INTERNAL MORPHOLOGY OF THE HYPOPUS OF *CALOGLYPHUS MYCOPHAGUS* (MÉGNIN) (ACARINA : ACARIDAE)

BY

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Abstract.

The internal morphology and histology of the hypopial stage of *Caloglyphus mycophagus* is described from serial sections fixed in 10 % acrolein and embedded in glycol methacrylate. There is no oral opening or salivary glands ; the alimentary tract is rudimentary ; there is the anal opening ; and only one epithelial cell type is present in each region. The rectum appears to have many vacuoles in the lumen. The nervous system shows very little change from that of the adult. The gonad tissue is paired but rudimentary and insufficiently developed to determine the sex. Some muscles are present in both adult and hypopus, others occur in the hypopus only and are characteristic of this stage. The cuticle is considerably thicker in the hypopus than in other stages and appears to have numerous ' pore canals '. Attention is drawn to the anatomical differences between the hypopus and the adult and between the hypopi of other species. Their functional significance is also discussed.

Introduction.

Caloglyphus mycophagus (Mégnin), like other acarid mites, is oviparous ; its life cycle normally consists of an egg, a hexapod larva, a tetrapod protonymph, a tritonymph and an adult. Under certain conditions, however, it, like most acarids, has a totally different form appearing between the protonymph and tritonymph, the hypopus or deutonymph. This form is essentially a dispersal phase.

In external morphology and behaviour the hypopus is quite different from other postembryonic developmental stages. It has an ovate body with distinct dorsoventral flattening ; four pairs of legs which are much shorter and stouter than those of other stages ; no mouth parts and a unique sucker plate located just behind the fourth pair of legs. By means of these suckers the hypopi attach themselves to the surface of passing arthropods, rodents and other migrants who may pass by, to be transported from one ecological niche to another.

Whilst many species of hypopi have been described from external anatomy, very little is known about the internal morphology of this or any other species of mite. The only available fragments of information in the literature are from the work of HUGHES (1939) on *Glycyphagus domesticus* (De Geer) ; and WALLACE (1960) on *G. domesticus, G. destructor* (Schrank) and *Histiostoma* (Oudemans). Both HUGHES and WALLACE fixed animals in alcoholic Bouin's solutions and embedded in wax. The inadequate methods then available limited the usefulness of these observations.

The present study uses a new thin-section technique (KUO and MCCULLY 1969) to study the internal morphology of the hypopial stage of *C. mycophagus* in detail. Not only the size of the body, about 400 μ in length, but also the cellular structures of the tissues in the hypopial stage are small. Because of the limitations of the light microscopy resolution, it is very difficult to observe the histological details of certain tissues. As a result our description of these must of neccessity be curtailed until they can be examined by the higher resolution of the electron microscope.

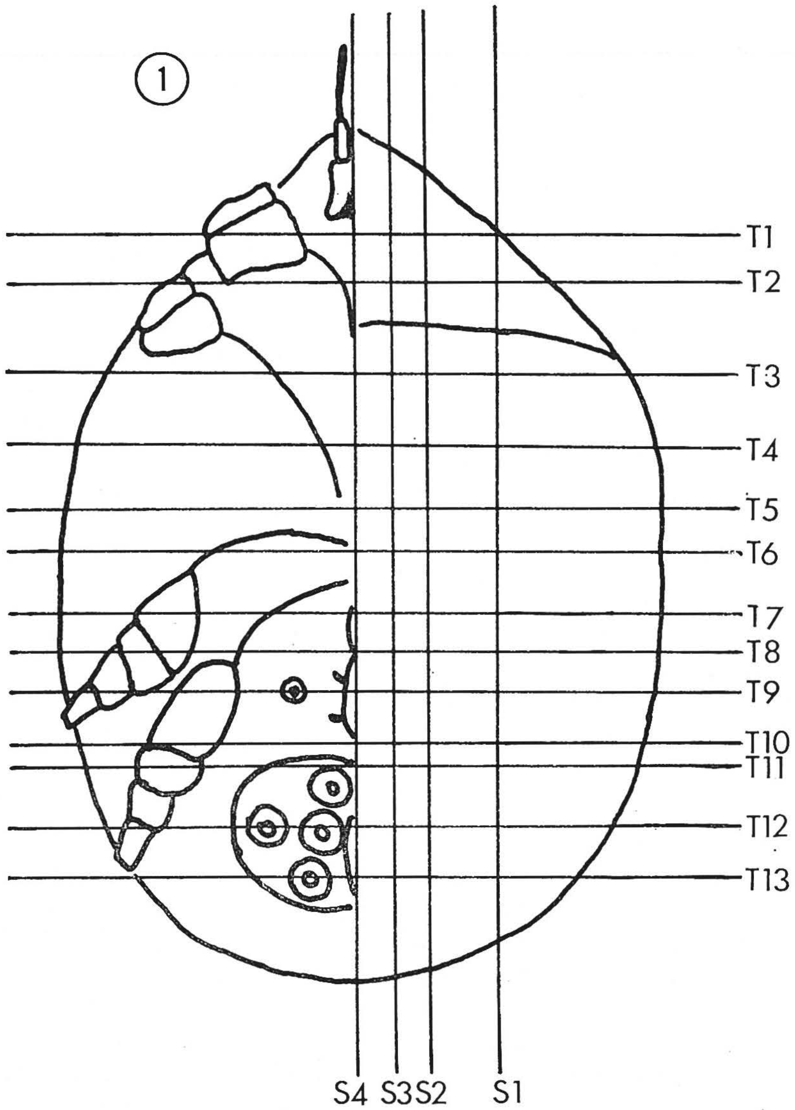

FIG. 1 : Key to section through sagittal and transverse planes of hypopus.

Materials and methods.

All hypopi were grown on Bacto Czapekh agar medium at 20°C. The active hypopi were removed from the culture tubes and fixed in 10 % acrolein and embedded with glycol methacrylate (see Kuo and McCully 1969). At least eight complete sets of serial sections 1 µ to 2 µ thick were cut through the entire animal in each of two planes, transverse and sagittal.

Typical section from these are shown (Figs. 2-18). The exact position in the animal from which all of these sections have been taken can be determined by reference to Fig. 1. Not all the figures were necessarily taken from the same specimen.

Observations.

