The Development of the Malpighian Tubules in *Dysdercus koenigi* (Hemiptera, Pyrrhocoridae)

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With two plates (figs. 5 and 7)

Summary

Dysdercus koenigi has two pairs of Malpighian tubules, each pair arising from a vesicle and forming a closed loop. The vesicles are connected dorsally, and the tubules are differentiated into proximal and distal regions with refractive granules in the latter and brush border in both.

In winters at Allahabad the embryonic period lasts about a fortnight, and on the 9th day, 4 small buds arise apparently from the anterior end of the proctodaeum. These grow rapidly and become tubular; their tips fuse, but the lumina remain separate. At this stage the met-enteric membrane (Henson, 1946*a*) stretches over the anterior end of the proctodaeum and an interstitial ring is differentiated, so that the zone of the origin of the tubules lies anteriorly to it and posteriorly to the met-enteric membrane. Meanwhile the basal part of each tubule swells and the two swellings of each side fuse, thus forming a vesicle on each side. A dorsal outgrowth from each vesicle meets its fellow of the opposite side. The tubules continue to elongate, at the same time becoming narrower, and ultimately only 3 cells can be seen in a cross-section. The tubules grow in 3 phases—(1) active mitosis, (2) of cell multiplication and cell rearrangement, and (3) of cell rearrangement and cell enlargement. Finally, the tips of each pair fuse completely and their lumina become continuous.

In the newly hatched first nymph the tubules are uniform throughout, but in the later part of this stage they become differentiated into proximal and distal regions and the striated or brush borders appear. Thus the embryonic development continues for some time in the first nymph. Once this condition has been reached, cyclical changes involving increase in the diameter of the tubules, in the size of the nuclei, and in the number of cells in the proximal region occur in each nymphal stage, but before each ecdysis the diameter decreases slightly. Thus the adult condition is reached in 5 distinct steps, corresponding to the 5 instars.

Henson's view that Malpighian tubules are endodermal is considered plausible, but his view regarding the primitive number of tubules is not accepted.

Introduction

AMONG the more important works on the embryonic development of the Malpighian tubules of insects are those of Wheeler (1889) on *Blatta germanica* and *Doryphora decemlineata*, Carrière (1890) on *Chalicodoma*, Nelson (1915) on *Apis*, Samson (1908) on *Heterogenea limacodes* and Ikeda (1913) and Ito (1921) on *Bombyx mori*. Later, Henson in a series of papers (1937, 1944, 1946) described the development of Malpighian tubules in *Pieris brassicae*, *Blatta orientalis*, and *Forficula auricularia*. Contradicting the earlier opinion, he stated that they are endodermal in origin. Drummond (1936), Thomas (1936), Paterson (1936), Mellanby (1937), and Butt (1949)

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referred to the development of the tubules in the course of their embryological works on *Ephestia*, *Carausius*, *Corynodes*, *Rhodnius*, and *Oncopeltus* respectively. Of these, except Butt, who is uncertain, all regard the tubules as ectodermal. Recently Savage (1956) has described the development of the tubules in *Schistocerca gregaria* and supported Henson.

It is thus clear that we are still far from certain regarding the fundamental nature of the tubules; and in the Hemiptera there has been no exhaustive study so far. In the present paper, therefore, an attempt has been made to furnish observations on the embryonic and post-embryonic development in a typical Heteropteran, *Dysdercus koenigi* (Fabr.) and to compare the results with such facts as we possess about other insects.

Material and Technique

Specimens of *D. koenigi* were reared under normal laboratory conditions during the months of December and January, when the outside temperature ranged from 6·5° C to 27° C. The eggs were removed soon after they were laid, kept separately, and fixed at regular intervals. Among the fixatives used, cold Carnoy's gave the best results. To ensure complete penetration of the fixative, the shell was carefully removed with fine needles after the egg had been kept in the fixative for some time. Transverse and longitudinal sections were cut at 5 to 8 μ; they were stained in Delafield's haematoxylin and sometimes counterstained with eosin.

Observations

The adult

D. koenigi has two pairs of Malpighian tubules, each pair arising from a small, laterally placed vesicle and forming a closed loop with the distal ends joined with each other (fig. 1). The vesicles are connected with each other dorsally, and each vesicle opens into the lower part of the mid-gut. The tubules are differentiated clearly into distal and proximal regions, with abundant granules in the cells of the former. Both the regions show an inner brush border.

