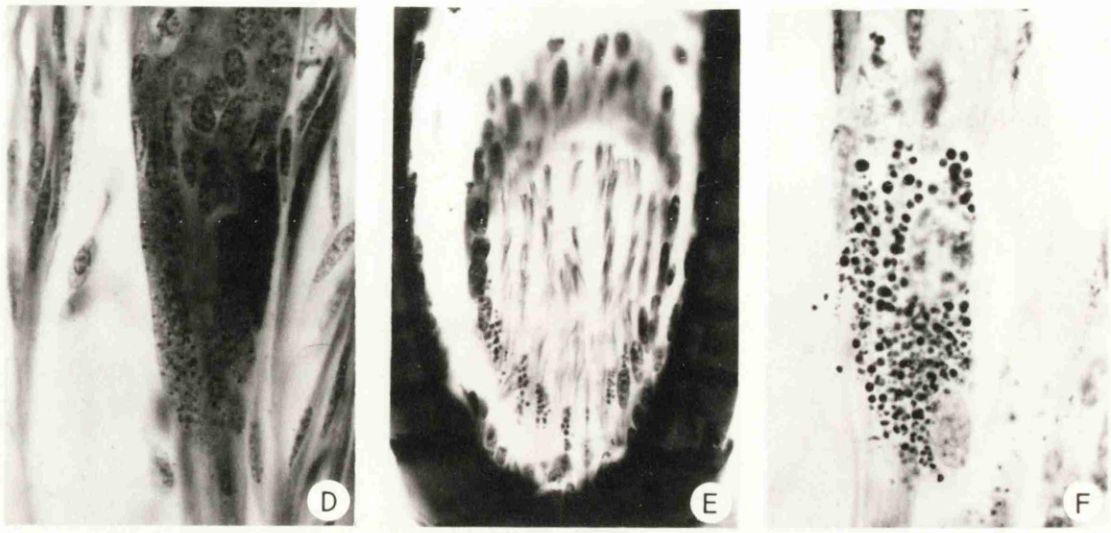
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Chapter III

(J. Morph., Submitted to Editor Feb. 16, 1962)

EXUVIAL SHEATHS OF SENSORY NEURONES IN THE LARVA OF *CTENICERA DESTRUCTOR* (BROWN) (COLEOPTERA, ELATERIDAE)[1]

by R. Y. Zacharuk

Research Station, Research Branch, Canada Agriculture, Saskatoon, Saskatchewan

INTRODUCTION

Fine cuticular tubules attached to the inner surfaces of hairs or hair-like structures in exuviae of insects were first described by Plotnikov (1904) in *Tenebrio molitor* (L.). He believed them to be the discarded ducts of moulting glands. Sihler (1924) showed that tubules of this type actually were shed by the distal processes of sense cells in hair organs of grasshoppers. This was corroborated by Feuerborn (1927), Hsü (1938), Richard (1952) and Slifer *et al.* (1957, 1959) in various types of sensilla of other insects. However, there is still some confusion in the literature regarding the morphology and terminology of these and associated structures (see reviews by Snodgrass, 1926; Hsü, 1938; Richard, 1952; Slifer *et al.*, 1957). Because each author usually based his findings on a different type of sensillum in a different insect, it is difficult to decide if the discrepancies in the literature

[1]Part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Zoology, University of Glasgow, Scotland.

Cuticular sheaths similar to the tubules noted above were observed in exuviae of some species of wireworms in a previous study (1962). A more detailed study revealed that these sheaths were shed at each larval moult not only by the distal processes, but apparently also by the cell bodies and axons of the sensory neurones in the seven types of cutaneous sensilla that were examined. These are described, compared among the various types of sensilla, and discussed in relation to the previous findings.

METHODS

The following descriptions are based on preparations of larvae of Ctenicera destructor (Brown), most of which were in the process of moulting to the 9th, 10th, or 11th instar.

Some specimens were stained intra-vitally with Methylene blue and mounted whole or as serial sections in paraffin, as previously described (1962). For most of the whole mounts the exuviae were removed from the larvae during or just after fixation. Other specimens were fixed in aqueous Bouin's fluid or 10% Formol, sectioned serially at 5 or 6μ in Ester wax, (Steedman, 1947), and stained by Mallory's Trichome, Heidenhain's Haematoxylin, McManus' Periodic acid-Schiff, or Adams and Sloper's Performic acid-Alcian blue methods, the latter two as outlined by Pearse (1960).

Specimens at different stages in the moulting process were examined. The periods to completion of the moult given below for some of these stages are only estimates. They are based on an average duration of about one week for the entire moulting process.

COMPARATIVE STRUCTURE

General

Late in the moulting process, at about the time the exuviae splits along the dorsal midline, the length of the exuvial sheath of a sensory neurone corresponds closely to that of the terminal region of the distal process from which it was shed (Zacharuk, 1962). At certain earlier stages in the moulting process these exuvial nerve sheaths are considerably longer (compare Figs. 12 and 13). Because of their delicate nature, varying lengths of many of the sheaths were undoubtedly broken off and lost during the removal and subsequent processing of the exuviae. Also, their free proximal parts were often considerably coiled (Fig. 8). Despite this, many instances were observed where the length of a sheath corresponded closely to the entire length of the neurone that shed it, from its termination in a sensillum peripherally to its entry into the ganglion of the central nervous system (CNS) proximally.

Such exuvial sheaths were shed only by the sensory

neurones of the cutaneous sensilla. None were evident from the neurones of the ocelli or of the efferent system. They were easily distinguished from the intima of tracheoles and the tonofibrillae of muscle insertions, which are also shed at each moult, by their appearance, staining, and points of attachment on the cuticula.

Each nerve sheath, or termed more specifically, exuvial sensory nerve sheath, consists of two morphologically distinct sections (Fig. 1). The tubular distal section has thick, rigid walls, and its length corresponds with that of about the terminal two-thirds of the distal process of the cell that shed it. It will be referred to here as the cuticular sheath, in accordance with the usage of Sihler (1924) and Slifer et al. (1957). The proximal section, which often corresponds in length with the basal part of the distal process, the cyton and the axon of the sense cell that shed it, will be referred to as the subcuticular sheath. It has very delicate, invariably collapsed walls, and tapers proximally to near and sometimes beyond the limits of resolution of the light microscope. There was no evidence of dichotomous branching on the proximal ends of this sheath. A slightly enlarged, darkly-staining junction body connects the two sections. The appearance of the nerve sheath in the 7 types of sensilla that were described previously in wireworms (1962) is as follows.

Thick-walled Hair, Companiform, and Scolopophorous Organs

In wireworms the sensilla of these 3 types are innervated by individual sensory neurones. The thick-walled hair and companiform organs are numerous, and are distributed generally over most of the head and body sclerites. The axons from their sense cells form, in part, a subhypodermal nerve net before they join one another and enter a ganglion of the CNS through a nerve trunk. The scolopophorous organs are few in number and, in the head, occur only in the tips of the maxillary and labial palps. Their axons enter directly into the nerve trunks that serve these appendages (Zacharuk, 1962).

In the exuviae most of the nerve sheaths of the thick-walled hair and companiform organs hang freely within the exuvial cavity from their points of attachment on the inner surfaces of the receptor cuticle (Figs. 2 to 4, 7). The interconnecting fibrils of the nerve net are not evident, so that this arrangement of the axons is not retained by the nerve sheaths. Probable remnants of the nerve net are seen occasionally as junctions of the subcuticular sheaths from 2 to 4 neighboring companiform and thick-walled hair organs (Figs. 7,8). The interrelationship of the sheaths at these junctions could not be determined. The cuticular sheaths can be easily distinguished from the cytonal and axonal parts of the subcuticular sheath, but there is often little difference between it and the distalmost region of the subcuticular

sheath that covered the basal part of the distal sense cell process. The junction body, a slight, darkly stained thickening of the walls at the base of the cuticular sheath (Figs. 1A, 3-5), delimits the cuticular from the subcuticular sheath. It also is not always distinct in the companiform and thick-walled hair organs. In rare instances, bulbous expansions are evident in the walls of the subcuticular sheath, in about the region previously occupied by the cell body (Fig. 6). The terminal apparatus of these sheaths has been described previously (Snodgrass, 1926; Zacharuk, 1962). At this stage, about one day before ecdysis, the structure of the sensory neurone appears to be definitive, and its distal process is already inserted in the thin, new cuticle of the sensillum. With the possible exception of the terminal apparatus, the new nerve sheath still cannot be distinguished from the darkly stained neural plasm.

In exuviae of scolopophorous organs, the distal half of the cuticular sheath is encased and capped by a heavy-walled, elongated cuticular structure (Fig. 10). This structure is described in detail elsewhere (Zacharuk, 1962). The junction body is a distinct node at the base of the cuticular sheath. The subcuticular sheath is thin-walled and collapsed along its entire length, unlike that of the companiform and thick-walled hair organs.

Pore Canal Organ

Six pore canal organs occur in each mandible of wireworms. Each is innervated by 2 sensory neurones (Zacharuk, 1962). A pair of sensilla of the same type, overlooked in the previous study, are situated on each side of the nasale in the anterior margin of the frontoclypeus, at about the position labelled (P) in figure 2. The heavily sclerotized, darkly pigmented exuvial cuticle of the mandibles obscured the nerve sheaths within, so the following description is based on the frontoclypeal pore canal organs only (Fig. 11).

One nerve sheath is shed by the 2-celled unit of each pore canal organ. The cuticular sheath is for the most part uniformly thick-walled and tubular. The distal extremity is slightly tapered where it traverses the pore canal. Its walls appear to be continuous with the surface layers of the cuticle surrounding the pore canal, and the cavity within the nerve sheath seems to open to the exterior at the surface. However, because of the poor resolution at this point, this could not be determined with certainty. The nodular junction body at the base of the cuticular sheath is darkly staining and distinct. The subcuticular sheath is very thin-walled and collapsed from the junction body inwards. It was impossible to determine by the methods used whether this part of the nerve sheath consists of a single tube that encased both neurones, or whether there is a septum that divides the cavity into two

compartments, one for each neurone.

Plate and Thin-Walled Hair or Peg Organs

There are 8 varieties of thin-walled hair or peg organs distributed primarily on the terminal portions of the antennae, galae, and the maxillary and labial palps. One plate organ is situated in each antenna, and there are 2 groups of 5 plate organs each situated in the dorsal wall of the oral cavity. Each of these sensilla is innervated by a unit of 4 neurones (Zacharuk, 1962).

One nerve sheath is shed by the unit of 4 neurones of each sensilla (Fig. 1B). The nerve sheaths of the sensilla on one appendage often are bundled loosely into a "nerve sheath trunk" (Fig. 12), retaining in a loose fashion the arrangement of the axons that shed them (Fig. 13). In rare instances, these loose bundles of nerve sheaths extend to about the point in the exuvial cavity where the brain had been. More often, the bundles are looped and coiled, or even separated into individual or smaller groups of sheaths.

There are no apparent differences in the structure of the nerve sheaths among the sensilla of these 2 types. In general, their appearance (Fig. 14) is similar to that of the nerve sheaths of the pore canal organs (Fig. 11). The walls of the cuticular sheaths are thicker in some sensilla (Fig. 16) than in others (Fig. 14), and the junction bodies also differ

in size or chromophilic properties among sensilla (compare Figs. 15 and 16 with Fig. 14). The cuticular sheaths are often thrown into tight coils, and the subcuticular sheaths are occasionally expanded near their distal ends (Fig. 17). It is not known if the subcuticular sheath envelopes the four neurones together or if there are four septa that separate the neurones in each sensillum.