1. *Digestive System.*

The alimentary tract of the hypopus is rudimentary and resembles a tubular structure. No mouth-parts or oral opening can be distinguished (Figs. 4-6). The region of the mouth-parts (gnathosoma) is represented by a tiny anteroventral body projection which may be the developing palpi (Fig. 4). As the mouth-parts are absent there is no trace of a buccal cavity or pharynx in the hypopus. The œsophagus, devoid of a lumen, can be traced in sections as a uniformly thin core of intensely strained tissue. On emerging from the ganglionic mass this rudimentary œsophagus is deflected dorsally to the point where it joins the stomach (Fg. 5). The stomach lacks the anterior lobe but bears one pair of lateral caeca (Fig. 9). The anterior limb of each caecum is reduced in size and difficult to locate ; the posterior, on the other hand, can be traced in sections by following the strongly stained tissue where it is disposed ventrolaterally of the main gut (Figs. 9-14) to end blindly just beyond the rectum. The lumen of neither the stomach nor caecum can be distinguished in the hypopus. The colon, which is much longer than the stomach, (Figs. 3, 5, 10 and 11) arises from the posterodorsal face of the latter and extends posterioly to lie in the dorsal part of the centre of the body. From it the rectum (Figs. 3-5, 13, 14 and 15) which is larger than any other portion of the alimentary tract in the hypopus, runs gradually downward as it extends posteriorly (Figs. 3-5) to terminate in the minute rudimentary anus formed by an invagination of the thick ventral cuticle in the middle line just posterad of the sucker plate (Fig. 16). A pair of Malpighian tubules enter the gut at the junction of the colon and rectum (Fig. 12). They run dorsolaterally and end blindly just beneath the dorsal integument. No salivary glands were found in any of the hypopi examined.

The epithelial cells of the alimentary tract of the hypopus appear to be totally different from those of the adult, particularly in the regions of the stomach and caecum. The walls of both the rudimentary stomach and caecum composed of one undifferentiated type of epithelial cell which is generally ovoid or round in the shape (Fig. 19). The cytoplasm is basophilic and encloses a large central nucleus in which, in most cases, the large and basophilic nucleolus is distinguishable. The epithelial cells of the colon (Fig. 20) appear to be similar to those of the stomach and caecum. They are ovoid in the shape, have a basophilic cytoplasm and contain a basal or central nucleus. The histological structure of the rectum appears to be somewhat different from the other regions of the alimentary tract ; the wall is composed of a layer of conical cells whose apices project into the lumen. These cells have a basophilic cytoplasm and a large ovoid basal nucleus which usually bears a large central nucleolus (Fig. 21). In addition, these cells appear to be unique in that the projecting apex of each is covered by a pale-staining material that resembles the developing

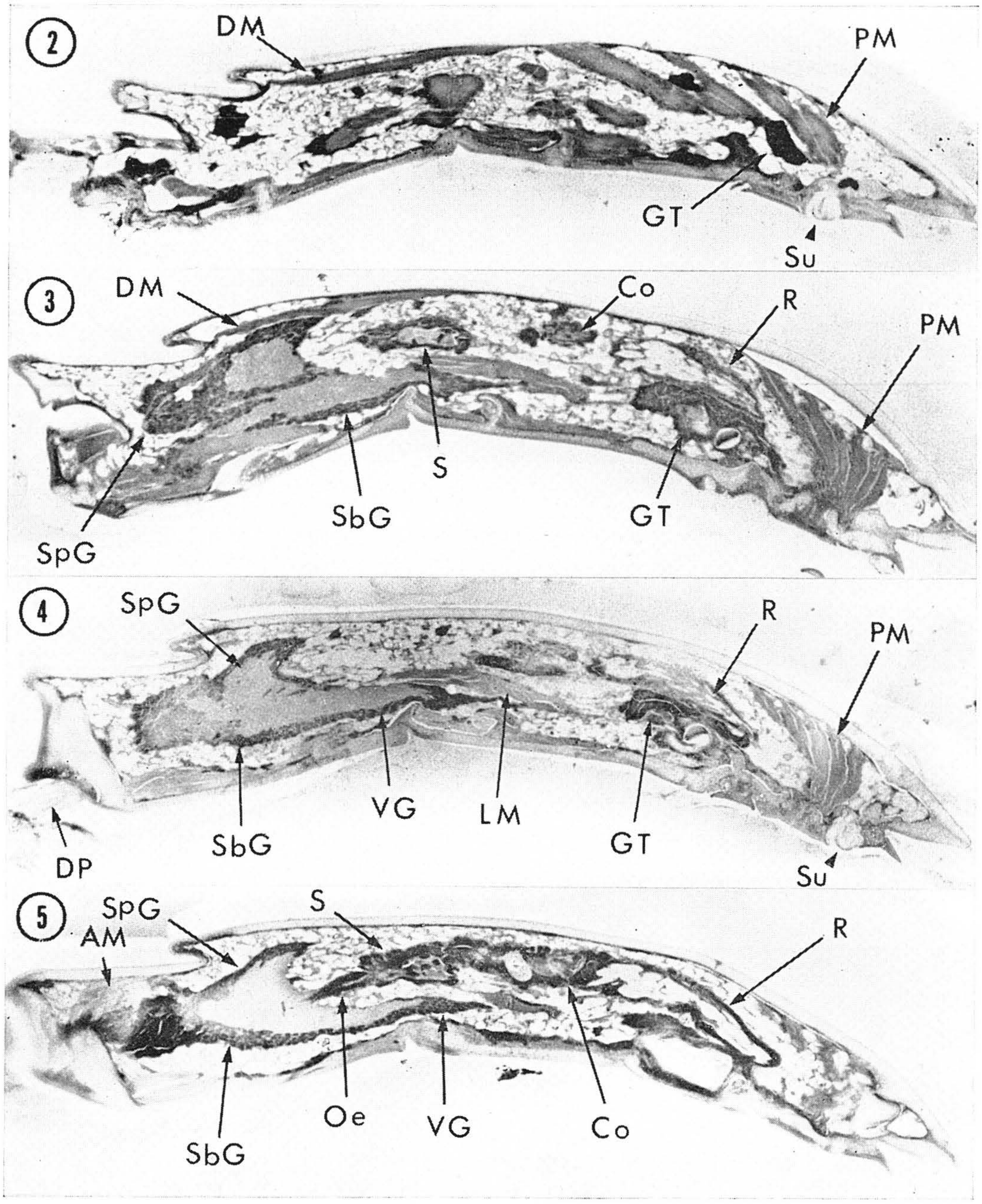

FIG 2-5 : *Caloglyphus mycophagus* (Mégnin).

