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DIGESTION IN SEA ANEMONES

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(Plate I and Text-fig. 1)

A sea anemone, normally a passive-looking animal, reacts to suitable foodstuffs by a series of fairly complicated activities. When its tentacles encounter solid food there is, first of all, a discharge of cnidae, which poison living prey and adhere to the food mass. These cnidae are independent effectors, responding directly to external excitation (Pantin, 1942). Next, the tentacles clasp the food and bend towards the mouth; they push the food into the mouth, and the pharynx draws it down into the coelenteric cavity where it is digested (Pantin & Pantin, 1943).

The food bolus lying in the coelenteric cavity gradually disintegrates, and the particulate and soluble products of digestion are absorbed. The indigestible residue is finally expelled from the mouth. Batham & Pantin (1950) have recorded the phasic activities of *Metridium* following feeding: these include elongation of the column, peristaltic movements and, finally, defaecation and shrivelling after about 48 h. The faecal pellets which are extruded are covered with thick mucus.

According to Yonge (1931, 1937, 1954), some preliminary digestion of protein takes place extracellularly in coelenterates, whereas the digestion of carbohydrates and fats, and the final degradation of proteins are all carried out intracellularly. Previous workers have had difficulty in demonstrating an extracellular protease in actinians, since fluid drawn from the coelenteron usually possesses little or no proteolytic activity (Mesnil, 1901; Bodansky, 1924). Many lines of evidence show, however, that extracellular digestion does take place (Boschma, 1925; Yonge & Nicholls, 1930; Krijgsman & Talbot, 1953). There is, seemingly, a paradox between the low proteolytic activity of coelenteric fluid, and the demonstrated efficacy of coelenteric digestion, and some experiments were made to investigate certain aspects of digestive secretion in sea anemones.

METHODS AND EXPERIMENTS

Most of the observations and experiments were made on the anemone *Calliactis parasitica* (Couch). For *in vitro* studies mesenteric filaments, together with neighbouring regions of the mesenteries, were removed from

anaesthetized sea anemones. Roughly weighed samples of these tissues were used for investigations of protease activity.

For estimating protease activity, the photoelectric method of Riggs & Stadie (1943) was used. In this method, changes in the turbidity of a suspension of homogenized boiled egg white during the course of digestion are measured in an absorptiometer. From suitable dilutions of the substrate, a curve is obtained relating concentration to density. The initial concentration is taken as 100%. Density readings made of the digest at suitable times are converted to concentrations by means of this curve. Concomitantly with the digests, blanks are run that contain fluid to be tested and preservative, but no substrate. Values for the blanks are subtracted from the digests. Digests were carried out at pH 7·4, using boric acid–borate buffer. This hydrogen-ion concentration was chosen somewhat arbitrarily after reviewing the literature. Previous workers have shown that the optimal pH for intracellular proteases and the hydrogen-ion concentrations of coelenteric fluids of diverse Anthozoa range from pH 6·7 to 8·75 (Yonge & Nicholls, 1930; Krijgsman & Talbot, 1953).

Digestive activity of extracts

Tests were made of the proteolytic activity of crude extracts of mesenteric filaments of *Calliactis*. Filaments from an animal were ground with sand, 10 ml. of water were added, and the suspension was centrifuged and filtered. Results are given in Table 1. The rate of digestion, computed as a monomolecular reaction, is shown in Text-fig. 1. The velocity constant, k, falls off with time. The extract contains a strong protease acting at pH 7·4.

Proteolytic activity of coelenteric fluid

Samples were drawn of coelenteric fluid of some anemones to test for proteolytic activity. At most a few ml. were obtained from each animal. Out of 14 specimens (2 *Tealia felina* and 12 *Calliactis parasitica*), only 1 (*Tealia*) showed slight digestive activity (substrate concentration reduced to 73% in 18 hours).

Rate of digestion of a meal in a normal animal

The rate at which *Calliactis* can digest a meal was investigated as follows. Animals were fed weighed pellets of denatured gelatine. At selected intervals animals were sacrificed, the residual pellets (if any) recovered and weighed. Kjeldahl analyses were made of the latter.

