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SYNCHRONY IN THE DIGESTIVE DIVERTICULA OF *MYTILUS EDULIS* L.

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(Plate I, Text-figs. 1 and 2)

Four tubule types can be recognized in the digestive diverticulum of *Mytilus edulis* L. These represent (I) a normal or holding phase, (II) an absorptive phase, (III) a disintegrating phase, and (IV) a reconstituting phase. In mussels collected from mid-tide level, the type I and II tubules made up 90% of the total with most tubules in the absorptive phase. With exposure the percentage of type I tubules increased in relation to the type II tubules. On submersion the percentage of type II tubules increased again and the percentage of type I tubules fell. Type III and IV tubules showed no tidal rhythm. A mechanism is proposed for the change in the digestive cells from stage II to stage I without replacement or reconstitution of the cells through stages III and IV.

Chi-square tests showed that changes in the digestive tubules were synchronized throughout the diverticulum in only half the mussels examined. There was no obvious relationship between synchrony within the digestive diverticulum and the tidal rhythm of intracellular digestion.

Mussels were fed discontinuously in the laboratory with a 6 h-ON and 6 h-OFF feeding regime. During the unfed period the percentage of type I and II tubules were approximately equal (37–44%). After feeding for 6 h the number of tubules in the absorptive stage II had increased to approximately 70% of the total. When the mussels were starved for 2–6 weeks the percentage of type I tubules, in the normal or holding phase, increased relative to those of type II.

In *Mytilus edulis* the digestive diverticulum is not in a steady state but is a dynamic system which is modulated by exposure and submersion on the shore and by feeding and starvation in the laboratory.

INTRODUCTION

The organization, cellular composition and function of the digestive diverticula has been described for a large number of molluscs (Nakazima, 1956; Owen, 1966; Purchon, 1968). Changes in the digestive cells which are associated with intracellular digestion have also been widely recognized (Graham, 1938; Carriker, 1946; J. E. Morton, 1955, 1956, 1959; Owen, 1955, 1966, 1970, 1972; Sumner, 1965, 1966*a*, *b*, 1969; Morse, 1968; McQuiston, 1969; Walker, 1970; Merdsey & Farley, 1973). In general the digestive cells show three cyclic phases, (1) absorption, (2) digestion, (3) fragmentation and excretion, and these phases, with only minor modifications, are typical of most molluscs (Owen, 1966). In many groups, especially gastropods, the changes in the digestive cells within the tubules of the digestive diverticula often appear to be synchronized and consequently the tubules have a uniform appearance throughout the diverticula. In the Bivalvia, however, this type of synchrony has not generally been recognized. Since bivalves have been considered to feed more or less continuously (Purchon, 1971; Winter, 1973) it is not surprising that a homogenous appearance was only exceptionally observed, e.g. J. E. Morton (1956), J. E. Morton, Boney & Corner (1957). On the other hand recent work has suggested that under certain conditions bivalves may show a co-ordinated

cycle of feeding, extracellular and intracellular digestion (B. S. Morton, 1970*a*, *b*, 1971, 1973; Owen, 1972; Langton & Gabbott, 1974).

The purpose of the present investigation was to provide a *quantitative* evaluation of the synchrony between the digestive tubules of *Mytilus edulis*, in individual mussels, in relation to the tidal cycle, and in response to a discontinuous feeding regime and starvation.

METHODS

Mytilus edulis were collected from a mid-tide level at Gallows Point, Beaumaris, Anglesey. Mussels ranging from 5 to 6 cm in length were put in a tray at a similar mid-tide position at Menai Bridge, Anglesey.

Field experiment

Several weeks after the mussels had been collected and transferred to Menai Bridge, two animals were removed from the tray at hourly intervals, over two consecutive tidal cycles, beginning at 18.00 h on 24 October 1972. At low tide the tray was exposed to air while at high tide standard SCUBA equipment was used to collect the mussels.

Starvation experiment

In November 1972, 5–6 cm mussels were taken from the shore at Beaumaris, cleaned and put in a bucket of filtered sea water (Acropor, pore size 0·20 μm) at 8–10 °C. The sea water was continuously aerated and replenished daily. Mussels were sampled at 2, 4 and 6 weeks.