2. — Sagittal section (S1) of the hypopus × 430. 3. — Sagittal section (S2) of the hypopus × 470.
4. — Sagittal section (S3) of the hypopus × 450. 5. — Sagittal section (S4) of the hypopus × 450.

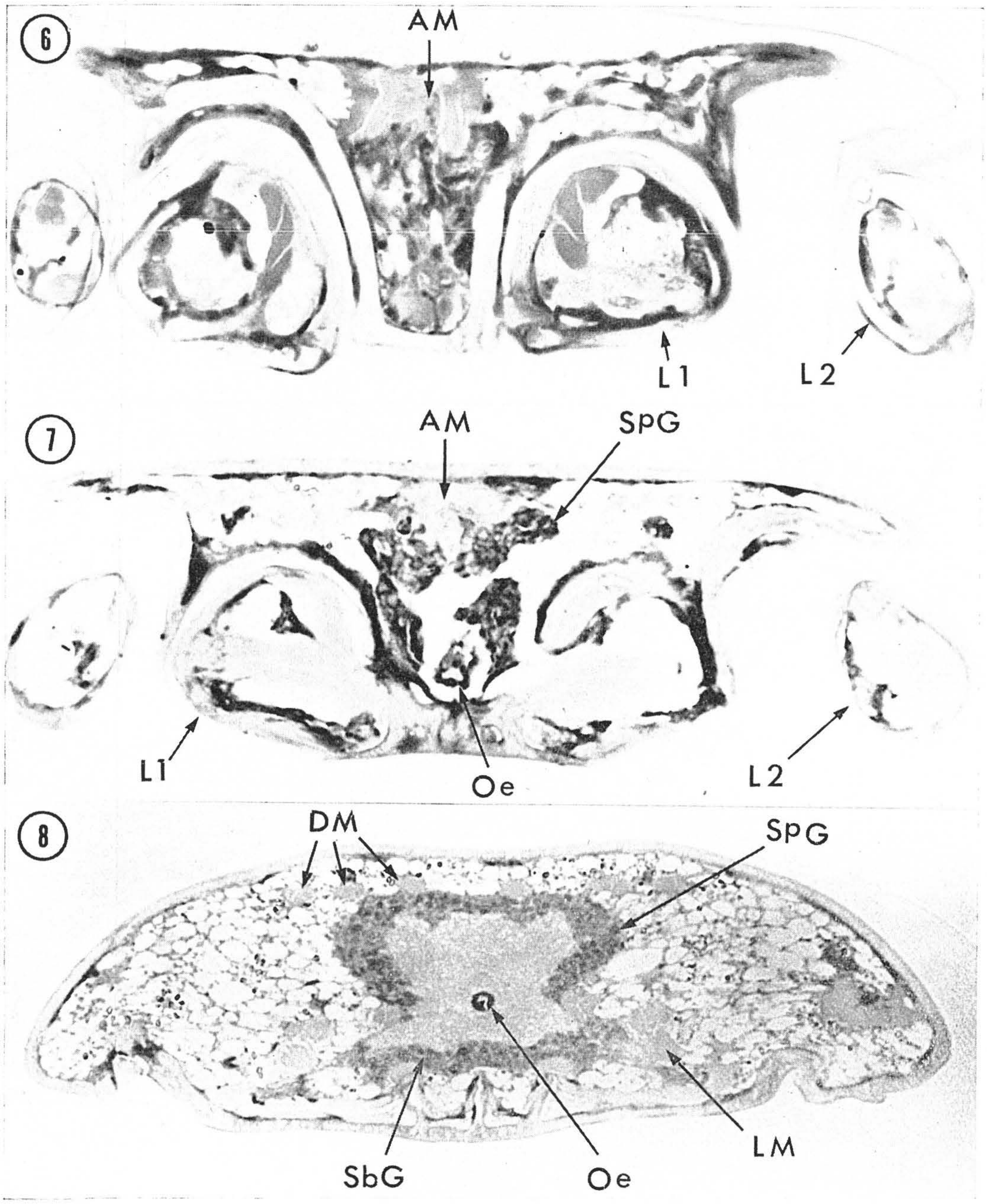

FIG. 6-8 : *Caloglyphus mycophagus* (Mégnin).
6. — Transverse section (T1) of the hypopus × 540. 7. — Transverse section (T2) of the hypopus × 500.
8. — Transverse section (T3) of the hypopus × 600.

brush border, this becomes confluent with that of neighbouring cells to produce a vacuoled packing of the lumen of the rectum (Fig. 21). In the histological structure, the Malpighian tubules of the hypopus and the adult appear to be very similar. Their walls consist of a group of very small conical epithelial cells whose apices project slightly into the lumen, and whose basophilic cytoplasm contains a large basal or central nucleus which sometimes has one or two large basophilic nucleoli or an aggregation of chromatin material (Fig. 22). The surface of these cells is covered by a pale-staining material that projects into the lumen of Malpighian tubule.

2. *Nervous System.*

Superficially, the nervous system of the hypopus appears to be similar to that of the adult in structure, size and shape. Since the size of the hypopial body is much smaller than that of the adult, and the hypopus is dorsoventrally flattened, the nervous system appears to occupy a disproportionately larger part of the anterior region of the hypopus than it does in the adult or other developmental stages (Figs. 3-5, and 8). The central nervous system consists of a dorsal lobe, or supraœsophageal ganglion and ventral lobe, or subœsophageal ganglion. There is, however, no clear distinction between these two nerve masses (Gigs. 3 & 4). At the point where the œsophagus appears to separate the ganglionic mass, there is laterally a slight depression or furrow (Figs. 5 & 8).

The dorsal lobe or the supraœsophageal ganglion runs anteriorly just beneath of the anterior oblique muscles (Fig. 3), and gradually decreases in size, to end beneath the anterior cuticle. Posteriorly it expands as a bulbar enlargement, just beneath the dorsal body muscle (Fig. 5) ; and laterally it extends as far as the second pair of dorsal muscles (in cross section — Fig. 8). It terminates, as seen in the sagittal section, at about 25 μ beyond the level of the dorsal transverse groove, as a tripartite fantail structure.

