A New Sporozoan in Amphioxus.

By

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With Plate XXIX.

My work has been carried on in the Laboratory of Comparative Anatomy at Oxford; and I wish at the commencement of this brief notice to offer my best thanks to Professor Ray Lankester, both for allowing me to work here and for his valuable suggestions and help.

Quite recently I had occasion to cut a series of transverse sections through Amphioxus lanceolatus for the purpose of demonstrating the working of the microtome, rather than with any idea of obtaining sections of any particular interest.

To my surprise, however, the series, although cut without any particular care and in a hot atmosphere, proved to be well worth examining, chiefly from the fact that the cochineal stain which was used had differentiated the tissues to a remarkable extent. This differentiation is chiefly noticeable in the intestine, which is stained a deep violet, in strong contrast to the cochineal red of the notochord and other tissues.

On examining the sections a peculiar structure in the intestine at once attracted notice.

In various parts of the posterior region of the gut the epithelium appeared to differ from that of the rest of the wall, the cells seeming to contain much larger nuclei, and being apparently curved round to form small cup-shaped organs (figs. 1—4).

As will be at once seen from the figures, these cups present
on cursory observation an appearance very similar to that of Vertebrate taste-bulbs.

Appearances, however, proved absolutely deceptive, for in thinner sections examined with a higher power the nuclear-like bodies (figs. 1—9, p.) were seen to be not nuclei, but each in itself a minute cell parasite, evidently a stage in the life history of a Sporozoon.

Each of these parasites is elongate, tapering at each end, and on an average only '01 mm. in length.

The protoplasm presents a coarsely granular appearance (figs. 8 and 9), the granules staining more deeply than the rest of the cell protoplasm.

Centrally there is a clearer space, and within this again a deeply staining round body, which I at first took to be the nucleus.

After careful examination of my slides with Zeiss's homogeneous immersion lens I am, however, inclined to regard the deeply stained body (fig. 9, n.) as the nucleolus, and the clearer protoplasm (N.) in which it is situated as the nucleus.

It is extremely difficult to make certain of this point in such minute organisms, and I have not been able to absolutely satisfy myself as to the presence of a nuclear membrane.

Nevertheless the forms depicted in fig. 9 give one the impression that the part marked N. is a large nucleus whose chromatin is all collected into the globular nucleolus (n.), and this appearance gains confirmation when compared with the nucleus of other Sporozoa.

Close to the nucleus in many of the specimens a clear round structure may be observed (v. in fig. 9, b and c). This structure is refringent and well defined, but is not visible in every specimen. It has the appearance either of a centrosome or a vacuole. One would not, however, expect to find the former, since there is no sign of nuclear division; whilst if it be a vacuole it is of great interest from the fact that, so far as I am aware, such a structure has never been previously described in a Sporozoon.

The parasites, though occasionally seen at the distal ex-
tremities of the epithelial cells, are usually situated in cavities at the proximal ends of the cells. Sometimes a single parasite is found in an isolated position in the digestive epithelium; but most frequently they occur in groups, and seem to exert some irritating influence over the epithelial cells, causing these to curve round to form the curious cup-like structures drawn in figs. 1 and 4. Not unfrequently the distal extremities of the cells containing parasites are broken away (figs. 7 and 8); but this by no means invariably occurs, and may be due to the preparation of the sections.

The extremity of the parasite nearest the free edge of the cell is in close proximity to the cell nucleus ($M$ in figs. 7, 8, and 9). The nucleus of the infected cell, moreover, differs considerably in form from that of the normal epithelial cell (figs. 7 and 8, $M'$). It seems probable that the parasite, by pushing against the nucleus, had altered its form until, as in figs. 7, 8, and 9, $a$ and $b$, it has the appearance of a granular cap to the parasite.

I have not seen stages showing the alteration of the nucleus; but Schneider, in his 'Tablettes Zoologiques,' figures such a change brought about by the pressure of a growing Sporozoon (vol. i, pl. ix and xxix). In the form described by Schneider the nucleus is situated at the proximal end of the cell, the parasite being more distal in position, whilst in the form now described the nucleus is nearer the free end of the cell than the parasite.

