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Histology of the Neurosecretory System and Neurohaemal Organs of the Spider, *Argiope aurantia* (Lucas)

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ABSTRACT Histological studies of the neurosecretory system during the post-embryonic development of a spider, *Argiope aurantia*, were made at the light-microscopic level.

Neurosecretory cells which are found in all stages are classified into type I and type II cells. The type I cells are present in the aboral region of the brain and in pedipalpal, ambulatory and abdominal ganglia of the subesophageal mass. The type II cells which appear from the seventh stage are confined to the cheliceral ganglia. Three stages of secretory activity (poor, medium and full) based on stainability are described in these cells.

In both types clear axonal transportation of neurosecretory material is observed. The discrete tracts and commissures formed by these neurosecretory axons are described in the brain and subesophageal ganglion. The complexity of some of these pathways is comparable to that of the ordinary neurons.

One pair of nerves from the brain and four pairs of nerves from the subesophageal mass enter a neurohaemal organ, the Tropfenkomplex. This is a paired structure, situated dorsally, on either side of the subesophageal mass. The neurosecretory axons branch extensively within the organ and on their course they form sacs or pools filled with secretory material.

The Tropfenkomplex is enveloped by a thin neural sheath which runs deep into the organ dividing it into a series of lobes. Glial cells are distributed within the organ. As in the neurosecretory cells, changes in stainability of secretory material were also observed in the Tropfenkomplex.

During intermolt periods two peaks of stainability have been noticed. The first peak lasts for 24 hours after the molt, and this is followed by a low activity period between second and fifth day. From the sixth to the tenth day after the molt the second peak commences. It is suggested that the second peak may be responsible for bringing about molting.

The cheliceral group appears (seventh stage) at a time when external indication of reproductive characters are visible. In the ninth stage, by the tenth day after the last molt, several of the type I and type II cells contain much secretion. This is followed by maturation of gonads and oviposition. Thus both type I and type II cells are believed to be involved in the reproduction of the animal.

Our knowledge of neurosecretory systems in spiders is due to the work of Gabe ('54, '55); Legendre ('54, '56a,b, '59, '64–'66); Kühne ('59); Streble ('66) and Eckert ('67). These histological studies were mostly descriptions of neurosecretory cells in the brain and subesophageal ganglion. In the brain the neurosecretory pathways were incompletely described and practically nothing is known about them in the large subesophageal mass. Moreover conflicting views were expressed regarding the functional significance of the neurosecretory cells. It therefore seemed opportune to study in detail the cytomorphology of the neurosecretory cells, the axonal

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pathways, the terminal depots for neurosecretory material and the probable functions of the neurosecretory system in the spider at various post natal stages.

MATERIALS AND METHODS

Female orbweb spiders, *Argiope aurantia* Lucas (Family: Araneidae, see Levi, '68) were collected at Raleigh, N. C. The life span of this spider ranges from eight to eighteen months (Reed et al., '69) which is advantageous for this kind of a study. Cocoons were collected in the field in the months of December and January. In February and March the spiderlings were released into cages by cutting open the cocoons. Each animal from the second stage ("stage" refers to an intermolt period; i.e., seventh stage after sixth molt) was kept separately in a glass jar and data sheets of the sex, age and time of molting for each animal were maintained. The younger ones, up to the fifth or sixth stage were fed on gnats and the older ones on houseflies. The laboratory provided a cycle of short (eight hours) cool (18°C) nights and long (16 hours) warm (24–28°C) days throughout for the animals.

The first stage was brief (approximately two days) and the young ones underwent their first molt within the cocoon. The total number of molts for females varied from seven to nine, the majority of them reaching adulthood in five to six months which is a normal period even for animals in the field. No significant differences between laboratory reared and field animals were noticed. The majority of animals became gravid and laid cocoons after the eighth molt. Females died normally about four to six weeks after laying the last of one to three cocoons.

The entire cephalothorax was fixed in Zenker's, Holly's or Bouin's, after the chelicerae, the legs and the abdomen had been cut off. A total of 240 animals of all age groups were used in this study. Twenty to thirty animals were fixed for each stage. During each intermolt period, animals were fixed at regular intervals starting immediately after the molt to before the time of the next molt.

Granular Histowax of M.P. 56–58°C was used throughout. Sections of the entire cephalothorax were cut at 8–10 μ in the three cardinal planes, very good paraffin ribbons, even of the cuticle, were obtained. Paraldehyde fuchsin-orange-G and chrome haematoxylin-phloxine were extensively used along with general stains like Heidenhain's haematoxylin, Azan and Mallory's phosphotungstic haematoxylin. Performic acid/Alcian blue, Feulgen, PAS with and without diastase and Gallocyanin were used for histochemical tests. A camera-lucida was used for making gross sketches. The finer details of the axonal pathways were filled into these sketches by directly incorporating observations from the slides. Making use of the horizontal, sagittal and transverse serial sections, neurosecretory pathways of the brain and subesophageal ganglion were reconstructed.

RESULTS

Neurosecretory cells

In the present investigation the serial sections stained in paraldehyde fuchsin-orange-G (PF) method gave very good results. Azan, Mallory's phosphotungstic haematoxylin methods also demonstrated the occurrence of neurosecretory cells. But the results with chrome haematoxylin were poor.

Even though four types of secretory cells were observed in the cephalothoracic nerve mass, histochemical and other tests show that only three of these are true nerve cells. The fourth one may be a glial cell type. Of the three secretory nerve cells, only two were identified as true neurosecretory cells. The third type may not be neurosecretory in the strictest sense, and this will be explained later. The neurosecretory cell possesses a nucleolus, prominent axon hillock and an axon often filled with neurosecretory granules. On the basis of size, cytomorphology and nature of the neurosecretory material, these cells are called Type I and Type II. Legendre ('56a,b, '59) named these two groups Type A cells.

In the life span of the spider *Argiope aurantia* relatively more groups of neurosecretory cells occur as the animals reach subadult stages. In the present study, only in the brief first stage were no cells observed. In the second stage, the cells are present in the aboral region of the brain and in the four pairs of leg ganglia. In the

later stages, besides the above mentioned regions, cells are also present in the ganglionic region (see fig. 1). Clear indication of the presence of these cells in the pedipalpal ganglia becomes evident from the fourth stage. In the cheliceral ganglionic region, the neurosecretory cells make their apppearance after the sixth molt or in the seventh stage. In general, the occurrence of neurosecretory cells in aboral, first leg, second leg, cheliceral and abdominal ganglionic regions, is more consistent and the stainability varies systematically not only in different age groups but also during intermolt periods. In the pedipalps, third leg and fourth leg, ganglionic regions, the stainability of the cells varied greatly and hence no functional significance could be attributed to their activity. All the cells of the cheliceral ganglia, first and second leg ganglia and several cells of the aboral and abdominal ganglia, can be identified easily because of their position, closeness to certain blood vessels and bilateral distribution (see fig. 3). During peak periods of stainability, the total number of active cells in different ganglia of subadult and adult stages remains nearly constant. The maximum number of cells found in different ganglia of subadults and adults is: Aboral 16; cheliceral six; pedipalpal six; first to fourth leg eight; abdominal 25. An oral group of neurosecretory cells was not observed.

Type I cells

The type I cells (Plate 1, A–D) are present in the protocerebral and in all subesophageal ganglia.

