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THE TIDAL RHYTHM AND ACTION OF THE DIGESTIVE SYSTEM OF THE LAMELLIBRANCH *LASAEA RUBRA*

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(Text-figs. 1–8)

It has been well established that some of the features of the feeding and digestive process in molluscs are rhythmic in character. Notably Hirsch (1915, 1917, 1931) has demonstrated a rhythmic periodicity of secretion in the salivary glands and digestive glands of some carnivorous Gastropoda, and Krijgsman (1925, 1928) has done similar work on the land pulmonate *Helix*. In all of these animals—and in cephalopods, too, where there is a more elaborate nervous control of secretion—discontinuous feeding is the rule. In the other and perhaps larger category of molluscs—those continuously feeding on fine particles—the central mechanism of the gut is the crystalline style, or its forerunner the protostyle. Here the need is, in Yonge's words (1937), 'to the extent to which they depend on extracellular enzymes for digestion, continuous secretion'. In Yonge's view, now classical, the style was regarded as 'an ideal mechanism for the continuous liberation of small quantities of an amylolytic enzyme'. Graham (1939), in his work on style-bearing gastropods, showed that the style is in general confined to animals with a continuous feeding habit, whether by ciliary means or by using the radula to graze on and rasp off fine particles. It is known that style secretion stops and the style is frequently dissolved when animals are removed from the water and cease feeding (see Yonge, 1925).

It has also been suspected in recent years that the digestive gland in continuous feeders displays a well-marked periodicity, with phases of absorption and of ejection of cell contents into the stomach. To some extent the action of the digestive gland in molluscs is still a subject of controversy. Yonge (1926*a*, *b*) made the first thorough study of this gland in a lamellibranch, and held it to be an absorbing organ, ingesting fine solid particles for intracellular digestion. In the opisthobranch gastropods, Fretter (1939) and Graham (1938) elucidated the nature of the digestive gland in detail, and found it to be an organ which, in addition to its ingesting role, had an important accessory role in excretion. Numerous authors, too, have found it to secrete into the stomach. This has always been assumed in carnivores, for example from the work of Hirsch (1915); and Millott's observations (1937) on the nudibranch *Jorunna* showed

the formation of what the present writer (1955*a*) later called 'fragmentation phagocytes'. It was further established that the digestive gland in the primitive pulmonates had a secretory role, which, it was suggested, might be general in microphagous as well as macrophagous gastropods.

In the lamellibranchs, Mansour (1946) made claims for the secretory action of the digestive gland which seemed to go to the length of denying its ingesting role. In this class the problem is simpler, for we are concerned with a gland whose single function appears to be digestive. Several writers on gastropods have suggested an alternation of absorption and secretion in the digestive gland; but the present view, most generally accepted by workers on lamellibranchs, is that such extracellular digestion of proteins and fats as may occur in the stomach is performed by the enzymes from burst or cytolysed phagocytes, and that the 'secretory' role of the digestive gland is confined to the elimination of effete and rejected particles. In a recent investigation of *Lasaea rubra* (Ballantine & Morton, 1956) the authors noted the extreme suitability of that species for a contribution to this problem. *Lasaea* was found to carry out preliminary extracellular digestion of naked or thin-walled flagellates, *Phaeodactylum* (*'Nitzschia'*) and diatoms. It furthermore has no gut phagocytes. *Lasaea* lives at a high tidal level, and the periodicity that must thus be imposed on its feeding was thought to offer good scope for an investigation of possible periodicity, both in crystalline style secretion and in the action of the digestive gland. Oldfield (1955) has recently completed a detailed account of the structure of *L. rubra*, including a full description of the gut; and Owen (1955) has published a review of certain aspects of the lamellibranch digestive gland, which is principally concerned with other problems than are discussed here. The writer has benefited greatly by discussion with Mr Owen and from being allowed to read his paper in manuscript.

This work was done while the author was the holder of the University of London table at the Plymouth Laboratory of the Marine Biological Association. His thanks are due to Dr Mary Parke for the generous provision of some of the organisms used in experiments, to Dr G. Y. Kennedy, for much assistance in the investigation of pigments, to Mrs E. Oldfield for a helpful discussion and to Miss D. Ballantine for useful criticisms and suggestions. Finally, the writer has had the great assistance of many discussions with Prof. C. M. Yonge, to whose knowledge of the lamellibranchs and kindly encouragement and criticism of this manuscript he is especially indebted.

The Ingestion of Food and the Secretion of the Crystalline Style

The specimens of *Lasaea rubra* used in this work were gathered from crevices and between barnacle shells near the Hoe (Tinside) bathing pool, at a point that is covered by normal tides for approximately 3 h out of 12. During this

time they were able to filter and feed. The relation of the condition of the gut to the tidal cycle was investigated by the daily collection of animals over fortnightly periods and by making routine observations during these periods at the same time each day. An alternative method was to collect animals at hourly intervals during a single day's tides, and also to examine animals experimentally fed after known periods of filtering.

The presence and position of food in the gut was first accurately determined by the fixing, clearing and examining by transparency of sufficiently large samples of animals. This feature was found to show a very constant relation to the position of the tide, so that the immediate past history of a given animal could be fairly reliably ascertained by such an examination of its gut. The nature of the contents of the gut have already been discussed by Ballantine & Morton (1956). Table I sets out the relation between the tidal level and the gut contents of some of the animals considered.

TABLE I. POSITION OF FOOD IN THE DIGESTIVE TRACT OF *LASAEA RUBRA* AT VARIOUS TIMES OF THE TIDAL CYCLE

Sample no.	Hours submerged	Stomach only	Stomach and anterior intestine	Loop of intestine only	Rectum only (faeces in pallial cavity)	Gut empty	No. of specimens examined
1	1	.	16	2	1	5	24
2	2	.	8	4	3	5	20
3	3	3	27	.	.	.	30
4	3½	.	24	1	1	1	27
	Hours exposed						
5	0·3	.	.	11	4	3	18
6	1	.	.	6	7	6	19
7	2	.	.	8	5	.	13
8	8½	.	.	1	1	14	16
9	9	.	.	3	3	16	22

Equally predictable, in relation to the tide, was found to be the size of the crystalline style, and these two features, gut contents and style size, are treated in the next section as the foundation for a third rhythmic feature of the digestive process, the condition of the digestive gland.

