Alkaline Phosphatase in Protonephridia of Terrestrial Nemertines and Planarians

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With one Plate

SUMMARY

1. Application of the Gomori method to sections of the terrestrial nemertine Geonemertes dendyi shows that there is no alkaline phosphatase in the flame cells or in the distal glandular canal of the protonephridia. But the proximal ciliated convoluted canal and the branching ciliated terminal ducts are rich in this enzyme.

2. Sections of the terrestrial triclad Rhynchodemus terrestris show the presence in the parenchyma of numerous ciliated convoluted canals resembling those of the nemertine.

3. A study of the protonephridial system of R. terrestris shows that this consists of numerous flame cells connected by branching unciliated ducts with the phosphatase-rich ciliated convoluted canals. The latter connect with unciliated distal canals which probably connect with the numerous exit ducts. These exit ducts are apparently confined to the ventral surface, particularly along the ciliated creeping sole.

4. The convergence between these nemertine and turbellarian protonephridia, and their analogy with the differentiated tubule system of the vertebrate kidney, are noted.

In an earlier paper (Pantin, 1947) it was shown by one of us that the excretory system of the terrestrial nemertine Geonemertes dendyi Dakin consisted of many thousands of protonephridia in a layer immediately beneath the muscular body-wall. The whole surface is covered by numerous minute nephridial openings. Each opening communicates by an efferent duct with a convoluted glandular canal with thick walls of radially striated protoplasm. Passing back, this glandular part suddenly opens into a highly convoluted end-canal from whence terminal branches serve the numerous flame cells. The end-canal and its branches have relatively thin walls and are ciliated, which the glandular canal is not. The differentiation of the system is seen in Pl. 1, fig. a.

There is evidence that the protonephridial system is concerned with water regulation, and the histological differentiation of sections of the canal system call to mind the differentiation of the kidney tubule in vertebrates. In these a striking feature is the rich supply of alkaline phosphatase localized in the proximal tubule leading from the glomerulus and its absence in the distal tubule. Accordingly it seemed of interest to investigate the distribution of this enzyme in protonephridia.

METHODS

For ordinary micro-anatomical studies, paraffin sections were stained with Mallory, and with Masson's haematoxylin–ponceau–light-green method. For the detection of alkaline phosphatase Gomori's (1939) method was used. The worms were fixed for 2 hours in ice-cold 80 per cent. ethyl alcohol. They were then passed through three 20-minute changes of absolute alcohol and two of cedar oil, followed by two changes of 52°C paraffin wax of 10 minutes each. The subsequent treatment followed the plan given by Danielli (1946). The sections were incubated for 2 hours in the presence of the glycerophosphate substrate at 27°C or 37°C. The presence of the enzyme was rendered evident in the usual way by the ultimate deposition of black cobalt sulphide. The sections were counter-stained with light green.

Controls, in which the glycerophosphate substrate was omitted, showed no blackening except where the calcareous bodies in the ectoderm reacted directly with the cobalt sulphide.

RESULTS WITH GEONEMERTES DENDYI

Pl. I, fig. c, shows a tangential section through the nephridial layer just below the dermal musculature. The presence of abundant alkaline phosphatase in the whole end-canal system, in its convolutions and in the branched end ducts, is very evident. Controls (Pl. I, fig. d) show no deposition of the sulphide.

A careful study of the distribution of the enzyme shows that it is confined to the end-canal system and is quite absent both in the flame cells and in the glandular canal. This is seen in Pl. I, in which fig. b shows the distribution of the enzyme in the same nephridium as that figured in its entirety in fig. a.

RESULTS ON RHYNCHODEMUS TERRESTRIS

(O. F. Müller)

This worm lives in the same humid environment as G. dendyi. Despite the phyletic distinction between nemertines and Turbellaria there are certain functional parallels in their grade of organization. Both possess a protonephridial system, though little is known of the organization of this in terrestrial triclads.

Sections of R. terrestris treated by the Gomori method at once brought to light the existence of localized convoluted ciliated tubules heavily stained with cobalt sulphide owing to the presence of large amounts of the enzyme. Pl. I, fig. e, shows such a convoluted canal. It is noteworthy that the black sulphide is deposited not only in the wall of the canal, but also strongly upon the cilia. A similar deposition upon the cilia is to be seen in the convoluted canals of G. dendyi, though it is less marked. Pl. I, fig. f, shows a similar convoluted tubule in the control.

Similar convoluted canals can be seen with active ciliary movement in the
tissues of *R. terrestris* during life. They, and the flame cells, are the only parts of the nephridial system visible in living tissues.

These convoluted ciliated ducts, rich in the enzyme, strongly resemble those of *G. dendyi*. They are, however, scattered all through the parenchyma and are not confined to a sub-dermal layer, as is the case in the latter. No other parts of the excretory system are rich in the enzyme, not even the terminal branches leading to the flame cells. Because they are isolated, connexion of these enzyme-rich coiled ducts with the excretory system must be established by other methods.

**TEXT-FIG. 1.** Protonephridia of *Rhynchodemus terrestris*. (a) Isolated flame cells and end-canals. (b) Groups of flame cells opening into end-canals. End-canals opening into convoluted ciliated canal. Ciliated canal opening into final ducts. (c) Final ducts opening into exit canals through ventral surface.