These cells show a continuous growth from 2nd to the post cocoon stages. In the second stage, the average cell diameter is 6 μ with a nucleus of 4 μ. In the post cocoon stage, the cells increase to 12 μ with a nucleus measuring 6.8 μ across. Thus, the nuclear-cytoplasmic ratio of 0.66 in the second stage is reduced to 0.56 in the adults. The gradual increase of the cytoplasmic area of the cells leads to a corresponding increase of the amount of neurosecretory substance synthesized in the cell. Hence in the younger animals the neurosecretory substance forms only a thin ring around the nucleus. As the animal grows older and as the cytoplasmic area of the cell increases, there is a corresponding increase in the amount of the neurosecretory material. In adult animals, the neuro-

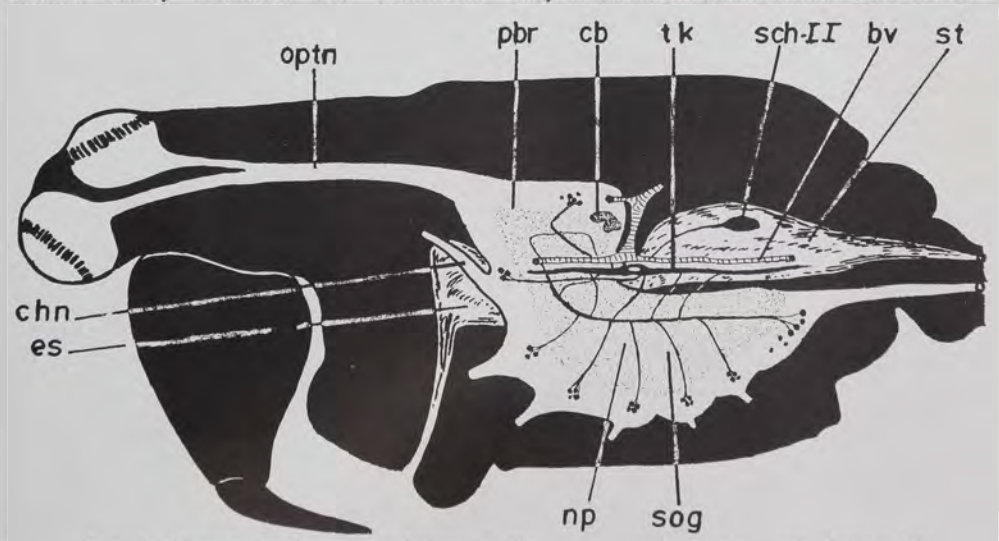

Fig. 1 Diagram to show interrelations of the neurosecretory system with the cephalothoracic nerve mass and the retrocerebral endocrine organs in *A. aurantia*. bv, blood vessel; cb, central body; chn, cheliceral nerve; es, esophagus; np, neuropile; optn, optic nerve; pbr, protocerebrum; Sch II, Schneider organ II; st, stomach; sog, subesophageal ganglion; tk, Tropfenkomplex.

secretory substance fills up the entire cytoplasm of the cell.

The contour of the cell is greatly dependent upon the quantity of the secretory product present in the neurosecretory cell. The stainability, as an indication of the activity of the cells in different age groups, can be divided into three important stages. In the least active state (poor), the cell is circular or oval and has a smooth outer contour. The secretory material which forms a ring around the nucleus is mostly in the form of fine granules with a few clumps here and there (Plate 1, A). During this stage it is difficult to see any secretory material beyond the axon hillock. In the second stage of the cells, the amount of granular material and the number of clumps increase and fill up the entire cytoplasm, including the axon hillock. At × 1000 magnification it is possible to observe the material beyond the axon hillock of the cell. The neurosecretory cells develop irregular outer contours which might be due to the increased quantities of neurosecretory material within the cytoplasm (Plate 1, B). During the maximal periods of secretion (full), both the granular and clumped secretory material fill the entire cytoplasm (Plate 1, C, D). The irregular contour of the cell increases. The secretory material is prominently seen to the axon hillock and also in the axons. During this stage, within the proximal part of the axon, the neurosecretory material is evident because of the small pools of the secretory material formed at irregular intervals, giving a beaded appearance to the axon. In the fibrous central mass, along with the smaller pools, here and there larger pools of accumulated secretory material were also found (Plate 2, A). As the axons leave the ganglion more of the larger pools are formed. These reach their maximum size and number in the Tropfenkomplex.

Type II cells

The type II neurosecretory cells (Plate 1, E) are larger and fewer (3 pairs) and confined to the cheliceral ganglia. In the seventh stage, at which time they begin to secrete, the cells measure 16 μ with a nucleus 8 μ across. In the adult stage these cells measure 30 μ and possess a nucleus 12 μ across. Thus, as in type I cells, the nuclear-cytoplasmic ratio decreases, in this case from 0.5 in the seventh stage to 0.4 in the adults. During the maximal period of stainability, there is only a slight change in the contour of the cell. The secretory material stains lighter with PF even during maximal production and is in the form of small bodies distributed evenly within the cytoplasm. When there is maximum secretion, vacuoles of different sizes are readily observed in the perikaryon. The secretory granules adhere or form a ring on the margin of the vacuoles. The droplets can be traced over long distances within the prominent axons. Unlike in the type I cells, even during low periods of activity, neurosecretory material even in axons is readily observable. Small granules of the secretory substance can be seen prominently close to the axon hillock. The swellings or pools of secretion along the course of the axons are almost absent. The gradual increase of the axon diameter distally, found in type I cells, is not observed here.

The secretory product of both type I and type II cells is acidophilic. Preliminary oxidation of the sections with $KMnO_4$ gives the product a very definite basophilia. Histochemical tests show that this might be a proteinaceous material since it stains intensively with performic acid/alcian blue. Sections treated with PAS gave negative results indicating that the product may not be a polysaccharide. The neurosecretory product also lacks affinity for gallocyanin and therefore contains no ribonucleic acids.

The third type of secretory nerve cells mentioned earlier are the largest cells in the central nervous system of the spider. These occur in the cheliceral and subesophageal ganglia. In adults, the cell diameter varies from 30–50 μ with a nucleus of 10–14 μ. There are three nucleoli, a prominent axon hillock and an easily observable axon that can be traced over long distances into the neuropile and in several cases right into the peripheral nerves. These are the typical unipolar motor neurons found in all invertebrates. The secretory material in these cells appears from the seventh stage and is present in increasing quantities as the animal grows older. These motor

neurons show densely stained areas of rolled membranes within the perikaryon (Plate 1, F). The number of such lobed areas varies from one to several, located at different places in the cytoplasm. From the lobed areas, granular secretory material was seen released into the cytoplasm, which reaches a maximum in gravid females. In gravid females the secretory material can be seen not only in the cytoplasm but also in the axon hillock and axons.

These granules do not stain with performic acid/alcian blue, but they give an intensive PAS positive reaction. When treated with diastase prior to the PAS reaction, the stainable material disappears completely. This indicates that the granular material stained with PF is most likely glycogen. Such chemical compounds are widely distributed in the animal cell and thus cannot afford a histochemical definition of a neurosecretory product (Gabe, '66).

The fourth type are clearly glial cells as pointed out by Kühne ('59). Legendre ('56a,b) claimed these to be neurosecretory and called them type B cells. In the present investigation it was found that these cells have no clear nulceoli, that the nucleus is smaller than in nerve cells, and that there are no axons. A number of processes radiate from the periphery of the cells, which occur at the edge of the neuropile where glial cells are found normally.

Neurosecretory pathways

The characteristics of neurosecretory neurons and their staining affinities to PF made it possible to trace the neurosecretory pathways. The axons which are loaded with secretion, characteristically stain darker with PF. This has made possible the tracing of the course and extent of the neurosecretory axons. The majority of these axons travel in bundles both in the brain and subesophageal ganglion, and this makes the task of tracing much easier. During the peak period of stainability, the swellings and pools of secretory material found along the course of neurosecretory axons were of additional help. The neurosecretory tracts described below were reconstructed from serial sections cut in the three planes. The results were pooled by observing serial sections from second to post cocoon stages.

The paths followed by the neurons are diverse. There are neurosecretory axons that have a simple and straight course from the soma to the neurohaemal organ. But there are others whose pathways are highly complex. These run through several layers of neuropile and ganglionic regions ipsilaterally or contralaterally and even descend or ascend, before they finally terminate in the neurohaemal organ.

Protocerebral neurosecretory pathways

The neurosecretory paths in the protocerebrum present a picture of confusion. This is primarily because of the single fibres which run at random within the neuropile. Hence no attempts were made to follow the course of these individual fibres. However, there are some well defined tracts and the following description applies to them.