As in the pore canal organs, the resolution at the distal ends of the cuticular sheaths was poor. The walls of the sheaths seemed to be continuous with the surface layers of the surrounding cuticla, and the cavity within the sheaths seemed to be open to the exterior at the surface (Fig. 1B). If this is the case, it would be similar to the more detailed findings of Slifer et al. (1957, 1959) in basiconic pegs of grasshoppers.

Antennal Sensory Appendix

The antennal sensory appendix is a large, complex sensillum situated on the second segment of each antenna in the majority of wireworm species. It is innervated by 8 or more neurones (Zacharuk, 1962).

Each neurone in this sensillum is a separate unit with a large distal nerve process. Numerous fine fibrils are evident in the terminal part of this process, and these are inserted in the cuticular covering. Each neurone sheds a

typical nerve sheath during moulting (Fig. 1C). The cuticular sheath is two or more times as wide as those of the other sensilla described above. The fine terminal neural fibrils were not seen in the exuvial sheaths. The manner in which the sheath remains attached to the cuticular covering of the sensilla in the exuviae could not be resolved by the methods used. The junction body is more chromophilic and more constricted than is the cuticular sheath. The subcuticular sheath usually is not completely collapsed in the region where the large cell body had been.

Process of Moulting

Between moults, the nerve sheaths of the cutaneous sensory neurones could not be differentiated clearly from the surrounding and enclosed tissues by any of the histological and histochemical stains used. During the moulting process, the cuticular sheaths are strongly positive to stains specific for disulfide groups (cystine), and thus could be demonstrated clearly.

Early in the moulting process, when the hypodermis starts to separate from the cuticla, the cuticular sheaths extend deeply into the hypodermis towards the sense cell (Fig. 18). As the space between the hypodermis and the cuticle widens, the cuticular sheath, anchored distally to the cuticle of the sensillum, is slowly pulled out of the hypodermis. It

is outside the hypodermal layer probably 2 or 3 days after the moulting process began (Fig. 19). With further contraction of the larval body, the hypodermal layer separates further from the cuticle and, in the head, moves posteriorly. Thus, corresponding points on the two layers become separated in the longitudinal plane as well as in the transverse plane. This causes the subcuticular section of the nerve sheath to be pulled through the hypodermis after the cuticular section. Movements of the larva within the exuvial case perhaps further facilitate this process.

About 2 or 3 days before ecdysis, when the new cuticle over the hypodermis is not yet distinct, a long length of nerve sheath, much of which is subcuticular, is coiled in the moulting fluid within the ecdysial cavity (Fig. 20). A proximal part of it still extends into and probably medially beyond the hypodermis. The cuticular sheath is shed with the exuviae (Zacharuk, 1962), but there is no evidence of the subcuticular sheath at ecdysis. It appears to be histolyzed by the moulting fluid during the last day of the moulting process. The subcuticular sheaths seemed to be intact in exuviae that were removed from larvae before then. Their collapsed condition undoubtedly resulted from the pull exerted on them during moulting or when the exuviae was forcibly removed from the larva for the preparations.

The neural plasm appears to be withdrawn from the part of the nerve sheath that lies in the ecdysial cavity at some time before the new cuticle is laid down. At these early stages short plasmic processes are sometimes seen extending into the proximal ends of the extra-hypodermal parts of the sheaths (Fig. 21). Perhaps these are vestiges of the distal processes of neural plasm that had not yet been withdrawn into the uncovered hypodermal layer. No neural plasm was evident in any of the exuvial nerve sheaths that were examined at stages after the new cuticle had appeared over the hypodermis.

DISCUSSION

Homologies Among Sensilla

The findings from this and the previous study (1962) indicate that there are some differences in the structure of the cuticular and subcuticular sheaths and of the junction bodies of the exuvial sensory nerve sheaths among the seven types of sensilla in wireworms. The differences are greatest in the structure of the terminal apparatus of the cuticular sheath and in the manner in which it is connected to the covering cuticle. However, each of the above 3 parts of the nerve sheath is undoubtedly homologous among the various types of sensilla examined.

The earlier literature seems to deal only with the cuticular sheath or the terminal part of it; the subcuticular sheath and the junction body were not recognized as such. The cuticular sheath has been referred to specifically by various terms: Stift or Stiftkörperchen by early German authors; chitinartige Hülle by Sihler (1924); sense rod or scolopala by Snodgrass (1926); chitineurium by Feuerborn (1927); corps scolopoïde by Hsu (1938); and others. Snodgrass (1926) indicated that although the form and complexity of the sense rods varies much in different sensilla, all are homologous structures. He saw no reason to differentiate between the scolopalae, a term often used specifically for the "sense rods" of chordotonal organs, and the "sense rods" of other sensilla. He used these terms interchangeably. However, Hsü (1938) differentiated between the two and referred to the former as a corps scolpal, and to the latter as a corps scolopoïde.

The scolopophorous organs in the maxillary and labial palps of wireworms have a cuticular sheath which is homologous with those of the other types of sensilla. In addition, there is a large accessory cuticular structure which encloses and caps the terminal part of the nerve sheath, and which is connected distally to the cuticle of the integument. If the term scolopala or scolopale is retained, it is suggested that it be applied only to such accessory subcutaneous cuticular

structures. These seem to be specific to scolopophorous sensilla.

In the sensilla that are innervated by a group of sense cells, Snodgrass (1926) describes minute sense rods that are removed a considerable distance from the covering cuticle. They are attached to the latter by their long individual terminal filaments. In comparing his descriptions and illustrations with the findings in similar types of sensilla of wireworms, these seems little doubt that the structures he refers to as sense rods are homologous with the junction bodies, and their terminal filaments are homologous with the nerve processes and enveloping sheaths that extend distally from the junction bodies. Slifer et al. (1957, 1959) describe and illustrate the cuticular sheath of a basiconic peg in grasshoppers as ending a short distance above the neurones. Its open end is flared and may be slightly thickened here. This flared, thickened proximal end also may be homologous with the junction body of similar types of sensilla in wireworms.

Origin and Nature of Exuvial Nerve Sheaths

The origin of the cuticular sheath has not been demonstrated. Some of the early workers suggested that it is a product of the sense cell (see review by Snodgrass, (1926)). Hsü (1938) and Slifer et al. (1957) believed that it is secreted by the trichogen cell which envelopes it, and which

also secretes the cuticular covering to which the sheath is attached. The latter mode of origin seems to be the more plausible one, and is in agreement with some of the findings in the present study.

There is general agreement in the literature that the so-called cuticular sheath is of a cuticular nature. It is based on the observation that it is shed with the cuticular exuviae at ecdysis (Sihler, 1924), that it stains and resists histolysis the same as the cuticle does (Feuerborn, 1927), and that it appears to be continuous with the cuticular covering of the sensilla and is secreted by the same cell (Slifer et al., 1957). Hsü (1938), on the basis of Hensen's findings (1866) that it resists treatment with NaOH, concluded that the cuticular sheath is of a chitinous nature. The cuticular sheaths of wireworms resist treatment with a weak solution of KOH (Zacharuk, 1962). But Slifer et al. (1957) could find no trace of the pegs or of the cuticular sheaths that are attached to them after applying Campbell's chitin test. Richard (1952) was uncertain about the composition of the sheaths, but thought it was not the same as that of the cuticle. Slifer et al. (1957) observed certain differences in permeability between the walls of the sheath and those of the epicuticla with which it is continuous. An important difference that was observed in the present study is that the cuticular sheaths are strongly chromophilic to methylene blue when

administered intra-vitally, unlike the surrounding layers of the cuticle. They lose this property, generally considered to be specific to nerve tissues, after ecdysis. Thus, these sheaths appear to consist of a special cuticular material, which is similar in some respects to the surrounding cuticle and differs from it in other respects, and which may contain chitin.

If the trichogen cell forms the cuticular sheath, it would be logical to assume that the subcuticular sheath is also formed by an accessory cell of the sensillum. The neurilemma cell that is closely associated with each sensory neurone could be the one involved. According to Haffer (1921), Wigglesworth (1953), and others, the cytoplasm of this cell is spread out thinly over the sense cell body and extends at least partly along its distal and proximal processes. Perhaps the neurilemma cells (termed glial or Schwann cells by some authors), which occur medially among the groups of axons bundled within the neural lamella in nerve trunks, also add material to these sheaths. Such a function would be similar to that of the tracheoblasts that secrete the intima of the tracheoles. The ramifications of the cytoplasm of these neurilemma cells among the axons are described in detail by Hess (1958) and Wigglesworth (1959).

The junction body appears to be the point of fusion between the cuticular and subcuticular sheaths. There are

certain differences in the form and staining properties of these bodies among the various types of wireworm sensilla between moults (Zacharuk, 1962). These suggest the presence of internal complexes within these points of junction, which may have some significance in the processes of reception.

In the larva, the exuvial sensory nerve sheath may function as a selectively permeable membrane, which "insulates" each neurone or unit of neurones from one another and from the other tissues and fluids in the larval body. Whether it also occurs around the sensory neurones in the adult stage is not known.

Fate of the Sensory Processes During Moulting

According to Haffer (1921), the "Terminalstrang" (apparently comprising the nerve sheath and the axial neural plasm) retains its connection with the old cuticle for some time after the hypodermis separates from it in moulting Saturnid caterpillars. It becomes elongated during this period by stretching. Before ecdysis, however, the connection with the old cuticle is broken, and the entire "Terminalstrang" is withdrawn into the new parts of the sensillum where it is reinserted. Sihler (1924) and Hsü (1938) claimed that, in various insects, the terminal filament of the sense cell is not shed with the nerve sheath, but is withdrawn from it before the moult occurs. On the other hand, Richard (1952)

believed that the axial nerve filament breaks at the base of the cuticular sheath in a termite and an antlion, and that the distal part is thus discarded with the exuviae. The results of Slifer et al. (1957) suggest that in grasshoppers the nerve plasm is usually withdrawn from the cuticular sheath before ecdysis, but that terminal portions apparently are broken off and left behind in the occasional sheath. In moulting wireworms, neural plasm was never evident within the nerve sheaths at stages after the new cuticle had appeared over the hypodermis. It is believed that the neural plasm is withdrawn and reused by the sense cell. However, the possibility that at times distal portions of the nerve process were broken off and subsequently histolyzed within the sheaths before ecdysis could not be discounted.

The manner in which the cell body and axon are withdrawn from the subcuticular sheath could not be determined by the methods used. Further, more detailed studies, particularly with the aid of the electron microscope, are required to resolve this and other relations of the nerve sheath to the enclosed and surrounding elements before and during the moult. Perhaps these sheaths may aid in differentiating between sensory and motor axons in electron microscope sections.

SUMMARY

1. Tube-like sheaths are shed at each moult from the cell bodies and the distal and proximal processes of the sensory neurones that innervate the cutaneous sensilla of wireworms, as exemplified in Ctenicera destructor. The neurones of the ocelli and of the efferent system do not shed such exuvial sheaths.