Discontinuous feeding experiment

Mussels were taken from the tray and placed in a 120 l laboratory tank at 10 ± 1 °C. Coarsely filtered sea water was continuously recirculated in the tank and replaced via an overflow system at the rate of approximately 500 ml min^{-1}. The animals were fed discontinuously using a 6 h-ON, 6 h-OFF feeding regime. *Isochrysis galbana* was metered into the tank to give a concentration of approximately 40 cells ml^{-1} during the feeding period. The cell count was calculated from nephelometer readings of the algal culture and the volume of culture added to the experimental tank. It does not include the level of particulate matter in the sea-water supply. No attempt was made to match the feeding periods with the cycle of exposure and submersion that the animals would have experienced on the shore.

Histological methods

In all of the experiments the mussels were opened and the digestive diverticula removed within 10 min of collection. The diverticula were fixed in Zenker's fluid, embedded in ester wax, sectioned at 5 μm and the sections stained with Heidenhain's haematoxylin.

Sampling procedure

The digestive diverticulum from each mussel was sectioned in four randomly chosen areas. A ribbon of nine sections was taken from each region and the four ribbons were mounted together on a slide. Four photographs, of areas containing a high percentage of tubule cross-sections, were taken of one of the sections from each ribbon, i.e. 16 pictures from each mussel. The sections were chosen by reference to a table of random digits (Snedecor & Cochran, 1967). The entire section was examined under low power ($\times 64$) and when a region of tubule cross-sections was found the magnification was increased ($\times 256$) and a picture taken using a Zeiss photomicroscope II. The individual tubules in each picture were graded into one of four categories (Type I, II, III, IV – see text). The pictures were thoroughly mixed, so that the grading was not influenced by the order in which they were examined.

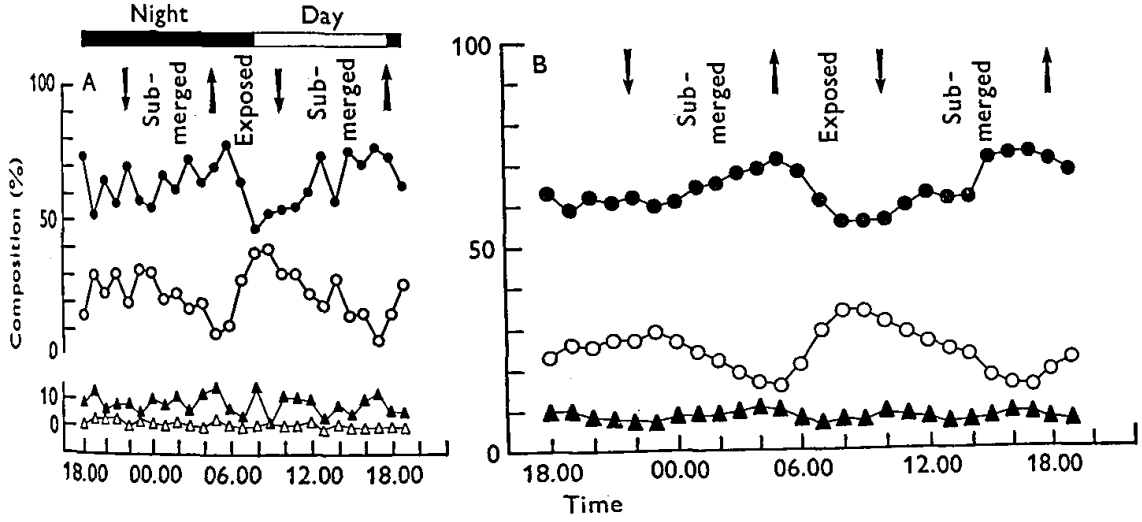
Statistical treatment of results

Chi-square values were calculated using general contingency tables to test the co-ordination between the tubule types for individual mussels and for homogeneity over two tidal cycles. For individual mussels the chi-square values were based on a 4 (gland areas) $\times 2$ (tubule types I and II) contingency tables. The observed values for tubule types III and IV were small and consequently not used in the calculations. A 3 (tubule types I, II and III) $\times 26$ (total number of samples) contingency table was used to evaluate the homogeneity of the data with respect to tidal changes.

In order to examine any tidal dependence of the data it is necessary to reduce the short period variation and to accentuate the longer term low frequency changes. This was accomplished by using a low pass digital filter. The filter is a smoothing technique which replaces each data point by a value obtained from a weighted running mean of several neighbouring data points. For further details of the method see Bendat & Piersol (1971).