From Fig. 1 it becomes clear that during the normal digestive cycle the crystalline style becomes partly dissolved, after the tide has withdrawn, to be rapidly secreted again on the return of the tide. When the stomach is filled with food the style is large and robust, filling the whole length and breadth of the style sac, in which it rotates, with a coiled food string attached to its free end. The style performs perfectly in *Lasaea* the role of a capstan first suggested by Orton (1923, p. 54) and later observed by Yonge (1949) in the Tellinacea, and by Morton (1952) in a number of gastropods. An observation sometimes possible in *Lasaea* strikingly illustrates this role: the style with the coiled string attached could by dissection be slipped from the grip of the

style sac, whereupon it was rapidly rotated in the reverse direction to its previous movement by the uncoiling of the tight twist it had imparted to the food string.

The style is reduced to a very small size in the last hours before the return of the tide, and sometimes—though not often—it seems to disappear completely. This was so with the animals in column 12 of Fig. 1. These were at the top of the tidal range during a neap tide, and the visit of the tide must have been too short to re-establish the style. This cannot happen often, and as a rule a style of full dimensions reappears in less than 1 h.

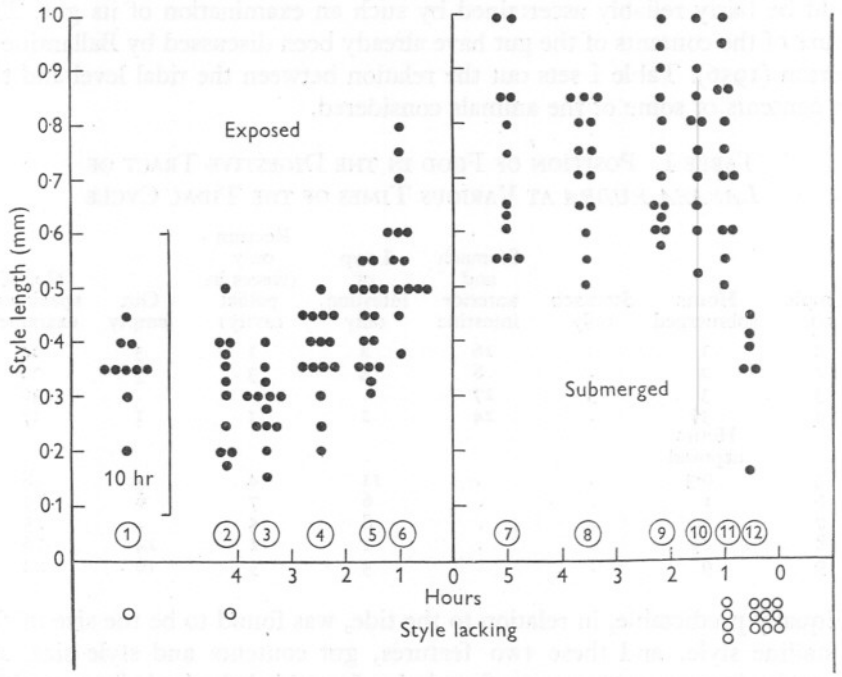

Fig. 1. Diagram showing the relation of the length of the crystalline style to the duration of exposure or submersion of the animal. Clear circles below the zero line represent animals in which the style was found to be absent.

From such observations as were made, the stimulus to the resecretion of the style appears to be the mere resubmersion of the animals. The presence of food in the stomach seems unnecessary, for the style was found to be restored to full size after 1½ h in filtered sea water (Fig. 1, column 10). Here, there may have been some small entry of particles such as debris dislodged from the animal's shell; but even after submergence in cultures of the toxic flagellate *Gymnodinium veneficum*, in which (see Ballantine & Morton, 1956) filtration is prevented, the resecretion of the style was not impeded (Fig. 1, column 11).

Some Experiments on Feeding and Digestion

Lasaea rubra is without gut phagocytes at any stage. During this work serial sections have been carefully examined from more than 200 animals, and these amoebocytic cells, which in other lamellibranchs emerge into the lumen to take part in digestion, could never be found. This is possibly a consequence of the small cell size of a minute animal, which may render the phagocytic mode of ingesting particles unsuitable. In this feature *Lasaea* is almost certainly exceptional among the eulamellibranchs. There is as yet no reason to suppose that other lamellibranchs of the Erycinacea may share in this peculiarity; as well as being of small size, *Lasaea* has further specializations peculiar to itself in this group, in particular its extremely high upper tidal limit and the periodicity thus imposed upon its feeding and digestion.

Extracellular digestion of carbohydrates has long been known to take place by the action of the enzymes of the crystalline style. In *Lasaea rubra* there is evidence, from two sources, of extracellular digestion of other substances as well. First, Ballantine & Morton (1956) have already reported in detail the preliminary digestion of *Phaeodactylum* ('*Nitzschia*'), the flagellate *Isochrysis*, and—more slowly—the diatom *Thalassiosira*. All these organisms were digested extracellularly within the lumen of the stomach. With *Phaeodactylum*, digestion could be quantitatively estimated at intervals over a 2 h period by direct counting of organisms in the stomach. The thin cell wall appears to require no cellulase to break it down, and the cell contents, consisting in large part of protein and lipoid reserves, were completely digested out. The cell wall was left as an empty but recognizable 'ghost', sometimes containing traces of the complex carbohydrate leucosin, which appeared to resist digestion longer. The cellulose-armoured dinoflagellate *Peridinium trochoideum* was also fed to *Lasaea*, but was not digested at all.

Evidence of extracellular digestion was also gained from feeding experiments with dogfish erythrocytes. This food, like flagellates, could easily be fed in filterable suspension and had the advantage that it could be readily recognized in the gut after sectioning, and stages in the digestive breakdown of the corpuscles could be followed over the succeeding 8 h, until absorption was complete. Digestion could be carefully localized, and was found to take place in the lumina both of the stomach and of the digestive diverticula. Table II sets out the histologically visible signs of erythrocyte digestion over a period of 10 h after feeding.

For the first 30 min after feeding, the cytoplasm of the erythrocytes stained blue with azan. With the onset of digestion, the cytoplasm was attacked first, and during the first 2 h the outlines of the cells became more irregular until, at length, the nuclei were alone recognizable. Azan staining had the great advantage that its reaction with the erythrocyte changed from light blue to yellowish brown or orange as digestion proceeded. Eroded corpuscles were

TABLE II. PROGRESS OF DIGESTION OF DOGFISH ERYTHROCYTES BY *LASAEA RUBRA* DURING 11 H, AFTER FEEDING WITH A PALE SUSPENSION OF BLOOD IN SEAWATER

(Animals were removed after feeding for various lengths of time, and in some experiments were kept in filtered sea water for varying times before fixing. Fixed Bouin's, stained azan.)