2. Each exuvial sensory nerve sheath consists of a short, rigid-walled distal section, and a long, delicate-walled proximal section, termed the cuticular and subcuticular sheaths, respectively. These are joined by a minute, strongly chromophilic junction body near the base of the distal sensory process.

3. Each of the 3 parts of the nerve sheaths are homologous among the different types of sensilla. The scolopophorous organs have an additional accessory cuticular structure for which the term scolopale is retained.

4. Of the sensilla that are innervated by groups of neurones, the neurones have individual sheaths in some, as in the antennal sensory appendix; in others, units of two or four neurones have a common nerve sheath, as in the pore canal, plate, and peg organs.

5. It is suggested that the cuticular sheath is formed by the trichogen cell, the subcuticular sheath by the neurilemma

cell or cells, and that the junction body is the point of fusion between the two sheaths.

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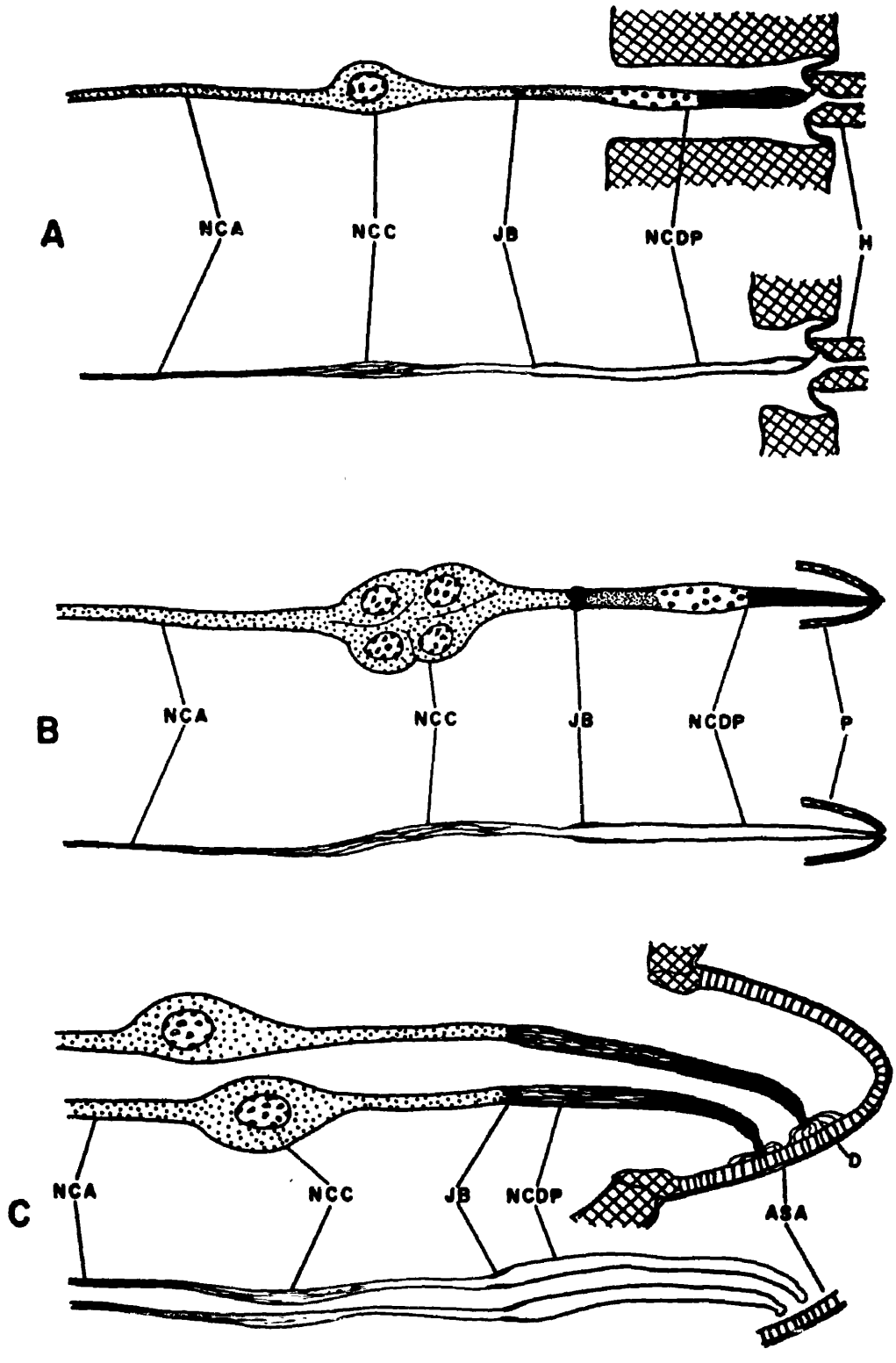
Fig. 1. Sensory neurones (upper) and the exuvial sheaths shed by them at ecdysis (lower) in larvae of C. destructor. Reconstructed from whole mounts stained intravitally with Methylene blue to show the cuticular sheath (NCDP), the subcuticular sheath from the cell body (NCC) and axon (NCA), and the junction body (JB).

A, tactile hair (H) innervated by a single neurone;

B, basiconic peg (P) innervated by a unit of four neurones;

C, two of the 8 or more neurones that innervate an antennal sensory appendix (ASA) through numerous dendritic fibrils (D).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Figs. 2 - 8, 10 and 11 are medial views of whole mounts of exuvial sensory nerve sheaths attached to exuviae that were removed from moulting larvae about 30 hours before ecdysis; stained intra-vitally with Methylene blue.

Fig. 2. Sheaths of tactile hair and campaniform organs on the frontoclypeus around the nasale. (P) denotes the approximate positions of one of the two pairs of pore canal organs on the frontoclypeus.

Fig. 3. Sheath attached to a tactile hair on the epicranial plate. The cuticular sheath is to the right and the subcuticular sheath to the left of the junction body (arrow).

Fig. 4. The same of campaniform organs on the ligula.

Fig. 5. The same of a tactile hair on the epicranial plate. Note the chromophilic junction body (arrow), and the absence of neuroplasm within.

Fig. 6. Sheaths under the epicranial plate. Note the bulbous expansion of the subcuticular sheath in the region previously occupied by the cell body (arrow), and the coiled axonal portions of the same sheaths.

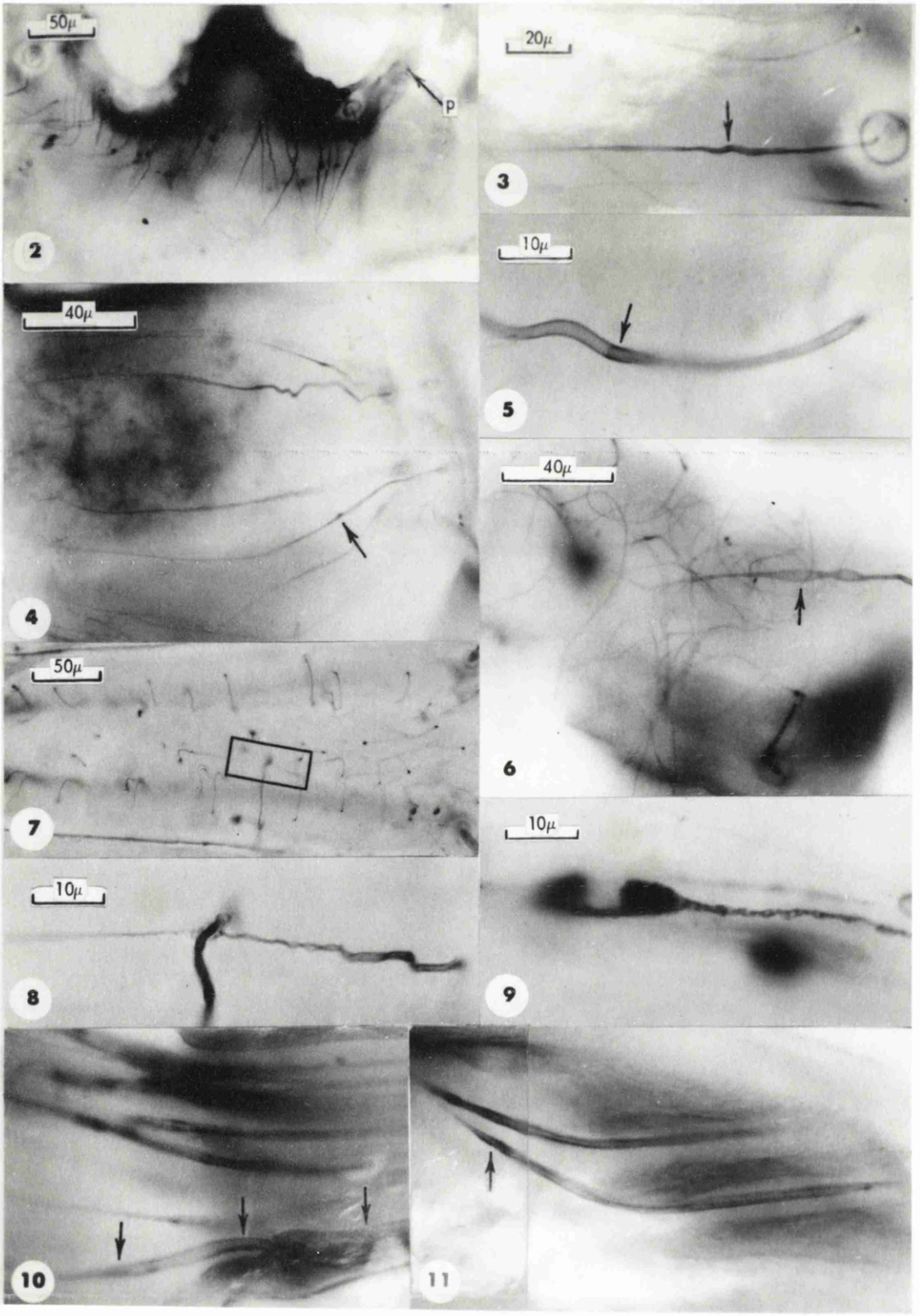
Fig. 7. Sheaths of campaniform organs and the tactile hairs on the postmentum. Note the union (enclosed area) of the subcuticular sheaths from a tactile hair (middle) and two campaniform organs.

Fig. 8. Enlargement of the enclosed area shown in Fig. 7.

Fig. 9. A nearly definitive sensory neurone of a tactile hair organ in the moulting larva about 30 hours before ecdysis, stained intra-vitally with Methylene blue. The distal process extends to the right.

Fig. 10. Sheaths of a scolopophorous organ (bottom) and of basiconic pegs (above) in the terminal region of the labial palp. Note the junction body (left arrow), the cuticular sheath (centre arrow) and the scolopale (right arrow).

Fig. 11. Sheaths of pore canal organs at the anterior margin of the frontoclypeus. The cuticular sheaths are to the right of the junction body (arrow).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Figs. 12 - 17 are of whole mounts stained intravitally with Methylene blue and fixed about 30 hours before ecdysis. Except for Fig. 13, they are of nerve sheaths attached to the exuvial skins.

Fig. 12. Nerve sheaths from sensilla on a labial palp bundled into a loose 'nerve' (arrows). The arrow on the right denotes the region where most of the junction bodies occur.