The ventral lobe is composed of the subœsophageal ganglion, *sensu-stricto*, plus the fused and indistinguishable ganglia of the ventral cord. The ventral lobe extends anteriorly to the anteroventral body cuticle (Figs. 3-5), and posteriorly as a gradually diminishing fillet ; rat-tail in shape when viewed in sagittal section (Figs. 3-5) and somewhat dorsoventrally flattened when seen in the cross sections (Figs. 9 & 10). It continues posteriorly to pass through the space between the longitudinal muscles of the ventral body wall (Fig. 9). Finally, this ganglion terminates in a fine nerve that diminishes so rapidly in size that it cannot be traced in the subsequent sections (Figs. 3-5 & 11). In addition to the structures just described, the ventral lobe gives off anteriorly a nerve to the anteroventral body projection or the developing palpi, and laterally, at the level of the dorsal transverse groove, two pairs of nerves to legs I and II, and at the level of the oblique muscles, two further pairs of nerves which run to legs III and IV.

The medulla of the central nervous ganglionic mass is a uniformly pale-staining matrix whose cellular structures could not be identified. The medulla is surrounded by many deep-staining cells which form the cortex (Figs. 3-5 & 8). Each ganglion cell contains a large, ovoid, deepstaining nucleus (Fig. 23).

3. *Reproductive System.*

The sexes are indistinguishable in the hypopus because both the gonadial tissues and external genitalia are rudimentary.

In the mid ventral line at about the level of the first coxa, there is a furrow or groove that appears to be the anlagen of the inner and outer valves of the adult (vide KUO and NESBITT, 1970) (Fig. 13). As in that stage two pairs of weakly sclerotized valve-like structures can be

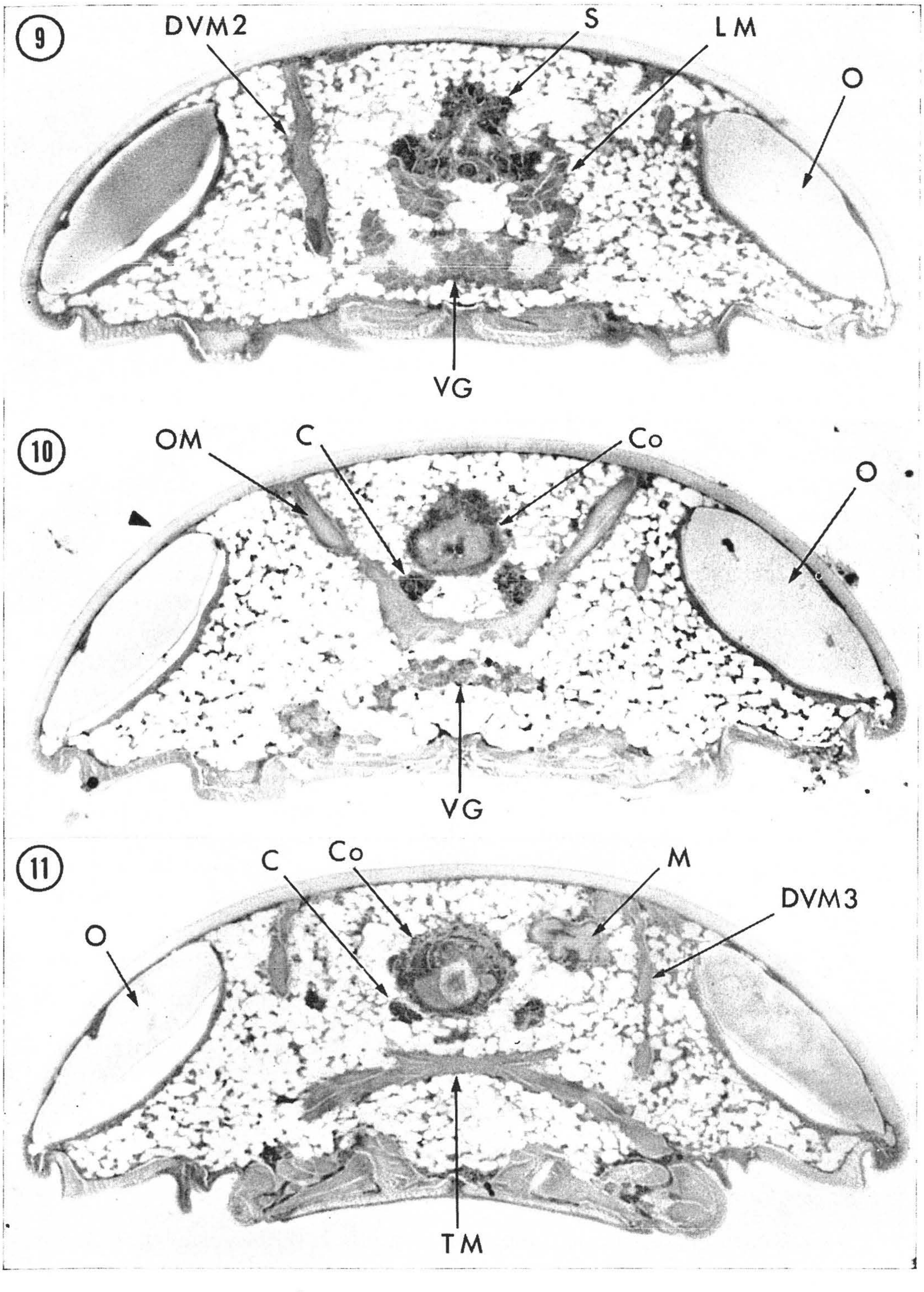

FIG. 9-11 : *Caloglyphus mycophagus* (Mégnin).
9. — Transverse section (T4) of the hypopus × 620. 10. — Transverse section (T5) of the hypopus × 620.
11. — Transverse section (T6) of the hypopus × 620.

seen in this furrow, an outer and larger pair located near the lateral margin and an inner and smaller pair arising close to the mid-line in the dorsal or bottom of the furrow. The free edges of both pairs hang in the furrow and between the inner and outer on each side a tubular " sense organ " projects obliquely into the cavity.

The gonadial tissues (Figs. 2, 3, 4 & 13) first appear just above the inner valve of the furrow where two cords of gonadial tissue meet and fuse. They then separate and run obliquely dorsally to be reflected back along either side of the rudimentary rectum, (Figs. 14 & 15), to end blindly at the level of the posterior dorsoventral muscle, anterad of the anus (Fig. 16). The tissue of the cords consists of many relatively ovoid cells with strongly staining cytoplasm and large, faintly-staining ovoid nuclei (Fig. 24).