Specimen no.	Time in blood suspension	Time in filtered sea water after feeding	Condition of erythrocytes after azan staining of sections	Condition of digestive gland (see pp. 571 *et seq.*) I	II	III	IV
1	30 min	—	Many in the oesophagus and stomach, all intact, with cytoplasm blue. Majority in diverticula intact and blue, though a few eroded, and cytoplasm yellowish	×	×	–	–
2	30 min	—	Only a few in diverticula, none digested. Many in stomach not digested.	××	×	–	–
3	1 hr	—	Majority in stomach and diverticula undergoing digestion, cytoplasm orange yellow, margins usually much eroded. Nuclei persist longest. Final stage is of dispersion of yellowish granular cytoplasm released from the cells. Free nuclei visible (See Fig. 2)	××	–	–	–
4	1 h 50 min	—	All in stomach staining yellow to orange; much erosion in diverticula, and dispersion of amorphous yellow material. Yellowish droplets appearing in distal cytoplasm of digestive cells	×	×	×	–
5	1 h 50 min	—	Very eroded in diverticula, intensely orange. Much diffused yellowish material in lumen, some being absorbed by digestive cells. No free nuclei detected	××	–	–	–
6	2 h	1 h	All now in the diverticula, much more digested than previously; very few intact, but released yellowish material freely taken up along upper third of digestive cells	×	××	–	–
7	2 h	1 h	Much diffuse yellow from digestion. No whole corpuscles. Digestive cells absorbing	×	××	×	–
8	2 h	1 h	A few erythrocytes, almost wholly eroded in diverticula, and a great deal of diffuse yellow	××	×	–	–
9	2 h	1 h	Identical with (8)	×	××	–	–
10	2 h	2 h	No corpuscles and little diffuse yellow material in lumina of diverticula, but the upper half of the digestive cells loaded with orange-red droplets. Digestion and absorption appear complete	–	××	×	–
11	2 h	3½ h	A few areas of yellowish material remain in the lumen, but digestion is mostly completed. Orange-red granules now deeper in the digestive cells, with a superficial layer of blue-staining vacuoles (? material absorbed after blood)				
12	2 h	3½ h	Similar to (11)	–	××	××	–
13	2 h	8½ h	No trace of yellowish material in lumina of diverticula. Digestive cells and cut off spherules filled with reddish brown vacuoles	–	××	××	–
14	2 h	8½ h	Identical with (13)	–	××	××	–

always yellowish brown, and after the complete breakdown of the cytoplasm, large amounts of yellowish granules appeared in the lumen. Absorption by the digestive gland could also be traced by staining with azan. At 3 h from first feeding, droplets of yellowish material were being taken up, after 4 h the vacuoles of the upper layer of the epithelium were strongly reddish brown after azan staining. After $5\frac{1}{2}$ h these vacuoles lay more deeply and there was a superficial layer of light-blue staining vacuoles, which had evidently arisen from material later absorbed (see Fig. 2).

Azan staining may not be ideal for the tracing of erythrocyte digestion, since its coloration is apt to be unpredictable and uncertain in interpretation. In *Lasaea*, however, orange or reddish stained digestive vacuoles were never obtained save after feeding with blood, and the method by good fortune allowed digestion and absorption to be reliably traced. Another staining technique that might be attempted after digestion of erythrocytes is the Prussian blue detection of iron after its possible release as 'haemosiderin'. While haemoglobin specific stains are sometimes lacking in definiteness, the writer has found Van Gieson's to be a general stain that picks out haemoglobin rather distinctively (see also Dunn & Thompson, 1945).

Although occurring *in vivo*, these experiments were subjected to as careful as possible control. It was suspected that the cells might have been disintegrated by the mechanical action of the surrounding mucus or ruptured by the effect of the lower pH of the stomach contents, rather than by enzyme action. Cells were therefore examined that had passed through the mucus of pseudofaeces, and were found to show no erosion. Comparison was made with blood cells in other locations in the gut of ciliary feeders where enzymes were not active. It was impossible to measure the stomach pH of *Lasaea*, but this was assumed (see Yonge, 1925), to be somewhat greater than 5. Suspensions of dogfish erythrocytes and of *Phaeodactylum* were therefore immersed in salt solutions isotonic with sea water and buffered at pH 5·3, 5·5 and 5·9. Inspection at hourly intervals over the period of the experiments never showed any evidence of rupture or disintegration. Smears of dogfish blood were also subjected to digestion by mammalian trypsin at pH 7, after which the erythrocytes showed erosion in a similar way to those fed to *Lasaea*.

References to 'extracellular digestion' in this paper must clearly be taken to imply digestion in its widest sense. Preliminary digestion of relatively large cellular particles in the stomach proceeds at least as far as the breakdown of the cell wall; the cell contents are thus liberated in finely divided form, able—with or without further digestion—to be ingested by the free surface of the digestive cells. No one to-day would doubt that the digestive gland has an important function of intracellular digestion.

