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MICROSCOPICAL ANATOMY OF LARVA OF CHELADONTA COSTULATA (ACARINA: TROMBICULIDAE). I. GLANDS

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Abstract. The larva of *Ch. costulata* possesses seven paired glands which can be differentiated according to their position and histological structure. In five of them, the dynamics of their secretory or excretory activity may be studied histologically during the feeding process, when not only the deposition of secretion in the gland cells, but also the size of the whole gland is changed. The morphology of the remaining glands is not affected by feeding.

As far as it is known, there has been no paper dealing with the anatomy of salivary glands of chigger mite larvae of the genus *Cheladonta*. Even the papers concerned with this subject in general with the larvae of Trombiculidae are few (Thor 1904, Jones 1950, Obata 1954, Voigt 1971).

The lesion produced by feeding larva of *Ch. costulata* (Willmann, 1952) in the skin of *Microtus arvalis* Pallas, 1778 is remarkable in its structure, but its origin has not yet been sufficiently elucidated. Since a detailed analysis of ulcerous changes in the skin revealed that their formal genesis is closely associated with the mode of sucking, the anatomy of the larva was studied with a special regard to the structure and function of salivary glands.

MATERIAL AND METHODS

The material used in the present study was collected by Daniel and Šlais during the investigation of natural focus of haemorrhagic nephroso-nephritis in eastern Slovakia in 1957.

Ulcers at various stages of development produced by larvae of *Ch. costulata* in the skin of bank voles were found at the end of summer and at the beginning of autumn (Daniel and Šlais 1957). The ulcers containing larvae commencing to feed up to fully engorged larvae were used for the detailed studies. Skin samples with ulcers and parasites were fixed in the following fixatives: 1% neutral formol, Susa, Zenker, 100% alcohol, Carnoy's fluid, sublimate. The excisions were embedded in paraffin and histological sections were stained using the following methods: Weigert's phosphotungstic haematoxylin, Böhmer's haematoxylin, Mallory's haematoxylin, Gomori's method for impregnation of reticular fibres, Masson's trichrome and Goldner's trichrome.

Mucosubstances were detected by staining with Best's carmine in combination with saliva test, and PAS reaction in combination with acetylation, desacetylation and saliva test.

The following methods were used for the detection of some amino acids: Sakaguchi's method modified after Baker (1947 — ex Müller and Chytil 1962) for the detection of arginine; copulatory tetrazonium reaction modified after Müller and Chytil (1962) for the detection of tyrosin and tryptophan; Morel-Sisley diazotization method after Pearse (1960) for the detection of tyrosin; Adam's (1965) method for the detection of tryptophan;DDD method of Barrnett and Seligman (1952) controled by a blockade with N-ethylmaleinimide for the detection of SH-groups; Pearse's (1960) reaction with performic acid and alcian blue for the detection of SS-groups.

RESULTS

The chigger mite *Ch. costulata* was found to possess seven paired glands differing in the size, histology and staining (Fig. 1).

In the anterior part of the idiosoma there is a pair of marked salivary glands surrounding the brain ganglia in form of a saucer. Their cells are large, of a characteristic conical shape (Plate I, Fig. 1) and their tips are directed to a common duct — opening of the gland around which they are arranged. A large nucleus with a nucleolus occupying two thirds of its body (Plate I, Fig. 1) is located in the widened basal part of the cell. The nucleus measures 9×6 μm and the diameter of the nucleolus is 4 μm. The activity of these glands, as followed at various stages of feeding, may be divided into two phases. During the resting phase the cell cytoplasm is densely filled with basophilic granules in basal two thirds, while the apical third near the gland openings is without granules, appearing like a light space where probably the secretion has been accumulated previously. During the phase of secretion the apical third of cells is filled with drops of secretion (Plate I, Fig. 2) with a high arginine content. The drops of the secretion of paranuclear origin seem to penetrate through the cell cytoplasm coalesce into larger drops and eventually accumulate in the narrow portion of the cell. After a certain amount of the secretion has been accumulated, it is expelled all at once. When the feeding is finished, the glands are completely depleted. The opening of these glands, measuring 0.5 μm in lumen, is formed by a smooth, eosinophilic membrane gradually thickening and the cellular structure of the opening is visible in the end (Plate I, Fig. 3).