Embryonic development

In December and January the embryonic development of *Dysdercus* is completed in about 15 days. When the embryo is about 9 days old, the Malpighian tubules make their first appearance as 4 minute buds (fig. 5, A) from the lateral and ventro-lateral sides of the anterior region of the hind-gut. This may be designated as the embryonic zone, as active cell-division takes place in it for some time (figs. 3, A; 5, B). The buds consist of 17 to 20 columnar cells measuring about 14 μ tall and arranged in a single layer (fig. 2, A). The cell-walls are not distinct. The lumen of the bud is only 7 μ wide. There is no indication of a striated border on the inner margin of the cells. The nuclei, which are large and basiphil, measure 5 by 4 μ. Many mitotic figures are seen

in longitudinal sections; this shows that active longitudinal cell-division is in progress.

In about an hour the length of the bud increases to about 64 μ, but its diameter and the number of cells in a cross-section remain nearly the same.

In an embryo 9 days, 8 h old, each tubule is about 90 μ long and about 36 μ in diameter. The tips of the two tubules of each side now join, although their

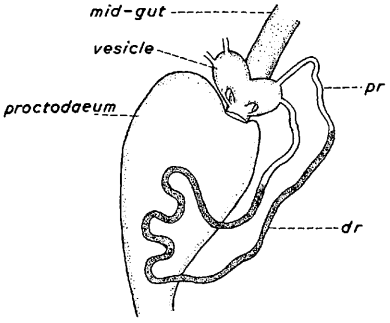

FIG. 1. Diagrammatic representation of the posterior part of the alimentary canal of adult *Dysdercus koenigi*, showing the arrangement of the Malpighian tubules. *dr*, distal region of the Malpighian tubule; *pr*, proximal region of the Malpighian tubule.

lumina remain separate (fig. 5, B). The tubules retain the histological character described in the earlier stage. Longitudinally arranged mitotic figures are still observed; this is correlated with increase in the length of the tubules. Sections at this stage show that the anterior walls of the tubules are continuous with the wall of the mid-gut and the posterior wall with the wall of the hind-gut. A thin membrane stretches across the anterior end of the proctodaeum (figs. 3, B; 5, F). It marks the posterior end of the mid-gut and is called the met-enteric membrane (Henson, 1946*a*). In an embryo 9 days, 6 h old, the region of the union of the posterior walls of the tubules and proctodaeum differentiates in a region distinct histologically from the mid-gut as well as from the proctodaeum: this is called the posterior interstitial ring (figs. 4; 5, H). Later, it develops into the proctodaeal valve. Mitotic division occurs in this ring. It may be noted that the tubules now lie anteriorly to this ring.

In an embryo 9 days, 12 h old, the basal part of each tubule has swollen on account of active cell-division (figs. 3, C; 5, G), and subsequently, on account of greater growth in the dorsal and ventral walls of the swellings of the upper and lower tubules of the same pair, they have come close and finally fused, thus forming a single vesicle from which both the tubules of a pair now appear to arise (fig. 5, B, C). Later an outgrowth arises towards the median line from the dorsal side of each vesicle. The two outgrowths meet each other. The

met-enteric membrane cannot be identified at this stage and the cells forming the vesicle resemble those of the rest of the Malpighian tubule. The tubules are straight, about 108 μ long and 28 μ in diameter, with 12 to 15 cells in a cross-section. Mitotic division still continues (fig. 2, B). The decrease in the number of cells in cross-sections, without cessation of mitosis, shows that the