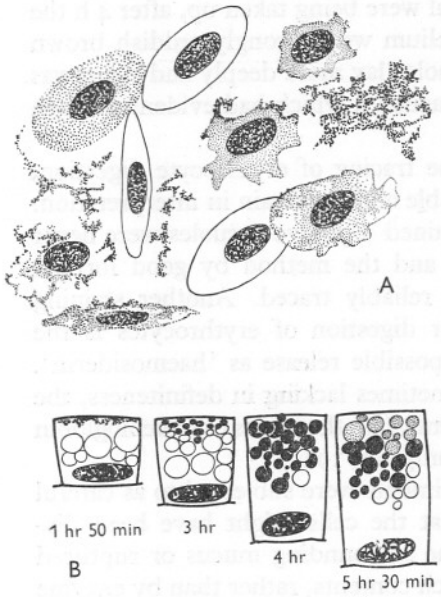

Fig. 2

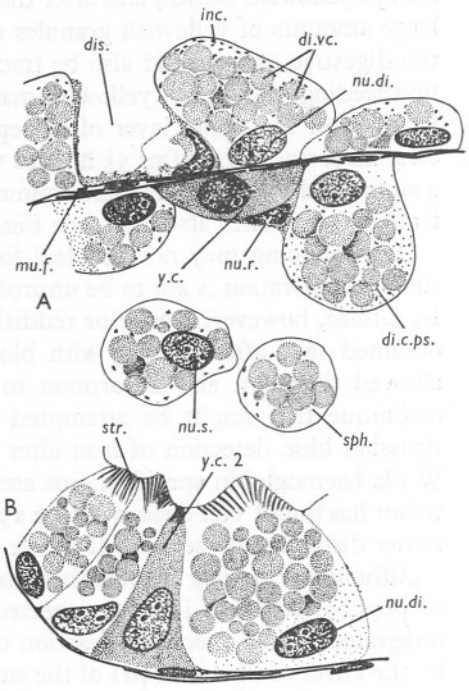

Fig. 3

Fig. 2. A group of dogfish erythrocytes in the lumen of the digestive diverticula of an animal that had been fed for 1 h with a suspension of blood corpuscles, and fixed in Bouin's fluid immediately. Erythrocytes with clear cytoplasm stained blue in azan, the nuclei red. After digestion an orange colour was shown by the cytoplasm, stippled in the figure, of both the intact and the eroded erythrocytes. Digestive cells, represented diagrammatically at 1 h 50 min, 3 h, 4 hr, and 5 h 30 min, respectively, after feeding with a suspension of blood. Absorbed material staining orange or brown with azan is represented black.

Fig. 3. (A) Portion of the wall of two adjacent tubules of the digestive gland. The digestive cells are at the beginning of the fragmentation stage, following absorption, and two spheres released into the lumen are shown in the lower part of the figure. Fixed Flemming's without acetic. (B) A group of cells from a tubule of the digestive gland during or after absorption, but before fragmentation. The striated layer at the free border is well shown. Fixed Bouin's fluid. Both drawings are from sections stained with azan. *di.c.ps.*, digestive cell which has become pseudopodial prior to the formation of a sphere; *di.vc.*, vacuole of absorbed material within a digestive cell, blue-staining with azan; *dis.*, space left in the epithelium after the discharge of a sphere; *inc.*, small inclusions, red-staining in azan, in the cytoplasm of a digestive cell; *mu.f.*, muscle fibre; *nu.di.*, nucleus of a digestive cell; *nu.r.*, nucleus, (?) remaining after discharge of a sphere, displaced to the surface of the epithelium; *nu.s.*, nucleus of a free sphere, lying in the lumen; *sph.*, sphere within the lumen, with no nucleus apparent; *str.*, layer of striations at the free surface of the digestive cell; *y.c.*, young cell; *y.c.* 2, young cell, compressed to a narrow shape between adjacent digestive cells.

The Cycle of the Digestive Gland

The structure of the digestive gland was studied in two ways: by the examination of stained serial sections, of which many were cut; and by the viewing of the whole gland in living condition immediately after removal from the animal. For this purpose digestive glands were isolated from other tissues, quickly mounted in sea water, lightly covered and immediately sketched under $\frac{1}{12}$ in. oil-immersion objective. For quickness and accuracy this method was by far the more useful: the whole gland could be carefully scrutinized, while avoiding all fixation artifacts and allowing reliable interpretation of the living cell. This is another of the advantages of working with a small animal. Furthermore, large numbers of preparations could be made with no laborious procedure.

On the whole, the best stained preparations were obtained after fixing with Flemming's without acetic. Though penetration of Flemming's was usually poor and microanatomy distorted, yet in cell detail the best preparations with Flemming's were the finest of any obtained. Both aqueous Bouin's and Susa were found suitable for microanatomy, and both penetrate well. In spite of what is sometimes alleged against it as a cytoplasmic fixative, I have not found Bouin's, in this or in other molluscs where I have used it, much inferior to Flemming's. It has the great incidental advantage that it perfectly removes the shell. Ten per cent. formalin always gave poor results with *Lasaea*, penetrating badly and yielding poor staining. Heidenhain's azan was used for staining throughout.

The tubules or follicles of the digestive gland are lined wholly with glandular cells, which, as usual in bivalves, are essentially of one kind. No cilia were found in the tubules anywhere beyond the exit of the diverticula from the stomach, and in this feature *Lasaea* differs, probably again on account of its small size and functional simplification, from the numerous lamellibranchs recently studied by Owen (1955).

The digestive cell is a versatile structure and passes through several forms during its history. We should first refer to the nests of small cells, which correspond to the 'crypts of young cells' described by Yonge (1926*a*, *b*) and by Owen (1955). These cells in *Lasaea* occupy the tips of the older tubules of the digestive gland, and have a very different appearance from the absorbing cells. They are coloured pale yellow in life and appear to lack all forms of visible inclusions. They generally form groups of half a dozen or more cells at the tubule tip (see Figs. 5 and 6); and small spherical clusters of young cells are usually also to be found, forming smaller club-shaped branches that evidently represent the rudiments of new tubules. Owen describes the 'young cells' in eulamellibranchs in general as extending from the tubule tip in two or more tracts or 'crypts' along the whole length of the lumen of the tubule. Isolated young cells could often be seen in *Lasaea*, mingled with the more mature

absorbing cells, along the length of the tubule, but their most usual position is always in clusters at the tip. In correlation with the small size of the animal, and the relatively small number of cells constituting the whole tubule, replacing cells would seem as a rule to be concentrated at the tip of the tubule rather than diffusely scattered. A well-known parallel to this distribution of young cells is found in the nests of cells at the tips of the digestive tubules in the crayfish, *Astacus*, described and figured by Jacobs (1928).

Difficulty was encountered in *Lasaea* with the fixing and staining of the contents of the terminal cells. Oldfield (1955) states that, on fixing with Susa, the cell contents contract from the periphery and form a sphere of heterogeneous consistency. With Bouin's and Flemming fixation, the writer's results were generally unsatisfactory, the cells appearing as almost empty vesicles with their cytoplasmic contents barely recognizable or wholly shed. The isolated young cells dispersed irregularly among the digestive cells (see Fig. 3, *y.c.*) were, however, usually well fixed, and their cytoplasm stained pinkish brown with azan.