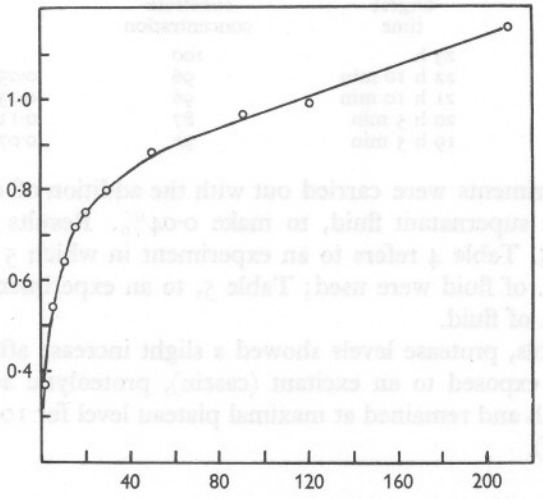
The pellets were prepared from a 10% solution of gelatine, denatured with formalin, and washed in sea water.

Fed sea anemones were sacrificed at 2, 4, 8, 16 and 24 hr. At least 3 animals were tested for each of these periods. Sea-water temperatures were about

TABLE 1. PROTEOLYTIC ACTIVITY OF EXTRACT OF MESENTERIC FILAMENTS OF *CALLIACTIS*

Period (time after mixing) (min)	Concentration of egg white (%)	k*
1	70·2	0·364
6	57·8	0·091
11	52·3	0·059
16	48·3	0·045
21	46·9	0·036
31	44·8	0·026
51	41·4	0·017
91	38·0	0·011
121	37·2	0·008
211	31·6	0·005

* $k = 2{\cdot}3/t \log_{10} C_0/C_t$ (proportion of protein hydrolysed per min.)



Text-fig. 1. Rate of digestion of egg white substrate by an extract of the mesenteric filaments of *Calliactis*. Abscissae, $2{\cdot}3 \times \log_{10} C_0/C_t$; ordinates, time in min after mixing extract and substrate.

TABLE 2. DIGESTION OF DENATURED GELATINE PELLETS BY *CALLIACTIS*

Digestion time (h)	No. of trials	Original wet weight (g)	Percentage of pellet digested	
			Basis wet weight	Basis N
2	5	0·766–1·035	44·5	45·4
4	5	0·592–0·847	65·9	79·1
8	8	0·906–1·022	53·9	48·9
16	5	0·663–1·019	76·5	80·7
24	3	0·687–1·013	98·7	98·6

12°–13° C. The results are summarized in Table 2. The gelatine blocks were about half digested in the first 8 h and completely digested in 24 h.

Progress of secretion with time

The temporal course of secretion *in vitro* was determined by the following procedure. In control experiments, 5 g of *Calliactis* tissue (isolated mesenteries and filaments) were placed in a vessel and covered with 25 c.c. of sea water. Samples of 1 c.c. were drawn from the supernatant fluid at the start and at 1 h intervals and were tested for protease activity. Room temperature was 18° C. The results appear in Table 3.

TABLE 3. PROTEASE ACTIVITY BY *CALLIACTIS* FILAMENTS *IN VITRO*

Time of drawing sample	Digest time	Final substrate concentration	k
0	23 h	100	—
1 h	22 h 10 min	96	$0{\cdot}030 \times 10^{-3}$
2 h	21 h 10 min	96	$0{\cdot}032 \times 10^{-3}$
3 h	20 h 5 min	87	$0{\cdot}117 \times 10^{-3}$
4 h	19 h 5 min	92	$0{\cdot}072 \times 10^{-3}$

Similar experiments were carried out with the addition of casein (B.D.H. soluble) to the supernatant fluid, to make 0·04%. Results are shown in Tables 4 and 5. Table 4 refers to an experiment in which 5 g of *Calliactis* tissue in 25 c.c. of fluid were used; Table 5, to an experiment with 10 g of tissue in 50 c.c. of fluid.

In the controls, protease levels showed a slight increase after 3 h. In the experimentals, exposed to an excitant (casein), proteolytic activity became marked after 4 h and remained at maximal plateau level for 10 h (duration of the experiment).