The descriptions of protocerebral neurosecretory tracts by Gabe ('55), Legendre ('59) and Streble ('66) were incomplete. Hence a detailed account of these tracts is presented below. Neurosecretory axons from each half of the aboral group of cells, form a major tract called the protocerebral neurosecretory tract (figs. 2, 3, pnt). The tract from its dorsal position moves anteriorly and downwards into the neuropile. In the middle region of the protocerebrum there is crossing over of several fibres between the left and right tracts. After decussation, which is here recorded for the first time, the protocerebral neurosecretory tract moves further downwards in an aboral direction. This entire tract with six to ten secretion-filled axons can be seen clearly. Under high power and oil immersion, the axons show swellings and occasionally small pools of secretory material. On either side of the esophagus, the protocerebral neurosecretory tract joins with two subesophageal and one pedipalpal neurosecretory tract. These tracts together (10–25 fibres) move up along the posterior margin of the brain to emerge out at the junctional region of the trito- and protocerebrum, as the principal nerve (figs. 2–4, pn) which enters the Tropfenkomplex near the first organ of Schneider.

Another major neurosecretory tract in

the brain whose origin is mainly from neurosecretory cells of the subesophageal ganglion is the subesophageo-protocerebral tract (figs. 2, 3; spt). The subesophageal neurosecretory tract in the pedipalpal ganglionic region below the esophagus divides into the subesophageo-protocerebral and esophageal tracts. The subesophageo-protocerebral tract from its central position moves orally and dorsally up into the protocerebral region (fig. 3; spt). In front of the central body, the tract moves downwards and joins the protocerebral neurosecretory tract as the posterior region of the esophagus.

Cheliceral neurosecretory tracts

As described by Gabe ('55), Legendre ('59) and Streble ('66) the neurosecretory cells in the tritocerebrum form a single tract on either side of the esophagus. Each bundle of fibres runs aborally along the esophagus and joins the pharyngeal nerve posteriorly. The nerve along with the secretory fibers ends in the II organ of Schneider (figs. 1–3). The secretory product in these fibres is granular and the beaded appearance or pools were not found here.

Neurosecretory tracts in the subesophageal ganglion

The earlier workers (Gabe, '55; Legendre, '59; Kühne, '59; Streble, '66) were content with description and distribution of the neurosecretory cells in the subesophageal ganglion. Until now, the organization of neurosecretory pathways and their eventual termination in a neurohaemal organ have been unknown. A nearly complete description of the organization of the neurosecretory pathways is presented below.

The neurosecretory axons from each of the ganglionic regions form discrete bundles and well-organized tracts and commissures that can be followed in PF-stained serial sections (fig. 1). In each half of the subesophageal ganglion the single largest tract formed by neurosecretory fibres from different ganglia is the longitudinal subesophageal neurosecretory tract (figs. 2, 3; snt). The position of this tract corresponds to the central tract of *Poecilotheria* (Babu, '65). The subesophageal neurosecretory tract increases in size gradually from posterior towards the anterior end of the ganglion. In the pedipalpal region, close to the esophagus it bifurcates into the subesophageo-protocerebral and the esophageal tract (figs. 2, 3; spt, ot). The path of the subesophageo-protocerebral tract was described earlier. The esophageal tract, on the other hand, runs aborally along with the esophagus within the pedipalpal ganglia and finally joins the principal nerve on the same side.

The subesophageal neurosecretory tract is formed from axons contributed by neurosecretory cells in the abdominal, ambulatory (4 pairs) and pedipalpal ganglionic regions. Fibres enter the tract ipsilaterally and even contralaterally from all the ganglia. The majority of the axons are of ascending rather than descending type.

Neurosecretory tracts of the first leg ganglion

The distinctive cell groups in the pedipalps, legs and abdominal ganglia form identical tracts and commissures before joining the subesophageal neurosecretory tract or the neurohaemal organ (fig. 2). Since these tracts and commissures are more pronounced in the first and second leg ganglionic region, the present description applies mostly to one of these regions. The three to four compactly placed neurosecretory cells in each half of the ganglion can be identified easily because of their distinctive staining reactions. It is normally difficult to find the proximal parts of these axons, but during maximal periods of secretion the beaded appearance makes it possible to trace the axons from the cell body right into the neuropile. The axons

Fig. 2 Neurosecretory cells and pathways in the brain and subesophageal ganglion of *A. aurantia*. The neurohaemal organs, with their interconnections, are also shown in the diagram. abnc, aboral neurosecretory cells; abdnc, abdominal neurosecretory cells; chnc, cheliceral neurosecretory cells; chnt, cheliceral neurosecretory tract; ot, esophageal tract; pdnc, pedipalpal neurosecretory cells; pn, principal nerve; pnt, protocerebral neurosecretory tract; Sch. I; first organ of Schneider, Sch. II, second organ of Schneider; snt, subesophageal neurosecretory tract; spt, subesophageo-protocerebral tract; tk, Tropfenkomplex; 4amc, neurosecretory commissure of the fourth leg ganglion; 1–4 amn, ambulatory neurosecretory nerves corresponding to four leg ganglia; 1–4 amnc, neurosecretory cell groups corresponding to four leg ganglia.

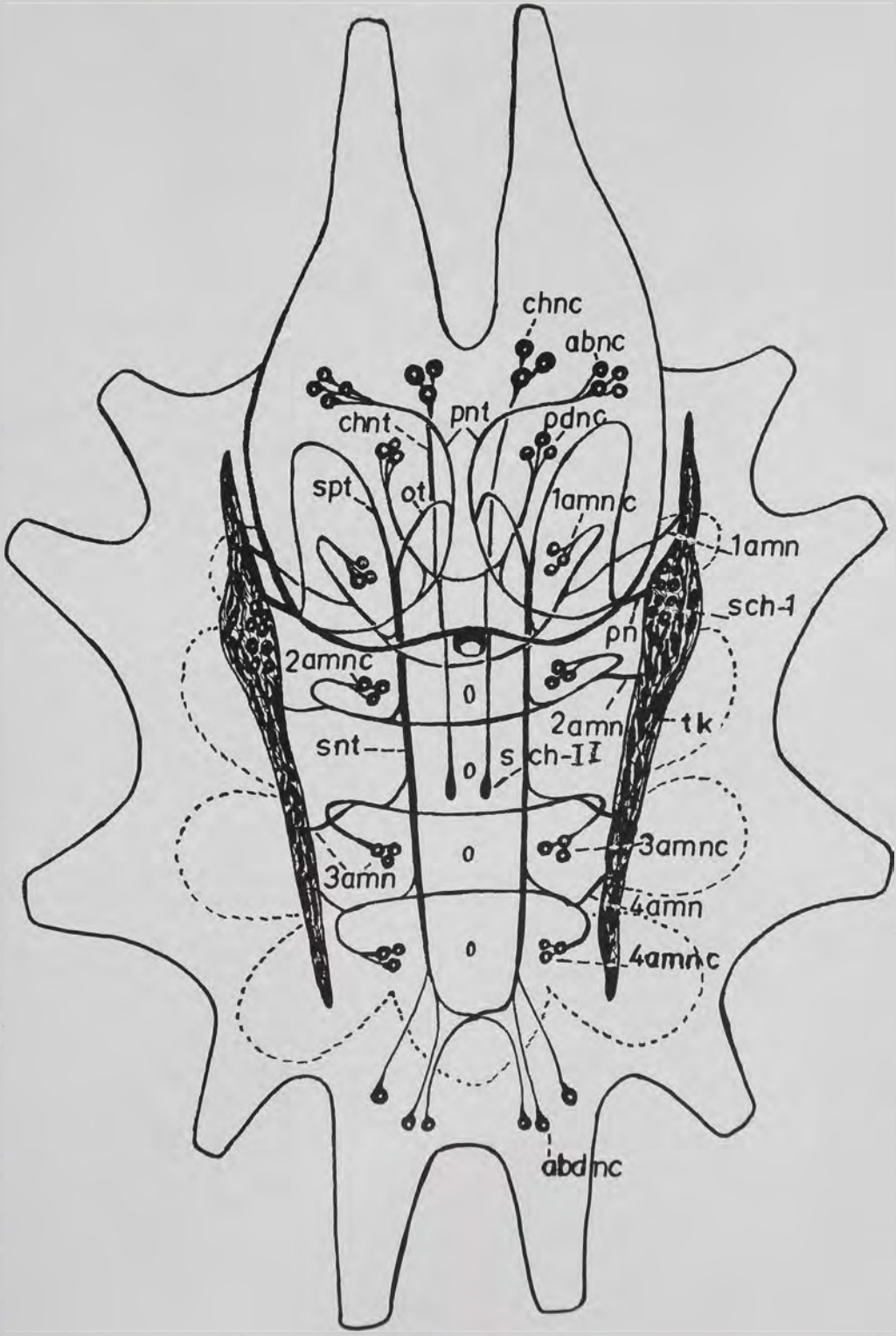
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Figure 2