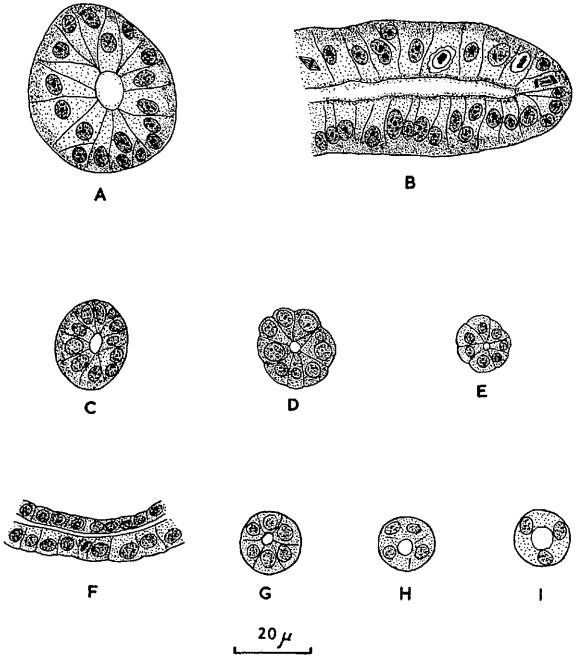

FIG. 2. T.S. and L.S. of the Malpighian tubules of embryos of different ages. A, T.S. at 9 days, 3 h stage. B, L.S. of apical part at 9 days, 12 h stage. C, T.S. at 9 days, 16 h stage. D, T.S. at 10 days, 12 h stage. E, T.S. at 11 days, 12 h stage. F, L.S. at 11 days, 20 h stage. G, T.S. at 12 days, 20 h stage. H, T.S. at 13 days, 12 h stage. I, T.S. at 14 days, 3 h stage.

processes of cell arrangement and cell production are now progressing simultaneously.

In an embryo 9 days, 6 h old, the length of each tubule has increased to about 136 μ and it has become slightly twisted in the form of an inverted S. Its diameter has decreased further to about 25 μ and there are only 10 to 12 cells in a cross-section (fig. 2, C). The vesicles have become further enlarged and their outer walls have become covered by thin muscles.

By the time the embryo is 10 days, 10 h old, each tubule measures about 256 μ long and about 19 to 22 μ in diameter. It is now coiled to form a few loops. The lumina of the two tubules of a pair are still separate at their tips.

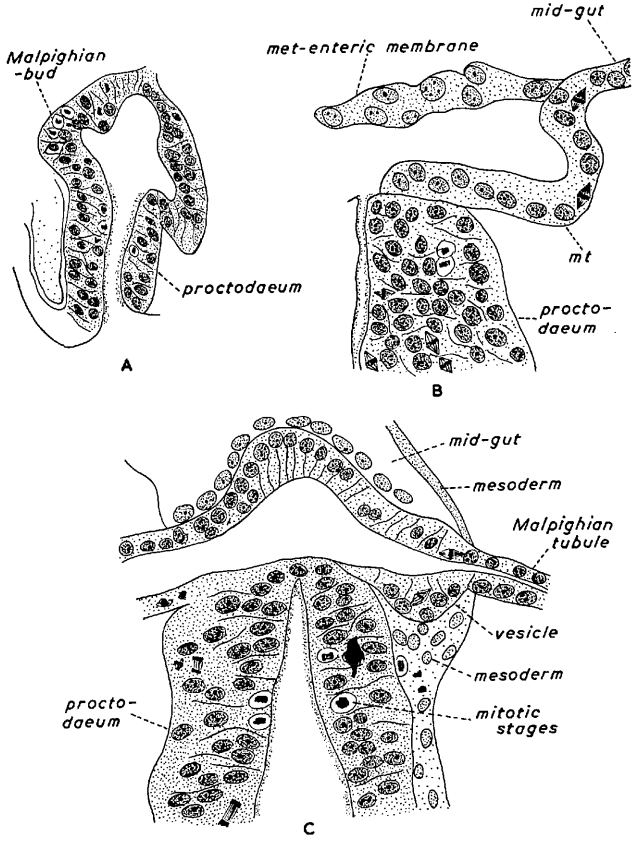

FIG. 3. L.S. of the proctodaeum of different stages. A, Malpighian buds at 9 days, 4 h stage. B, met-enteric membrane at 9 days, 8 h stage. C, formation of vesicle at 9 days, 12 h stage.

In an embryo 10 days, 12 h old, the tubules have 8 to 10 cells in a cross-section (fig. 2, D). Mitotic figures are still seen and the vesicles are better differentiated.

In an embryo 11 days, 12 h old, the length of the tubules has increased to

about 0·8 mm and the diameter has decreased to 14 μ. Only 6 to 8 cells are seen in cross-sections (fig. 2, E). There is no other change in the histology of the tubules or the vesicles.