In vitro *studies with various excitants*

The results obtained in the experiments described in the previous section suggested the possibility of testing the relative efficacy of various excitants. Accordingly, a long series of experiments was carried out, using 1 g samples of mesenteric filaments in 5 c.c. of sea water. Excitatory substances tried were proteins, proteose, peptones, glutathione and many amino acids (0·02%). Test samples were drawn after 4 h (temp. 17°–19° C). Digest time was 19 h. Since the results were very variable, it is not proposed to describe them in detail. Controls (mean of 12 tests) showed a final substrate concentration of 86%; proteins (casein and egg albumen, 12 tests), 66–73%; peptones (bacteriological, 14 tests), 55–83%; proteose, glutathione, and amino acids (52 tests), 83–100%. Proteins and peptones were secretagogues, producing stronger secretory activity than that occurring in the controls.

TABLE 4*

Time of drawing sample	Digest time	Final substrate concentration	k
0 h	23 h 30 min	100	—
1 h	22 h 35 min	100	—
2 h	21 h 40 min	100	—
3 h	21 h	83	$0{\cdot}170 \times 10^{-3}$
4 h 20 min	19 h 40 min	66·5	$0{\cdot}343 \times 10^{-3}$

TABLE 5*

Time of drawing sample	Digest time	Final substrate concentration	k
0 h	23 h	95	—
2 h	23 h	66	$0{\cdot}298 \times 10^{-3}$
4 h	23 h	61	$0{\cdot}358 \times 10^{-3}$
6 h	23 h	59	$0{\cdot}383 \times 10^{-3}$
8 h	23 h	63	$0{\cdot}335 \times 10^{-3}$
10 h	21 h 5 min	62	$0{\cdot}370 \times 10^{-3}$

* Details in text.

DISCUSSION AND CONCLUSIONS

There is now clear evidence, both from previous observations and the present work, that a protease is secreted into the coelenteron of sea anemones. Earlier studies are reviewed by Boschma (1925) and Yonge (1931). Especially pertinent are the experiments of Jordan (1913), who enclosed fibrin in sacs of filter-paper and fed them to anemones (*Anemonia sulcata*). Digestion of the fibrin occurred and, since the mesenteric filaments could not come into contact with the food, he concluded that extracellular protease diffused into the sacs and caused hydrolysis of the fibrin. Krijgsman & Talbot (1953) fed *Pseudactinia flagellifera* with pieces of sponge soaked in meat extract. The sponges were removed, the juice expressed and tested for proteolytic activity (casein precipitation method and alcohol titration). With casein substrate, there was pronounced digestion after 3 h. With gelatine substrate, marked digestive activity was found after 3 h (increase of acidity measured by alcohol titration method of Waldschmidt–Leitz).

Studies dealing with excitation of digestive secretion in lower animals are rather infrequent. In the experiments of Krijgsman & Talbot (1953), cited above, the coelenteric fluid collected from anemones which were fed pieces of sponge containing meat extract showed pronounced proteolytic activity, but, as no similar experiments were recorded of feeding sponge alone, it is difficult to draw any conclusions concerning the stimulatory effect of meat extract on the secretory mechanism. Ishida (1936) collected coelenteric fluid from *Actinia mesembryanthemum* by cutting off the pedal disc; starved and fed animals were compared for proteinase activity. Three hours after

feeding cooked egg white there was a marked increase in the proteinase ('tryptase') activity of the collected fluid.

Only a small amount of coelenteric fluid can be collected from sea anemones by suction or by cutting open the animals, and this fluid shows little proteolytic activity, even in fed animals (Bodansky, 1924). Yet there is secretion of protease, extracellular digestion proceeds rapidly and large food masses are quickly cleared. How are these apparent contradictions to be explained? Extracellular protease has been detected in fluids collected from the intact animal (*Hydra*, anemones), and secretion of protease has been demonstrated by using *in vitro* preparations (anemones). Krijgsman & Talbot (1953) believed that a food mass becomes coated with stiff mucus in the coelenteron of an anemone. The mucus coating is impregnated with proteinase, and forms a protective barrier against dilution by sea water; digestion takes place within the mucus coating. The particulate products of extracellular digestion are then phagocytized and digestion is completed intracellularly.