Fig. 13. Labium of the larva from which the exuviae shown in Fig. 12 was removed. Note the positions of the cell bodies and the nearly definitive structure of the distal and proximal processes.

Fig. 14. Sheaths of basiconic pegs of the labial palp. The cuticular sheaths are to the right of the junction bodies (arrows).

Fig. 15. The same of the fine plate organs on one oral sensory plate.

Fig. 16. The same of a basiconic peg on the galea.

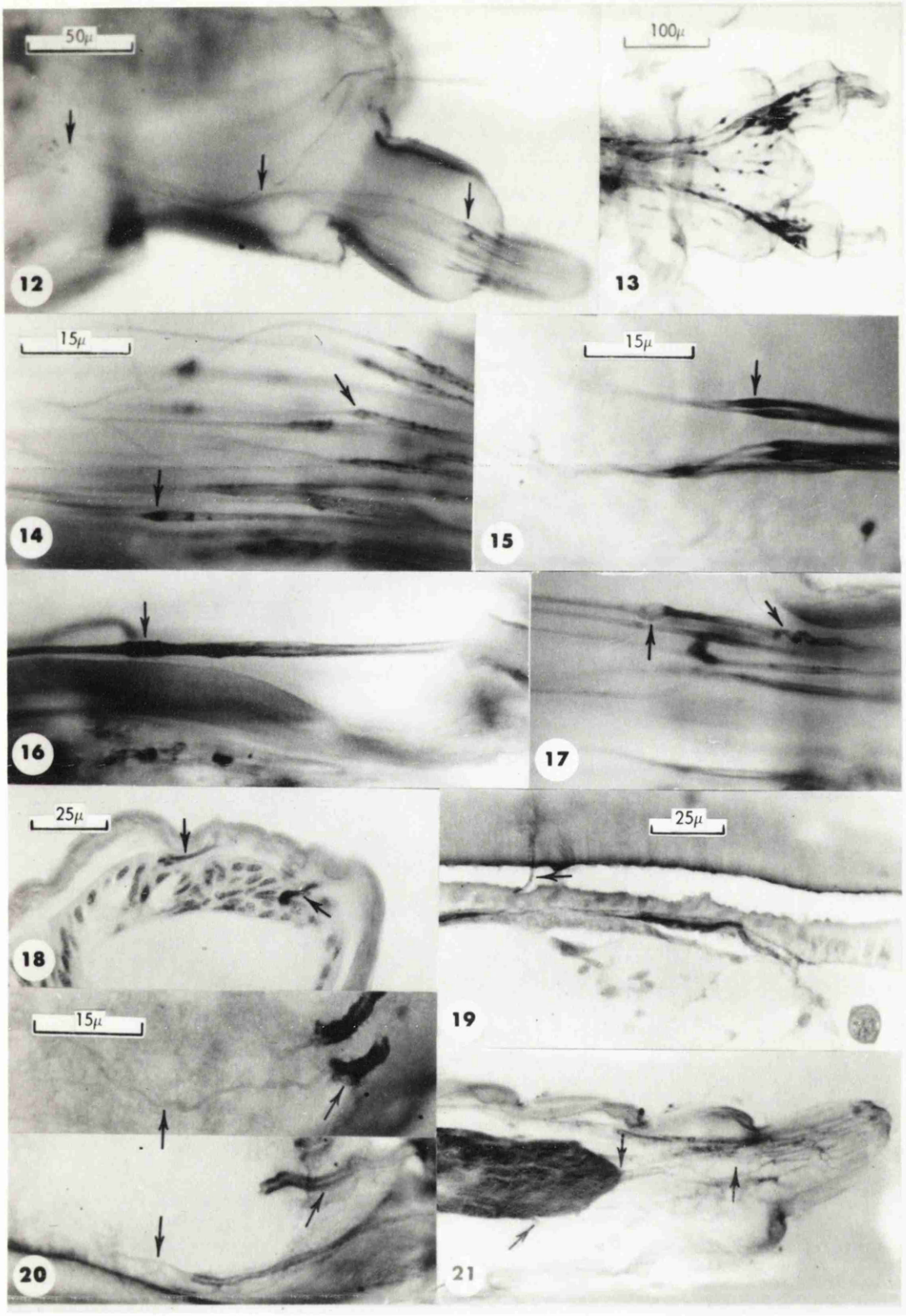
Fig. 17. The same of the basiconic pegs on a maxillary palp. Note the bulbous expansion of the subcuticular sheath (left) and the coils in the cuticular sheath (right arrow).

Fig. 18. The strongly cystine-positive cuticular sheaths of the peg-shaped campaniform organs on the ligula, about 7 days before ecdysis. (Performic acid-alcian blue).

Fig. 19. The same of a campaniform organ on the frontoclypeus, about 4 to 6 days before ecdysis. The basal part of the cuticular sheath (arrow) is almost completely withdrawn from the hypodermis (lower layer) and lies in the space between it and the old cuticle (upper layer).

Fig. 20. The same of campaniform organs on the ligula, from two successive serial sections, about 2 or 3 days before ecdysis. The entire cuticular sheath (right) and a large part of the subcuticular sheath (left arrow, cystine-negative) have been withdrawn from the hypodermis and lie in the moulting fluid of the ecdysial cavity.

Fig. 21. Nerve sheaths of sensilla on the maxillary palp (right arrow) being withdrawn from the hypodermis about 2 or 3 days before ecdysis. Short plasmic processes (left arrows) still extend into the nerve sheaths at this stage. (Periodic acid-Schiff).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Chapter IV

(Submitted to Editor of Can. J. Zool. April , 1962)

SOME HISTOCHEMICAL CHARACTERISTICS OF TISSUES IN LARVAE OF CTENICERA DESTRUCTOR (BROWN) (COLEOPTERA, ELATERIDAE), WITH SPECIAL REFERENCE TO CUTANEOUS SENSILLA[1]

by R. Y. Zacharuk[2]

Abstract

The sensory axons from the cutaneous sensilla and some of those in the recurrent nerve stain strongly with S-specific stains. The axons of the efferent system and those from the ocelli lack this staining characteristic. This difference among axons possibly is related to the origin of their precursors in the ontogenetic sequence.

Some of the metabolites involved in the synthesis of cuticular structures are demonstrated and discussed. The following sequence in the synthesis of the cuticula is suggested: glycogens → more complex, diastase-fast polysaccharides → chitin → a carbohydrate-protein complex containing SS groups → a complex (procuticle)

Footnotes

[1]Manuscript received

Part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Zoology, University of Glasgow, Scotland.

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The mechanisms of the histochemical reactions are discussed, with particular reference to staining with aldehyde-fuchsin after oxidation with potassium permanganate. This method may serve to differentiate histologically certain afferent from efferent axons in insect nervous systems.

Introduction

Sensory neurones of cutaneous sensilla in wireworms shed delicate sheaths during each moult (21). Each sheath consists of a cuticular section from the distal process, a subcuticular section primarily from the cyton and axon, and a junction body which connects the two sections near the base of the distal process. Other neurones, such as those of the ocelli, the stretch receptors, and the efferent system, do not appear to have such exuvial sheaths. Also, numerous argyrophil granules have been demonstrated in the trichogen cells of primarily those sensilla that are innervated by more than one neurone (20). Some histochemical charateristics of these and other components of the nervous system and of related parts of the integument are presented here.

Materials and Methods

Larvae of Ctenicera destructor (Brown) that were in the 7th to 10th instars were selected to provide

preparations of various stages between and during the moulting processes. The head and prothorax were removed and fixed in aqueous Bouin's fluid or 10% neutral formalin, usually with equal results. For most of the staining procedures the material was embedded in Ester Wax (17) directly after final dehydration in absolute ethyl alcohol, and was sectioned at 2 to 10 μ.

The following staining procedures were employed, most of them basically as described by Pearse (14).

(1) The aldehyde-fuchsin stain of Gomori, after oxidation with acidified potassium permanganate (PPPF). Sections were stained for 2.5 mins., differentiated in acid alcohol for 3 mins., and counterstained the Groat's haematoxylin and picro-indigocarmine. Positive tissues stained brilliant purple; nuclear chromatin granules were blue-black; other tissues were bright to olive green.

(2) Owen's (13) aniline blue method, counterstained with acid fuchsin in picric acid (PPAB). Tissues stained deep blue were considered positive; other tissues were red, or lighter shades of blue depending on the extent of differentiation.

(3) Bargmann's chrome-haematoxylin counterstained with phloxin (PPCH). Structures that were positive to the preceding two methods stained deep black; other tissues were red or various shades of grey, blue-black or black.

(4) Adams and Sloper's performic acid-alcian blue method for disulphide (SS) groups (PFAB), without counterstain or counterstained with picro-acid fuchsin. Tissues with demonstrable quantities of SS groups stained steel blue.

(5) Barnett and Seligman's dihydroxy-dinaphthyl -disulfide reaction for sulphydryl (SH) groups (DDD), with maleimide-blocked sections as controls. To demonstrate SS and SH groups together, sections were reduced with thioglycollate, and similarly reduced sections blocked with maleimide were used as controls. For SS groups only, maleimide-blocked sections were reduced with KCN or thioglycollate before staining. Tissues stained dark red to blue were considered positive. Other tissues were colourless or pink to pale brick-red.

(6) Chèvremont and Frederic's ferric-ferricyanide method for SH groups.

(7) Baker's modification of the Millon reaction.

(8) McManus' periodic acid - Schiff reaction without counterstain or counterstained with Groat's haematoxylin and picro-indigocarmine (PAS). Substances stained rich red or magenta were considered strongly positive. Pink to pale red tissues were considered weakly positive.

(9) Steedman's alcian blue method for acid mucopolysaccharides.

(10) Standard toluidine blue method for metachromasia.

(11) Sudan black B method of McManus for lipids in paraffin sections, after fixation in formalin-calcium-cobalt and post-chroming.

For brevity, the first five staining methods will be referred to collectively as S-specific, although as discussed in the next section, all of them may not necessarily be specific for S-containing tissues.

Principles of the Histochemical Reactions

The mechanisms of most of the histochemical reactions used here are discussed extensively by Pearse (14).

With the PAS test, considered specific for aldehydic groups liberated mostly by controlled oxidation of 1:2 glycol groups, Pearse lists polysaccharides, neutral mucopolysaccharides, muco- and glycoproteins, glycolipids, unsaturated lipids and phospholipids as giving a positive reaction. The polysaccharides (glycogens) are diastase-labile. Acid mucopolysaccharides are PAS-negative, but are demonstrated clearly by Steedman's alcian blue method. They display weak β- (violet) or strong γ-metachromasia (pink-red) with toluidine blue. The lipids, including those listed above, are sudanophil.

A positive Millon's reaction is given by any

phenolic compound containing the hydroxy-phenyl group. Tyrosine is the only known amino acid containing this group (14). Various compounds besides those containing SH groups, including phenols, promptly reduce ferric-ferricyanide mixtures to Prussian blue (11). Results with the Chèvremont and Frederic ferric-ferricyanide test were therefore interpreted by comparisons with the DDD reaction. The latter has a high selectivity and sensitivity for SH groups. Disulfide groups can also be demonstrated selectivèly and with a high sensitivity by this reaction, after existing SH groups are blocked and the tissue disulfides are reduced to sulphydryls. The PFAAB test provided an additional check on tissues containing demonstrable quantities of SS groups. Although the selectivity of this reaction is high, its sensitivity is low. Thus, a negative PFAAB reaction may be given by tissues containing small quantities of cystine demonstrable by other histochemical reactions.