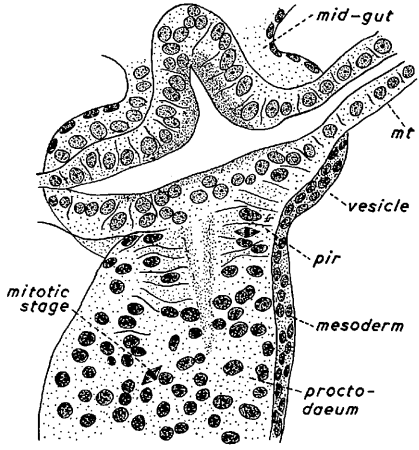

FIG. 4. L.S. of the proctodaeum at the 9 days, 12 h stage, showing the posterior interstitial ring. *mt*, Malpighian tubule; *pir*, posterior interstitial ring.

In an embryo 12 days old, the two tubules of a pair have fused completely and their lumina have become continuous, thus forming a complete loop (fig. 5, D), about 1·92 mm long. The diameter is reduced to about 13 μ and only 6 cells are seen in cross-sections. Cell production now ceases. The striated border has not developed yet. Therefore the tubules are now anatomically similar to, but histologically dissimilar from, those of the adult.

In an embryo 13 days, 12 h old, each loop of the Malpighian tubules measures about 3·2 mm long and 13 μ in diameter, and there are only 3 or 4 cells in cross-sections (fig. 2, H).

FIG. 5 (plate). A to D, photomicrographs of whole mounts at different stages.

A, Malpighian buds at 9 days, 3 h stage (under phase contrast).

B, Malpighian tubules and proctodaeum at 9 days, 16 h stage, showing vesicles and the fusion of tubules at the distal ends.

C, origin of the tubules from the vesicles at the same stage.

D, Malpighian tubules at the 12 days stage, showing complete fusion at the distal ends.

E to H, photomicrographs of longitudinal sections of the proctodaeum.

E, at 9 days, 4 h stage, showing the Malpighian buds.

F, part of L.S. at 9 days, 8 h stage, showing the met-enteric membrane.

G, at 9 days, 12 h stage, showing the formation of a vesicle.

H, part of L.S. at 9 days, 16 h stage, showing the posterior interstitial ring.

lp, loop; *m*, mitotic stage; *mb*, Malpighian bud; *mem*, met-enteric membrane; *mt*, Malpighian tubule; *pir*, posterior interstitial ring; *v*, vesicle.

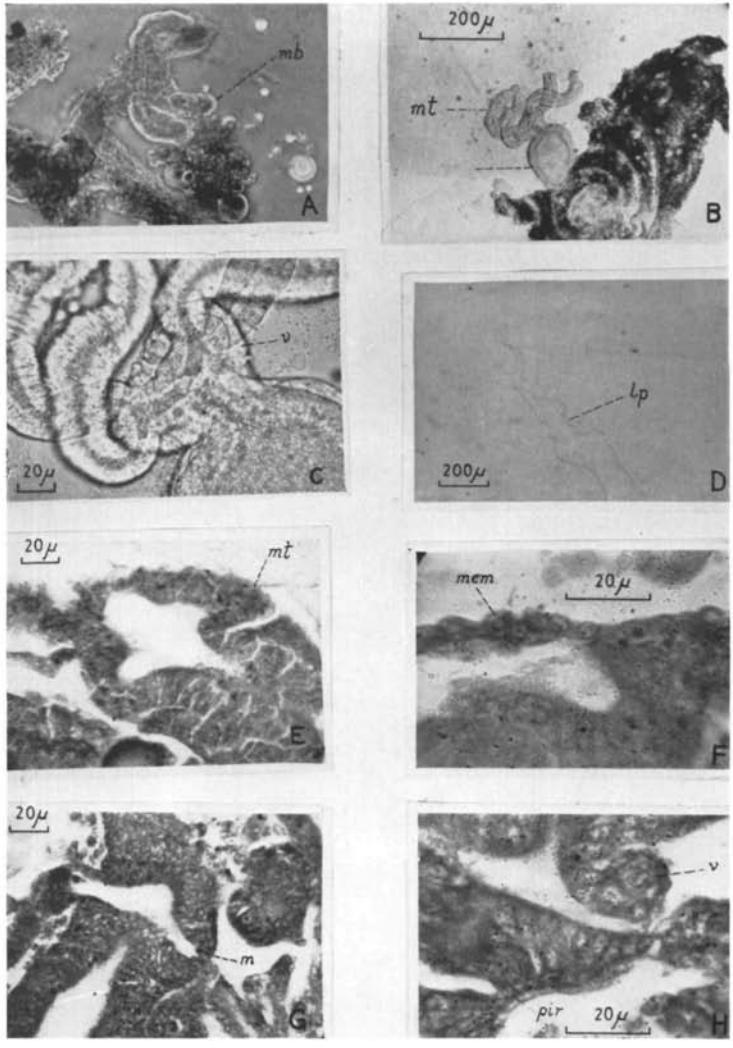

FIG. 5

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By the time the embryo is 14 days, 3 h old, the loops become about 3·52 mm long and about 13 μ in diameter, with only 3 cells in cross-sections (fig. 2, I).