As for the distribution of these parasites in Amphioxus, they occur all round the intestinal epithelium, but are limited to the posterior region of the gut from the atriopore to the anus. In this post-atrioporal region they may be extremely numerous, the epithelium, as in fig. 3, being crammed with them.

The epithelium of the hepatic caecum is so similar to that of the intestine that I had every expectation of finding these cells also infected.

Although, however, I have sought with the utmost care, both in my own sections and in some excellent series, for the
use of which I am indebted to Mr. Willey, I have been unable to find any trace of the parasite in the caecum; and, as a matter of fact, I have never seen it so far anteriorly in the digestive tract.

Further, I regret to be obliged to state that I have not yet succeeded in obtaining any other stage in the life history of this form.

Methods.

From its differentiating properties cochineal is an extremely useful stain for Amphioxus, and the parasites are easily seen in sections prepared with this; but the details of protoplasmic and nuclear structure I have made out best in specimens stained with a mixture of borax-carmine and picro-carmine as prepared by Bolles Lee.¹

The use of the homogeneous immersion lens was essential, and figs. 6—9 were drawn under this with the aid of a Zeiss's camera.

In conclusion, my sincere thanks are due to Dr. G. H. Fowler, who first drew my attention to an unusual appearance in my sections of Amphioxus, which I investigated with the results now published; to Mr. Willey for the most kind loan of his sections, and to Dr. Benham and Mr. Minchin for ever ready advice and help.

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Addendum.

In fig. 10 I have figured specimens of a ciliate parasite which occurs in considerable numbers in the atrium of Amphioxus.

Professor Lankester noticed these years ago, and has recently drawn my attention to them, with the suggestion that the cell parasite described above might possibly be a stage in the life-history of this ciliate form.

¹ Bolles Lee, 'Vade Mecum,' p. 192.
Whether this be so or not, a brief description of this Protozoan may not be without interest, no parasite having been previously described in Amphioxus.

This member of the Ciliata is one of the Aspirotrichous Trichostomata, varying in length from 0.4 to 0.7 mm. There is a definite mouth and short pharynx in a lateral position (fig. 10, a, O). A large nucleus is situated more or less posteriorly, and is not unfrequently lobed in a curious fashion (fig. 10, c), an appearance which is, however, probably due to shrinkage, since it is not of invariable occurrence.

The cell protoplasm is very granular, the granules sometimes, as in fig. 10, d, being extremely large.

These parasites occur in considerable numbers, and are found in the atrium both anterior and posterior to the atriotricle.
EXPLANATION OF PLATE XXIX,

Illustrating Miss E. C. Pollard’s notice of “A New Sporozoön in Amphioxus.”

Reference Letters.

M. Nuclei of epithelial cells, altered in form by pressure of parasites. 
M’ Typical nuclei of normal epithelial cells. 

Figs. 1—3.—Transverse sections through post-atrioporal region of the intestine of Amphioxus, showing the parasites in groups.

Fig. 1. Zeiss, oc. 2, obj. A.
Fig. 2. Zeiss, oc. 4, obj. B.
Fig. 3. Zeiss, oc. 2, obj. B.

Fig. 4.—Group of the parasites within cells, giving cup-like appearance. Zeiss, comp. oc. 4, obj. D.

Fig. 5.—Small portion of intestinal epithelium, with cells containing parasites. Zeiss, oc. 4, obj. D.

Fig. 6.—Some of the parasites from Fig. 5 under a higher power. Zeiss, comp. oc. 4, homog. imm. lens.

Figs. 7 and 8.—Groups of parasites in intestinal epithelium.

Fig. 7. Zeiss, oc. 4, homog. imm. lens.
Fig. 8. Zeiss, comp. oc. 4, homog. imm. lens.

Fig. 9.—d—f, drawn from parasites found isolated from the cell, probably due to the breakage of the cell by the razor; a—c, drawn from groups of parasites seen in Fig. 7. Zeiss, oc. 8, homog. imm. lens.

Fig. 10.—a—d. Ciliate parasites occurring in the atrium of Amphioxus. 