The excretory system of these terrestrial triclads is far harder to determine than that of *G. dendyi*. Unlike the latter, the system is not confined to a definite layer, but is diffused throughout the parenchyma. Further, although the system is extensive, the flame cells are small and the ducts narrow; and the ill-defined structure and thinness of the walls of the ducts make them hard to distinguish in the complex pattern of cell types and other structures in the parenchyma. Once observed, however, the different elements of this
system can be detected and are seen to be abundant at all points. But the connexion between the elements is difficult to determine except in an occasional lucky section.

The flame cells are about 10μ long and 4μ broad (Text-fig. 1a). They possess a single lateral nucleus, and the ciliary flame arises from a simple cap of protoplasm. The lower part of the flame cell is apparently supported by some strengthening material which stains with iron haematoxylin. This recalls the supporting structures which Schröder (1918) found in the flame cells of G. palaensis, though the elaborate ribs of the latter have no counterpart in these triclad flame cells.

The flame cells open singly and in groups into branched terminal ducts the walls of which are often almost impossible to distinguish. In favourable sections, particularly when using Masson’s iron haematoxylin–ponceau–light-green method, the sudden passage of these ducts into ciliated convoluted canals, identical in structure with those seen with the Gomori method, is seen (Text-fig. 1b). From these ciliated convoluted canals a duct with thicker glandular walls descends through the parenchyma towards the ventral surface (Text-fig. 1, b and c). The ducts are numerous and they run a long way. Like the end-canals they are very difficult to trace through the varied structures of the parenchyma. The path of any single duct has not yet been followed by us continuously to the exterior, but ducts of similar structure and running in the same direction are found connected to exit canals. These exit canals are very numerous, opening on to the ventral surface, particularly on the curious ventral ciliated sole along which the animal glides (Text-fig. 1c).

CONCLUSION

These results show a striking convergence between the protonephridia of a species of terrestrial nemertine and a terrestrial triclad. In both there are numerous flame cells, though in the triclad these are not confined to the sub-dermal layer. In both, the end-canals from these lead to a convoluted canal rich in alkaline phosphatase, which is absent in the rest of the system except that both ciliation and the phosphatase extend back into the terminal ducts in G. dendyi. Distally, beyond the convoluted canal the histology changes. In R. terrestris there is a thicker walled duct. In G. dendyi there is the curious glandular canal. Finally, in both, the excretory canals open to the exterior by very numerous minute efferent ducts. These are distributed over the whole ciliated surface in G. dendyi whilst in R. terrestris they are found only in the neighbourhood of the ventral strip to which the ciliation of these animals is confined.

We have made no studies of the extent to which our studies of phosphatase are complicated by diffusion phenomena. Consequently we cannot claim that the detail of the distribution of cobalt sulphide observed corresponds to the detail of phosphatase distribution. All that is claimed is that there is a high concentration of alkaline phosphatase in the end-canal system of the protonephridia of G. dendyi, and in the convoluted tubules of R. terrestris. We can-
not, for example, be certain that the blackening of the tubule cilia indicates
that the cilia in this region are rich in phosphatase.

With the above reservation there is an interesting functional resemblance
between the nephridia studied here and the nephrons of fish, amphibia, and
mammals. In the fish having glomerular kidneys, in amphibia, and in mammals
one unit, the glomerulus, produces a passage of fluid into a tubule system
(see review, Smith, 1937). This fluid passes down the proximal and distal
tubules, and its composition is modified during its passage by secretory
activity of the tubule cells. The tubules proximal to the glomerulus have
brush borders which are rich in alkaline phosphatase (Gomori, 1939; Lorch
and Danielli, 1951), and even in the fish having aglomerular kidneys the
borders of the cells of the tubules corresponding to proximal tubules are rich
in phosphatase. In the same manner, the protonephridia are organized so that
one type of unit, the flame cell, produces a passage of fluid into a tubule
system: the cells of the part of the tubule system more or less proximal to the
flame-cell system are distinguished from other parts of the nephridia by being
rich in alkaline phosphatase. It seems very probable that in both nephrons
and protonephridia the phosphatase is connected in some way with the process
of modifying the composition of the fluid passing down the tubules.

REFERENCES


EXPLANATION OF PLATE I

Fig. a. Geonemertes dendyi. Camera lucida drawing of an entire protonephridium.
Fig. b. Geonemertes dendyi. Alkaline phosphatase distribution in end-canal system, and
its convolutions, from the same specimen. Note absence of enzyme in flame cells and in final
glandular canal. (Incubation 2 hours at 37° C.)
Fig. c. Geonemertes dendyi. Alkaline phosphatase in end-canal system. (Incubation 2 hours
at 27° C.)
Fig. d. Geonemertes dendyi. Control, showing absence of blackening in convoluted end-
canal.
Fig. e. Rhynchodemus terrestris. Alkaline phosphatase in ciliated convoluted tubule. (Incuba-
tion 2 hours at 27° C.)
Fig. f. Rhynchodemus terrestris. Control showing absence of blackening in ciliated convoluted
tubule.

For Figs. a and b, bar is 80μ. For Fig. c-f, bar is 40μ.