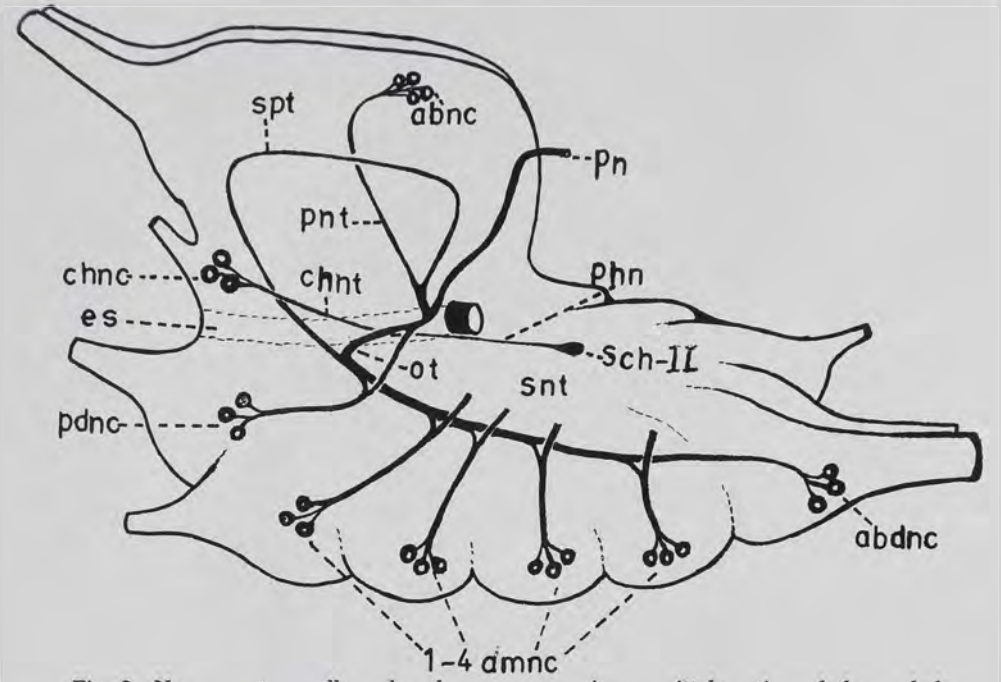

Fig. 3 Neurosecretory cells and pathways as seen in a sagittal section of the cephalothoracic nerve mass of *A. aurantia*. The Tropfenkomplex is not represented. abnc, aboral neurosecretory cells; abdnc, abdominal neurosecretory cells; chnc, cheliceral neurosecretory cells; chnt, cheliceral neurosecretory tract; es, esophagus; ot, esophageal tract; pdnc, pedipalpal neurosecretory cells; phn, pharyngeal nerve; pn, principal nerve; pnt, protocerebral neurosecretory tract; Sch II, second organ of Schneider; snt, subesophageal neurosecretory tract; spt, subesophageo-protocerebral tract; 1–4 amnc, neurosecretory cell groups of four leg ganglia.

from the ventral side of each half of the ganglion move up into the neuropile and join with the fibres coming from the cells on the other side. Thus, one semicircular commissure is formed in the fibrous core from neurosecretory cells of leg, pedipalpal and abdominal ganglia. But the position and extent of the commissure for each ganglion varies as shown in figure 2 because of the fusion of several ganglia into a single large mass.

From the commissure, laterally some fibers enter the subesophageal neurosecretory tract (figs. 2, 3). A group of three to six fibres leave the commissure at its dorsolateral region on either side and these move out peripherally through the fibrous mass. The fibres emerge out of the subesophageal mass at its dorsolateral region as ambulatory nerves (figs. 2, 3; 1–4 amn). Thus there are four pairs of nerves corresponding to the four pairs of leg ganglia which eventually terminate in the Tropfenkomplex.

The intra- and extraganglionic course of these four pairs of nerves varies considerably. The tract corresponding to the fourth leg, as it leaves the commissure, emerges from the subesophageal ganglion immediately between the third and fourth leg ganglion. In the case of the third nerve, the tract runs parallel, and often between the layers of neurilemma before it emerges

Fig. 4 Cephalothoracic nerve mass and retrocerebral neuroendocrine organs of *A. aurantia*. View from dorsal, caudal end at bottom of figure. end, endosternite; es, esophagus; esg, esophageal ganglion; pbr, protocerebrum; pn, principal nerve; phn, pharyngeal nerve; Sch I, first organ of Schneider; Sch II, second organ of Schneider; sog, subesophageal ganglion; tk, Tropfenkomplex; 1–4 amn, ambulatory neurosecretory nerves corresponding to four leg ganglia.

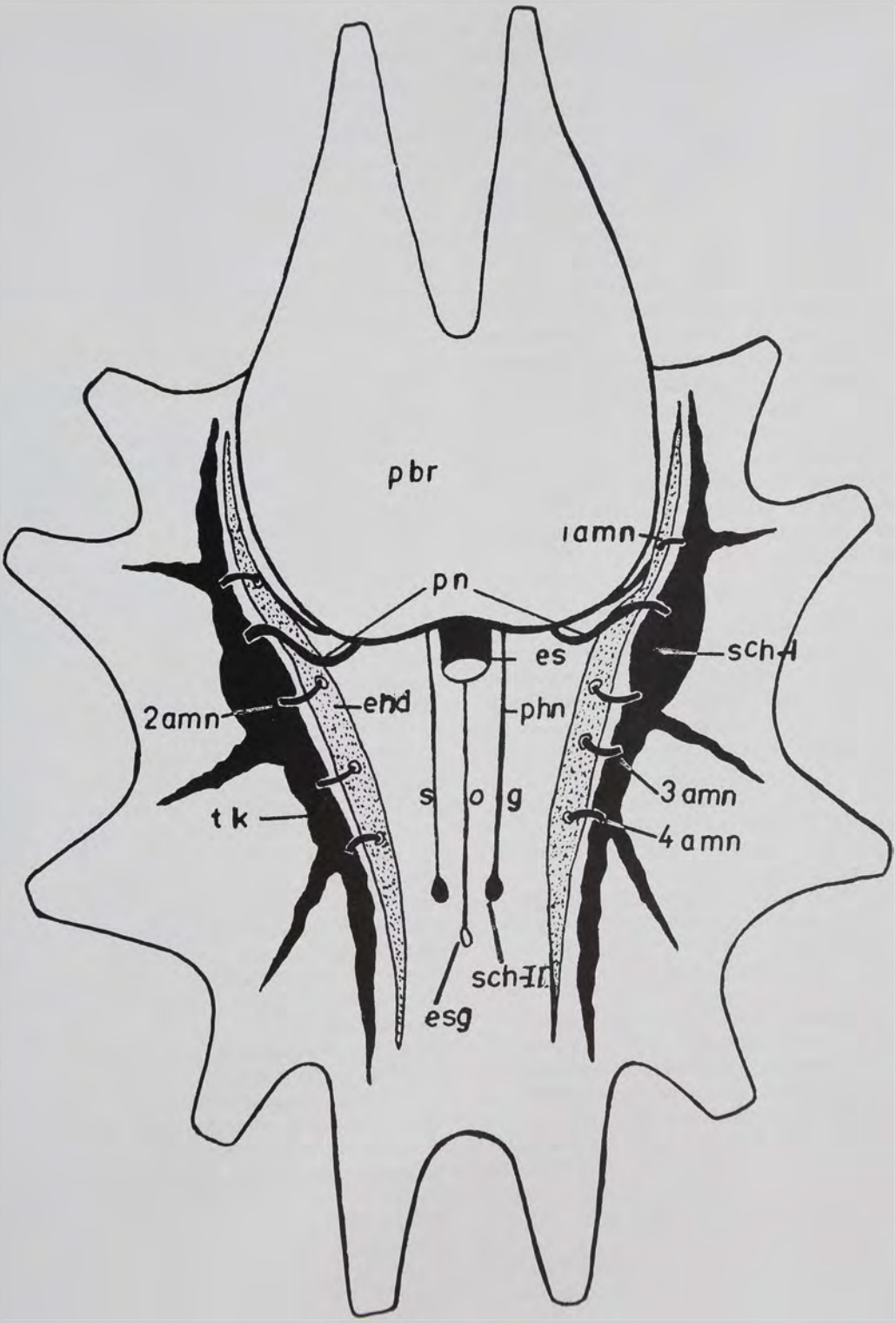

Figure 4

from the subesophageal ganglion between second and third leg ganglia. The second and first nerves, as they leave the commissure run for a long distance in the fibrous mass showing large swellings and great pools of colloidal secretory material on their way. The second nerve emerges just behind the brain after running through the neurilemmal layers (Plate 2, B) for a considerable distance. The first nerve also runs through the neurilemma of the subesophageal mass and the tritocerebral part of the brain. From there it emerges and cuts through the endosternite and enters the Tropfenkomplex anteriorly.

