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THE MALPIGHIAN TUBULES OF THE LARVA OF
HEPTAGENIA INTERPUNCTATA, SAY.

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The Malpighian tubules of the Ephemera, both of the larva and the imago, have been described by a number of workers; as far as I have been able to ascertain there has not been any paper published in which one can find a detailed description of their structure either in the immature or the mature stage of these insects. These tubules in the May-flies assume, according to different observers, many forms; several have been described as filiform, others as similar in a general way to the ones described in the present paper. From both descriptions and figures it has been shown that in several species of May-flies the tubules are short and bent or coiled, or, in some species they assume a spiral form. The more detailed descriptions have shown that in many species each tubule consists of a distal, thicker and secretory, portion from which a thinner tube passes and enters indirectly or directly into the intestine. Tubules opening separately have been figured by Dufour (3) and Drenkelfort (2) but in most species it has been shown that they open into several common receptacula and that these open directly into the digestive tract; Leue (8) and Vayssiere (13). The short account of most of the quoted authors gives but a meager description; many, however, show the two above mentioned parts of each tubule and the spiral or twisted shape is commonly mentioned or figured. From these various accounts the one which most closely resembles the tubules of *Heptagenia interpunctata* is the figure given by Vayssiere (13) of *Heptagenia longicauda*.

In *Heptagenia interpunctata* there are eight tubes opening into the intestine and carrying the products of the Malpighian tubules; each of these, soon after its origin from the intestine, branches into two or three short and fairly wide tubes and these receive the ducts from the Malpighian tubules; there may be a secondary branching before the ducts are received. The number of tubules was not counted but there are many of them surrounding the intestine more being lateral than either dorsal or ventral. These eight tubes probably open into the intestine equidistant from each other although a couple of sections had the appearance of a lateral arrangement, four tubes entering each side. Drenkelfort (2) found that in *Siphylurus lacustris* there were more than a hundred Malpighian tubules.

Each Malpighian tubule is divided into a distal, secretory, part, the glandular portion, from which a tubular duct, somewhat longer than the active part, leads to one of the receptacula into which, as described above, they all have their outlet. The duct leaves the widest portion of the tubule near the center, really the end of the central coil.

The glandular portion of the tubule can, for the sake of description, be divided into two parts; there is first a long curved and bent distal arm which leads into the thicker and shorter central section from the end of which the duct leaves. This first arm is different in nearly every tubule but begins with a hook-like curve which, in some specimens, is more abrupt than in others. The terminal part of this hook is a little thinner than the rest but from the end the tubule gradually increases in diameter to reach its widest part just proximal to the beginning of the abrupt hook-like curve. This thicker part continues for about one-third the length of the tube which then gradually decreases in diameter. While but a single bend is shown in either the diagram (Fig. 2) or the drawing (Fig. 1) of the entire tubule this part rarely lies in one plane, even the hooked end, besides bending on itself, may also turn in some other way. The narrower portion continues for a short distance with only a slight curve and then the tube again turns abruptly on itself and for a short distance this part is more or less parallel to that portion of which it is a continuation. Soon after making this second abrupt curve the tube starts to make a spiral-like loop bending even more abruptly than either of the other turns, it also suddenly becomes very much wider; this is the beginning of the central portion. The increase in width in this central portion is very pronounced and, as it begins to abruptly widen, the tube turns sharply back on itself and so continues until it returns to where the wide central portion started; then it suddenly decreases in diameter to form the beginning of the duct. The duct then forms a nearly circular loop lying on the surface of the central part of the tubule and passing out to the convex margin of this portion, runs along this margin and returns

to where it had its origin; here it leaves the tubule and goes to one of the receptacula already mentioned. These Malpighian tubules vary considerably in shape and size as also in their twists and turns so that it is extremely difficult to write a description of any one of them, they all, however, conform to the same general plan. Each tubule can be divided into a long distal arm, the wider central portion and the proximal part, the narrow duct. An examination of the drawing (Fig. 1) and the more diagrammatic representation (Fig. 2) will, aided by the description, give one an idea of the general shape and the relationship of the different parts.