In an embryo 14 days, 17 h old, i.e. a few hours before hatching, the tubules have all the characteristics of the tubules of the adult apart from the differentiation of the striated border and of the proximal and distal regions.

It will be seen that the entire period of embryonic development can be divided into 3 phases. In the first phase of short duration, the tubules appear and increase in length by active mitosis; in the second phase, both cell production and cell rearrangement occur, and increase in the length of the tubule is accompanied by reduction in diameter; in the third phase, cell production ceases and cell rearrangement and cell enlargement take place so that while the diameter of the tubules remains constant, their length increases and at the same time the number of cells in a cross-section decreases.

During the embryonic development of the Malpighian tubules the nuclei show a steady reduction in size. The nuclear size in the different stages, along with the length, diameter, number of cells in cross-sections of the tubules, &c., are given in table I (see appendix).

Post-embryonic changes

In the first nymphal stage the Malpighian tubules, in the newly hatched condition, are devoid of the striated border (fig. 7, A) and of differentiation into proximal and distal regions (fig. 6, A, B). There are 3 cells in a cross-section, each about 5 μ in height, and the lumen is only about 6 μ in diameter. The oval, basophil nuclei measure about 4 by 3 μ. In 2 days, 12 h the diameter and length have increased to about 25 μ and 4·48 mm respectively. As the nymph advances further in age, the size of the cells and the length of the tubules increase further, and at the same time the tubule is differentiated into proximal and distal regions on account of the deposition of certain refractive granules in the cells of the latter.

Meanwhile, the inner margin of the cells becomes frilled, and in a nymph of 3 days it is broken to form the brush border.

In the fully developed first nymph, the diameter of the tubules has increased to 25 μ; but shortly before ecdysis, it is again reduced to about 22 μ. The size of the nuclei remains more or less the same throughout the life of the first nymph.

In the second nymphal stage for some time after the ecdysis, the length and diameter of the Malpighian tubules remain the same as at the time of the ecdysis, but later they increase rapidly to a maximum of 7·36 mm and 40 μ respectively. Both the length and the diameter of the tubules will now remain stationary until before the next ecdysis. The diameter of the nuclei also increases from 5 μ to 6 μ in 2 days, and this diameter persists for the rest of this nymphal stage.

In the 1 day, 12 h second instar, the differentiation between the proximal and distal regions is more distinct (fig. 7, B). The latter region, besides being

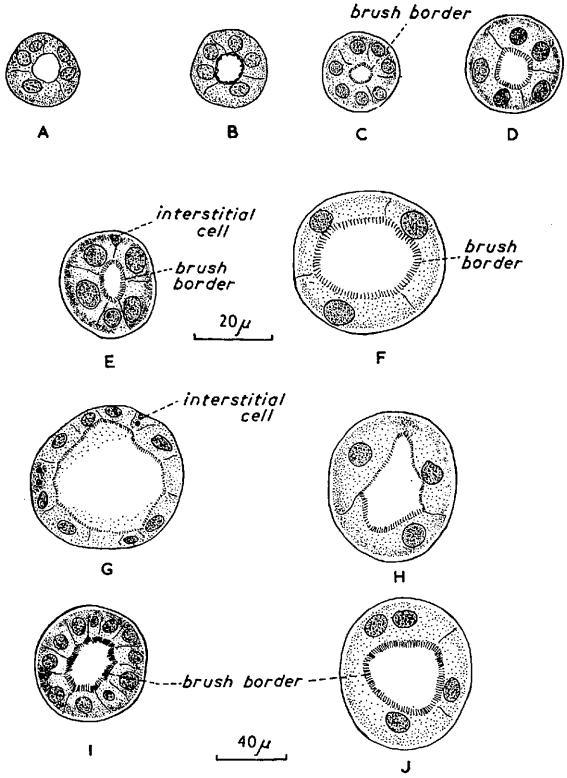

FIG. 6. T.S. of the Malpighian tubules of different stage nymphs. A, of 12 h old first nymph. B, of 3 days old first nymph. C, of proximal region of second nymph. D, of distal region of second nymph. E, of proximal region of third nymph. F, of distal region of third nymph. G, of proximal region of fourth nymph 4 h before the next moult. H, of distal region of fourth nymph 4 h before next moult. I, of proximal region of fifth nymph. J, of distal region of fifth nymph.