We have found no stiff masses of mucus about partially digested food boluses in *Calliactis*. In an expanded fed animal very little fluid can be collected from the base of the coelenteron. When fed sea anemones are quickly frozen (solid carbon dioxide in acetone) and cut open in the frozen state, the food bolus is found completely invested by mesenteric filaments at the base of the coelenteron, below the stomodaeum, and there is no fluid filled cavity in this region (Pl. I). This would explain why it is difficult to draw a sample of uncontaminated coelenteric fluid through the stomodaeum. Fluid which is obtained comes mostly from the upper regions of the coelenteron, or contains sea water drawn down the stomodaeum.

Mesenteric filaments of anemones are very active structures which move over, and closely adhere to the food bolus. Boschma (1925) and Yonge (1930) have made similar observations on corals (*Astrangia* and *Euphyllia*): they found that mesenterial filaments close quickly over plankton organisms, completely obscuring them. These filaments are in continual motion, one replacing another as soon as the first becomes gorged with food. The gastrozooids of *Physalia* behave in an analogous manner, closely enveloping the prey. Digestive enzymes are secreted, and partially digested food material passes up the lumina of the gastrozooids (Wilson, 1947).

The structure of the coelenteron favours enzymic activity. The food bolus is enveloped in a sac-like mass of mesenteric filaments which adhere closely to the surface of the food (Pl. I). Proteolytic secretion is stimulated by protein components in the food mass. The enzymes act on the food practically at the surface of the filaments, and suffer little dilution; as the food is dissolved and absorbed the filaments continue to press against the shrinking mass. This situation is in contrast to that found in many higher Metazoa, notably vertebrates, which discharge extracellular enzymes into large sacs or tubes, in the open lumina of which digestion takes place. The apparently feeble

proteolytic activity of gastric fluids or *in vitro* samples, therefore, should be compared with the *in vivo* condition, in which the enzyme is concentrated in small volume, immediately over the food mass.

Corals and anemones digest food masses quickly. In the present experiments it was found that *Calliactis* would dissolve sticks of insoluble gelatine, weighing 0·6–1 g and containing 5–9 mg N, within 24 h (12° C). Yonge & Nicholls (1930) found that the corals *Fungia*, *Symphyllia* and *Favia* digested large plankton organisms (copepods, mysids, etc.) in 4–13 h (around 25° C). Masses of coagulated blood were digested by *Fungia* in 4 h.

It is generally believed that the extracellular protease of coelenterates hydrolyses some part of the protein fraction of the meal to polypeptides; the disintegrated food bolus is then phagocytized, and digestion is continued intracellularly (Yonge, 1937).

The mesenteric filaments of corals and anemones contain a powerful proteinase. This is regarded as being of the trypsin type (i.e. an endopeptidase acting in an alkaline medium on peptide linkages adjacent to arginine or lysine); it has the same pH optimum as extracellular protease, and may be identical with the latter (Krijgsman & Talbot, 1953). It would be reasonable to find the precursor of the extracellular protease in the secretory tissue. If digestion of proteins proceeds beyond the polypeptide stage, then other intracellular proteases must be involved.

SUMMARY

Proteolytic activity of *Calliactis parasitica* was investigated by a photoelectric method.

The filaments contain a strong protease; the coelenteric fluid shows little or no proteolytic action.

Gelatine pellets (up to 1 g wet weight and 9 mg N) were digested within 24 h (12° C).

Secretion by mesenteric filaments *in vitro* was followed. Proteins and peptones acted as secretagogues. Secretion reached a maximum in 4 h.

The mesenteric filaments closely invest the food bolus. Digestion, initiated by extracellular protease, takes place at the surface of the filaments, and partially decomposed food material is absorbed.