RESULTS AND DISCUSSION

The recognition of different tubule types in *Mytilus edulis* is dependent upon the state of the digestive cells since these are the most abundant cells in the digestive diverticula. In *Mytilus*, as in *Lasaea* (J. E. Morton, 1956; McQuiston, 1969) and *Cardium* (Owen, 1972, 1974), the sequence of changes in the digestive cells in any one tubule are generally synchronized so that, in a cross-section, the tubule has a uniform appearance. As the cells pass through the various stages of intracellular digestion four distinct tubule types may be identified (Pl. 1A–D). These types are the same as the four stages, (I) normal or holding phase, (II) absorptive, (III) disintegrating and (IV) reconstituting, described by Platt (Ph.D. Thesis, The Queen's University of Belfast, 1971), as representative of the digestive cycle in *Mytilus edulis*.



Text-fig. 1A. Changes in percent composition of tubule types I–IV with exposure and submersion on the shore. Tubule type I, ○; II, ●; III, ▲; IV, △. B. Percent composition of tubule types I-III corrected by weighted running mean (see Methods).

Digestive rhythm on the shore

In *Mytilus edulis*, collected from a mid-tide position on the shore, the changes in the condition of the digestive tubules can be related to the periods of exposure and submersion (Text-fig. 1, Table 1). The type I and II tubules made up 90% of the total with

most tubules in the absorptive phase. When the mussels were exposed the percentage of type I tubules increased in relation to those of type II. When the mussels were submerged the percentage of type II tubules increased again and the percentage of type I tubules fell. The remaining tubule types, III and IV, made up only 10% of all the tubules examined and showed no obvious changes with the tidal cycle.

TABLE 1. *MYTILUS EDULIS*. OBSERVED VALUES FOR NUMBER OF TYPE I–IV TUBULES FROM SHORE ANIMALS

Tide ...	Exposed				Submerged							Exposed	
Time ...	18.00	19.00	20.00	21.00	22.00	23.00	24.00	01.00	02.00	03.00	04.00	05.00	06.00
Tubule type													
I	59	87	89	121	69	102	97	64	79	57	58	27	39
II	277	147	242	218	239	179	167	195	203	222	181	194	232
III	35	38	22	30	27	15	32	24	36	19	35	42	20
IV	3	8	17	11	1	10	4	3	7	3	2	9	1

Tide ...	Exposed			Submerged							Exposed	
Time ...	07.00	08.00	09.00	10.00	11.00	12.00	13.00	14.00	15.00	16.00	17.00	18.00	19.00
Tubule type													
I	107	140	141	77	110	91	72	104	48	52	31	59	102
II	234	166	184	135	192	226	259	196	222	210	311	247	231
III	14	41	9	29	35	37	9	29	15	30	52	23	22
IV	1	1	5	1	1	8	3	6	1	0	1	1	1

Chi-square = 459·81 based on a 3 (tubule types I, II and III) × 26 (total number of samples) contingency table. d.f. = 50, $P < 0{\cdot}001$.

In his description of the digestive rhythm in *Cardium*, *Macoma* and *Ostrea* B. S. Morton (1970*a*, *b*, 1971) implies that each tubule goes through a complete digestive cycle, from stage I to IV and back to stage I again, during every tidal cycle. On the other hand J. E. Morton (1956) thought that in *Lasaea* it was unlikely that all the cells or the digestive tubules took part in each cycle of activity and McQuiston (1969) suggested that the digestive cells were replaced every two tidal cycles. From the data in Text-fig. 1, it seems that for *Mytilus edulis* the tidal rhythm may only involve tubule types I and II and that the replacement or reconstitution of the digestive cells, through stages III and IV, is unrelated to the tidal cycle. If this is true, then some means must exist for the absorptive stage II to revert back to stage I. A possible mechanism for this change can be envisaged by reference to the fate of residual bodies in various species of bivalves. Owen (1972) has shown that in *Mytilus* the residual bodies are released individually, and the apex of the cells is not totally shed as in *Cardium*, nor are the cells completely broken down as in *Lasaea*. Thus, in *Mytilus* the tidal rhythm for tubule types I and II could be independent of types III and IV and the tubules might only pass into stage III when the digestive cells can no longer shed their residual bodies individually. There is also the possibility that stages III and IV are passed through very rapidly, resulting in low numbers and an apparent lack of any tidal rhythm.