4. *The Musculature.*

As the hypopus is different in form from the other stages and as it has a unique ventral sucker plate, its musculature is somewhat different. In this study, eight groups of muscles are identified.

Anterior Oblique Muscles (AM).

Six to eight muscles (Figs. 6 & 7) originate on the dorsal integument just anterad of the dorsal transverse groove. They extend anteriorly and obliquely ventrally to insert on the ventral surface of the anterior portion of propodosoma (Fig. 5).

Dorsal Muscles (DM 1-3).

Three pairs of dorsal muscles (Figs. 2, 3 & 8), originate on the dorsal postpropodosomal apophysis. They extend posteriorly, parallel to the dorsal integument to be inserted on the integument at the level of the middle of the oil vesicle. Each muscle consists of two bands.

Dorsoventral Muscles (DVM 1-6).

Five pairs of dorsoventral muscles originate on the dorsum ; the first pair (DVM 1) arise at the level of the first coxa, extend directly ventrally to be inserted on the base of the first coxa ; the second (DVM 2) (Fig. 9) originate at the level of the anterior one-third of the oil vesicle, and have a ventral insertion on the posterior margin of the second coxa ; the third (DVM 3) originate just laterad of the oblique muscle (OM), extend ventrally to be inserted on the inner margin of the third coxa (Fig. 11). The fourth (DVM 4) originate at the level of the middle of the oil vesicle, extend slightly posteriorly and obliquely laterally to insert on the outer margin of the third coxa ; and the fifth (DVM 5) (Figs. 13 & 14) originate at the level of posterior margin of the oil vesicle, extend mesoventrally to be inserted on the base of the ventral furrow. The sixth (DVM 6) (Fig. 14) originate at the level just behind the oil vesicle, extend lateroventrally to be inserted on the outer margin of the fourth coxa.

Posterior Dorsoventral Muscles (PM).

There are about six pairs of muscles which have a fan-shaped origin on the posterior part of the dorsum, they extend ventrally to be inserted on the sucker disc (Figs. 2-4, 16-18).

Longitudinal Muscles (LM).

A pair of muscles, each of which originates on the anterior wing of the endosternum, extend posteriorly adjacent to the ganglionic mass at the level of the second coxa (Fig. 4). They divide into two groups (Fig. 8), each consisting of several bands of fibres, which re-unite at the level of mid-point of the oil vesicle (Fig. 9) and insert on the muscle-mass formed by the fused oblique and transverse muscles.

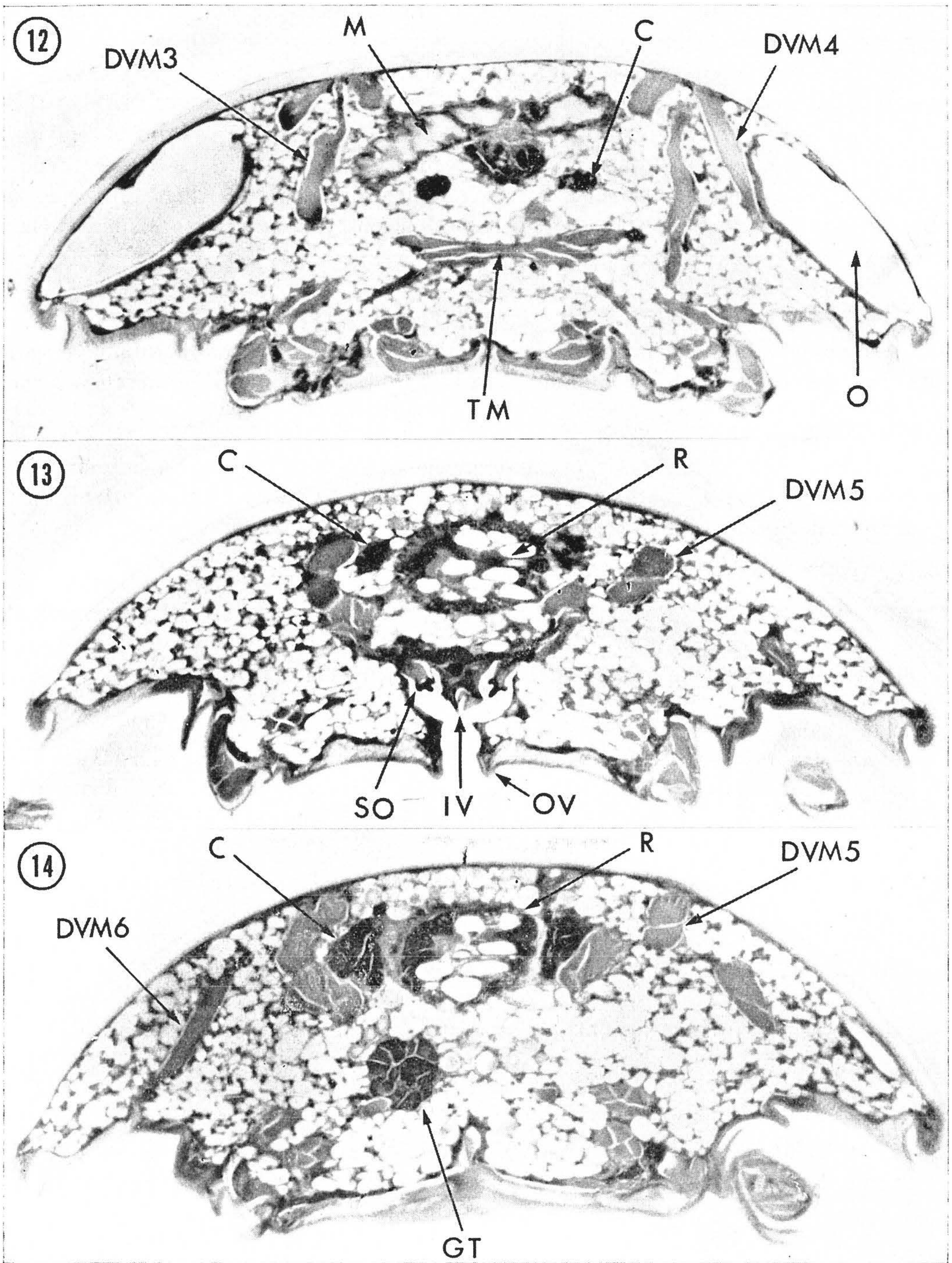

FIG. 12-14 : *Caloglyphus mycophagus* (Mégnin).