The mechanisms of the PPPF, PPAB and PPCH reactions are still not clearly defined.

The results of Halmi and Davies (8), Scott and Clayton (15), and Bangle (1,2) with aldehyde-fuchsin and Schiff's reagents and toluidine blue metachromasia suggest that there are possibly three reactive components involved in the staining with aldehyde-fuchsin. The first are aldehydes, such as the insoluble ones derived from periodic

acid oxidation of orthochromatic tissue polysaccharides. The reaction probably depends on the formation of Schiff's bases or azomethines between the derived tissue aldehydes and the dyes, such as basic fuchsin, possessing open amino groups. The second are specific mucopolysaccharides, such as the metachromatic, highly sulphated acid mucopolysaccharides. Tissues containing or associated with these components stain intensely with aldehyde-fuchsin without prior oxidation, but not with Schiff's reagent under the same conditions. The third are specific orthochromatic proteins with a high content of cystine. These react strongly with aldehyde-fuchsin but not with Schiff's reagent after prior oxidation. The mechanisms of staining the latter two components is a complex problem. Pearse (14) indicates that it involves a salt linkage between the aldehyde-fuchsin and the acidic groups in the tissues. These could be sulphuric groups in acid mucopolysaccharides, or sulphonic or sulphinic groups derived by oxidation of the dithio bonds of cystine or other S-containing complexes. Gabe (6) considered the aldehyde-fuchsin reagent to have an affinity for sulphydryl groups as well as for aldehydes, both liberated by oxidation with potassium permanganate.

In fresh preparations of aldehyde-fuchsin (about 3 days old), some of the free amino groups of the pararosanilin fraction of basic fuchsin presumably have

reacted with and are blocked by acetaldehyde derived from the gradual depolymerization of paraldehyde in the presence of an acid catalyst, producing the characteristic azomethines. The remaining amino groups are free to attach to tissue aldehydes by non-polar bonds. In preparations aged for several weeks, presumably all the amino groups are blocked by acetaldehyde, and such preparations no longer stain tissue aldehydes (1). Observations made in the present study suggest that there is a similar decrease in reactivity to S-containing tissues with ageing of the reagent.

The alcohol-soluble dye used in Owen's aniline blue method is, like basic fuchsin, a basic dye of the triphenyl-methane series. It has one free amino group, and is probably similar in this respect to slightly aged paraldehyde-fuchsin. The mechanism of staining with aniline blue is therefore presumably similar to that of aldehyde-fuchsin.

According to Sloper (16), the chrome-alum-haematoxyphil neurosecretory substance in the intercerebralis-cardiacum system of a cockroach is PFAAB-positive; the latter reaction is given by sulfonates derived from protein-bound cystine or cysteine. Similar comparative results were obtained with several other S-containing tissues in the present study. In this reaction, perhaps the chromium cation of the chrome-haematoxylin lake forms a new link in the peptide chains

where the dithio bonds of cystine are broken by oxidation. It may bond similarly to the oxidation products of two adjacent SH groups of cysteine molecules in protein. The selectivity would be low, however, as the mordant also bonds to certain hydroxyl and carboxyl groups in the tissues.

With carefully controlled staining and differentiation periods, the PPPF reaction appeared to be highly sensitive and selective for certain S-containing tissues. The procedures followed in this method resulted in little, if any, reactivity with potential aldehydic groups. The PPAB method was as sensitive to S-containing tissues as the PPPF method, but proper differentiation for selective staining was more difficult. The PPCH method was also highly sensitive for certain S-containing tissues, but the selectivity was low.

Neurones and Accessory Cells of Cutaneous Sensilla

Distal Nerve Processes

The neural plasm of the distal sense cell processes was negative to the histochemical reactions used. Small sudanophil granules, probably mitochondria, were scattered along the processes, with denser aggregations occurring in the region of the junction bodies. The cuticular sheaths also were unstained between moults (Fig. 1). However,

early in the moulting process they became positive to the S-specific stains. The reaction was stronger in sensilla innervated by a single neurone (Fig. 4) than in those innervated by more than one neurone (Figs. 2,3), and the junction bodies stained more intensely than the sheaths. They retained this characteristic until discarded at ecdysis. In newly moulted larvae the sheaths and sockets of the sensilla, or more probably a thin layer of adjacent cytoplasm, were usually weakly metachromatic and faintly positive to Steedman's method for acid mucopolysaccharides, but only traces of these components were demonstrated in heavily sclerotized larvae. The cuticular sheaths in heavily sclerotized larvae, particularly the terminal apparatus in campaniform and thick-walled hair organs, gave strong Millon's and ferric-ferricyanide reactions for phenols, similar in this respect to the exocuticula of the integument. These reactions were weaker in new sheaths being formed during the moulting process.

Cytons

The nuclei, with fine, scattered chromatin granules, and the cytoplasm of the sensory cytons invariably stained lightly with the counterstains used (Figs. 2,5). Fine sudanophil granules, probably mitochondria, were scattered through the cytoplasm. These or other granules like them were diastase-fast, PAS-positive (Fig. 12) and PPPF-positive

(Fig. 13) late in the moulting process and in newly moulted larvae, but failed to give these reactions in heavily sclerotized larvae. The reactive components involved were perhaps glycolipids and/or muco- or glycoproteins formed around the mitochondria during moulting.

The sense cell boundaries were usually indistinct between moults (Fig. 1), but were delimited by very delicate membranes which became positive to the S-specific stains during and just after ecdysis (Fig. 5). These membranes are believed to be the cytonal portions of the new subcuticular sheaths that were in the process of formation. The portions of the old subcuticular sheaths that were in the moulting fluid in the exuvial cavity did not react with the S-specific stains. Earlier in the moulting process portions of the old subcuticular sheaths that were still being pulled through the scattered, highly active hypodermal cells appeared to be weakly S-positive (Fig. 6). Interpretation at this stage was difficult, however, because of the disorganization of the hypodermal cells and the cell groups of the sensilla.

One preparation was made by the PPPF method of a newly moulted larva that was in an early stage of infection by an entomophagous fungus, believed to be Metarrhizium anisopliae (Metch.) Sor. The body cavities were filled with spores. The PPPF-positive membranes around the cytons,

normally continuous (Fig. 5), were disintegrated into globules of PPPF-positive material. These globules were either scattered or still arranged in chains along the periphery of the cytons (Fig. 7).

Accessory Cells

The argyrophil granules of the trichogen cells described previously (20) usually were negative to the S-specific reactions. Their positions in multiple-celled sensilla were usually indicated by distinct vacuoles (Fig. 8). Occasionally the periphery of the vacuoles were weakly PPPF-positive. The granules were PAS-positive. Some were diastase-labile and others were diastase-fast in moulting and newly moulted larvae (Fig. 9).

In one newly moulted larva that was fixed in formalin, the secretory substance in the trichogen cells of campaniform and thick-walled hair organs was massed into several large and small globules. These were diastase fast, and gave positive PPPF, PPAB, PPCH and PAS reactions (Figs. 14, 15). These globules are believed to be, in part, artifacts, involving some polar movement of the secretory substance and perhaps the formation of relatively stable potentially reactive aldehydic groups during fixation and an increased concentration of an S-containing fraction in the globules. This "artifact" was not evident in other

preparations, some of which were processed similarly. In moulting larvae the entire cytoplasm of the trichogen and tormogen cells in these sensilla appeared homogeneously but weakly PAS- and S-positive against the background coloration of the counterstains used (Figs. 11-13). The reactions were usually more intense in the region of the socket in thick-walled hair organs (Fig. 12).

PAS-positive, diastase-fast granules were often present at the bases of the sense cell bundles in multiple-celled sensilla (Figs. 9,10). It was difficult to determine if these were confined to the abapical ends of the cytons or were also in the neurilemma cells. There were none in the neurilemma cells of the campaniform and tactile hair organs at the same stage in the moulting process (Fig. 12).

Axons

The axons of the sensory neurones of all the cutaneous sensilla were positive to the S-specific stains used (Figs. 1-3, 16-26). They stained more strongly during the moulting process than between moults. The axoplasm rather than the subcuticular sheath seemed to be largely involved, because each axon stained solidly when the cut ends were viewed in transverse (Figs. 20,21) and oblique (Fig. 23) section. Also, they gave a positive S-specific reaction in all the developmental stages of an instar that

were examined. However, it was difficult to determine the specific structures involved with the methods used because of their small size.

The S-positive nerve fibres originated as irregularly globular nodes at the abapical poles of the cytons (Figs. 2,16). Smaller oval or elongated S-positive nodes also occurred along the fibres for a short distance medially from their points of origin (Fig. 17), but they were not evident further medially in the nerve trunks (Figs. 19,23). The axonal fibres in moulting larvae were often looped in the region of the cyton and neurilemma cell in campaniform and tactile hair organs (Fig. 18), but the exact relationship was not evident. The pathways of the axons in the nerve trunks were wavy, and individual fibres were frequently tightly coiled for short distances (Figs. 19,23). The S-positive fibres were easily traced into the supra- and suboesophageal ganglia, where they branched dichotomously at the periphery of the neuropile into fibres too fine to be stained sufficiently or to be resolved by the light microscope (Figs. 24-26). Globules of S-positive material also occurred among the four accessory neurones in the mandibular ganglia (Fig. 28), suggesting that some S-positive sensory axons also originated in these structures.

The S-positive material of the sensory axons was orthochromatic and PAS-negative. The axonal walls were weakly

sudanophil. Diastase-labile, PAS-positive granules were sparsely scattered among the axons, and the neural lamellae of all the nerve system was diastase fast, PAS-positive (Fig. 22). There was no distinctly S-positive component in the neural lamella. However, it stained a pale to dark brick-red with the DDD technique (Fig. 21), suggesting perhaps a low content of cystine or cysteine.

Neurones of Other Sensilla

Unlike the sensory neurones of the cutaneous sensilla, no part of those of the ocelli was S-positive. The outlines of the axons from the ocelli were only faintly indicated by the counterstains used (Figs. 29, 31, 32). In marked contrast, those from the sensilla on the antenna (Figs. 29, 30) and from the tactile hair and campaniform organs in the cuticula around the ocelli (Fig. 32) and axons of other cutaneous sensilla were strongly S-positive in the same sections. The axons from the cutaneous sensilla around the ocelli follow closely those from the ocelli to the brain, but in a separate nerve.

The distal process of the neurone that innervates a stretch receptor was usually S-negative (Figs. 27, 34), or only the basal region was faintly PPPF-positive. It was difficult to distinguish with certainty the axon of this

neurone from other surrounding axons, so that its reaction to the S-specific stains could not be ascertained.

Many of the axons in the recurrent nerve of the stomodaeal nervous system were S-positive (Fig. 39). It is believed that these are sensory axons from sensilla in the digestive tract. In contrast, the nerve fibres in the connectives to the frontal ganglion were S-negative.