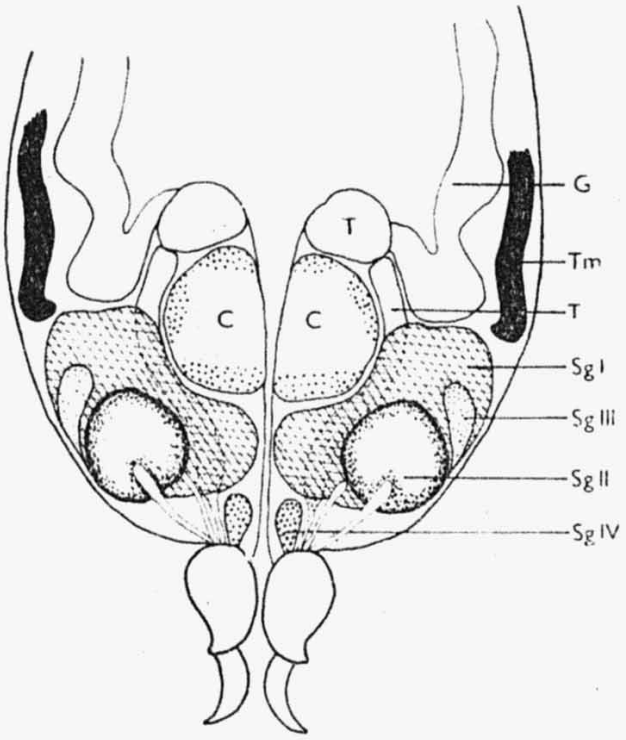

Fig. 1. Scheme showing individual gland types and their localization. G — intestine, Tm — tubular Malpighian glands, SgI — first pair of salivary glands surrounding the cerebral ganglia (C). SgII — second pair of salivary glands, sgIII — third pair of salivary glands, SgIV — fourth pair of salivary glands, T — fifth pair of salivary glands — tubular glands.

The second pair of glands is frontal to the above-described one adjoining its lobes laterally. It is much smaller than the first pair, reaching only one third of its size. The second pair of glands is composed of a small number of conical cells whose apical ends are concentrated around the gland opening (Plate I, Fig. 4). A nucleus with a conspicuous nucleolus is located in the distal, widened portion of the cell. The cytoplasm of these cells stains with haematoxylin and is pervaded by minute vacuoles. Around the gland opening there is a light space due to different tincturing character of the apical third of the cells. The opening of this gland is formed by a duct, the cell cytoplasm of which stains only lightly. It is connected with the opening of the first gland.

Another, third pair of glands are lateral to the two above glands. They are oval and the cytoplasm of their cells is strongly vacuolized (Plate I, Fig. 1). The opening of this gland could not be found due to its poor staining during the whole course of feeding. It was observed that these glands are almost inconspicuous in larvae starting to feed, but they become more distinct after some time of feeding.

Medially, immediately behind the gnathosoma are small saccular glands consisting of a small number of cells. There is a poor eosinophilic staining in their cytoplasm but intensive staining by haematoxylin in the nuclei.

Tubular glands run between cerebral ganglia and large salivary glands (Plate II, Fig. 1). At the site where the oesophagus passes to the intestine the intestinal wall is pressed by saccular glands which form an oval isle here and most probably an anlage of the tubular gland. The cells of the saccular gland are cubic, lying close to one another and their cytoplasm has no distinct granulation. Small nuclei are situated near the periphery of the cell forming a rosary on margin of the gland. Frontal to oval isles are tubular glands whose cells have the same character as those of the saccular portion of gland impressed into the gut wall. In the region where openings of the preceding glands were found, the tubular gland passes into a thin duct the wall of which consists of flat epithelial cells. The size and shape of these glands remain unchanged during the course of feeding even in fully engorged larva.

Another pair of glands is posterior to gnathosoma. These are excretory tubular glands running between the hypodermal cells and intestine (Plate II, Fig. 2). In the proximity of the excretory organ these two excretory tubular glands unite. The cells of these glands are spherical and their cytoplasm is filled with small granules increasing in number in the course of feeding. The nuclei are covered with the granulation and are therefore difficult to distinguish. We assume that Malpighian glands are concerned.