12. — Transverse section (T7) of the hypopus × 620. 13. — Transverse section (T8) of the hypopus × 675
14. — Transverse section (T9) of the hypopus × 640.

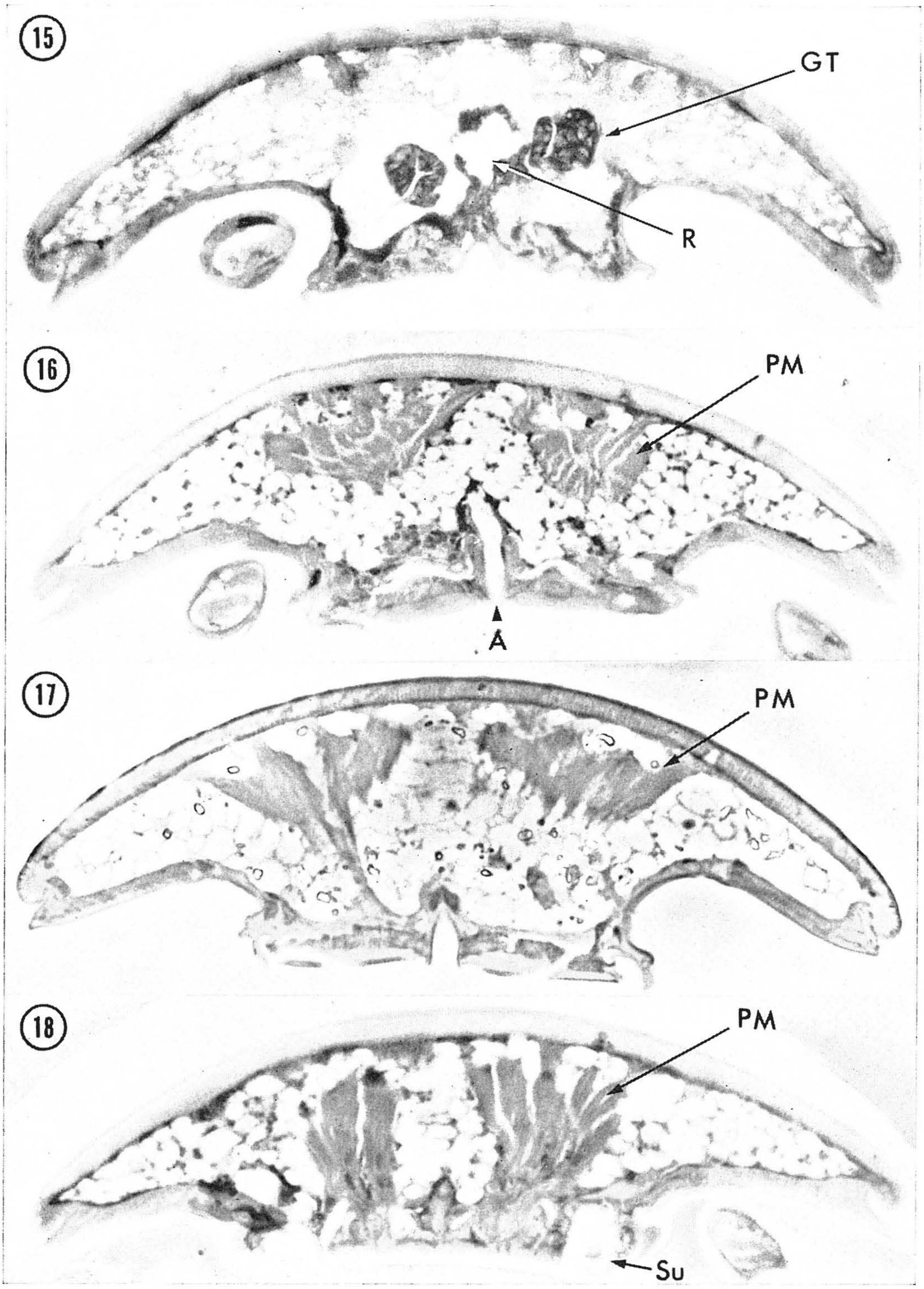

FIG. 15-18 : *Caloglyphus myophagus* (Mégnin).

15. — Transverse section (T10) of the hypopus × 695. 16. — Transverse section (T11) of the hypopus × 695.
17. — Transverse section (T12) of the hypopus × 695. 18. — Transverse section (T13) of the hypopus × 790.