Having thus attempted to describe the Malpighian tubule from a surface view we will give what the study of sections shows us about its structure. In nearly all of the tubules stained and mounted entire something of the lumen of the tube can be seen and it is noticed that this hollow is very much bent as it passes down the tube, it is large and irregular and varies greatly in diameter. Near the distal end of the tubule the form of the lumen is fairly regular and its structure that of a simple tube; the wall here, as in the other parts, is fairly uniform and of a single cell in thickness; the cell boundaries could not be seen (Fig. 3). From a surface view the nuclei, as in the cells of most Malpighian tubules, are quite large and are circular or irregularly so in outline; from an edge view they are oval and vary greatly in size. All the nuclei in this distal end are large.

In the next section of the tubule, from the commencement of the thinner part to the beginning of the second turn, the nuclei are smaller. In this part a surface view of the tubule often shows many of the nuclei of a kidney-bean shape and adjacent to their concave surface there can generally be seen a portion of the lumen. In a whole mount this section of the tubule shows for some distance the nuclei arranged in a single row; from a more careful study we see that there is really a double row of nuclei but that the alternate ones are in a different focus. In this section of the tubule the lumen shows a distinct wavy appearance the bend going from one side to the other; this is shown (Fig. 4) in section to be due to the fact that each nucleus with a small amount of surrounding cytoplasm projects out into the lumen and bends it towards the opposite surface; passing along the tube the next nucleus is seen to be on the opposite side of the tubule and the lumen bends in the other direction. This arrangement gives the tubule from a surface view the appearance of having the nuclei alternating with the clear spaces (Fig. 3, to the right).

Structurally the tubule shows no modifications until it widens into the central part; this change appears, in a whole mount, to be abrupt but in sections it is seen to be gradual (Fig. 9). The nuclei in that part of the tubule before it widens into the central portion are the same as in the preceding part and the lumen continues its course of alternating towards one side of the tube and then towards the opposite one. The lumen throughout this entire part of the tubule is not nearly so regular as is shown in the two figures (4 and 9), it often pushes out much nearer the wall and forms irregular pockets between the nuclei

giving to some parts of the tubules, internally, a very irregular appearance; this is partly shown in the transverse sections (Figs. 5, 6 and 7).

The irregularities we have just mentioned in the shape and width of the lumen do not compare to what is found in the large central part where these changes in diameter are much greater. Here in both surface view and in sections it appears as if the hollow part of the tubule occupies, in sections, a large portion of the interior, or, in surface view it appears as large cracks between the nuclei. The cells of this central portion are larger than in any other part of the tubule but their nuclei do not show a corresponding increase in size. Sections through this central part if cut away from the center show the lumen as pockets pushing out between the nuclei towards the wall of the tubule (Fig. 10) and no doubt outlining the cell boundaries; or, nearer the median portion (Fig. 11) the true width of the lumen can be seen. We have already said that in the distal end of this central part the change from one part to the other was a gradual one (Fig. 9); at its proximal end, from which the duct arises, this is not the case and the change from the secretory part of the tubule to the duct is an abrupt one (Fig. 12). This change takes place near the concave surface of the central portion from which place the duct, lying on the surface of the central portion, passes to its convex margin near which it starts to turn and passes for some distance along the edge, then it gradually turns back again to near the place from which it started; in doing this it forms a loop and then passes away from the tubule towards one of the receptacula into which it opens. The duct shows no variation, except in width, throughout its entire length. From a surface view the nuclei are seen to be circular in outline and, in any one view, they often alternate from one side of the duct to the other; in many specimens each nucleus is seen to have a clear ring surrounding it (Fig. 14). In sections the wall of the duct consists of a thin layer of cytoplasm in which the nuclei, now seen from an edge view, are disk-like. Inside of this layer there is a thin lining, it often presents a shiny appearance as if it were cuticular.