The last observed pair of glands is situated on both sides of body behind cerebral ganglia near the bend of the third coxa. These glands are oval and their cells are tightly packed. The nucleus displays strong staining with haematoxylin and occupies about two thirds of the cell size; therefore the glands have an appearance of a darkly stained organ (Plate I, Fig. 4). The openings of each of the glands are bordered by a smooth, basophilic cuticle and are connected in a common duct. No marked changes have been observed in these glands during the course of feeding.

DISCUSSION

As it has been mentioned in the introduction, there has been no paper dealing with the anatomy of salivary glands of *Ch. costulata*. Our results will be therefore compared with the records of other trombiculids, though they sometimes differ considerably even if the salivary glands of the same chigger mite order were studied. For example, according to Jones (1950) the salivary glands of *Neotrombicula autumnalis* possess two pairs of glands: small, oval glands with marked affinity to basic stains, and large glands lying on both sides of oesophagus, widening around cerebral ganglia and with cells containing markedly granulated plasm. On the other hand, Voigt (1971) described in *Neotrombicula autumnalis inopinata* five pairs of salivary glands and one pair of accessory glands like in larvae of *Neotrombicula zachvatkini*, *Ascoschoengastia latyshevi* and *Leptotrombidium intermedium*. The same number of glands was reported by Obata (1954) in *Trombicula akamushi*.

The large salivary gland described by Jones (1950) corresponds to the large salivary gland in *Ch. costulata*. Jones observed an accumulation of secretory granules in the basal portion of gland cells which may be explained by the fact that the glands were studied only in the resting phase. We managed to demonstrate that the secretory drops, which are formed juxtanuclearly, accumulate in the apex of conical cells during the phase of secretion and are expelled from the cells after some time. The small oval gland described by Jones (1950) corresponds to our third pair of glands in *Ch. costulata*.

The distribution of salivary glands observed in our specimens of *Ch. costulata* conforms

essentially to the descriptions of the species studied by Voigt (1971), though there are some morphological differences in the details. This author also described large salivary glands surrounding cerebral ganglia, but she only supposed that the secretion is accumulated in the apical portion of cells of this gland. This fact was clearly demonstrated in our material. We have also found that towards the end of feeding the gland is depleted and only fragments of cells remain. In the larva which was released and found free in the hair only small remainders of the gland pressed with the filled intestine were detected.

Also the second pair of salivary glands described by Voigt (1971) is comparable with the second pair of *Ch. costulata*. According to Voigt the opening of this gland is smooth, but we have found that it consists of cells and its lumen is covered with an eosinophilic cuticle.

The third pair of salivary gland in *Ch. costulata* is identical with that in the species studied by Voigt (1971). The fourth pair, however, somewhat differs. In *Ch. costulata*, these are small saccular glands with a small number of cells; their cytoplasm is feebly eosinophilic and the nucleus is conspicuous. In Voigt's description, this is the smallest bean-shaped gland with strongly granulated cells and indistinct borders between them.

The tubular salivary glands observed by Voigt to begin between the cerebral ganglia, oesophagus and intestine run laterally to the third coxa and cerebral ganglia. The cells are strongly vacuolized and contain few granules; the cell nuclei show poor stain. The tubular salivary gland of *Ch. costulata* begins also between the oesophagus and intestine and passes forward between cerebral ganglia and large salivary gland. In contrast to Voigt's observations, it does not reach the third coxa. In *Ch. costulata*, the cytoplasm of this gland is strongly eosinophilic and neither vacuolization nor marked granulation have been observed.

Another pair of glands in *Ch. costulata* are the excretory tubular glands which we suppose to be the Malpighian glands. The last gland found in this mite is a paired gland near the bend of the cuticle of the third coxa; we have managed to find its opening and to follow its course. This gland would correspond to the sensory gland in mites studied by Voigt, but this author also failed to find the opening of the gland.

The mites of the family Trombiculidae possess a large number of paired salivary glands, compared to ticks, in which the gland cells form a single giant paired salivary gland with several functional sections, as described by Chinery (1965) in *Haemaphysalis spinigera*. This difference in the structure of salivary glands in chigger mites and ticks seems to be associated with the different manner of sucking.

According to Chinery (1965), the secretion of salivary glands of ticks is of proteinaceous nature. It was determined to contain the amino acids tyrosine and tryptophan, and neutral proteins. The secretion of salivary gland in the larva of *Ch. costulata* is also of proteinaceous nature. It contains also the amino acids, tyrosine and tryptophan, but in contrast to tick secretion also a high percentage of arginine was detected in it.