Synchrony within the digestive diverticulum

Examination of numerous sections through the digestive diverticulum of individual mussels gave the impression of a lack of synchrony within the diverticulum as a whole. The tubules in some areas appeared to be co-ordinated with each other, but not with tubules in other areas of the diverticulum. Using the data from the shore experiment, chi-square values were calculated to test the association between the numbers of type I and type II tubules from four different areas in the diverticulum of individual mussels. In 25 of the 52 mussels tested the value of chi-square was significantly greater than the expected value if the numbers of type I and type II tubules had been evenly distributed throughout the diverticulum. This indicates that the changes in the digestive tubules were synchronized throughout the diverticulum in only half of the mussels examined. There was no obvious relationship between synchrony within the digestive diverticulum and the tidal rhythm of intracellular digestion (Text-fig. 1).

To understand the lack of co-ordination between the digestive tubules in individual mussels, it is necessary to consider the basic structure of the diverticulum. This consists of a large number of tubules connected to the stomach by a system of ducts, and in lamellibranchs these ducts are narrow and more frequently branched than in other molluscs (Owen, 1955; Nakazima, 1956). In the Filibranchia, the order to which *Mytilus* belongs, the tubules originate from the distal ends of ducts where the lumen is particularly narrow (Nakazima, 1956). This type of organization would allow food to be passed simultaneously to a small group of tubules served by the same duct, whereas in other areas of the diverticulum the food may not reach the tubules until some time later. Consequently the changes in the digestive cells in tubules in different areas of the diverticulum would not necessarily be co-ordinated with each other. This is very similar to the situation described by Owen (1972), in which he suggests that the tubules pass from a homogeneous 'holding phase' into an unsynchronized cycle. Individual tubules or groups of tubules pass through the digestive cycle at different rates and this is presumed to be dependent on the uneven distribution of food in the digestive tubules.

Recently B. S. Morton (1973) has suggested that shell valve movements, at the time of feeding, may serve to pump the fluid contents of the stomach into the digestive diverticula and may be more important in this respect than the counter current flow in the main ducts (see Owen, 1955; Mathers, 1972). If this 'pumping' hypothesis is correct it might lead to the appearance of synchrony between the digestive tubules because of the regularity, or rhythm, in the alternating processes of adduction and quiescence. In *Mytilus*, however, all four tubule types can be found at any time during the tidal cycle (Table 1) and there does not seem to be any definite synchrony between the tubules in different areas of the diverticulum. It has already been suggested that the lack of synchrony may be due to the uneven distribution of food to the tubules which would tend to preclude the pumping hypothesis. Brand (1972) has also suggested that the pumping hypothesis is unlikely because there may not be a significant pressure gradient between the stomach and the digestive diverticula, as they are in such close proximity to each other.

Discontinuous feeding

In experiments on the shore and when using tidal machines in which the animals are regularly exposed and submerged, no distinction can be made between the effect of the tidal cycle *per se* and the effect of differences in the availability of food. For this reason mussels were brought into the laboratory and kept totally submerged but fed discontinuously with a 6 h-ON, 6 h-OFF feeding regime. Although the food level was low the changes in tubule type were similar to those in the shore animals (Table 2). In the unfed period (10.00–13.00 h), the percentage of tubule types I and II were similar (37–44%). After three hours of feeding (16.00 h) the percentage of type I tubules increased. When the mussels had been feeding for 6 h, approximately 70% of the tubules were in the absorptive stage (II) while the number in the holding phase (I) fell to approximately 20% of the total. The results show that with feeding, as well as submersion, the absorptive stage (II) increases relative to the other stages.

TABLE 2. *MYTILUS EDULIS*. PERCENT OF EACH TUBULE TYPE DURING DISCONTINUOUS FEEDING: 6 h–OFF (7.00–13.00 h) 6 h–ON (13.00–19.00 h)

Time ...	10.00	13.00	16.00	19.00
Tubule type				
I	44	38	53	21
II	37	44	38	68
III	11	13	7	11
IV	7	6	2	0

