FORMATION AND BREAKDOWN OF SERPULID TUBES

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DURING an investigation of the blood systems of serpulid worms (Annelida, Polychaeta), opportunities occurred to make the following observations on the mode of formation and breakdown of the calcareous tubes in which these animals live.

Although much research has been carried out on the asexual reproduction of *Salmacina* and *Filograna* by Malaquin, Faulkner¹ and others, it is not known how a newly formed individual leaves its parent's tube and makes its own tube. During last summer, specimens of *Salmacina incrustans* Claparède settled on the sides of a glass aquarium in the Zoological Station at Naples, and daily observations were made on the growth of the colonies. The new individual is formed at the posterior end of the parent, and after separation it moves backwards down the parent's tube and makes a hole in the side of the tube from which it protrudes its branchial crown (Fig. 1, X). It may destroy the posterior end of the parent's tube and thus separate it from the rest of the colony (Y). It may also form a new tube which grows out from the original opening (Z). These colonies spread out over the wall of the aquarium. Colonies taken from the sea usually consist of masses of entangled tubes raised off the substratum to which the basal parts are attached. These three-dimensional colonies are built up of tubes with side-branches which are presumably formed in the same way as in the two-dimensional colonies growing in the aquarium.

It is not known how the newly separated bud makes the hole in the side of its parent's tube. One is reminded of the ability of the sabellid *Potamilla* to bore into calcareous rocks². At Naples *Potamilla* often lives inside the walls of the calcareous shell of the mollusc *Vermetus*. How *Potamilla* bores its holes is also unknown. Some observations of Fox^{3,4} are interesting in this connexion. He has found that when the anterior end of the tube of *Sabella spallanzanii* is artificially closed, the animal can make a hole in the side of the tube, from which it then protrudes its branchial crown. The tube of *Sabella* consists largely of mucus and is not calcareous.

Alkaline phosphatase is probably concerned in the calcification of bone and in the formation of the calcareous shells of molluscs⁵. It was therefore interesting to find this enzyme in *Pomatoceros triqueter* L. (Fig. 2) which, like the other serpulids,

Fig. 2. PHOTOMICROGRAPHS OF HORIZONTAL LONGITUDINAL SECTIONS THROUGH THE THORAX OF *Pomatoceros triqueter*. $\times 25$. (a) TREATED BY GOMORI'S METHOD FOR ALKALINE PHOSPHATASE (SITES OF ENZYME BLACK); (b) CONTROL

builds a calcareous tube. When *Pomatoceros* is removed from its tube, it begins to form a new one⁶. The new tube first appears on the ventral surface of the anterior part of the thorax under the ventral collar fold. It is not known how this new tube is produced; but it seems likely that it is secreted by glands in this part of the thorax.

One specimen of *Pomatoceros*, fixed immediately after removal from its tube, was sectioned and tested for alkaline phosphatase by Gomori's method⁷. The enzyme was found in the following places:

(1) The epithelium of the alimentary canal (Fig. 2, A) from the posterior end of the oesophagus to within a few segments of the anus. This is the part of the alimentary canal concerned with the digestion and absorption of food⁸. Phosphatase was concentrated at the inner and outer borders of the cells and was also found in the nuclei and in small granules in the rest of the cytoplasm. There was a thick deposit of phosphatase on the cilia. Bourne⁹ has shown that the enzyme is confined to the absorptive regions of the guinea pig's alimentary canal and is there concentrated in the brush borders of the epithelium. He has discussed the hypothesis that alkaline phosphatase is concerned in the phosphorylation of sugars being absorbed from the canal.

(2) The epithelia of the thoracic nephridia (Fig. 2, N) and their ducts. In the excretory sacs of the nephridia the phosphatase was distributed in the same fashion as in the gut epithelium, and was also present on the cilia. Alkaline phosphatase has been seen in the brush borders of the proximal convoluted tubules of the mammalian kidney⁷ and in the brush borders of the Malpighian tubules of the goat-moth caterpillar and a spider⁹. Danielli⁷ has discussed the suggestion that in the kidney alkaline phosphatase plays a part in the reabsorption of substances from the glomerular filtrate.