Neurones of the Efferent System

There was no S-positive material in any part of the motor neurones examined. Thus, the nerve fibres in the circumoesophageal connectives were unstained, in marked contrast to the sensory axons in the nerve trunks from the peripheral cutaneous sensilla (Figs. 24, 25). Similarly in the antennal (Fig. 30) and mandibular (Fig. 33) nerves, the small, deeply stained sensory axons could be easily distinguished from the larger, unstained motor axons. No S-positive material was evident in the smaller motor nerve branches to individual muscle fibres or in the terminal neuromuscular junctions (Figs. 35, 36).

The motor axons were usually dorsal to many of the sensory axons in the central portions of nerve trunks that contained both. However, some sensory axons were also dispersed among the motor axons (Figs. 21, 33). As exemplified

in the antennal nerve, bundles of motor axons remained discrete from the sensory for a short distance after a bundle of each joined into a single nerve (Fig. 30), but some interspersion occurred more medially (Figs. 20, 21). The motor and sensory axons appeared to be in discrete bundles again on or just after entry into a central ganglion.

Neurosecretory Substance

In the stages examined, S-positive neurosecretory substance was evident only in the ventrolateral regions of the suboesophageal ganglion (Fig. 37), and in storage vacuoles primarily in the peripheral cells of the corpora cardiaca. This substance was strongly PAS-positive in the ganglion and along the terminations of the nerve fibres leading from the supraoesophageal ganglion to the corpora cardiaca, but gave a weaker reaction in the storage vacuoles of the latter (Fig. 38). A PAS-positive substance was also demonstrated in cells in the dorsolateral regions of the supra- and suboesophageal ganglia, but this secretory substance was not distinctly S-positive. The components involved were diastase-fast. No secretory substance was demonstrated in the corpora allata in any of the stages seen.

Integument and Connective Tissues

During the moulting process a thin layer between the hypodermis and the developing new cuticle was strongly S-positive and very weakly PAS-positive (Figs. 14, 15). Fine, similarly stained fibrils extended from this layer into the pore canals of the developing cuticula and others extended partly into the hypodermis. The reactive components were diastase-fast. The hypodermal cells contained numerous PAS-positive granules, large diastase-labile ones basally and finer mostly diastase-fast ones distally, during synthesis of the cuticula (Figs. 12, 15). An inner layer of the old cuticle that was undergoing histolysis was S-positive and very weakly PAS-positive. The component involved was diastase-fast. Very weakly PAS-positive, diastase-fast and diastase-labile components were also present in the moulting fluid, but these were not demonstrably S-positive. In heavily sclerotized larvae, thin laminae in the procuticle were weakly PAS-positive. The procuticle gave a strong positive reaction to the DDD test for SH groups, but it was negative to this and the PFAAB tests for SS groups. Both the procuticle and exocuticle gave a positive ferric-ferricyanide reaction, the latter staining more intensely blue than the former. The positive reaction by this method may have been due to SH groups in the procuticle, but it

probably was given by phenols in the exocuticle because the latter was DDD-negative. The exocuticle and the pore canals gave a strong positive Millon's reaction for tyrosine.

An acid mucopolysaccharide, weakly positive to Steedman's alcian blue method and weakly metachromatic, occurred primarily between the laminae of the procuticle of intersegmental membranes, and especially where the membranes were folded. This reaction was similar to that of the substance along the pore canals and in the sockets of the cutaneous sensilla mentioned previously. In contrast, only the large globular inclusions (mucoid substance) in the midgut epithelium were strongly positive to Steedman's test for acid mucopolysaccharides and were distinctly γ-metachromatic.

In most of the muscle insertions a thin layer along the junction of the tonofibrillae with the myofibrillae was strongly PAS-positive and S-negative throughout the larval stadia. The tonofibrillae, which passed through the hypodermis into the cuticula, were PAS-negative and weakly S-positive in heavily sclerotized larvae, but became weakly PAS-positive and strongly S-positive during the moult. They were largely histolyzed and shed at ecdysis (Fig. 37). New tonofibrillae arose along the proximal PAS-positive band, and were themselves PAS-positive and S-negative initially. As they lengthened their reactions to the stains reversed

from the distal ends medially. Thus, one or two days after ecdysis a large portion of the tonofibrillae was S-positive and PAS-negative. The reactive components were diastase-fast. Numerous PAS-positive, mostly diastase-labile granules were in the cytoplasm of the epidermal cells around the tonofibrillae during their formation. These granules were absent in heavily sclerotized larvae. In contrast to the above insertions, the connective tissue that joins the ends of the skeletal intersegmental muscles to one another and to the cuticula was PAS-positive but SS-negative in all the preparations. The reactive component was diastase-fast.

The sequence of components involved in the synthesis of the intima of tracheae was basically similar histochemically to that of the integument. However, small PPPF-positive diastase-fast granules were evident in the cytoplasm of the tracheoblasts in newly moulted larvae. These did not appear to be the same as the diastase-fast, PAS-positive granules, because the latter were not as numerous and much smaller at this stage.

The basement membranes of many of the cellular layers, particularly the thicker ones such as that of the mid-gut epithelium (Fig. 39), were weakly PAS- and strongly SS-positive. Certain intima, such as that of the oesophagus and of tracheoles, gave similar but weaker reactions.

Discussion

In comparing the results from the PAS, PPPF, PFAAB and DDD reactions, it is evident that a strong positive reaction with the PPPF method involved primarily tissue components with potential acidic S-containing groups, rather than those with potential aldehydic groups. The latter groups may have been responsible for a weak PPPF reaction in some instances, as in the weak peripheral staining of the PAS-positive secretory granules of the trichogen cells. However, the cuticular sheaths, which are presumably synthesized at least in part from these secretory granules, contain a demonstrable quantity of S, some of which may have been carried there in the granules. The possibility could not be discounted that a small quantity of a S-containing fraction, rather than a carbohydrate fraction, was actually involved in the highly sensitive PPPF reaction in this instance.

The components of the neurosecretory substance apparently differed, with different origins, in the content of an S-containing fraction. All the secretions noted contained a PAS-positive polysaccharide fraction, but only those secretions that were PFAAB-positive were also PPPF-positive. Thus, aldehydic groups did not seem to be involved in the latter reaction with neurosecretory substances.

Similarly in the integumental structures during moulting, PAS-positive tissues were not strongly PPPF-positive, but a strong PPPF-positive reaction was given by tissues reactive to the PFAAB and DDD tests for SS groups. It is possible that the strong oxidant used in the PPPF reaction oxidized the potential aldehydic groups of tissue carbohydrates beyond the aldehydic stage, but made the S-containing fraction reactive to the staining reagent. However, the more probable explanation is that the non-polar bonds between the amino (dye) and aldehyde (tissue) groups are broken by the acid and the dye removed from the tissues during differentiation, while the amino (dye) - acidic (tissue sulphuric, sulphonic, sulphinic) salt linkages are unaffected.

With the above staining reactions it was possible to demonstrate parts of the metabolic sequence and components involved in the formation of cuticular structures in moulting wireworms. The PAS-positive, diastase-labile granules in the epidermal cells are undoubtedly glycogens. In the hypodermis they appear to build up and are stored temporarily in the larger vacuoles near the basement membranes of the cells. The finer apical granules near the developing cuticula were probably undergoing anabolism into the PAS- and S-negative neutral mucopolysaccharide, chitin, and perhaps other carbohydrate complexes. The PPPF-positive, PAS-negative granules observed by Cochrane (3) in the epidermal cells

that apparently were secreting the peritrophic membrane in larvae of Protomorphia, were not recognized in the hypodermis in the wireworm preparations, but they were evident in the tracheoblasts. Cochrane suggested that these granules may contain an intermediate metabolite in the synthesis of chitin from glycogen.

The next demonstrable metabolic stage was evident in the strongly PPPF-positive, very weakly PAS-positive material forming a thin layer between the hypodermis and the cuticula, with fine fibrils extending into the hypodermis and, on the opposite side, into the pore canals of the cuticula. An S-containing protein fraction (cystine?) apparently had been incorporated with the carbohydrate fraction in this layer, perhaps forming a muco- or glycoprotein. The latter would explain the weak PAS reaction observed here and in the laminae of the definitive cuticula, but this reaction could also be given by simpler polysaccharides enmeshed in a protein complex. The S-containing fraction apparently undergoes further metabolism in the cuticle. The procuticle is SS-negative but gives a positive reaction with the DDD and ferric ferricyanide tests for SH groups, while the exocuticle is negative to the PFAAB and DDD tests. The Millon's reaction indicated that tyrosine is incorporated in the outer layers of the cuticla towards the end of the metabolic sequence, in the tanning of the exocuticular layers.

In moulting wireworms, PPPF- and PAS-positive components were liberated during histolysis of the old cuticla in a reverse sequence to their occurrence in the synthesis of the new cuticula.

Ih the muscle insertions, the components involved in the metabolic and histolytic processes during moulting were similar histochemically to those of the cuticula, with two exceptions. An additional layer of strongly PAS-positive, diastase-fast material lacking S was present proximal to the layer with a demonstrable S-containing fraction. The tonofibrillae gave a weak Millon's reaction, indicating that little, if any, phenols were present. The connective tissue that joins the intersegmental muscles to one another and to the cuticula appeared to be even less complex than the other muscle insertions. Its synthesis apparently did not progress beyond a PAS-positive, diastase-fast stage.

The components involved in the formation of the cuticular sheaths of the sensory neurones and the cuticular coverings of sensilla were more difficult to demonstrate because of the fineness of the structures. The presence of diastase-labile and diastase-fast PAS-positive granules in the formative accessory cells during synthesis, and of an S-containing fraction in the cuticular sheaths during histolysis, suggests that the sequence of synthesis and the components involved are similar to those of the cuticula.

However, the granules in the active trichogen cells of multiple-celled sensilla differ from those of the hypodermal cells at the same stage in the moulting process, in that they are also argyrophilic (20). The basis for this difference is not known. The strong Millon's reaction indicated that the tanning of the cuticular sheaths, with the incorporation of tyrosine, was also similar to that of the exocuticula. This was particularly evident in the terminal armature of the nerve sheaths in campaniform and thick-walled hair organs. The cuticular sheaths of sensilla considered to be chemoreceptors, which are innervated by more than one neurone, gave a much weaker Millon's reaction.

Trim (18) recovered a carbohydrate from the proteinaceous fraction soluble in warm alkali in the larval cuticula of Sphinx. His findings suggested that it was present either directly combined with the protein or in the form of a polysaccharide, and that the S-containing component is more closely associated with the carbohydrate-rich proteinaceous fraction than with any other. In the wireworm, the histochemical results also suggest that the S-containing component is incorporated with a complex carbohydrate component soon after the latter is synthesized from glycogen. However, it is difficult to reconcile the positive histochemical tests for SS and SH groups obtained in wireworm cuticula with Trim's findings that, although the water-soluble protein fraction

of the larval cuticula of Sphinx contains organic S, this is not in the form of ethereal sulfate, cysteine, cystine, sulphydryl or methionine. Dennel (5) and de Haas et al. (4) also reported no S-containing amino acids in the larval cuticula and the puparium of Calliphora, and in the cuticles of mormon crickets, respectively. However, McFarlane (12) found cystine or cysteine present in the maternal, serosal and embryonic cuticles of the house cricket, and Johnson et al. (10) estimated a content of 4.6% cystine in protein from exuviae of the mormon cricket. It is of interest that lack of cystine in the diet interfered with the moulting process in Aedes, Blatella and Lucilia (7).