Oldfield (1955) remarks upon the great difference in appearance of the terminal cells from the absorbing cells, and gives evidence for the presence of two distinct types of cell in the digestive tubules of *Lasaea*. A short statement is thus necessary of the present writer's reasons for adopting a different interpretation on this point, and for taking a unitarian view of the terminal cells, as the youngest stages of a single versatile cell type, the absorptive and digestive cell. In specimens examined during this work, it seemed that the cell contents, 'of semifluid yellow material', rather than forming a vacuole, constituted in fact the whole of the cytoplasm of the cell, uniformly light yellow coloured and devoid of any stainable inclusions. As Oldfield points out, there is no response to tests for calcium, glycogen, fats or lipides. In the examination of digestive glands dissected living, these terminal cells can be shown to be wholly similar in form and appearance to the light-coloured 'young cells' interspersed with mature cells along the length of the tubule. Furthermore, in digestive glands examined alive immediately after feeding, pale-coloured cells in the terminal groups were seen to be absorbing droplets of pigmented food material along their free surface, while otherwise identical in appearance with non-absorbing cells alongside them. At times the whole cell group at the tip of a tubule was found to be carrying out absorption. In other specimens, soon after feeding, a single non-absorbing 'young cell' alone remained at the tip of the tubule; all its neighbours had been pressed into service as absorbing cells. This was the picture most generally seen within an hour or two of feeding; by contrast, in animals that had remained dry for some hours, the most conspicuous feature of the almost colourless digestive gland was the appearance of small rudimentary tubules, with no contents in their

lumina, and composed wholly of 'young cells' that had not absorbed (see sketches from life in Fig. 6).

The position of young cells at the tip of the tubule is in agreement with Owen's results on a wide range of eulamellibranchs, and also, as was pointed out above, with the condition in the digestive gland of *Astacus*. It would be desirable, but was not attempted during the present work, to investigate the incidence of mitotic divisions in these cells in *Lasaea*. Yonge (1926*a*) found numerous mitotic figures during histological studies of similar cells in other lamellibranchs. It is remarkable that the terminal cell groups should fix and stain so poorly as compared with the similar cells irregularly scattered among mature digestive cells. One must suppose that the uniform, semi-fluid cytoplasm of the groups of young cells is peculiarly susceptible to disruption by the tensions set up by diffusion currents in fixing and dehydrating. The isolated cells, surrounded by mature absorbing cells packed with contents, would thus be better protected against distortion arising during fixation.

The young cells are at first typically dome-shaped or pyramidal (see Figs. 5 and 6) and bulge from a flat base towards the lumen. Although they were never seen to be ciliated in *Lasaea*, either by the present writer or by Oldfield (1955), they seem with little doubt to be cells of the same type as were found in other lamellibranchs (see Owen, 1955; Potts, 1923) to bear long lash-like cilia at this stage of their history.

The digestive cells do not long remain dome-shaped or colourless, and the first transformation seems to be brought about by the flow of food material into the tubules. The lumina now become widely distended, and—though certainty is difficult on this point—the impression is gained that the young cells constituting some of the tubules are actually stretched by the mechanical pressure of contents, and that as the tubule is enlarged, so each cell becomes wider based and flattened. The surface appears at this stage to absorb material freely from the lumen, and there is often a continuity of staining between the cytoplasm of the cell and the contents of the lumen. At the same time there is a gathering of yellow vacuoles in the cytoplasm.

Absorption was studied by several experiments with natural and artificial foods. A suspension of colloidal graphite ('Aquadag') was fed to the animal, by mixing it with *Phaeodactylum*. After 2 h, absorption was freely in progress, and the surface of the digestive epithelium was dark grey. Closer examination revealed that graphite particles lay freely in the superficial cytoplasm, and were not at this stage contained in vacuoles. After feeding with *Phaeodactylum* alone for 1 h, immense numbers of tiny colloidal droplets could be detected both in the lumen and in the cytoplasm surrounding the vacuoles. They were being rapidly absorbed by the cells. Pigment introduced with food organisms, as well as probably indigestible waste materials, seems to aggregate in the vacuoles. After feeding for 2 h on *Phaeodactylum* made pink with neutral red, a pink coloration was found in the vacuoles in the upper half of the cell,

those at the base remaining yellow. In addition, minute red-stained granules lay freely in the cytoplasm between the pink vacuoles. These probably represent particles derived from the preliminary extracellular digestion of *Phaeodactylum* and now being taken up by the cells. A similar absorption of pigment by the vacuoles could be detected after feeding with a culture of *Dunaliella*, a thin-walled chlorophycean flagellate (6–12 μ) (Plymouth no. 83). After 1½ h feeding a long rope of flagellates was found attached to the head of the style and each digestive cell showed a conspicuous absorption of chlorophyll in all the vacuoles of its superficial half. Preliminary digestion of *Dunaliella* took place extracellularly. The cells—as with *Phaeodactylum*—are much too large for direct ingestion; in one case where the lumen of a tubule was clogged with undigested flagellates no ingestion took place.

In addition to the absorption of graphite and plant pigments, the ingestion of soluble and insoluble iron saccharate by the digestive cells of *Lasaea* has been well demonstrated by Oldfield (1955).

Absorbing cells increase rapidly in height to columnar or bulging form (Fig. 3). During absorption the cytoplasm becomes loaded with conspicuous orange-yellow vacuoles which impart their colour to the living digestive gland. Such vacuoles seem to occur very widely in the molluscan digestive gland. They are essentially similar in *Lasaea rubra* to those recently described in the ellobiid pulmonates by Morton (1955*a*) in an account of the cyclic changes of the digestive gland. Their staining reactions in various Mollusca seem very uniform. They are coloured blue after azan (except in some rather exceptional cases, as after feeding with blood, p. 569); they become green with Masson's trichrome and do not stain at all with haematoxylin, or with the mucus stains alcian blue or mucicarmine. They appear to be loaded with lipoid material which they probably store and which is responsible for their strong staining reactions with Sudan scarlet and with osmic acid. The vacuoles are not, however, simply droplets of lipoids: they have a permanence in the cell quite distinct from their occupation by fatty inclusions, and after treatment with acetone or ether they can be seen to persist as colourless spheres. Staining reactions with azan and with Masson's stain are well elicited in sections from which stored fat has been removed by routine treatment.

In the pulmonate *Otina* (Morton, 1955*b*) these vacuoles were studied in their 'empty' colourless condition towards the base of the cell, and were shown to be covered by a surface layer or membrane, stainable with thionin, but with no other stains tried. Attempts to detect it in *Lasaea* failed. This layer has many of the properties of that described by Barrington (1951) surrounding the 'chromophobe bodies' in the islet cells of the frog's pancreas. Colourless vacuoles are present in the digestive cells before the ingestion of food, and the writer earlier suggested (1955*a*) that the cell enzymes might be contained in or around them, or absorbed on the 'surface layer' when it occurs. There is as yet little positive evidence of this, and it would be of interest to examine

further the enzyme localization of the digestive cell. The small size of *Lasaea* does not, however, make it ideal for such tests.