The pedipalpal neurosecretory cells, which also form a commissure, give off a dorso-lateral tract which joins the principal nerve. The abdominal neurosecretory cells, similarly form a commissure and enter the subesophageal neurosecretory tract.

Tropfenkomplex

The first and second organs of Schneider constitute part of the retrocerebral neuroendocrine complex. The earlier workers, Legendre ('59), Kühne ('59) and Streble ('66), gave anatomical and histological descriptions of these two organs. An extension of the first organ of Schneider was called by Kühne the Tropfenkomplex. Schneider (1887/1892) who first found this organ called it marginal ganglion. But it is appropriate to call this structure the Tropfenkomplex since it does not contain any nerve cell bodies. The present study shows that the principal neurohaemal organ in *Argiope* is the Tropfenkomplex.

The Tropfenkomplex (figs. 2, 4; tk) is a paired structure, on either side of the cephalothoracic nerve mass. Each half of the organ lies in an anterio-posterior direction with the anterior end placed lateral to the supraesophageal ganglion and the posterior end lying dorsal to the subesophageal ganglion. Anteriorly, the organ ends near the cheliceral ganglion and posteriorly it terminates near the abdominal ganglion. Along its entire length, it rests on the endosternite and is partly encircled by extensions of endosternite and radiating muscles. The diameter of the organ varies considerably in the middle region (from 40–100 μ) tapering towards both ends. A series of transverse constrictions are found particularly in the middle part of the organ. The sheath runs inwards dividing the organ into a series of lobules. There are three main branches that are given off from the middle region of the organ. A blood sinus runs dorsally and is in close contact with the organ (fig. 1; bv). The first organ of Schneider is intimately attached to the Tropfenkomplex just behind the protocerebral region.

Innervation

The accessory nerve innervating the first organ of Schneider from the brain and the interganglionary nerves between first and second organs of Schneider, as described by Legendre ('54), could not be found. The Tropfenkomplex is innervated by the principal nerve which leaves the brain near the tritocerebral region as was mentioned earlier by Legendre ('54), Kühne ('59) and Streble ('66). The Tropfenkomplex, as the present study shows, is also innervated by four pairs of nerves from the subesophageal ganglion (fig. 4; 1–4 amn). The first pair of nerves whose path has been described earlier, joins the Tropfenkomplex anterior to the first organ of Schneider (fig. 4; 1 amn). The second pair of nerves enters the Tropfenkomplex in close proximity to the posterior end of the Schneider Organ I. The third pair of nerves joins the neurohaemal organ behind the Schneider Organ I (fig. 4; 2 amn) and near the middle of the subesophageal mass. The fourth pair enters the organ near its posterior bifurcation, anterior to the abdominal ganglionic region. All four nerves on their way from the subesophageal ganglion cut through the endosternite before reaching the Tropfenkomplex. The majority of the neurosecretory fibres in each of these nerves come from neurosecretory cells of the corresponding ganglion. Each nerve contains three to six fibres which are loaded with secretory material. There are also a few ordinary axons present in the same nerve bundle.

Structure of the Tropfenkomplex

The first organ of Schneider and the Tropfenkomplex even though inimately interconnected, are separated by a small

neck through which fibres of Schneider Organ I enter the Tropfenkomplex (Plate 2, C. The bulk of the neurosecretory fibres present in the organ come from the subesophageal (four pairs) and from the supraesophageal ganglia (one pair). The principal nerve merely passes through the outer margins of the first organ of Schneider, on its way into the Tropfenkomplex. The internal organization of the Tropfenkomplex merits the term "chaotic" (Plate 2, E, F). Description is rendered difficult because of the anatomical complexity which is mainly due to diversity of neurosecretory fibres and tinctorial properties of the secretory material. The neurosecretory fibres in the organ branch and swell repeatedly along their entire length. The size of the bulbs varies extensively, reaching at times a diameter of 30 μ. The innumerable drops of varying sizes might have prompted Kühne to call this organ the Tropfenkomplex. The homogeneous pools of secretory material in some fibres have the same staining property as those present in the axons and in the soma of the subesophageal ganglion. Most of these can be traced back to their cell bodies in the sub- and supraesophageal ganglia. The secretory material in other axons stains pink with PF, like the product present in neurosecretory cells of the primary organ of Schneider. But in the pools, the neurosecretory material is mostly in the granular form.

The first organ of Schneider and the Tropfenkomplex are covered by a thin membrane, the neurilemma. Neurosecretory cells are absent in the Tropfenkomplex. In sections, the organ presents a two structured appearance (Plate 2, E, F). The dorsal part is heavily loaded with secretion bearing sacs, and axons of varying sizes. The majority of them are arranged at right angles to the neurilemmal sheath and thus appear as though hanging from the dorsal wall of the ganglion. Thus, a kind of palisade arrangement close to the blood vessel was observed in the dorsal part of the ganglion (Plate 2, F).

The ventral half, in contrast, contains both secretion filled and ordinary axons. The two types of secretory axons mentioned above are present in this region also. The organization in this region is much simpler because the axons run mostly parallel to each other. Thus, the palisade appearance found in the dorsal area is absent here.

Amidst these structures, cells with a nucleus and refractory cytoplasm extending into the numerous processes are present. These are called the glial or Schwann cells. A relatively great number of these cells were found in the organ of Schneider and in the main lobe of the Tropfenkomplex.

Changes in stainability of the neurosecretory material

In the present study animals reared in the laboratory have been used so that the date of ecdysis preceding fixation was known. This provides an accurate indication of variations in neurosecretion in the course of the molt cycle.

From the second to the ninth stage the total number of neurosecretory cells in different ganglia during each intermolt period was counted. The NSC were arbitrarily graded, as mentioned earlier, into stage 1 (poor); stage 2 (middle) and stage 3 (full). At the same time, the amount of stainable material present in other regions such as neurosecretory axons and the Tropfenkomplex was also divided into these three stages.

In general, the cyclical accumulations of the stainable material of each neurosecretory cell is different from that of other nerve cells present in the same ganglion. This type of asynchronous activity is more common in type I neurosecretory cells of aboral and abdominal groups. But the type II cells in the cheliceral ganglia are mostly synchronous in their accumulations. On the basis of histochemical data collected from more than 100 animals, the intermolt period in all stages can be divided into an early, middle and late period. The early period begins immediately after the molt and ends by 24 hours later. During this period, cell counts in 20 animals had shown that 16–28% were poor; 55–69% were medium and 18–33% were full with secretory material. These animals also showed medium quantities of secretory material in the Tropfenkomplex and in neurosecretory fibres in the neuropile (fig. 5).