A more detailed description of the tubule brings out some interesting points. The striated layer, rhabdiorium, which is so characteristic for the mid-intestine and Malpighian tubules of insects is not present in the tubules of the larva of *Heptagenia interpunctata*, the adults were not examined and the presence or absence of this layer in the imago is not shown. Leue (8) in his work on the larva of *Heptagenia sulphurea* does not mention a striated layer and omits such a layer from his figure. McDunnough (9) working with *Chrysopa* found this layer in the tubules of the larva but not by the adult and Holmgren (6) records the absence of a striated layer in the Malpighian tubules of *Apion*. Most of the sections were stained only with Delafield's haematoxylin, a few were counter stained with Bordeaux red and in these it was quickly seen that the wall of the tubule, especially the central part, consists of two portions. The inner part of the wall bore the haematoxylin blue with a faint trace of the red but there was a fairly wide outer layer, widest in the middle of the central portion, which was of a much darker red; while best seen

in the wide central portion this darker layer extended to near the end of the arm. This darker layer has been shown on only four of the figures, 9, 10, 12 and 13. In the sections stained with Heidenhain's haematoxylin the outer border was also noticeable and darker than the remaining part of the protoplasm. This is the external striated layer but, as in this insect there is no inner one present, the word external could well be omitted. This layer showed a number of elongated clear spaces at right angles to the basement membrane; these pass through the darker layer and give to it its striated appearance; these have been described as the canaliculi. In *Heptagenia interpunctata* they are very distinct in some specimens but not seen in others. This may be due to the different preserving fluids and stains used or to the time, relative to feeding, at which the insect was killed; than a stage in the activity of the tubule.

In very many of the tubules examined a second and smaller tube accompanied the duct and could be traced as far as the glandular portion of the tubule. This was probably a trachea to supply the tubule but a careful examination failed to show the presence of a taenidium; this might have been a tracheole from which the cuticular lining is absent.

The largest nuclei showed a peculiarity in the presence in each of a large vacuole (Fig. 8) but never more than one in any nucleus.