The vacuoles of the digestive cell owe their coloration to mixtures of pigments taken into the cell from the materials fed upon. These pigments, originating in phytoplankton and fragments of plant detritus, included large amounts of chlorophyll. For extraction of the total pigments from *Lasaea*, pale or colourless low-tidal individuals were selected, with a minimum of algal

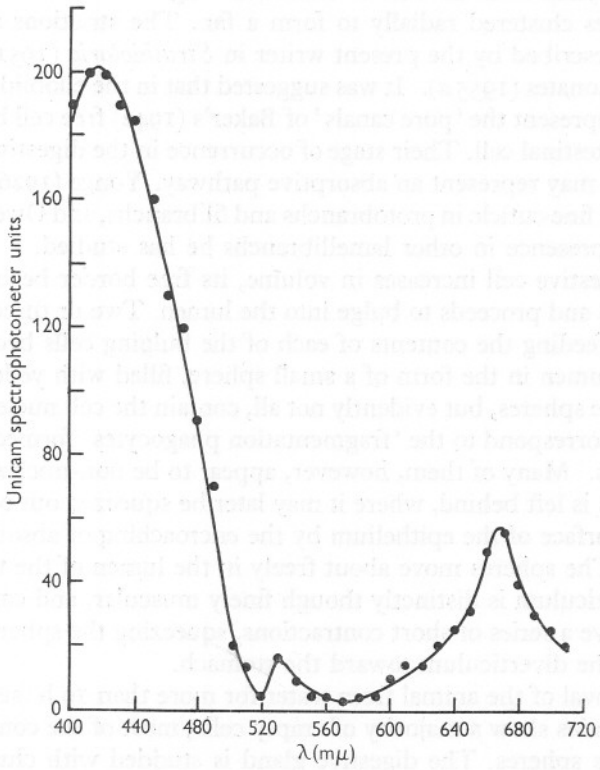

Fig. 4. Light-absorption curve obtained from the pigments extracted from the digestive gland, measured by the Unicam absorptiometer.

contamination of the shell. The spectral curve obtained after extraction with methanol of a greenish yellow solution from the soft parts is shown in Fig. 4. With peaks at 670, 535 and 420, it corresponds fairly closely with that of chlorophyll. Examination of macerated digestive glands with the spectroscopic microscope was difficult because of the animal's minute size; the 670 band, however, seemed fairly clearly identifiable. Pigments of the fucoxanthin type might also be expected to appear in the digestive gland, deriving with chlorophyll from the phytoplanktonic food (see Heilbron, 1942), and these are

probably chiefly responsible for the yellow coloration. No attempt has yet been made to extract separately the pigments present in *Lasaea*, and in the absorption curve shown in Fig. 4, fucoxanthin bands at *c.* 450 and 480 would—if present—be concealed (Strain, Manning & Hardin, 1944).

During the absorption of food the free border of the digestive cell may often be obscured by the passage of particles from the lumen. At other times, following soon after absorption, its free surface possesses a narrow border of fine striations, and when the surface of the cell bulges towards the lumen, these are sometimes clustered radially to form a fan. The striations are identical with those described by the present writer in *Struthiolaria* (1951) and in the ellobiid pulmonates (1955*a*). It was suggested that in the ellobiid *Leucophytia* they might represent the 'pore canals' of Baker's (1942) free cell border in the vertebrate intestinal cell. Their stage of occurrence in the digestive cell makes it likely they may represent an absorptive pathway. Yonge (1926*a*) speaks of this layer as a fine cuticle in protobranchs and filibranchs, and Owen (*in litteris*) confirms its presence in other lamellibranchs he has studied.

As the digestive cell increases in volume, its free border becomes bluntly pseudopodial and proceeds to bulge into the lumen. Two or three hours after cessation of feeding the contents of each of the bulging cells become nipped off into the lumen in the form of a small sphere, filled with yellow vacuoles. Some of these spheres, but evidently not all, contain the cell nucleus, and they would then correspond to the 'fragmentation phagocytes' formed in this way in gastropods. Many of them, however, appear to be non-nucleated, and the basal nucleus is left behind, where it may later be squeezed out or pressed up to the free surface of the epithelium by the encroaching of absorbing cells at either side. The spheres move about freely in the lumen of the tubules. The wall of diverticulum is distinctly though finely muscular, and can sometimes be seen to give a series of short contractions, squeezing the spheres and other contents of the diverticulum toward the stomach.

After removal of the animal from water for more than 10 h (see Fig. 5) the digestive tubules show a majority of empty cells, most of the contents having passed out as spheres. The digestive gland is studded with clusters of new tubules, consisting of young cells that have not yet absorbed.

At most times, spheres can be identified in the lumen (see Fig. 3*a*). They are generally present in some numbers during the absorbing phase of the digestive gland (Fig. 5*a*), though their time of formation appears always to be at the final stage of the cell's history, after absorption has been completed. They mark the end of the life of the cell, and their formation is a type of holocrine 'secretion'. The production of free spheres from the digestive cells has generally been regarded in lamellibranchs as a form of excretion. By this means, much pigmented material and indigestible remains are returned to the stomach for disposal as faeces. Owen (1955) has shown in detail how the arrangement of the typhlosole of the stomach and the excurrent tracts of