МИКРОСКОПИЧЕСКАЯ АНАТОМИЯ ЛИЧИНКИ *CHELADONTA COSTULATA* (ACARINA: TROMBICULIDAE). I. ЖЕЛЕЗЫ

Я. Шрамлова

Резюме. Личинка *Ch. costulata* имеет семь парных желез, которые можно дифференцировать по их положению и гистологической структуре. При помощи гистологических методов у пяти из них можно наблюдать во время сосания динамику их секреторной или экскреторной деятельности, причем происходят изменения не только в позиции секрета в железистых клетках, но и в размере железы. У других желез сосание не оказывает влияния на их морфологию.

REFERENCES

ADAMS C. W. M., Neurohistochemistry. Elsevier, Amsterdam, 1965.

BARRNETT R. J., SELIGMAN A. M., Histochemical demonstration for protein bound sulphydryl groups. Science 116: 323—327, 1952.

CHINERY B. A., Studies on the various glands of the thick *Haemaphysalis spinigera* Neumann 1897. Acta Trop. 22: 235—266, 1965.

DANIEL M., ŠLAIS J., To the question of intradermal parasitism and its morphology in larvae of chiggers *Euschöngastia ulcerofaciens* (Acari: Trombiculidae). Čs. Biol. 6: 365—371, 1957. (In Czech).

JONES B. M. The penetration of the host tissue by the harvest mite, *Trombicula autumnalis* Shaw. Parasitology 40: 247—260, 1950.

MÜLLER J., CHYTIL F., Úvod do histochemie bílkovin. (Introduction to the histochemistry of proteins.) Stát. zdrav. nakl., Praha, 1962. (In Czech).

OBATA J., Anatomical studies on *Trombicula akamushi* (Brumot), the vector of Tsutsugamushi disease. Ei sei Dobutsu 5: 11—143, 1954.

PEARSE A. G. E., Histochemistry theoretical and aplied. J. A. Churchill Ltd., London, 1960. (In Japanese).

THOR S., Recherches sur l'anatomie comparée des Acariens prostigmatiques. Ann. Sci. Nat. Zool. 19: 1—190, 1904.

VOIGT B., Anatomie und Histologie der Drüsen bei Trombiculiden-Milbenlarven. Zool. Anz. 186: 403—417 1971.

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TRYPANOSOMA (HERPETOSOMA) LEMMI SP. N. FROM THE NORWEGIAN LEMMING, LEMMUS LEMMUS (L.).

The first report concerning "*lewisi*-like" trypanosomes in Norwegian lemmings, *Lemmus lemmus* (L.) included information on the incidence of infection and morphological measurements of blood stream forms (Wiger R., Norw. J. Zool. 19: 83—87, 1971). Trypanosomes had not been reported earlier from the genus *Lemmus*. However, the results of cross infection experiments and certain biological aspects of this trypanosome imply that it is specific for *L. lemmus*, the only member of the genus in Fennoscandia.

The trypanosomes in the present study originate from *L. lemmus* which were collected near Standal (62° 15′N, 6° 15′E) in1 969. The blood stream forms of trypanosomes from *L. lemmus* were long and narrow, and generally S-shaped, although many possessed several undulations which resulted in double S-forms. The posterior end was long and pointed. The kinetoplast was generally oval and the undulating membrane had few undulations (Fig. 1). Trypanosomes from the reproductive phase of the infection were more variable in form and both long, slender "adult" forms (Figs. 1a, b), and "stubby" forms (Figs. 1c, d) could be found.