The component of the sensory axons from the cutaneous sensilla, which gave a strong positive reaction with the S-specific reagents, may be identical to the component that reacted similarly in the thin layer between the hypodermis and the developing cuticula. Part of the reactive component may be in the subcuticular sheath of the axon, which is shed with the cuticula at each moult. The solid staining of the axon and the appearance of the axonal nodes near the base of the cytons indicate that this component is also partly or possibly entirely in the axoplasm. The comparative histochemical results suggest that the reactive component may be a mucoprotein, consisting of a complex PAS-negative carbohydrate probably allied to chitin, and a

protein that contains cystine or cysteine.

The basis for the differential staining of the axons of the cutaneous sensilla from those of the ocelli and of the efferent system may be a product of their origin in the ontogenetic sequence. The precursors of the cutaneous sensilla are hypodermal cells, some of which are differentiated late in embryonic development (9), and others are differentiated to form additional sensilla during postembryonic development (19). Though more specialized in function, the cells of the cutaneous sensilla probably still retain to a certain extent the secretory processes of their parent hypodermal cells. The precursors of the ocelli and of the efferent system are generally differentiated from the primary ectodermal layers much earlier in the development of the embryo (9), perhaps at the same time as are the precursors of the hypodermis. They presumably do not possess or have not elaborated the metabolic processes of hypodermal cells for secreting cuticular material. Thus, the lack of staining of the ocellar and motor axons with the methods that strongly stain the axons of the cutaneous sensilla apparently reflects an absence of an intermediate metabolite in the synthesis of cuticle.

If the above characteristics of the various axons of wireworms are also valid for other insects, in larval and adult stages, the S-specific stains should prove valuable for differentiating histologically most of the afferent from

the efferent nerves in the peripheral and stomodaeal systems. Of the methods used in this study, the PPPF reaction appears to be the most suitable for this purpose.

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Fig. 1. Longitudinal section of antenna of a heavily sclerotized larva, distorted slightly during sectioning. The axons in the sensory nerve from the third segment (upper nerve) and those from the sensory appendix are darkly stained, while the cytons are unstained. PPAB.

Fig. 2. The same very early in the moulting process. The junction bodies (arrow) and cuticular sheaths are weakly S-positive. PPPF.

Fig. 3. The same after the hypodermis has separated from the cuticula during moulting. The cuticular sheaths (lower arrow) and a layer of the old cuticle undergoing histolysis (upper arrow) are S-positive. PFAAB.

Fig. 4. The strongly S-positive cuticular sheath of a campaniform organ on the ligula early in the moulting process. PPPF.

Fig. 5. Subcuticular sheaths of cytons in the cell bundle of the antennal sensory appendix. PPPF.

Fig. 6. Subcuticular sheaths (?) (right arrow) and axons of sensilla (left arrow) on labial palp about midway in the moulting process. PPPF.

Fig. 7. Globules of material from disintegrated subcuticular sheaths of cytons of the antennal sensory appendix in a newly moulted larva infected with an entomophagous fungus. PPPF.

Fig. 8. Cell bundles of sensilla on the maxillary palp of a newly moulted larva, showing fine S-positive axons, and vacuoles presumably containing secretory material in the cytoplasm of the trichogen cells. PPCH.

Fig. 9. Diastase-fast granules (arrow) in the trichogen cells of sensilla on labial palp of a newly moulted larva. PAS after diastase digestion.

Fig. 10. Diastase-fast granules in abapical poles of cytons or in neurilemma cells of the antennal sensory appendix in a newly moulted larva. PAS with counterstains after diastase.

Fig. 11. Weakly S-positive (?) cytoplasm of trichogen and tormogen cells of a developing thick-walled hair organ, and the unstained cyton (with nucleus), about mid-way in the moulting process. Note the strongly S-positive axons in the nerve at lower left. PPPF.

Fig. 12. Cells of two thick-walled hair organs late in the moulting process. The cytoplasm of the sense cells contains granules (lower left arrow) but that of the neurilemma cell (lower right arrow) does not. Note the large basal and small distal granules in the surrounding hypodermal cells, and the homogeneous positive band of cytoplasm near the socket (upper arrow). PAS.

Fig. 13. Section of the same, showing granules in the cyton. PPPF.

Magnifications: Figs. 2, 3, 6, and 8 - 11 same as Fig. 1;
Figs. 5, 7, 12 and 13 same as Fig. 4.

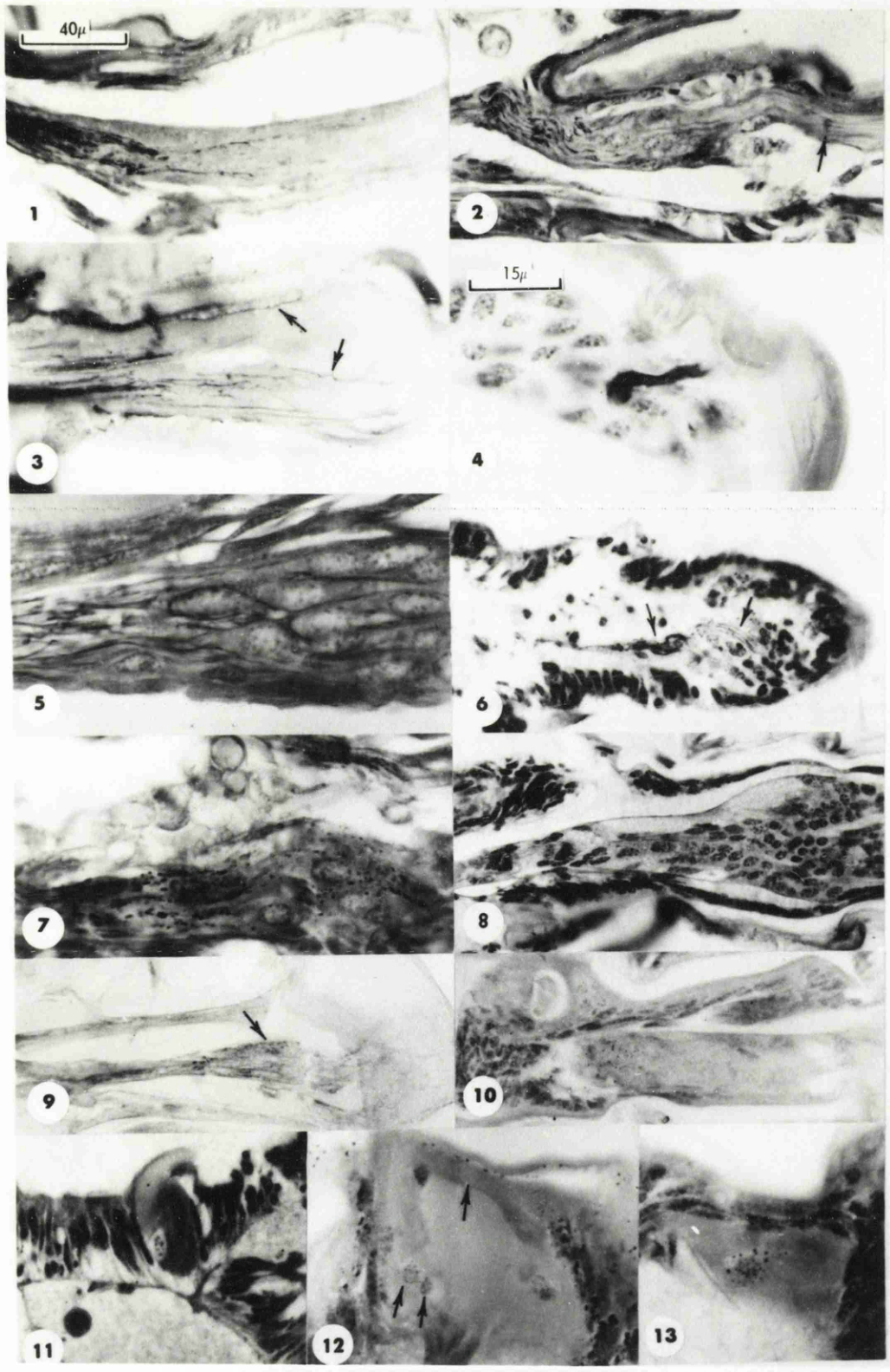
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Fig. 14. Partial artifacts (?) in trichogen cell of an integumental campaniform organ (right arrow). Note the S-positive layer between the hypodermis and the developing cuticula (left arrow). Newly moulted. PPAB.

Fig. 15. Section of same individual as figure 14, with positive globule of material in trichogen cell of a campaniform organ (arrow). Note strongly positive glycogen granules in hypodermis and weakly positive pore canals and layer between hypodermis and developing cuticula. PAS.

Fig. 16. Cell bundle of antennal sensory appendix. Longitudinal section; heavily sclerotized larva. PPPF.

Fig. 17. S-positive nodes of axons near base of cytons of sensilla in maxillary palp. Heavily sclerotized larva; longitudinal section. PPPF.

Fig. 18. Loops of axons in vicinity of neurilemma cell and cyton of integumental campaniform and thick-walled hair organs mid-way in the moulting process. Antennal nerve with S-positive axons to the right. PPPF.

Fig. 19. Bundles of axons in nerves from campaniform and thick-walled hair organs of the integument. Newly moulted. PPPF.

Fig. 20. Solid-stained sensory axons in the antennal (upper) and mandibular nerves. Newly moulted; transverse section. PPAB.

Fig. 21. Section of the same. Note the large, unstained motor axons scattered among the S-positive sensory axons in the dorsal parts of the nerves. DDD.

Fig. 22. Section of an antennal nerve, with the positive-staining neural lamella and occasional granules scattered among the axons. Newly moulted. PAS.

Fig. 23. Oblique section of the antennal (upper) and labral nerve trunks late in the moulting process. Arrow indicates a tight coil in a sensory axon. PPPF.

Fig. 24. Longitudinal section of the anterior part of the brain, showing stained sensory axons in the labral nerve (lower) and unstained contents of the suboesophageal connective late in the moulting process. PPPF.

Fig. 25. Same part of a section adjacent to that of figure 24.

Fig. 26. The fourth serial section from that of figure 24, showing the dichotomous branching of sensory axons of the antennal nerve within the brain.

Magnifications: Figs. 16, 18 - 20, 24 and 25 same as Fig. 15; Figs. 21 - 23 same as Fig. 17.