packed with granules in the cytoplasm, is now wider (about 24 μ), has 3 cells in cross-section (fig. 6, D), each about 8 μ tall and containing nuclei with a diameter of about 5 μ. The proximal region is narrower (about 18 μ in diameter), and has 7 cells in cross-section, each only about 6 μ tall (fig. 6, C). A brush border is seen in both the regions.

In the third nymphal stage and subsequent instars, there is no marked histological change in the tubules but the distal region is packed with more

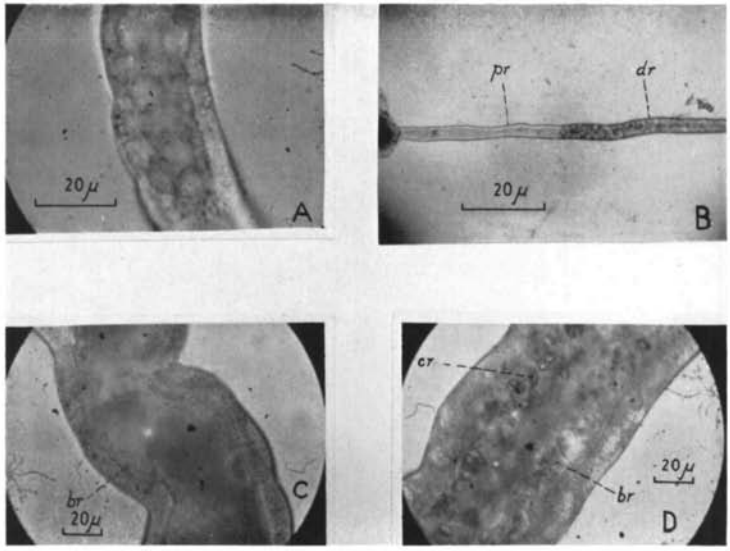

FIG. 7

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granules, and in the proximal region 5 to 8 cells, and a few interstitial cells, are seen in cross-section. Mitotic divisions and the replacement of main cells by interstitial cells were, however, not observed. The diameter of the distal part gradually increases and finally reaches a maximum of 61 μ in the 6 days, 12 h nymph. The lumen increases proportionately and the oval nuclei which measured 8 μ by 5 μ become spherical and have a diameter of 11 μ. The diameter of the proximal part increases likewise and the spherical nuclei measure 6 μ in diameter (fig. 6, E, F). During this stage the length of each loop increases to approximately 11·2 mm.

In the fourth nymphal stage the length of each Malpighian loop increases to about 15 mm, its maximum diameter to about 83 μ, and the diameter of the nuclei to 13 μ. In the distal region there are still 3 cells in a cross-section with numerous and larger granules in the cytoplasm (fig. 6, H), whereas in the proximal region there are 8 to 12 cells in a cross-section (fig. 6, G). Interstitial cells are present in the proximal region and brush border is seen clearly in both the regions (fig. 7, C, D).

In the fifth nymphal stage, which is much longer, considerable increase in the length and diameter of the tubules is brought about by enlargement of cells. Each loop reaches a length of about 30 mm and the diameter of the tubule, which was 72 μ after the last ecdysis in the distal region, reaches a maximum of about 101 μ. The spherical nuclei again become oval and have a size of 22 μ by 14 μ. The distal region has only 3 cells in a cross-section; these are 14 μ tall and have a lumen about 40 μ wide. The cells are stuffed with large refractive granules (fig. 6, J). The proximal region has 8 to 13 cells in cross-section, the cells being about 16 μ tall; the lumen is about 22 μ wide (fig. 6, I). The cytoplasm is dense and has only a few granules. Interstitial cells are seen here and there.

The increase in the length and maximum diameter of the Malpighian tubules in the different stages of the 5 nymphal instars are shown in tables 2 and 3 (see appendix).