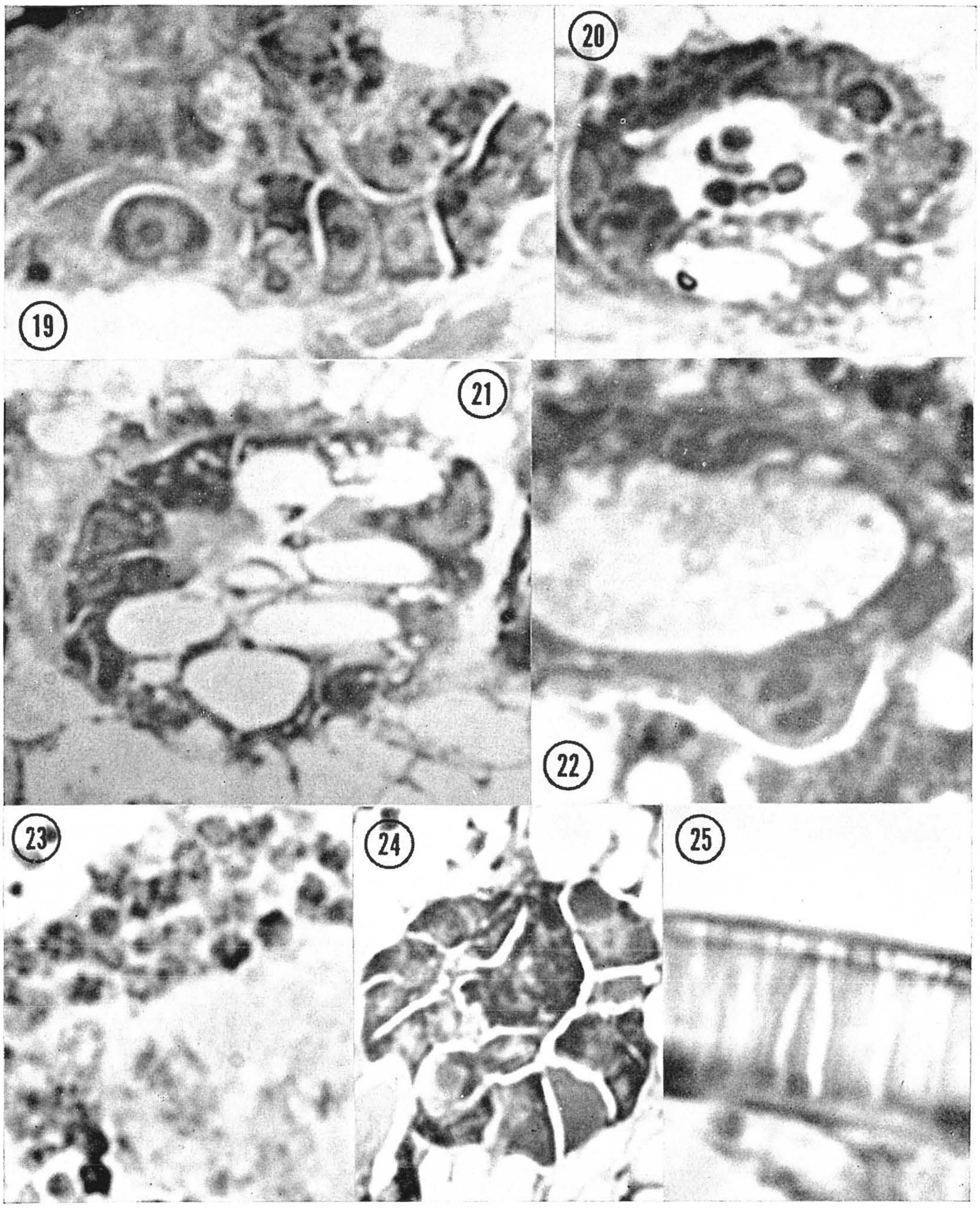

Fig. 19-25 : *Caloglyphus myophagus* (Mégnin).

19. — High magnification of the stomach and caecum × 4 650. 20. — High magnification of the colon region in transverse section × 3 930. 21. — High magnification of the rectal region in transverse section × 1 985. 22. — High magnification of the Malpighian tubules × 3 300. 23. — High magnification of the portion of the central nervous ganglionic mass × 3 150. 24. — High magnification of the gonad tissue × 4 800. 25. — High magnification of a portion of the dorsal cuticle × 3 820.

Oblique Muscles (OM).

At the level of the base of the third coxa or the middle of the oil vesicle, there is a pair of muscles originating on the dorsum slightly mesad of DVM 3. They extend mesoventrally (Fig. 10) and coalesce with the fibres of the transverse muscle (TM) in the centre of the body.

Transverse Muscle (TM).

At the level of the third coxa a single muscle traverses the body between the two coxae (Fig. 11). The muscle bifurcates at each end to be inserted dorsally on the inner and outer margins of the coxae. The common muscle band runs between the suboesophageal ganglion and the gut to join the oblique and longitudinal muscles.

Excretory Pore Muscles.

A single pair of muscles associated with the anal opening arises from the inner margin of the fourth coxa, and extends across above the ventral body cuticle, to be inserted on the lateral margin of the anal opening.

5. *The Integument and Parenchyma.*

Between the internal organs, the haemocoel contains a parenchymatous tissue of spherical or ovoid cells similar to the fat body of other arthropods. Closely applied to, or continuous with the epidermis, many of the cells loose their contents during the preparation for sectioning. In some, however, there are residual inclusions which are either spherical and slightly basophilic or crystalline and pale yellow ; their number is much less than in the similar tissue of the adult.

As in the other stages, there is a pair of lateral " oil vesicles " in the hysterosoma just beneath the dorsal cuticle. Each is surrounded by a very thin layer of chitin and a thin, but deep-staining epidermis. In sections the vesicle appears to be empty (Figs. 9-12) because the contents are dissolved or destroyed in preparation.

Unlike other stages in the life cycle, the hypopus is invested with a heavy, thick cuticle (Figs. 2-18), approximately 5 μ in thickness. It is very difficult to see layers in this thick cuticle, but, numerous fine vertical striations, possibly pore canals, are evident (Fig. 25). Underlying the thick cuticle, is a very thin epidermal layer of less than 1 μ which stains intensely with basic dye, but which shows no distinguishable cellular structure.

Discussion.

The internal anatomy of the hypopial stage of *C. mycophagus* is strikingly different from that of the adult, or any other post-embryonic developmental stage. These are fundamentally similar, except for the reproductive system which is undeveloped before adulthood.

There is no evidence of an oral opening in the hypopus under discussion or in any hypopus that has been studied. Hughes & Hughes (1939) found that there is an invagination which appears in the older hypopus of *Glycyphagus domesticus*, and they believe this to be the prospective buccal cavity and pharynx. Although the regions of the digestive tract can be recognized in the hypopus, the epithelial cells on the wall of the tract are very simple and apparently do not differentiate into the many different types found in the adult. The rudimentary lumen of the alimentary tract and absence of salivary glands are unique to this stage and all evidence suggests that the digestive tract is not functional during the non-feeding hypopial stage. There is no anal opening in the inert hypopus of *G. domesticus* nor in the intermediate type *G. destructor*

(Wallace 1960). Only mobile hypopi have an anal opening, and these also have a highly vacuolated rectal lumen. A possible explanation would be that the higher metabolic rate of more active hypopi necessitates the retention of excretory products by the rectum. On the other hand, it is possible that both the rectum and anus are functional, and the pale-staining materials (Fig. 19) which give the lumen a vacuolated appearance, may be the developing brush border of the rectal epithelial cells which are well developed in the adult stage.

The high degree of fusion of the central nervous system in the hypopus appears to be very similar to the condition in the adult and probably as well as to that of the other postembryonic developmental stages. According to Hughes (1939) and Wallace (1960), further, there is no change taking place in the central nervous system during the metamorphosis. From these it is evident that the central nervous system unlike all other internal organs, is not modified in this particular stage. This is probably a reflection of a free living form on a highly developed nervous system. Furthermore, the nervous system develops so much more slowly than the other organs in ontogeny that the short duration of the moult precludes extensive rearrangements of its tissue. However, additional study on moulting in different species is necessary to confirm this speculation.

