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The structure and development of the Malpighian tubules of an aquatic bug: *Sphaerodema rusticum* LINNAEUS

(Hemiptera)

With 4 textfigures

Introduction

The embryonic developments of the Malpighian tubules of insects have already been described by various workers and most of them have considered the tubules to be of ectodermal origin. But HENSON in a series of papers (1937, 1944, 1946) described the development of the Malpighian tubules in *Pieris brassicae*, *Blatta orientalis* and *Forficula auricularis* and considered the tubules to be of endodermal origin. His finding has been further strengthened by the works of SAVAGE (1956) on *Schistocerca gregaria* and SRIVASTAVA & BAHADUR (1961) on *Dysdercus koenigi*.

In the aquatic heteropterans the number of the tubules has been considered to be two (LOCY, 1884; HAMILTON, 1931) and even in *Sphaerodema rusticum* PRESSWALLA & GEORGE (1936) believed that there are only two Malpighian tubules. Recently BAHADUR (1961) has shown in a few aquatic bugs that morphologically the number of the tubules is four and not two. The views of different workers are, therefore, conflicting.

The present paper not only deals with the developmental sequence of the Malpighian tubules but also furnishes embryological evidence on the origin and the number of the tubules which are themselves controversial issues.

Material and Technique

Specimens of *Sphaerodema rusticum* were collected from the ponds and kept in large aquaria containing pond water. The females laid eggs which were attached over the abdomens of the males. The entire embryonic development, therefore, takes place under parental care. The eggs were removed from the clusters and fixed at regular intervals. The eggs of the various clusters were kept separately. Among the fixatives used, cold Carnoy's fluid gave the best results. Before treating the materials for sectioning, the upper corions were removed under stereoscopic binocular microscope. Transverse and longitudinal sections were cut at 5 to 8 μ and stained with Delafield's haematoxylin and counter stained with eosin. Living eggs were also dissected out under normal saline to follow the sequence of the development.

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Observations

The adult Malpighian tubules

In the adult *Sphaerodema rusticum* the Malpighian tubules arise from the extreme posterior end of the barrel shaped fourth region of the mid gut. In a longitudinal section, the proctodael valve marks the beginning of the hind gut and the Malpighian tubules lie anterior to it. The tubules run in a coiled manner around the alimentary canal. They are apparently four in number and are arranged in pairs and join each other at their distal ends, near the basal portion of the rectal caecum, to form a cross (Fig. 1).

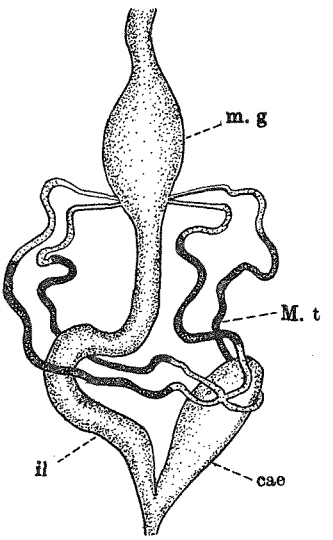

Fig. 1. Diagrammatic representation of the posterior part of the alimentary canal of *Sphaerodema rusticum* to show the arrangement of the Malpighian tubules. (*cae*, caecum. — *m.g*, mid gut. — *M.t*, Malpighian tubule. — *il*, ileum)

Each tubule, which shows four distinct regions (Fig. 2A), measures about 63.7 mm in length from the opening into the gut to the cross. The first region, nearest to the gut, is transparent and measures about 4.9 mm in length. It has a thin wall and hence shows a wider lumen but the diameter of the tubule is smaller. This part of the Malpighian tubule is known as the capillary tube. In a transverse section (Fig. 2B) it shows four cells, each with a small nucleus. The cytoplasm is dense, eosinophil but small in quantity. This portion does not take up vital dyes in contrast to the rest of the portion. Neutral red, however, stains the part feebly. The inner margin of the cells is devoid of a striated border and is therefore smooth. A few muscle fibres are observed around the basal part of the tubule.