TABLE 3. *MYTILUS EDULIS*. PERCENT OF EACH TUBULE TYPE AFTER STARVATION

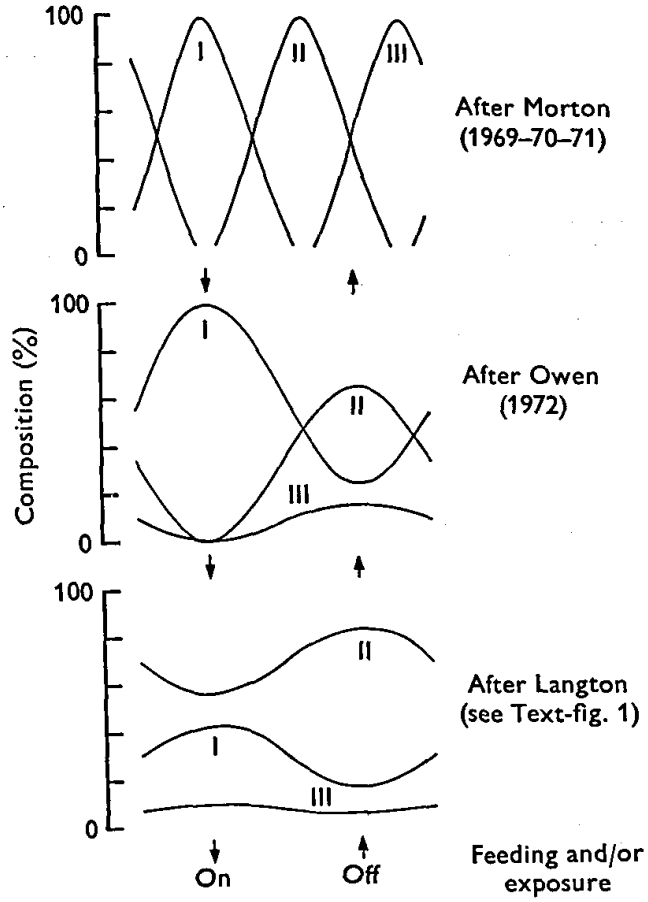
Starved ...	0	2	4	6	weeks
Tubule type					
I	25	67	59	57	
II	65	25	32	37	
III	9	4	6	3	
IV	1	3	4	3	

Starvation

In the digestive cycle described for *Mytilus* by Platt (Ph.D. thesis, The Queen's University of Belfast, 1971) she suggests that the tubules are most frequently in stage I, the normal or holding phase. Platt's mussels were collected from the mid-tide level on the shore and then placed in a tidal machine. After allowing the mussels 24 h to settle, the sea water was enriched with 0·5 l of an algal culture prior to each period of immersion. In the present study, when mussels were taken directly from the shore, the majority of the tubules were in stage II (see Table 3; week 0). After 2–6 weeks starvation in the laboratory, however, the condition of the digestive tubules was similar to that described by Platt with most tubules in stage I (Table 3). It is clear from this and from the results in the previous section (Table 2) that proper attention must be paid to the feeding regime when describing the condition of the tubules in the digestive diverticula of laboratory animals and that the results may not be typical of animals on the shore.

CONCLUSION

Two previous models describing the co-ordination between the different tubule types in the digestive diverticula of bivalves are schematically represented in Text-fig. 2. In the first model (top) tubule types I–III are pictured as passing from stage I to stage III and back to stage I again by replacement or reconstitution of the digestive cells. In this model all the tubules in the digestive gland would be synchronized and only two of the three tubule types should be found at any one time. Such a situation has been described as being typical of *Dreissena*, *Cardium*, *Macoma* and *Ostrea* by B. S. Morton (1969, 1970*a*, *b*, 1971). However, this model is clearly inappropriate for *Mytilus* since all the tubule stages may be identified at any time during the tidal cycle (Text-fig. 1; Table 1).



Text-fig. 2. Schematic representation of models describing the co-ordination between different tubule types in the digestive diverticula of bivalves.

A second model (Text-fig. 2, centre) in which the tubules go from a more or less homogeneous appearance or 'holding phase' into an unsynchronized cycle, eventually returning to the holding phase when the food supply is exhausted or the animals are exposed by the tide, has been proposed by Owen (1972). Although there is a short period during which the tubules may appear more or less uniform, most of the time it would be possible to find all three tubule types. This seems to be the best generalized representation of events for *Mytilus edulis*. The difference between Owen's model and the results for *Mytilus* (Text-fig. 2, bottom) is probably due to differences in exposure time or in the level of

feeding. Whereas Owen suggests that during the low tide period all the tubules will assume the homogeneous appearance of the holding phase, this does not occur at mid-tide level on the shore. Instead, there is a gradual change over the tidal cycle, in the relative proportions of tubules in stages I and II.