Discussion

Wheeler (1889) stated that the Malpighian tubules of *Blatta* and *Doryphora* arise from the proctodaeum and are thus ectodermal, and Carrière (1890) and Nelson (1915) reported that in *Chalicodoma* and *Apis* respectively they arise from the ectoderm before the proctodaeum is formed. But Tirelli (1929) showed that these tubules arise from an undifferentiated zone between the hind-gut and mid-gut, and in 1932 Henson postulated that the proctodaeal

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Fig. 7 (plate), Photomicrographs of parts of Malpighian tubules at different stages.
A, in the first nymph, showing absence of striated border.
B, in the second nymph, showing two regions of a tubule.
C, in the distal region of the fourth nymph, showing brush border.
D, in the proximal region of the fourth nymph, showing brush border and crystals.
br, brush border; *cr*, crystal; *dr*, distal region of Malpighian tubule; *pr*, proximal region of Malpighian tubule.

invagination of insects, like the blastoporal lip of *Peripatus*, is a complex structure. In *Pieris brassicae*, he stated that the posterior interstitial ring and the proctodaeum behind it are ectodermal, while the tissue anterior to the former is endodermal, and so the Malpighian tubules arising from the former are also endodermal. In *Blatta* he (1944) showed that the membrane covering the anterior end of the proctodaeum represents the junction of mid- and hind-gut and therefore the Malpighian tubules, arising anteriorly from this zone, are endodermal. He also homologized the embryonic zone with the posterior interstitial ring of higher Holometabola and labelled the region lying between the mesenteron proper and the hind-gut as the met-enteron and the membrane covering the hind-gut as the met-enteric membrane.

Recently Savage (1956) has supported the idea of endodermal origin of Malpighian tubules in *Schistocerca*, but Drummond (1936) in Lepidoptera, Thomas (1936) in Orthoptera, Paterson (1936) in Coleoptera, and Mellanby in Hemiptera had all described an ectodermal origin. Drummond and Mellanby found that the Malpighian tubules arise from the anterior end of the proctodaeum; Thomas regarded the partition membrane between the mid-gut and hind-gut itself to be ectodermal, and Paterson did not observe the interstitial ring and so regarded the entire proctodaeum as ectodermal. Butt (1949) described the development in the bug *Oncopeltus*, which is similar to Mellanby's, but he considered Henson's view possible.

The present study does not provide sufficient material to permit a detailed evaluation of Henson's views, but it may be mentioned that we did observe in the proctodaeum of *Dysdercus* a specially active zone from which the Malpighian tubules arise, and a region posterior to this zone and behind the Malpighian tubules is later differentiated into a ring which is similar to the interstitial ring of *Vanessa* and the embryonic zone of *Blatta*, although it does not bud off secondary tubules. Besides, the met-enteric membrane covering the proctodaeum anteriorly and the Malpighian tubules are histologically similar to the mid-gut. Therefore Henson's views regarding the endodermal origin of Malpighian tubules appear highly probable.

As regards the sequence of the division, rearrangement, and enlargement of the cells, Savage (1956) concluded that the sequence noted in *Pieris* (Henson, 1937), in which the phase of cell proliferation precedes the phase of cell rearrangement and enlargement, is more advanced than that of *Blatta* (Henson, 1944) and *Schistocerca* (Savage, 1956) in which cell proliferation and rearrangement occur before cell enlargement. This view appears to be based on the ground that the former type of sequence is seen in more advanced insects. In *Dysdercus* 3 phases are noted—those of cell production, of cell production and rearrangement, and of cell rearrangement and enlargement. Thus the processes are overlapping and somewhat similar to those of *Pieris*. But *Forficula* (Henson, 1946*a*) also resembles *Pieris* in this respect, and so this type of sequence cannot be considered advanced. The question really seems to be related to the presence or absence of secondary Malpighian tubules: in insects in which secondaries are absent (e.g. *Pieris*, *Dysdercus*), the Malpighian tubules

continue to elongate for a long time and cell rearrangement and enlargement continue together, whereas in insects in which secondaries make their appearance to meet the increasing excretory requirement, cell enlargement occurs only for a short time after cell rearrangement has been completed.

Wheeler (1893) regarded the primitive number of Malpighian tubules in insects to be 6, but Henson (1944) believed that the primitive condition consists of 6 primary and numerous secondary tubules, the development of the latter being suppressed in the higher Pterygota. Savage (1956) thought that *Schistocerca* shows the latter condition. It is true that the secondaries are seen in lower insects, but when present, they develop much later than the primaries. Besides, *Schistocerca* possesses a larger number of secondaries than *Blatta* and *Forficula*. Therefore it seems possible that the primary and secondary tubules do not have an identical nature, and that the primaries, appearing much earlier in ontogeny, represent the primitive tubules. If so, Wheeler's concept regarding the primitive number of tubules would be correct, and the addition of the secondaries may be related to the increased excretory requirement which cannot be met by the primaries.