The capillary tube is followed by the tubule proper, differentiated into three regions, namely proximal, middle and distal (Fig. 2A). In a cross section only three cells are seen (Fig. 2C), each with a large nucleus. The inner margin throughout is lined by a striated border of the honeycomb type. The striations are joined to each other so that the inner margin appears to be continuous. The cytoplasm is dense and eosinophil and includes some granules which are heavily concentrated in the middle region. The three regions measure about 9.8, 38.5 and 10.5 mm respectively. Since the nature of the striated border is the same

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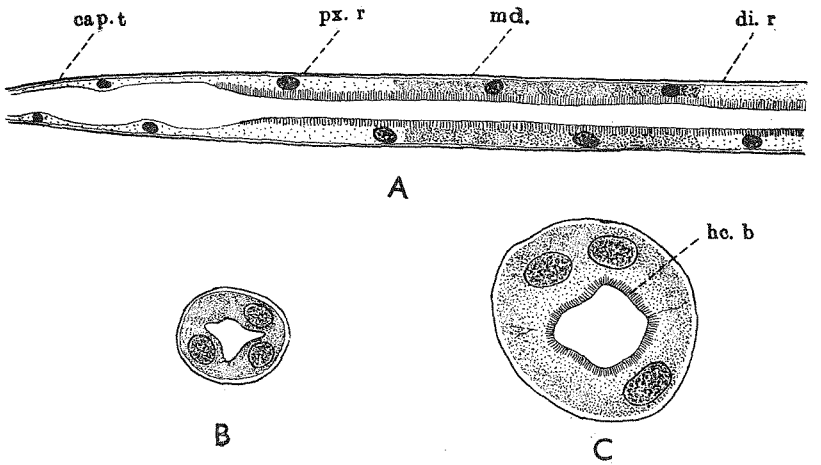

Fig. 2 A. Diagrammatic longitudinal section through the Malpighian tubule to show four regions and their borders. — Fig. 2 B. T.S. of the Malpighian tubule of the adult through the capillary tube. (× 450). — Fig. 2 C. T.S. of the tubule through the tubule proper. (× 450. *cap.t*, capillary tube. — *di.r*, distal region. — *hc.b*, honeycomb border. — *md.*, middle region. — *px.r*, proximal region)

throughout, it is not possible to isolate different regions in the sections but they can be well demarcated in living condition either with or without vital staining.

The embryonic development

The embryonic development of *Sphaerodema* lasts for about 11 days during the months of August to November. When the embryo is about 4 days old, the Malpighian tubules appear as four, minute, 0.07 mm long buds (Fig. 3A) from the lateral sides of the anterior portion of the hind gut. In a transverse section each bud, with a diameter of 21 μ, shows 13 columnar cells arranged in a single layer (Fig. 4A). The cell walls are not very distinct and there is no trace of any border. In longitudinal sections, the bud shows a number of mitotic figures suggesting active cell division. At 4 days 8 hours stage, the bud increases to attain a length of about 0.21 mm but the diameter is reduced to about 18—19 μ and the number of cells in a cross section also appears to be 10. The mitotic figures are still visible.

In an embryo 4 days 12 hours old, each tubule is about 0.42 mm long and about 15 μ in diameter (Fig. 4B). The tips of the two tubules of each side now join each other by means of some tissue (Fig. 3B). The lumina of the two tubules, however, remain completely separate from each other. The number of cells in a cross section is further reduced to 7—8. By this time, the mid gut is not formed but in a 5 days old embryo the mid gut differentiates posteriorly. All the four tubules are observed to converge over the rectal caecum and remain connected with each other by some tissue (Fig. 3C). Each tubule at this stage measures about 1.26 mm in length and 15 μ in diameter. The mitotic figures

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are not observed in the longitudinal sections at this stage and that suggests that the cells required for the formation of the tubules have already been formed. It is thus obvious that up to 4 days 12 hours stage, there is cell production and since a reduction in the diameter of the tubules and number of cells is observed,