The sex ratio of adult *C. mycophagus* which have passed through the hypopus stage is about 1 : 1 (unpublished data). The sexes in the hypopus, however, cannot be distinguished. The gonad tissue enlarges and differentiates into either oocytes or spermatocytes in the tritonymphal stage (Hughes, 1939). Parts of the female reproductive system apparently are well developed in the tritonymph, because copulation can take place between the male adult and female tritonymph.

As Wallace (1960) has pointed out, the muscles of the hypopus can be divided into two groups : those that are present in all stages and those unique to the hypopus. The muscles of *C. mycophagus* common to both adult and hypopus are : dorsal, oblique, in part dorsoventral, transverse, excretory pore, and, according to Wallace (1960), the coxal and leg-segment muscles. Those peculiar to the hypopus are : anterior oblique, posterior dorsoventral, longitudinal and transverse. There is evidence that the anterior oblique muscles of the hypopus become modified to form the gnathosomal muscles in the following stage. The posterior dorsoventral muscles which are associated with the sucker plate are homologus with the copulatory muscles in the adult male. The unusual arrangement of the transverse, longitudinal and oblique muscles that meet in the centre of the body is probably associated with the locomotion of the hypopus which is capable of more rapid and active motion than other stages. In addition to the above-mentioned groups of muscles there are several pairs that are present in the adult stage but do not occur in the hypopus : viz., the ventrolateral, dorsolateral, and genital muscles. The absence of the first two pairs of these might be attributed to the dorsoventral flattening and the heavy sclerolization of this form but as no trace of any of these muscles, nor anything that could be construed as a rudiment, could be found in our sections it is difficult to understand how muscles that were present in the nymphal stage can be reconstituted in the adult. Many features of the hypopus are more than reminiscent of the pupal stage of insects.

Since the oral opening is absent in this stage, the fat body cells of the haemocoele probably supply much of the nutritional requirements of the hypopus. In appearance, the haemocoele and fat body are similar in the adult and hypopus. Wallace (1960), however, claimed that during the protonymphal-hypopial moult a new parenchyma is formed. Differences in the composition and volume of reserves in the fat body between the non-feeding hypopial and other feeding stages can be anticipated. However, further study and analysis are required.

There is no evidence of tracheae or spiracles in either the adult or the hypopus. The small, lightly chitinized body of the adult apparently respires by direct diffusion through the thin and frequently damp integument (HUGHES, 1944). On the basis of insect studies it would appear that air would pass less readily through the thick hypopial cuticle. It is possible that the paired " oil vesicles " on the dorsum are in some way connected with respiration, as claimed by HUGHES (1959).

The hypopi of *C. mycophagus* are sensitive to differences in relative humidity because they will not transform from the hypopial stage into the tritonymph until they are in a region of high humidity (KUO & NESBITT, 1970 B). A similar effect was demonstrated by BAKER (1964) in the studies on the hypopus of *Histiostoma feroniarum*.

The thick sclerotized cuticle in the hypopus may aid water conservation. In this respect the presence of what appears to be pore canals is very interesting. LEE (1947) suggested that cytoplasmic filaments within pore canals were important in the uptake of water in ticks. In insects, such canals are related to the presence of a wax layer (LOCKE 1964) which is essential in preventing dessication. The histochemical method used in this study (Sudan Black B) did not indicate whether there is a wax layer on the cuticle. This may be due to the thin sections which could retain only a small amount of wax on the cuticle and were not picked up by the stain. It is also possible that the cuticle of this stage has an unknown layer, not indicated by any of the histochemical methods used, similar to the wax layer of insects. This is probably necessary because hypopi are normally found in nature adhering to insects, other arthropods and small mammals that can withstand great variation in humidity and temperature as they disperse the hypopi from place to place.

REFERENCES

BAKER (R. A.), 1964. — The further development of the hypopus of *Histiostoma feroniarum* (Dufour, 1839) (Acari). — Ann. Mag. Nat. Hist. Ser. 13, **7** : 693-695.

HUGHES (T. E.), 1943. — The respiration of *Tyroglyphus farinae.* — J. Exp. Bio., **20** : 1-5.

HUGHES (T. E.), 1959. — Mites or the Acari. — The Athlone Press, London.

HUGHES (T. E.) and HUGHES (A. M.), 1939. — The internal anatomy and postembryonic development of *Glycyphagus domesticus* DeGeer. — Proc. Zool. Soc. London B., **108** : 715-733.

KUO (J. S.) & MCCULLY (M. E.), 1969. — Preparation of thin sections of mites for high resolution light microscopy. — Can. J. Zool., **47** : 737-739.

KUO (J. S.) & NESBITT (H. H. J.), 1970. — The internal morphology and histology of the adult of *Caloglyphus mycophagus* (Mégnin) (Acarina : Acaridae). — Can. J. Zool., **48** : 505-518.

KUO (J. S.) & NESBITT (H. H. J.), 1970 b. — Termination of the hypopial stage in *Caloglyphus mycophagus* (Mégnin) (Acarina : Acaridae). — Can. J. Zool., **48** : 529-537.

LEES (A. D.), 1947. — Transpiration and the structure of the epicuticle in ticks. — J. Exp. Biol. **23** : 379-410.

LOCKE (M.), 1964. — The structure and formation of the integument in insects. *IN* The physiology of insecta. — Vol. **3**, *Edited by* M. Rockstein. Academic Press, New York., pp. 380-470.

WALLACE (D. R. J.), 1960. — Observations on hypopus development in the Acarina. — J. Insect Physiol., **5** : 216-229.

Abbreviations used in Figures.

A	—	Anus
AM	—	Anterior oblique muscle
C	—	Caecum
Co	—	Colon
DM	—	Dorsal muscle
DP	—	Developing palpus
DVM1	—	First Dorsoventral muscle
DVM2	—	Second dorsoventral muscle
DVM3	—	Third dorsoventral muscle
DVM4	—	Fourth dorsoventral muscle
DVM5	—	Fifth dorsoventral muscle
GT	—	Gonad tissue
IV	—	Inner valve
LM	—	Longitudinal muscle
L1	—	First pair of legs
L2	—	Second pair of legs
M	—	Malpighian tubules
O	—	Oil vesicle
Oe	—	Oesophagus
OM	—	Oblique muscle
OV	—	Outer valve
PM	—	Posterior dorsoventral muscles
R	—	Rectum
SbG	—	Suboesophageal ganglion
SpG	—	Supraoesophageal ganglion
S	—	Stomach
So	—	Sense organ
Su	—	Sucker
TM	—	Transverse muscle
VG	—	Ventral ganglion