In *Blatta*, *Pieris*, and *Schistocerca* the striated border develops on the inner margin of the cells during embryonic development, while in *Dysdercus* this development and the differentiation of the proximal and distal regions takes place in the first nymphal stage. Evidently the true embryonic life does not terminate with emergence, but continues in the first nymph. Such a view was expressed earlier by Henson (1929, 1946). Further changes involving growth and differentiation are cyclical and repeated in each nymphal stage, as observed already in *Blatta*, *Forficula* (Henson, 1944, 1946), and *Schistocerca* (Savage, 1956). But while the increase in the size of the nucleus is gradual in *Dysdercus* and *Schistocerca*, it occurs suddenly in the third instar in *Forficula* and in *Pieris* it is accompanied with lobulation and ramification. These differences are not properly understood, but it is not unlikely that the increase in the nuclear size or surface area is related to the increase in the size of the cells.

A few words may be added here about the appearance of granules in the distal region of the tubules in each nymphal instar as it starts feeding. These are flushed into the lumen towards the end of each instar, pass into the hindgut and contribute to the formation of the meconium. The appearance of the granules in the active, feeding nymph and their elimination at each moult show that they are excretory products.

APPENDIX

TABLE 1

Condition of the embryonic Malpighian tubules at various stages of development of the embryo

Age of embryo	*Length of tubules* (mm)	*Diameter of tubules* (μ)	*No. of cells in a T.S.*	*Size of nucleus in each cell* (μ)	*Appearance of vesicles*	*Whether tubules free or fused*
9 days, 3 h	0·04	36	17–20	5·4×3·6	vesicle absent	free
9 days, 8 h	0·09	36	up to 20	,,	,,	joined at tips but lumen separate
9 days, 12 h	0·108	28	12–15	4·8×4·8	formation starts	,,
9 days, 16 h	0·136	25	10–12	4·8×4·8	vesicle formed	,,
10 days, 10 h	0·256	19 to 22	8–10	4×4	vesicle present	,,
10 days, 16 h	0·288	,,	,,	,,	,,	,,
11 days	0·352	,,	,,	,,	,,	,,
11 days, 12 h	0·800	14	6–8	,,	,,	,,
12 days*	1·92	,,	,,	,,	,,	fusion complete; lumen continuous
12 days, 22 h	2·88	13	6	3·2×3·2	,,	,,
13 days, 12 h	3·20	,,	3–4	,,	,,	,,
14 days, 3 h	3·52	,,	3	3·2×2·4	,,	,,
14 days, 17 h (a few h before hatching)	,,	,,	,,	,,	,,	,,

* At this stage the two tubules of a pair have united to form a loop. Therefore subsequent figures relate to the measurement of a loop.

TABLE 2

Gradual increase in the length of the Malpighian tubules in various nymphal stages

Nymphal stage	*Length of a loop of Malpighian tubules in the young nymph* (mm)	*Length of a loop in the middle-aged nymph* (mm)	*Length of a loop in the old nymph* (mm)
1st nymph	3·5	4·2	4·5
2nd nymph	4·5	6·4	7·4
3rd nymph	8·1	10·6	11·2
4th nymph	12·8	14·7	15·0
5th nymph	15·2	22·7	30·2

TABLE 3*

Diameter of the Malpighian tubules at different stages of life-history

1st nymph	μ	*4th nymph*	μ
Newly hatched	16	After last ecdysis	58
12 h after hatching	22	After 12 h	79
2 days and 12 h old	25	3 days later	83
Immediately before ecdysis	22	1 day before ecdysis	79
		Immediately before ecdysis	72
2nd nymph			
Soon after last ecdysis	22	*5th nymph*	
2 days later	29	After last ecdysis	72
4 days later	40	1 day later	83
6 days later	29	2 days later	94
Immediately before ecdysis	29	Old nymph	101
3rd nymph			
Soon after last ecdysis	40		
3 days later	54		
After 6 days, 12 h	61		
Immediately before ecdysis	58		

* All measurements have been taken from whole mounts and in the distal region.

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