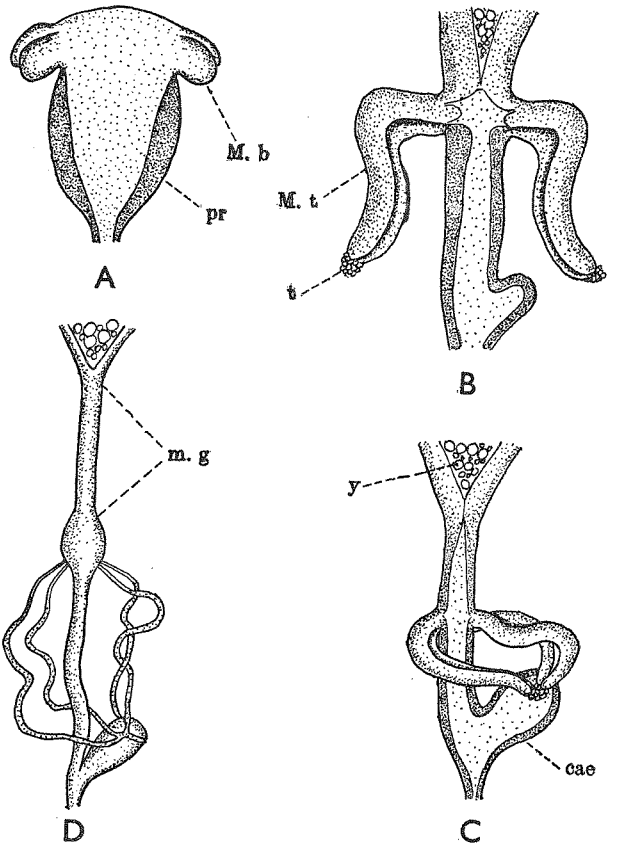

Fig. 3. Diagrams of the hind guts of the embryo at various stages to explain the sequence of development of the Malpighian tubules:
A. Origin of the tubules as four buds in 4 days old embryo. — B. Tubules arranged in two pairs in 4 days 12 hrs. old embryo. — C. Tubules converging distally over the caecum in 5 days old embryo. — D. Typical arrangement arrived at after the tenth day. (*cae*, caecum. — *m.g*, mid gut. — *M.b*, Malpighian bud. — *M.t*, Malpighian tubule. — *pr*, proctodaeum. — *t*, tissue. — *y*, yolk)

with increase in length, it is apparent that with cell production there goes on cell rearrangement.

In a 7 days embryo, each Malpighian tubule attains a length of 3.64 mm and a breadth of 12 μ (Fig. 4C). The number of cells in a cross section is seen to be only six. There is further reduction in the diameter of the tubules, so that in

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a 9 days embryo it is reduced to 9 μ only (Fig. 4D). The length, of course, continues to increase and each tubule attains a size of 4.48 mm. In a transverse section only five cells are seen. The developmental sequence shows that there is substantial increase in the length of the tubules with reduction in the number of the cells and the diameter of the tubules. This only suggests an active period of cell rearrangement and enlargement. The tubules, though distally attached, have no luminal continuity but on the tenth day it is observed that the lumina become continuous and thus a cross is formed over the rectal caecum (Fig. 3D).

On the eleventh day, just a few hours before hatching, the diameter of the tubule becomes 18 μ and the cells are reduced to 4 in a cross section (Fig. 4E). Each tubule increases in length to attain a size of 5.32 mm. This shows that from the ninth day onwards, cell enlargement becomes more pronounced than cell rearrangement.

The Malpighian tubules actually arise at the junction of the mid gut and hind gut. In a longitudinal section of the hind gut at a 4 days 12 hours stage, the valve is undifferentiated. It, however, differentiates at a later stage but actually develops posterior to the point of origin of the tubules, a feature which is retained even in the adults.