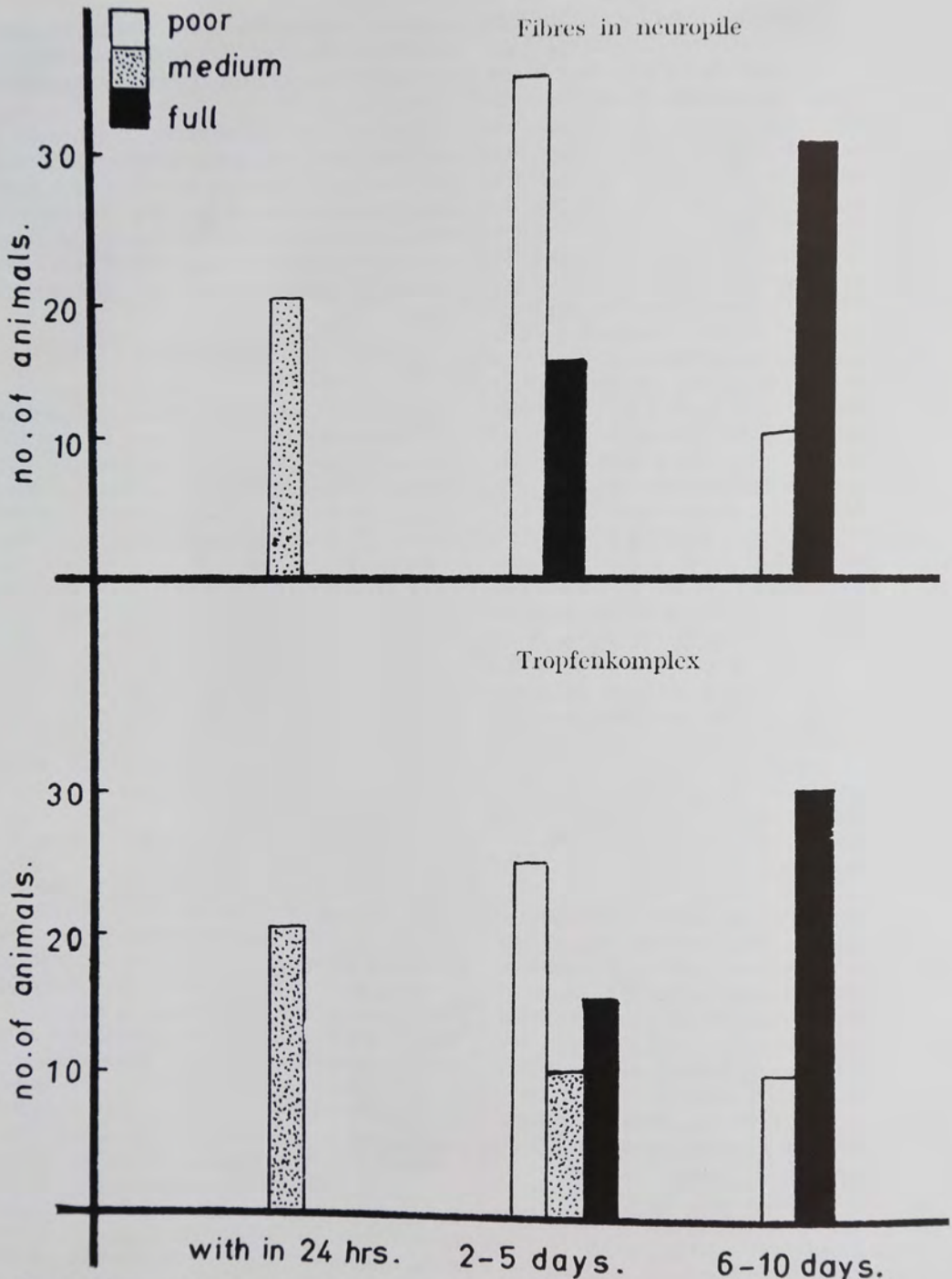

Fig. 5 Changes in stainability of the neurosecretory axons of *A. aurantia* during the intermolt periods. Abscissa, time after last molt; ordinate, amount of stainable material in neurosecretory cells in number of animals.

The middle period falls between two to five days after the molt. Sixty animals were observed during this period. Cell counts in these animals had shown that 47–90% were poor; 2–45% were medium; 0–20% were full. The neurosecretory material in the Tropfenkomplex of 25 animals was poor; in ten it was medium and in 15 it was full (fig. 5). The neurosecretory material in axons of 35 animals was poor and in 15 it was medium. An exception was found in five animals. In these 55% of the cells were full, 44% were medium and only 1% were poor. A corresponding high degree of activity was also noticed in the Tropfenkomplex and fibres in the neuropile.

The late period starts by the sixth day after the molt and in the majority of cases ends before the fifteenth day. Cell counts in 40 animals showed that 8–29% were poor; 30–41% were medium; 52–68% were full. The neurosecretory material in axons and Tropfenkomplex of 30 animals was full and only in ten it was poor (fig. 5).

A graph plotted on the molting pattern of these laboratory reared animals of all the stages shows that the intermolt periods last between 10 and 15 days (fig. 6). The shortest period in some cases is six days and the longest intermolt period may extend, in some cases, beyond 20 days.

DISCUSSION

The present study shows that in *Argiope aurantia* the neurosecretory paths are formed from metamerically arranged perikarya situated in all the ganglia of the cephalized nerve mass, and the neurosecretory product travels along these axons which terminate in neurohaemal organs.

The occurrence of neurosecretory cells in all the postembryonic stages and the stagewise appearance of certain groups has also been reported in other animals. In Opiliones (Naisse, '59), the oral group can be identified from the time of hatching, whereas the lateral neurosecretory cells were identified from the third larval stage. Jones ('56) in *Locusta;* Sharan and Sahani ('60) in *Dysdercus;* Khan and Fraser ('62) in *Periplaneta* found protocerebral neurosecretory cells even during embryonic development. The lateral neurosecretory cells in some insects appear in the last part of the larval life (Arvy and Gabe, '53, '54).

Protocerebral neurosecretory pathways are known for all arthropods studied so far (Gabe, '66). The organization of these pathways, particularly in insects and spiders, are strikingly similar. In both groups, they arise from a mass of neurosecretory cells in the protocerebrum and decussate intraganglionically. The nerves emerge from the brain as nervi corpora cardiacii in insets and as principal nerves in spiders. The former terminate in the corpora cardiaca and the latter in the Tropfenkomplex which are the principal neurohaemal organs. The tritocerebral groups characteristically do not decussate in both groups: in insects they terminate in the corpora cardiaca, whereas in spiders they terminate in the second organ of Schneider. In insects and crustaceans the organization of these pathways in the subesophageal and other ganglia of the ventral nerve cord has not received the same attention as those of the protocerebral tracts. The detailed study of these tracts in the subesophageal ganglion of the spider reveals that the neurosecretory fibres aggregate in discrete metamerically arranged bundles forming commissures and longitudinal tracts. Some of the tracts identified are as complex as those of the ordinary neurons in their course towards the terminal organs.

The only account available on the neurosecretory tracts of a phalangid (Juberthie, '64) lacks detailed mapping of the pathways. The partially represented neurosecretory tract in the subesophageal ganglion is strikingly similar to the subesophageal neurosecretory tract described in the spider. In spiders all free abdominal ganglia have migrated forwards and fused into a single, large subesophageal mass. Perhaps, because of this anatomical specialization, the neurosecretory material from several ganglia migrates into the single large Tropfenkomplex. But in insects and crustaceans which have a long ventral nerve cord, other neurohaemal organs were also reported. The median nerve neurohaemal organs in insects (Brady and Maddrell, '67; Raabe and Ramade, '67; Smalley, '70) and pericardial organs in crustacea (Alexandrowicz, '52, '53) are some of