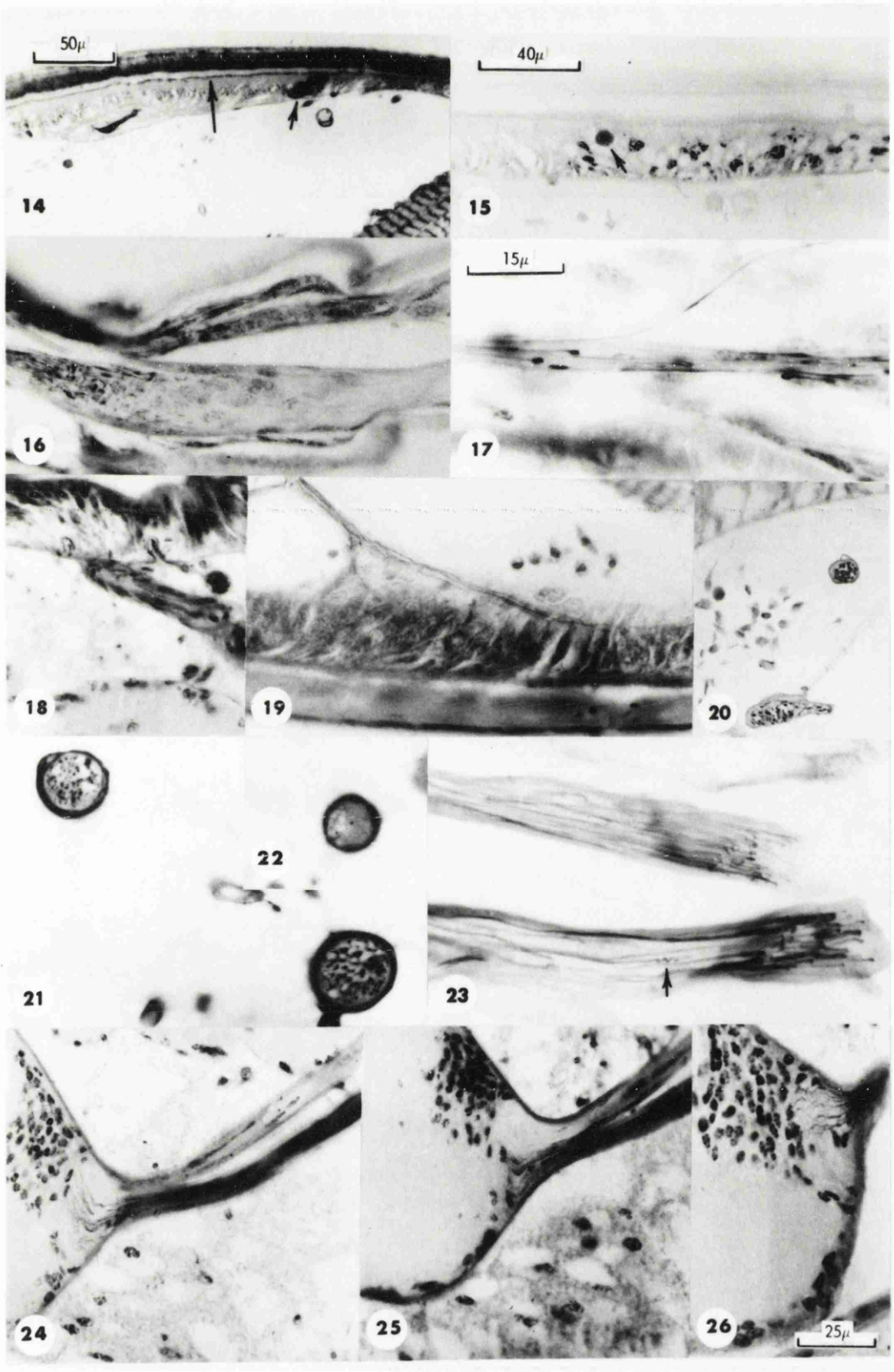
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Fig. 27. Oblique section of maxillary nerve through the group of four accessory neurones. One neurone is evident in the section. Also shown are globules of an S-positive substance (upper arrow) and the tubular tendon enclosing an unstained distal process of the maxillary stretch receptor (lower arrow) Newly moulted. PPPF.

Fig. 28. Longitudinal section of the mandibular ganglion 2 days after a moult. PPPF.

Fig. 29. Transverse section of the antennal (upper arrow) and ocellar nerves 2 days after a moult. PPCH.

Fig. 30. The same of the antennal nerve in section next to that of figure 24, near the junction of a motor nerve with the main trunk. Note the large, unstained motor fibres, the small, stained sensory fibres, and 2 neurilemma nuclei.

Fig. 31. The ocellar nerve of figure 24 enlarged. Note the unstained axons and the neurilemma nucleus.

Fig. 32. Transverse section of the ocellar nerve with unstained axons (left), and the closely associated nerve from cutaneous sensilla around the ocellus with darkly stained axons. Newly moulted. PPPF.

Fig. 33. The same of a mandibular nerve, with large, unstained motor axons dorsally, and small, darkly stained sensory axons ventrally, some of which are also interspersed among the motor axons.

Fig. 34. Terminal portion of the tendon and distal process of the maxillary stretch receptor, which terminates among the tonofibrillae of a muscle insertion (left arrows). The larger tubular tendon (right arrow) connects this muscle insertion to the base of the hypopharyngeal rod. Two days after a moult. PPPF.

Fig. 35. Portion of labial nerve containing stained sensory axons, with 2 motor nerve branches to muscle fibres (arrows) in which axons are unstained. Longitudinal section; 10 days after a moult. PPPF.

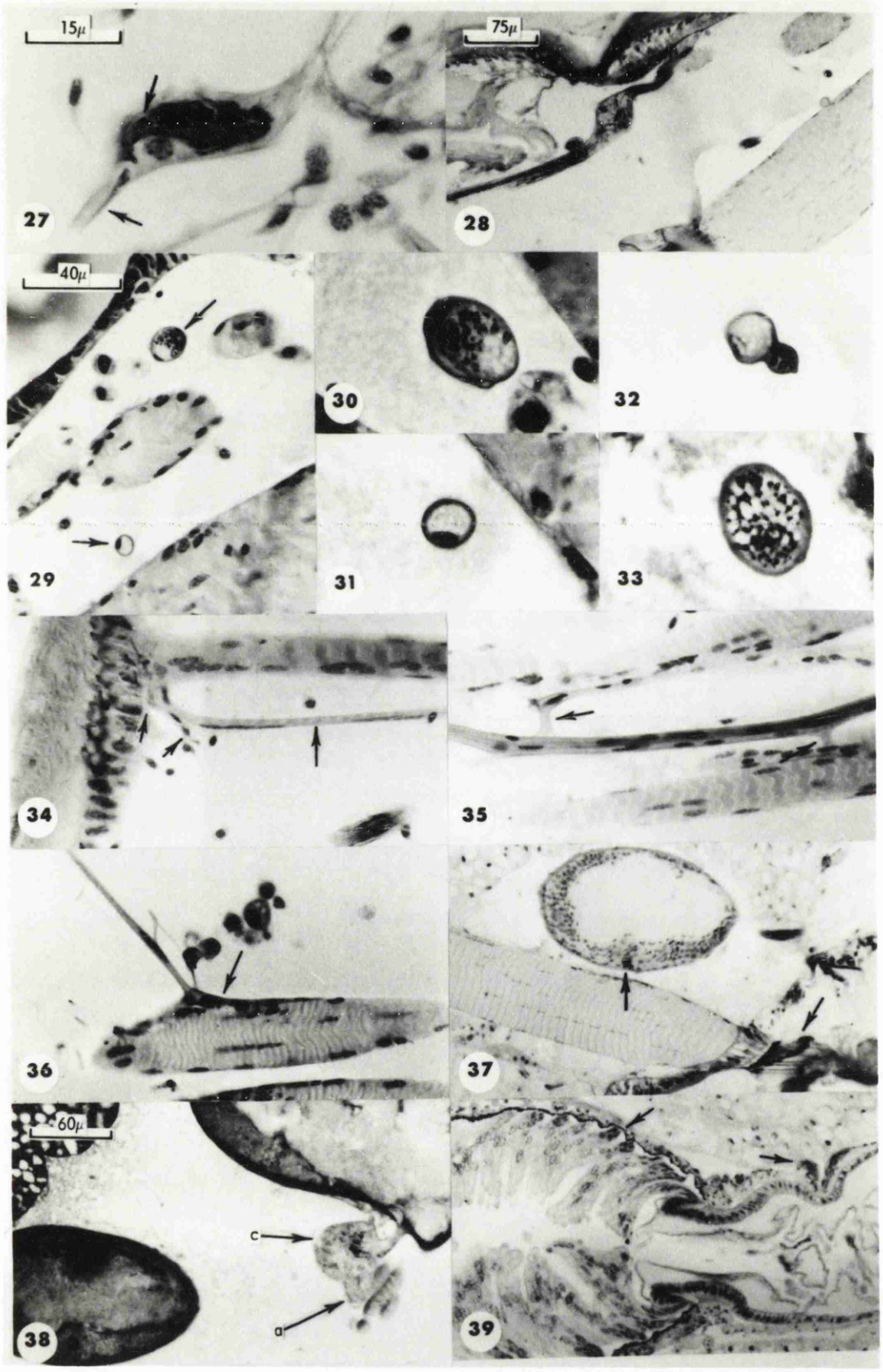
Fig. 36. Motor nerve branch to a muscle fibre and a neuromuscular junction (arrow) containing Schwann cells but no apparent S-positive material. Longitudinal section; 2 days after a moult. PPCH.

Fig. 37. Neurosecretory material in ventrolateral region of brain (top arrow) and S-positive old tonofibrillae undergoing histolysis (bottom arrow). The new tonofibrillae are still largely S-negative at this stage. Longitudinal section; mid-way in the moulting process. PPPF.

Fig. 38. Neurosecretory substance in ventrolateral and dorsolateral cells of the suboesophageal (lower) ganglion and in the corpus cardiacum (c) but none in the corpus allatum (a). The neural lamella and numerous fine granules in the perineurium are also positive. Longitudinal section; newly moulted larva. PAS.

Fig. 39. S-positive sensory axons in the recurrent nerve (right arrow) and basement membrane of midgut epithelium. Longitudinal section; newly moulted larva. PPPF.

Magnifications: Figs. 30 - 33 same as Fig. 27; Figs. 37 and 39 same as Fig. 28; Figs. 34 - 36 same as Fig. 29.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Summary of New Findings

1. The number and pattern of certain thick-walled hairs in the post-gular region of the neck and on the antennae and labial palpi are useful characters in the major classification of larval Elateridae.

2. Three varieties of thick-walled hairs and four varieties of campaniform organs are differentiated on the basis of structure, distribution, and probable differences in function. The pore canal organ is a new type of sensilla not previously described from insects. The complex structure of the antennal sensory appendix indicates that it also is not closely related to any type of sensilla in current classifications.

3. The various types of sensilla may be innervated by single or groups of individual neurones, or by units of two or four neurones, but the three regions of their distal nerve processes are homologous among the sensilla.

4. Individual neurones or units of neurones shed sheaths at each moult. The portion from the distal process is a heavy cuticular sheath, while that from the cyton and axon is a delicate subcuticular sheath. A junction body connects the two near the base of the distal process. The exuvial sheaths are basically homologous among the various types of sensilla.

5. Wireworms possess a peripheral mandibular association centre (ganglion) not previously described from insects.

There is also a complex of interconnections among the main peripheral nerves and between these and the stomodaeal nervous system.

6. The sensory axons from the cutaneous sensilla and some of those in the stomodaeal nervous system are strongly positive to histochemical tests for cystine (eine). The axons of the efferent system and those from the ocelli lack this characteristic.

7. There is a similarity in the histochemical characteristics of the metabolites involved in the synthesis of parts of the sensilla and their neurones, and those involved in the synthesis of the cuticula. The synthesis of some of the former does not include some of the later stages involved in the synthesis of the latter.

8. Paraldehyde fuchsin used after oxidation of tissues with potassium permanganate serves to differentiate histologically certain sensory nerve fibres from other sensory fibres and from those of the efferent system.