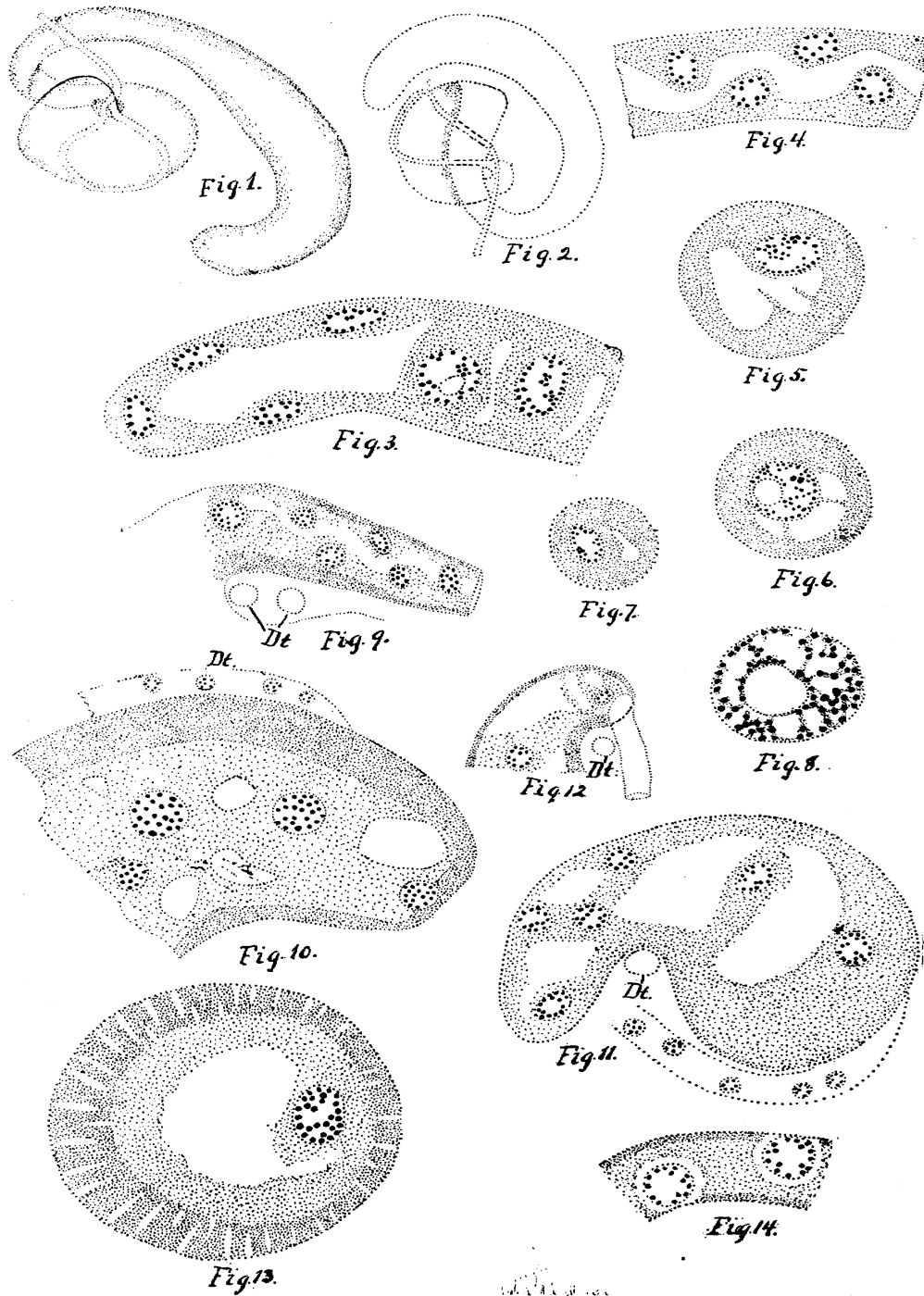
A basement membrane is present as also a peritoneal covering, the latter can easily be seen especially so where the duct runs along the margin of the central body as this membrane lies external to the duct; it has not been drawn in any of the figures.

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EXPLANATION OF PLATE VIII.

- Fig. 1. Surface view of a Malpighian tubule. $\times 280$.
- Fig. 2. Diagrammatic view of another tubule. In this and the preceding figure only a short piece of the duct is shown. Both figures show the long distal arm, the thicker central part and a portion of the duct including that part which forms a loop over the surface of the central body.
- Fig. 3. Longitudinal section through the distal end of the arm. To the left the section is cut through the center of the tubule, to the right the section is almost a surface view. $\times 740$.
- Fig. 4. Longitudinal section of a portion of this arm taken from the narrower part. $\times 740$.
- Figs. 5, 6 and 7. Transverse sections through different parts of this armlike part of the tubule. All $\times 740$.
- Fig. 8. View of one of the large nuclei to show the contained vacuole. $\times 1700$.
- Fig. 9. Section through the proximal end of the arm where it enlarges to form the wider central part of the tubule, this latter portion is only in part outlined. Dt., duct. $\times 740$.
- Fig. 10. Longitudinal section of a part of the large central portion of the tubule. This section is cut between the center and outer surface and shows only in part the lumen which is represented by the five areas not stippled. Dt., duct. $\times 740$.
- Fig. 11. Another section, more median, of the same part. To the left the section is cut through the center of the tubule, to the right it is more of a surface view. $\times 740$.
- Fig. 12. The proximal end of the central portion showing the origin of the duct. Dt., transverse section of another part of the duct. $\times 740$.
- Fig. 13. Transverse section through the larger central region. This section as well as sections 9, 10 and 12 show the darker external striated layer. In this section, which is a higher magnification than the others, the canaliculi can be seen in this outer layer. $\times 1100$.
- Fig. 14. Surface view of a small portion of a duct. $\times 1700$.



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