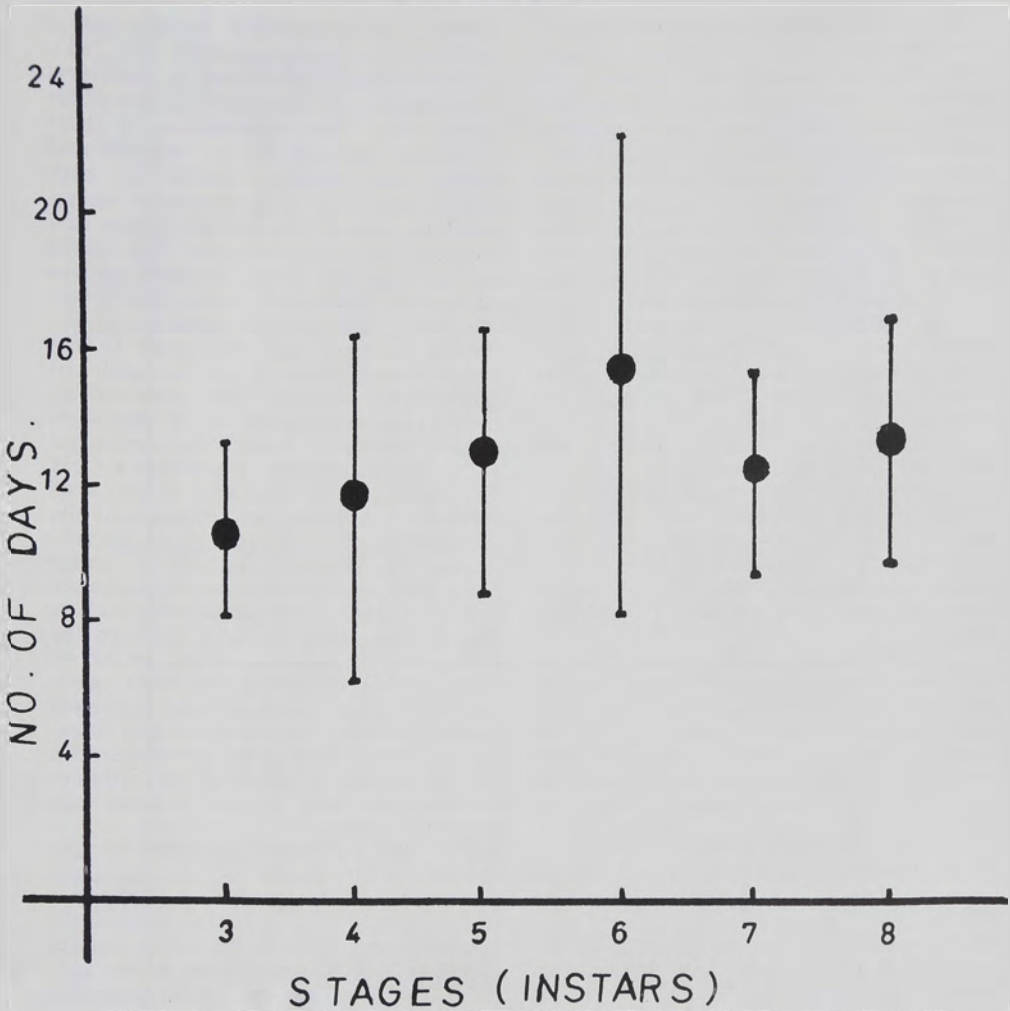

Fig. 6 Length of molting cycle (third to eighth stages) in laboratory reared animals of *A. aurantia*.

those present outside the cephalized anterior masses.

The light microscopic anatomy of the spider (Argiope) Tropfenkomplex provides evidence as in other animal groups (Bern and Hagadorn, '65; Gabe, '66; Novak, '66) suggesting that this structure may be a neurohaemal organ. The evidence is as follows: (1) Secretion bearing axons enter the organ from the protocerebral and subesophageal ganglia. A blood sinus on the dorsal side of the organ is present. (2) The axons branch extensively and along their course show swellings of various dimensions. (3) These swellings or sacs are filled with homogeneous colloidal material which has the same staining properties as in the soma. (4) The Tropfenkomplex is enveloped by fibrous sheaths which run into the organ. (5) The accumulations histochemically show cyclical changes which correspond to the stainability

changes in the perikarya. (6) Neuroglial cells are present in the neurohaemal organ. Further studies, especially at the electron microscope level, are highly desirable.

Kühne ('59) stressed the absence of any relationship between the morphology of the neurosecretory perikaryons and the period that lapsed since the last ecdysis. In the present study two peaks of stainability during the intermolt periods were found. The first peak during the intermolt period, regardless of stage or instar, is suggested to be responsible for postmolt development and differentiation of adult characteristics. The second peak which falls in the later part of the intermolt period is prior to molting time. Since the average time taken to complete a cycle of ecdysis is between 10 to 15 days, the second peak of activity comes between six to ten days after the last molt. A corresponding peak of accumulations in axons and the Tropfenkomplex was observed. In Opiliones, Naisse ('59) reported two peaks for the oral group and one peak for the aboral group of neurosecretory cells. The peak was closer to the time of ecdysis, and thus, it was linked to the phenomenon of molting. The stainability of the perikarya in the pars-intercerebralis of insects undergoes cyclical changes in the course of each larval intermolt period (Rehm, '51). Herlant-Meewis and Paquet ('56) in *Carausius*, and Steel and Harmsen ('71) in *Rhodnius* described two peaks of stainability in the fifth larval intermolt where the second peak of secretion in the perikaryons was before the next ecdysis. In *Bombyx* (Bounhiol et al., '53) there was only one peak of secretion before the next ecdysis. Thus, the peak period of staining before the molt as presented in spiders may also be related to inducement of molting. In spiders one or more cells in each group of neurosecretory cells in the brain and subesophageal ganglion which show the rhythm may be involved in the process of molting. Eckert ('67, '68), after elimination of the oral neurosecretory cells and the stomodeal bridge, reports that there is either delay or complete elimination of molting in *Coelotes*. Since an oral group is absent in *Argiope*, experiments designed to eliminate other groups of N.S.C. in the brain and subesophageal ganglion of this spider might prove useful.

In *Argiope aurantia*, the females show external indications of sexual characters in the form of epigynum only after the sixth molt. During this seventh stage, the neurosecretory cells in the cheliceral ganglia make their first appearance. From this stage, they grow in size and in the amount of secretory material synthesized and in the amount transported along the axons. Thus from the seventh stage there is morphological and functional development of the female reproductive system which can be correlated with the appearance and increased stainability of the cheliceral neurosecretory cells. Maximal staining and axonal transport was observed in these cells during the ninth stage, during which maturation of gonads occurs. Females deposit their eggs in cocoons at 20–25 days after the ninth molt. A corresponding increase of neurosecretory material in several cells of the aboral, first and second leg and abdominal groups was noticed. The stainability and transport along the axons of these type I cells also reaches its maximum while the females are gravid. Hence, it is suggested that both, type I and type II neurosecretory cells may play a role in the process of reproduction. Since it is dangerous to draw conclusions only from the stainability of N.S.C. (for further discussion see Highnam '65), the best other evidence of activity is furnished by the axonal transport of neurosecretory material (Lea and Thomson, '62).

Thus the present results confirm the earlier report of Legendre ('59); Kühne ('59) and Streble ('66.) In adult phalangids (Naisse, '59) the neurosecretory cells of the oral, aboral and lateral groups which are rich in the secretory product are linked with reproductive functions. A similar relationship between neurosecretory activity and reproduction was reported in insects (Bounhiol, Gabe and Arvy, '53; Highnam, '61; Hoffman, '70).

According to Kühne ('59) after oviposition the neurosecretory cells became progressively impoverished of secretion as the animals aged. But in the present study it was found that maximal accumulation continued until the time of death. The laboratory-reared animals and some from

the field, laid two or even three cocoons with and without eggs. This may be responsible for the continued presence of high stainability of the neurosecretory cells during this stage.

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PLATE 1

EXPLANATION OF FIGURES

A Type I neurosecretory cells. The two cells show very small quantities of the stainable material. Hence these are at stage I in synthesis of the secretory material. × 1000.

B Type I neurosecretory cells. The three cells show medium quantities of the secretory material. Hence these are at stage II in their secretory activity. × 1000.

C-D Type I neurosecretory cells (from ninth stage). The cells show maximum quantities of secretory material. Hence these are at stage III in the production of stainable material. C, aboral group of cells. D, neurosecretory cells from the first ambulatory ganglion. × 1000.

E Type II neurosecretory cell from cheliceral ganglion. × 1000.

F Densely stained and lobed areas (lm) in the perikaryon of large nerve cells. These masses are PAS positive but when treated with diastase they disappear completely. Hence the stained material is most likely glycogen. × 1000.