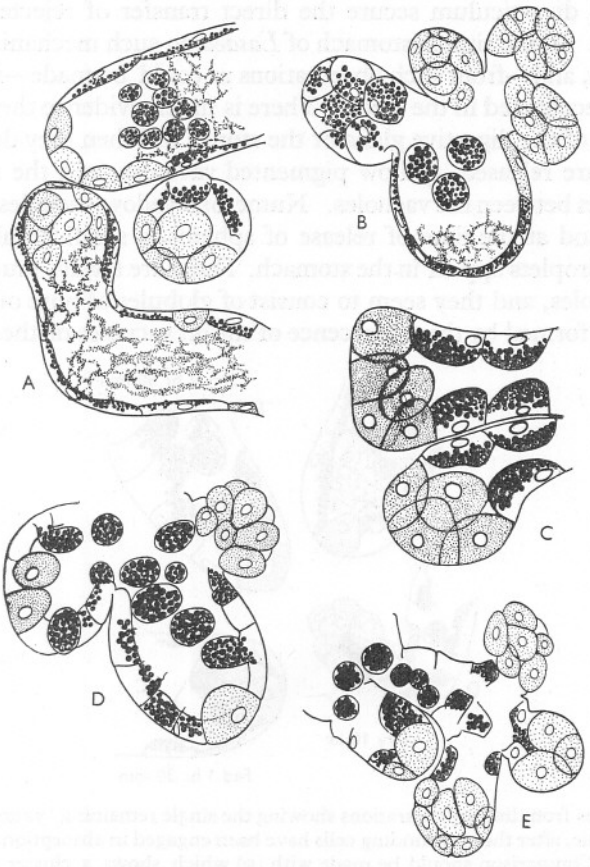

Fig. 5. Sketches made from living digestive glands, showing the features typical of various stages in the cycle. The portions of tubules illustrated are drawn from preparations examined as transparent objects under 1/12 objectives, the cover-slip resting lightly to prevent distortion of the cells. Young cells are present singly, in groups, or as young tubules and are lightly stippled in the figures; the coloured vacuoles in the mature cells are represented black. (A) Stage of absorption, with wide stretched tubules and flattened cells. Some free spheres are still present intact within the lumen of one of the tubules. Fed with *Phaeodactylum* for 1 h and examined immediately. (B) A later stage, from an animal exposed for 3 h after tidal withdrawal. The sketch shows three immature tubules composed of young cells, and, rather untypically for 3 h exposure, a tubule containing one young, non-absorbing cell, and the rest of its cells stretched flat and taking up contents from the lumen. (C) Stage of intact cells filled with absorbed contents, from a specimen exposed for 3¼ h after feeding with *Phaeodactylum*. The two tubules show very typically the appearance of groups of young cells at the rounded tips. (D) Stage of fragmentation of digestive cells, to produce free spheres. The majority of the digestive cells have discharged their contents, and are represented by colourless spaces. One cell is shown as a pseudopodial projection about to be cut off into the lumen. (E) Stage of fully discharged epithelium, from a specimen exposed for more than 12 h after feeding on *Phaeodactylum*. The majority of the cells are represented by empty spaces, and there are numerous spherules lying freely in the lumen. Three immature tubules are shown, composed of colourless young cells.

the digestive diverticulum secure the direct transfer of rejected material to the intestine. In the simple stomach of *Lasaea* no such mechanism appears to be developed, and—from such observations as could be made—intact spheres were never recognized in the faeces. There is much evidence they disintegrate in the lumen of the digestive gland or the stomach. When they do so, two sorts of contents are released—yellow pigmented vacuoles, and the residual cytoplasm that lies between the vacuoles. Numerous yellow vacuoles are found in the lumen, and at the time of release of spheres, a small number of golden brown, oily droplets appear in the stomach. These are always much larger than the cell vacuoles, and they seem to consist of globules of pure oil or fat. They are probably formed by the coalescence of the oil vacuoles of the disintegrated spheres.

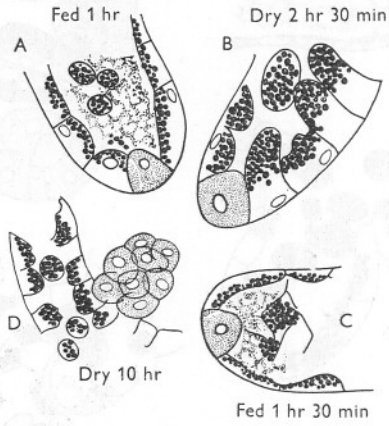

Fig. 6. Sketches from living preparations showing the single remaining 'young cell' at the tip of the tubule, after the surrounding cells have been engaged in absorption for 1 h or longer (A, B, C). Comparison should be made with (D) which shows a cluster of 'young cells' forming the rudiment of a tubule, in a specimen that has been several hours exposed; and with the group of young cells forming the whole tip of a tubule in Fig. 5 (*c*).

These large droplets were occasionally found in living preparations in such proximity to a recently discharged digestive cell as to suggest that they had been formed *in situ* by the coalescence of vacuoles before discharge. Such large vacuoles would then resemble those of the 'excretory cells' found in the digestive gland of gastropods. They were never found in sections of *Lasaea*.

Disintegration of the spheres may also release traces of free enzymes for extracellular digestion. It is suggested that these enzymes originate in the residual cytoplasm of the cells after the vacuoles are shed, and are responsible for the preliminary digestion of flagellates, *Phaeodactylum* cells and dogfish blood corpuscles shown to occur before absorption by the digestive gland. In other lamellibranchs, the free enzyme action detected in the stomach has been suspected to be due to the action of amoebocytes. In *Lasaea*, there would appear to be a different mechanism whereby food is able to be broken

down by preliminary digestion to particles minute enough to be absorbed by the digestive cells. The digestive cycle is primarily one of absorption and excretion; but from the contents of previously fragmented cells there appear to remain sufficient free enzymes to initiate a first phase of extracellular digestion.

Tidal Rhythm

The sequence of activity of the digestive cells was investigated by the examination of 225 animals, at known times after feeding or exposure by the tide. Some of these were fed experimentally, with *Phaeodactylum*, *Dunaliella*, colloidal graphite ('Aquadag') or dogfish erythrocytes. Others were collected at regular intervals from the field, and examined after natural feeding. Examination was by dissection of the living digestive gland, as described above (p. 573) or from stained serial sections. Table III presents in condensed form the methods used in this survey, and its results; while Fig. 7 shows graphically the condition of the digestive gland from hour to hour during feeding and subsequent exposure. Three stages of activity of the digestive gland have been recognized, namely, (I) *absorption*, with flattened epithelial cells of low height and widely distended tubules; (II) *digestion*, with the majority of cells fully loaded and columnar; (III) *excretion*, with the production of free spheres by rounding off and liberation of the epithelial cells. 'Secretion' may be regarded as a subordinate activity taking place together with excretion. A further condition (IV) may be recognized where the epithelium is almost completely discharged of coloured cells, after several hours' exposure, and the whole digestive gland is pale or colourless.

Tables III and IV and Fig. 7 disclose a periodicity of the digestive gland, in relation to feeding and exposure. Like the presence of contents in the stomach and the size of the crystalline style, this is under ultimate tidal control. It is thus possible to speak of a tidal rhythm in the digestive system of *Lasaea*. Processes that may take place more or less continuously in bivalves mostly covered by the tide are, in high-tidal *Lasaea*, compressed into the short period of 3 or 4 h out of the 12, the period to which filtering is restricted.

There is no period when it is possible to say that all the cells of the digestive gland are at a particular stage, and it is perhaps unlikely that all the cells or tubules of a digestive gland ever take part together in one cycle of activity. Nevertheless, it is possible to recognize a well-marked *predominance* of activity, during various times of feeding and exposure. Thus, immediately after feeding the cells are with few exceptions at the flat, actively absorbing stage. And later in the tidal cycle, subsequent stages can be very satisfactorily followed, each having a maximum period, varying in length with the duration and amount of feeding. Successive phases overlap, and in all cases young cells are present in clusters at the tips of the tubules.