(3) The contents of the alimentary canal.

(4) The chloragosomes (yellow- or brown-coloured globules) in the chloragogen cells which in serpulids lie on the thoracic ring vessels. Globules of similar appearance, also giving a positive phosphatase reaction, are found in the epithelium of the excretory sacs of the thoracic nephridia. According to Liebmam¹⁰ chloragosomes contain food substances, and he has found that in the chloragosomes of the oligochaete *Eisenia foetida*, phospholipids are transformed into fats, lipoproteins appearing as transitional substances. Alkaline phosphatase may play some part in this process.

Fig. 1. FREEHAND DRAWING OF ONE PART OF A COLONY OF *Salmacina incrustans* ON FIRST, THIRD AND EIGHTEENTH DAYS OF OBSERVATION

A second specimen of *Pomatoceros* was fixed three days after it had been removed from its tube; it had not begun to form a new tube. Alkaline phosphatase was again found in the same four sites as in the other specimen, and also in the following places (Fig. 2):

(5) The borders of small irregular cavities (C) situated in the thick epithelium on the ventral surface of the thorax under the ventral collar fold. This is the tissue which probably secretes the calcareous tube, and alkaline phosphatase is perhaps concerned in this process, as it seems to be in the calcification of bone and in the formation of the calcareous shells of molluscs⁵.

(6) The epithelium of the dorsal and ventral lips of the mouth.

(7) The nuclei of the endothelium in the walls of the blood vessels in some of the pinnules of the branchial crown. Bourne⁵ has found the enzyme in endothelial nuclei of vertebrate capillaries.

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⁴ Fox, H. Munro, *Proc. Roy. Soc., B*, **125**, 554 (1938).

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⁷ Danielli, J. F., *J. Exp. Biol.*, **22**, 110 (1946).

⁸ Thomas, J. G., "Pomatoceros, Sabella and Amphitrite" (Liverpool, 1940).

⁹ Bradfield, J. R. G., *Nature*, **157**, 876 (1946).

¹⁰ Liebmann, E., *J. Morph.*, **70**, 151 (1942).

PLANT VIRUS PROTEINS AND ANTIBODY-ANTIGEN REACTIONS

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PROTEIN mixtures such as those found in blood have been widely used in the study of antibody-antigen reactions. It is difficult to prepare pure samples of single proteins from such naturally occurring mixtures. On the other hand, preparations of certain plant virus proteins, derived from a single infectious unit, can be isolated in quantities sufficient for experimental work in a state which fulfils all the usual criteria of homogeneity. As they are also good antigens, they would appear to be especially suitable objects for the study of antibody-antigen reactions.

The method used by Boyd¹ has been applied to the study of the turnip yellow mosaic virus antisera produced in rabbits. Successive doses of 0.01, 0.1, 1.0, 10.0, and 100.0 mgm. of turnip yellow mosaic virus² (prepared by the method of Markham and Smith³) were given intravenously to a single rabbit. Bleedings were taken two weeks after each injection, followed by a rest period of a few days before the next injection.

The antiserum produced by the 0.01 mgm. injection was of low titre with marked suppression of precipitation in the region of antiserum excess. This antiserum would be of the H type according to Boyd's classification. With increasing dosage of antigen the antiserum produced gave progressively higher titres,

Fig. 1

and the type of precipitating system produced tended progressively towards Boyd's R type, with less-marked inhibition in the region of antiserum excess.

Fig. 1a shows the precipitating system for the antiserum produced after the 0.01 mgm. plus 0.1 mgm. injections. Fig. 2 shows the system for the antiserum from the same rabbit after the full course of injections.

If this antiserum obtained after the full course of injections is diluted with normal serum to make its titre approximate to one of the earlier antisera obtained, then the type of system produced approximates fairly closely to that produced by the earlier bleeding. For example, the antiserum obtained after the full course of injections (with a titre of 1:8192) was diluted to 1:256 with normal serum and a precipitation diagram prepared (Fig. 1b). This antiserum precipitated to a titre of 1:64, and there was fairly marked inhibition in the region of antiserum excess similar in amount to that found for the antiserum of Fig. 1a with a titre of 1:128.

Fig. 2