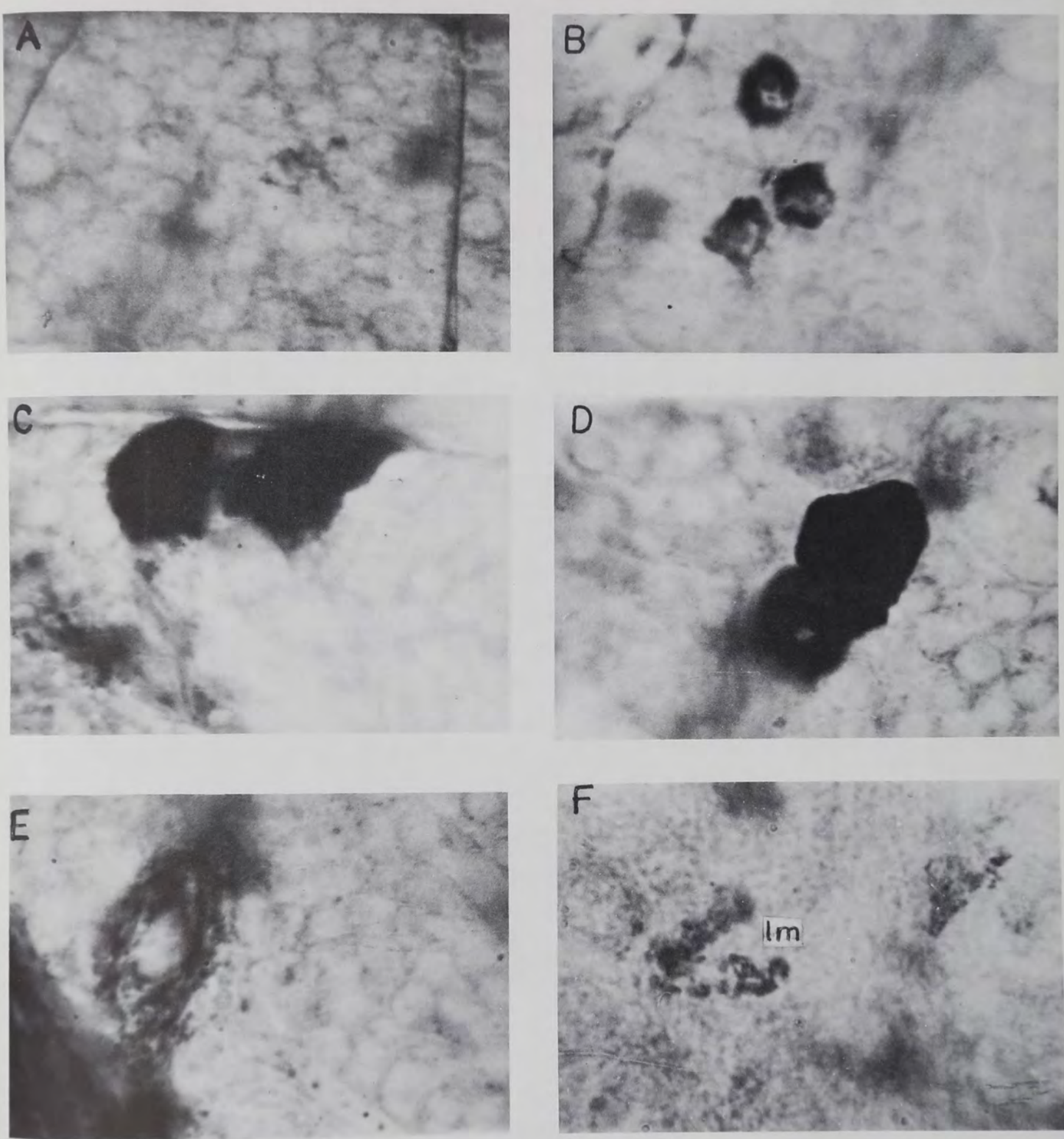

A
B
C
D
E
F
lm

PLATE 2

EXPLANATION OF FIGURES

A Pools of neurosecretory material within the neuropile. These fibres arise from the neurosecretory cells of the first ambulatory ganglion. × 1000.

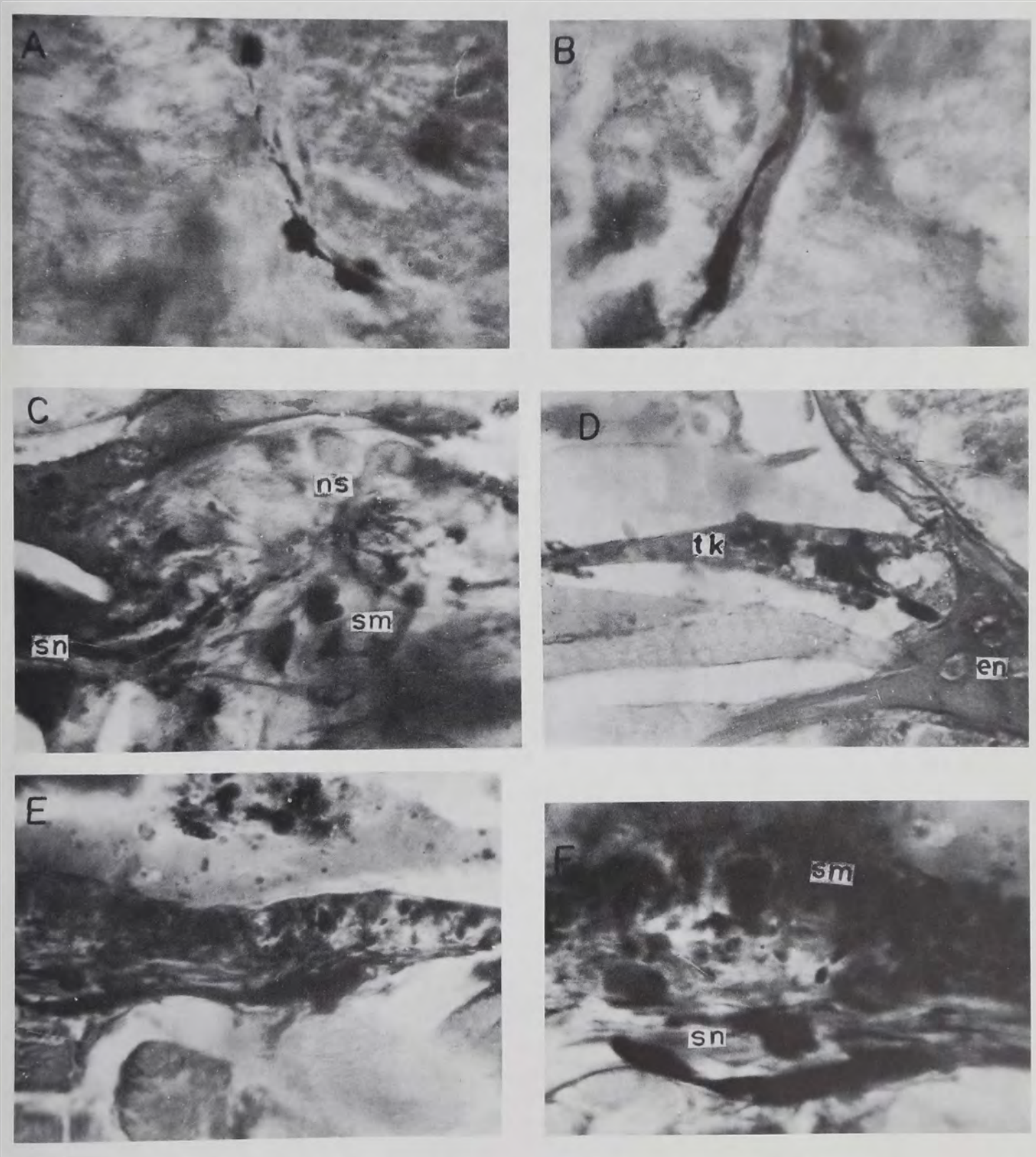
B Extraganglionic course of a secretion filled axon on its way to the Tropfenkomplex from neurosecretory cells of the first ambulatory ganglion. × 1000.

C Sagittal section of first organ of Schneider. Note the nerve cells (ns) in the upper half and pools of secretory material (sm) in the lower half. Several secretion filled axons (sn) are present at the lower left hand corner through which the Tropfenkomplex is connected with the first organ of Schneider.

D Transverse section of the Tropfenkomplex (tk) at one of its lateral extensions. Note the entry of two secretion filled axons into the neurohaemal organ. en, Endosternite. × 400.

E Sagittal section in the middle part of the Tropfenkomplex. × 400.

F Tropfenkomplex. Note greater accumulation of the secretory material (sm) in the dorsal part. The ventral part contains mostly secretory fibres (sn) running parallel to each other. × 1000.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