Morphological data reveal that blood stream forms of trypanosomes from *L. lemmus* have bodies that are both longer and wider than either *Trypanosoma microti* from *Microtus agrestis* and *T. evotomys* from *Clethrionomys glareolus*. The mean body length and width in microns of "adult" forms of *T. microti* are 18.0 and 1.3, of

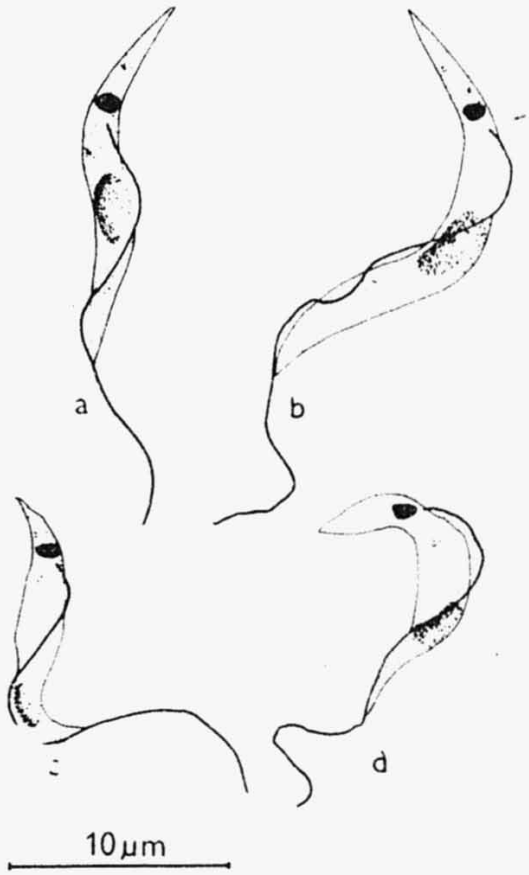

Fig. 1. Blood stream forms of *Trypanosoma* (*Herpetosoma*) *lemmi* sp.n. from the Norwegian lemming, *Lemmus lemmus* (L.). "Adult" forms (a and b) and "stubby" forms (c and d).