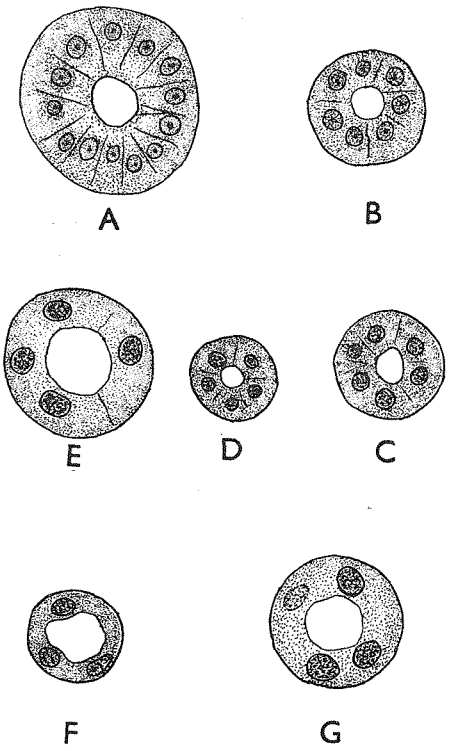

Fig. 4. T.S. of the embryonic tubules at various stages of development:
A. Through bud of 4 days old embryo. — B. Through tubule of 4 days 12 hrs embryo. — C. Through tubule of 5 days embryo. — D. Through tubule of 7 days embryo. — E. Through tubule of 9 days embryo. — F. Through the capillary tube of the I nymph. — G. Through the tubule proper of the same. (× 1000)

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Table 1

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

Age of the embryo	Length of the tubule (mm)	Diameter of the tubule (μ)	Number of cells in a T.S. of the tubule
4 days	0.07	21	13
4 days 8 hours.	0.21	18–19	10
4 days 12 hrs.	0.42	15	7–8
5 days	1.26	15	8
6 days	1.68	12	8
7 days	3.64	12	6
9 days	4.48	9	5
11 days (A few hours before hatching)	5.32	18	4

The entire embryonic development can be divided into two phases. In the first phase of short duration, there is both cell production and cell rearrangement and in the second phase there is cell rearrangement and enlargement since increase in the length is accompanied by reduction in the diameter and number of cells. But in the late embryonic life, the enlargement becomes more pronounced as shown in table 1.

A striated border does not differentiate during the embryonic period and there is absolutely no differentiation into various regions but just before hatching a differentiation of the capillary tube from the rest of the portion is observed (Fig. 4F, G). The capillary tube measures 0.70 mm in length and 12 μ in diameter. There are only 4 cells in a cross section. The rest of the portion which measures 4.62 mm in length and 18 μ in diameter has typical structure of the Malpighian tubule with 4 cells in a cross section. Further differentiation into various regions is attained only in the later part of the life of the first nymph.

Discussion

In the present century, NELSON (1915) in *Chalicodoma* and *Apis*, DRUMMOND (1936) in Lepidoptera, THOMAS (1936) in Orthoptera, PATERSON (1936) in Coleoptera and MELLANBY (1937) in Hemiptera assigned ectodermal origin to the Malpighian tubules but HENSON (1932, 1944, 1946) in *Pieris* and *Blatta* has shown the endodermal origin of the tubules. He has been supported by SAVAGE (1956) in *Schistocerca* and SRIVASTAVA and BAHADUR (1961) in *Dysdercus*. The latter workers have shown that since the tubules lie anterior to the interstitial ring, they are endodermal in origin. SRIVASTAVA and BAHADUR have shown that it is the interstitial ring which at a later stage develops into the proctodael valve, so that the tubules lie anterior to the valve in the adult. In *Sphaerodema* also all the Malpighian tubules lie anterior to the proctodael valve and hence can be considered to be of endodermal origin. The present study, however, does not provide sufficient material to make a detailed evalution.

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As regards the phases in the development of the embryonic Malpighian tubules, HENSON (1937) in *Pieris* showed two phases, one of cell production and the other of cell rearrangement and enlargement. In *Blatta* (1944) he again noted two phases; the first being of cell production and rearrangement and the second exclusively of cell enlargement. In *Schistocerca*, SAVAGE (1956) found similar phases but considered the *Pieris* type of phase to be more advanced. In *Dysdercus*, however, SRIVASTAVA and BAHADUR (1961) observed three phases — that of cell production, of cell production and rearrangement and of cell rearrangement and enlargement. They dismissed the idea of *Pieris* type of development to be more advanced because of the occurrance of such a sequence in *Forficula*. In *Sphaerodema rusticum* there are two phases, firstly that of cell production and rearrangement and secondly that of cell rearrangement and enlargement. Such a type of sequence is, however, unique and does not fit in with any described so far. Since all the phases, described uptil now, differ from each other, it is apparent that no uniform rule can be applied to consider one type as either primitive or more advanced.