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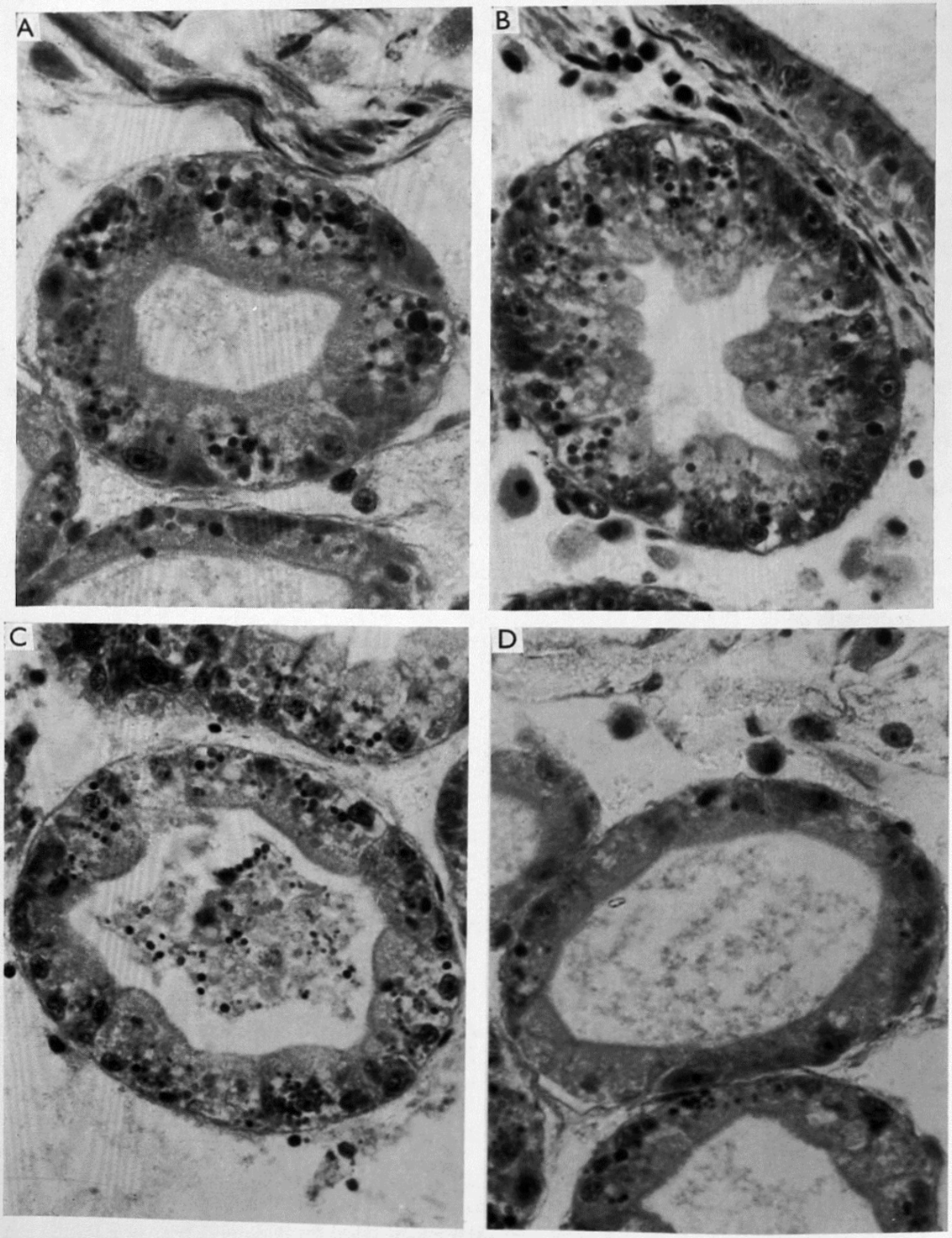

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EXPLANATION OF PLATE I

Tubule types in the digestive diverticula of *Mytilus edulis*. A, Type I, normal or holding phase; B, Type II, absorptive phase; C, Type III, disintegrating phase; D, Type IV, reconstituting phase. Magn. A, B and D ×750; C, 563.