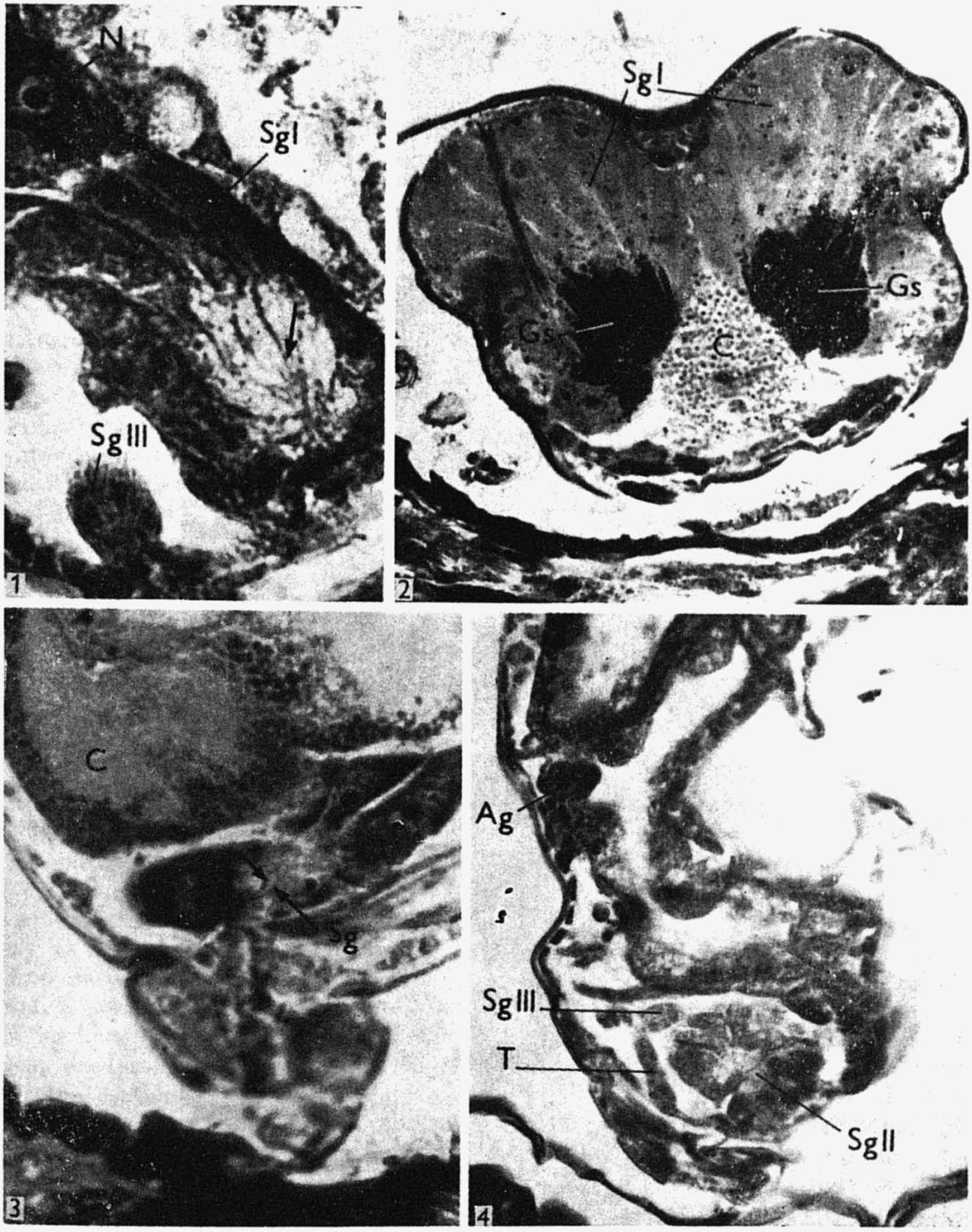

Fig. 1. Tangential section through the large salivary gland (SgI) with a light field formed after the secretion is released (arrow). Note nucleus (N) with nucleolus in the basal part. Lateral to this gland is the third pair of salivary glands (SgIII). (Haematoxylin-eosin, 800×).
Fig. 2. Tangential section through large salivary glands (SgI). The secretion (Gs) starts to accumulate in apical parts of cells; its droplets are dispersed even in the cytoplasm of cells. C — cerebral ganglia. (Bromphenol blue, 400×). **Fig. 3.** Opening of large salivary gland (SgI). Note thickening of the wall (arrows). (Giemsa, 1000×). **Fig. 4.** Tangential section through engorged larva. Note salivary gland of the second pair (SgII), a portion of the salivary gland of the third pair (SgIII) and tubular gland of the fifth pair (T). Ag — accessory gland. (Haematoxylin-eosin, 400×).

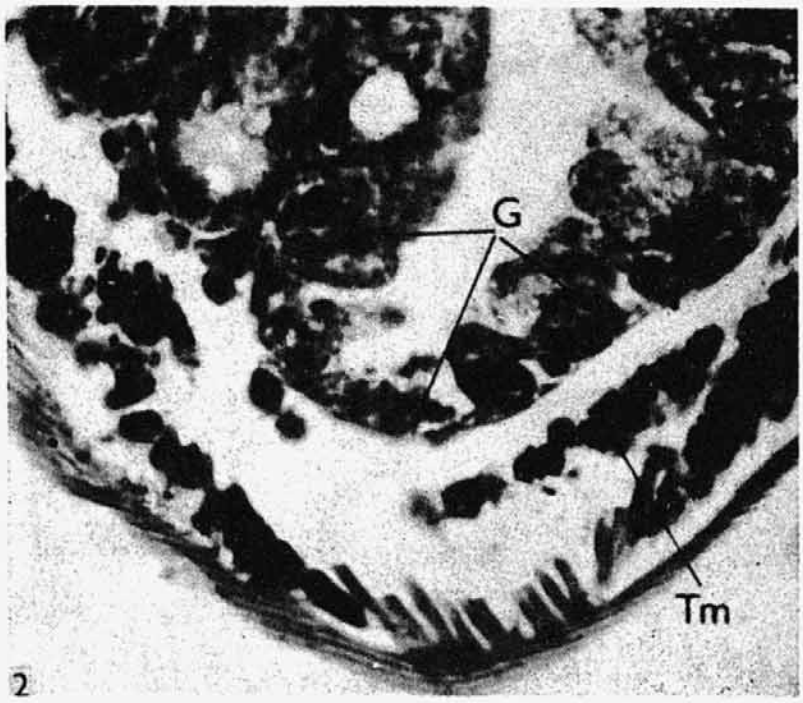

Fig. 1. Tangential section through larva at the beginning of feeding. Note cerebral ganglia (C) and tubular glands (T) around them. Oesophagus (Oe) passes between cerebral ganglia into the intestine (G). (Haematoxylin-eosin, 800×). **Fig. 2.** Excretory tubular gland (Tm) runs between the wall of intestine (G) and the cuticle. (Haematoxylin-eosin, 800×).