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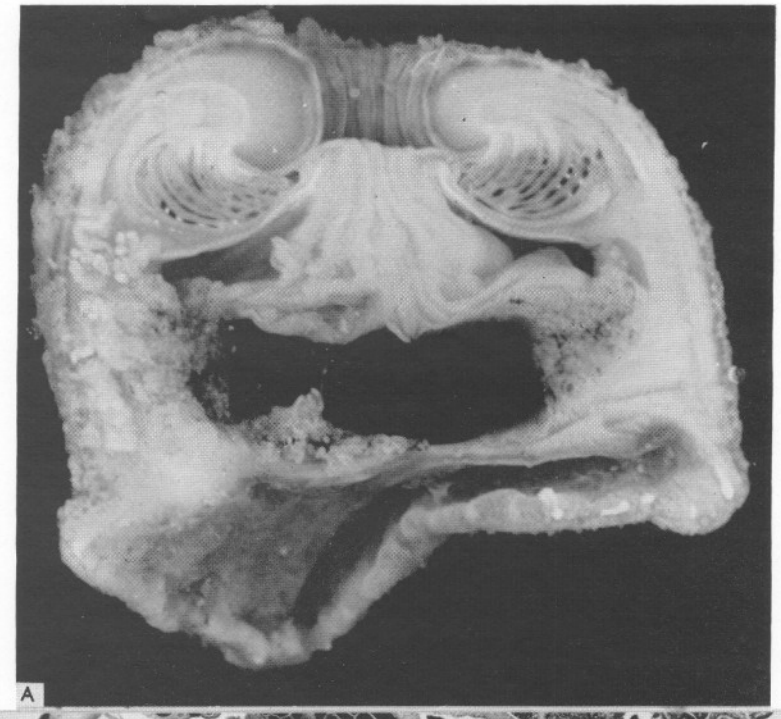
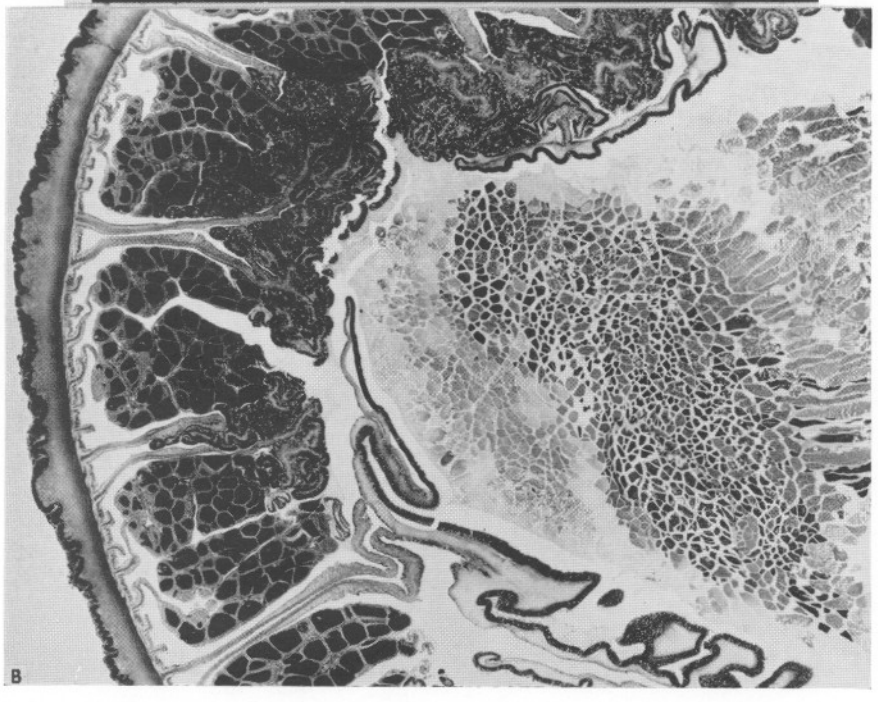
EXPLANATION OF PLATE I

A. A frozen *Calliactis*, cut vertically through the centre. The black rectangle, below the stomodaeum, is a gelatine pellet coloured red with carmine. It is closely invested by mesenteric filaments (× 5).

B. Horizontal celloidin section across the body of a fixed *Calliactis* that had been digesting a meal of plaice muscle. A mass of muscle lies in the coelenteron; note the change in density at the margin, due to partial digestion. Mesenteric filaments press closely against the muscle mass (a little separation has occurred during fixation) (× 12·5).





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