LOCY (1884) in *Belostoma*, HAMILTON (1931) in *Nepa cinerea* and PRESSWALLA & GEORGE (1936) in *Sphaerodema rusticum* have described only two Malpighian tubules. They considered that after arising from the gut each tubule turns back and opens again into it, thereby forming a loop. But BAHADUR (1961) has shown in a few aquatic bugs that the number of the tubules is four and not two in each insect. His observations are, however, purely morphological and lack embryological support. In the embryonic development of *Sphaerodema* it has been clearly observed that the tubules arise as four small buds which elongate and fuse distally at a later stage to form a cross, thereby obscuring the true nature. The present work establishes beyond doubt that there are four Malpighian tubules in the aquatic forms too and not two as reported by earlier workers.

In *Sphaerodema rusticum* each tubule consists of four regions as described in certain aquatic heteropterans (BAHADUR, 1961). The first region has been designated by him as the capillary region. His findings do not give any clue whether this part is a modified region of the Malpighian tubule or an additional development but presently, it has been observed that the capillary tube is in fact a part of the Malpighian tubule which differentiates into this region at a later stage of development. The absence of rich tracheation with failure to take up vital dyes confirms its function as a conducting tube. In the other three regions, BAHADUR described a honeycomb border throughout the tubule in *Ranatra* and *Micronecta* but in *Laccotrephis* and *Enithares* he found a brush type of border in the distal region and honeycomb in the rest of the two regions. In *Belostoma*, but for the middle region which is without a border, the rest of the portion is lined by a honeycomb border. In *Sphaerodema*, however, the entire tubule but for the capillary part is lined by the honey comb border and approaches a condition reported by BAHADUR (1961) in *Micronecta* and *Ranatra*.

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Summary

In adult *Sphaerodema rusticum* LINNAEUS there are four Malpighian tubules which join distally to form a cross. Each tubule shows four regions, namely capillary tube, proximal, middle and distal regions. The capillary tube is a part of the Malpighian tubule though it is conducting in function. Internally it is smooth and has no striated border. The rest of the regions are lined by a honeycomb-striated border. The embryonic development has established beyond doubt that the number of the tubules is four and not two as reported earlier for aquatic bugs. The entire developmental sequence has been described and the period divided into two phases: (i) cell production and rearrangement and (ii) cell rearrangement and enlargement. This type of sequence is unique and does not fit in with any described so far. The Malpighian tubules have been considered to be of endodermal origin.

Zusammenfassung

Das ausgewachsene *Sphaerodema rusticum* LINNAEUS hat vier malpighische Röhrchen, die distal zusammenkommen und ein Kreuz bilden. Jedes Röhrchen zeigt vier Regionen, nämlich die Haarröhre, die proximale, mittlere und distale Region. Die Haarröhre ist ein Teil des malpighischen Röhrchens, obwohl sie ihrer Funktion nach ein Leiter ist. Innen ist sie glatt und hat keinen gestreiften Rand. Die übrigen Regionen werden von einem wabenartig gestreiften Rand begrenzt. Die embryonale Entwicklung hat eindeutig ergeben, daß die Anzahl der Röhrchen vier beträgt und nicht zwei, wie es früher für Wasserwanzen angegeben wurde. Der gesamte Verlauf der Entwicklung wurde beschrieben und der Zeitraum in zwei Phasen eingeteilt: 1) Produktion und Anordnung der Zellen; 2) Neuordnung und Vergrößerung der Zellen. Dieser Typ des Ablaufs ist einzigartig und paßt zu keinem bisher beschriebenen Typ. Es wird angenommen, daß die malpighischen Röhrchen endodermischen Ursprungs sind.

Резюме

Взрослый *Sphaerodema rusticum* LINNAEUS имеет четыре мальпигиевых сосуд, которые дистально образуют крест. Каждый сосуд имеет четыре областей, волосную трубу, проксимальную, среднюю и дистальную область. Волосная труба часть мальпигиевых сосудов, по функции однако она только проводник. Внутри она гладкая и не имеет полосатый край, другие области ограниченны сетообразным полосатым краем. Эмбриональное развитие несомненно показало, что количество сосудов четыре и не два, как это раньше указалось. Описывался полный ход развитии и разделился на две фазы: 1) производство и расположение клеток; 2) новая организация и увеличение клеток. Этот тип развитии единственный и не имеет сходства с никаким до сих пор описанным типом. Принимается, что мальпигиевые сосуды происходят из ендодерма.

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