Table III. Condition of Digestive Gland after Feeding

(The condition of the digestive gland is given in relation to time of feeding or exposure in 232 specimens of *Lasaea rubra*. The stages recognized are (I) absorption, (II) intracellular digestion, (III) formation of spheres, (IV) post-spherulation (see pp. 579 *et seq.*). A bold numeral indicates a *predominance* of a given stage. A bracketed numeral indicates a stage that was also frequent.)

Series 1 (4/54). Fed *Phaeodactylum* 1½ h, transferred to filtered sea water. Fixed at intervals shown. Examined in stained sections. (20 specimens.)

Hours after feeding	I	II	III	IV
		(numbers of specimens)		
1	**8**	3 (3)	1	.
2	(2)	**5**	1 (2)	.
3	1 (1)	3 (2)	1 (2)	.

Series 2 (4/54). Fed erythrocytes up to 2 h, transferred to filtered sea water, and fixed at intervals shown. Examined in stained sections. (43 specimens.)

Hours after feeding	I	II	III	IV
.	**12** (1)	4 (10)	(3)	.
1	1 (2)	**5**	(1)	.
2	.	2 (4)	1 (4)	.
3½	(2)	5 (1)	(3)	.
6½	1	2 (2)	2 (2)	.
8	(1)	4 (2)	3 (3)	(1)

Series 3 (4/54). Fed *Peridinium trochoideum*, 1¼ h. No digestion. Fixed immediately. (5 specimens.)

I	II	III	IV
1 (2)	**5**	1	.

Series 4 (4/54). Fed *Isochrysis galbana*, removed into filtered sea water. Fixed after intervals shown. (5 specimens.)

Hours after feeding	I	II	III	IV
7	1	1 (1)	1 (1)	.
8½	1	1	(1)	.

Series 5 (4/55). Fed 'Aquadag',* *Phaeodactylum* with neutral red,† and *Dunaliella*,‡ for times shown, and examined alive immediately after feeding. (7 specimens.)

Hours fed	I	II	III	IV
2*	3	.	.	.
2†	2	(1)	.	.
2‡	1 (1)	(1)	.	.
3‡	.	.	1	.

Series 6 (7/54). Fixed in the field daily over 8 days. Examined in stained sections. (43 specimens.)

Hours after feeding	I	II	III	IV
½	**5**	(4)	.	.
¾	1 (2)	5 (1)	3	.
1	2 (2)	3 (2)	1)	.
3	(1)	2	1 (2)	.
3½	1 (1)	2 (1)	1(1)	.
6	(2)	6	(1)	.
7	1 (1)	4 (2)	1 (3)	.
7½	(1)	4 (2)	3 (1)	.

Series 7 (8/54). Fed *Phaeodactylum* for times shown and fixed while still feeding. Examined in stained sections. (38 specimens.)

Hours fed	I	II	III	IV
2½	6 (4)	8 (3)	1 (5)	.
2¾	(1)	(3)	4 (1)	.
3½	2 (4)	3 (2)	1 (2)	.
4	.	.	1 (2)	.
5	1 (1)	4 (1)	2 (4)	.

Series 8 (8/54). Examined alive after feeding with *Phaeodactylum* or after exposure in the field for the periods shown. (42 specimens.)

Hours after feeding	I	II	III	IV
1¾	(1)	1 (2)	2 (6)	1 (3)
2½	.	.	1 (5)	(4)
3½	(1)	1	2 (2)	1 (1)
4½	.	(1)	4 (2)	2 (2)
5⅝	.	.	2 (3)	1 (4)

Series 9 (4/55). Examined alive after feeding with *Phaeodactylum* or after exposure in the field for the periods shown. (42 specimens.)

Hours fed	I	II	III	IV
1	2	.	(1)	.
1–2	1	.	.	.
2–3	(1)	1 (1)	.	.
3–4	2	(2)	(2)	.
4–5	.	2 (1)	1 (2)	1
Hours since feeding	**I**	**II**	**III**	**IV**
2–3	.	(5)	5	1 (1)
3–4	1 (1)	2 (4)	3 (4)	4 (3)
4–5	.	1 (2)	5	2
12–13	.	.	2 (6)	9 (1)

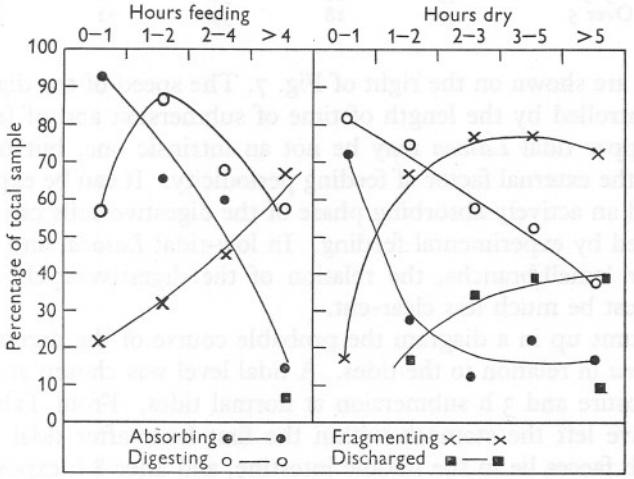

Fig. 7. Graph of the data set out in Tables III and IV showing the relation between the condition of the digestive gland, and the hours for which the animal had fed (left) or the hours for which it had remained exposed, following upon feeding (right).

Fig. 7 makes a comparison between two series of experiments in which animals were rather differently treated.

In the first series (results shown on the left) animals had been experimentally fed with *Phaeodactylum*, or with dogfish erythrocytes, in much greater abundance than that of natural food. After 4 h, the stages of 'absorption' and 'digestion' have generally been passed through, and 'fragmentation' predominates. Although the animals were still immersed in a suspension of food particles, the digestive cycle had—after overfeeding—advanced to a further stage than is normally found when animals in the field are left dry by the retreat of the tide. Animals which had spent 2 h feeding in natural conditions in the field were examined in a second series of experiments, and

TABLE IV. CONDITION OF DIGESTIVE GLAND

Percentage of occurrence of stages I to IV of the digestive gland, in relation to time after feeding or exposure, from results obtained with 225 animals. (In many cases a single animal is represented under more than one stage, and the calculations have been made by adding together the bold and bracketed entries in Table III.)

Hours	Total no. of animals	Percentages I	II	III	IV
Fed					
0–1	14	93	57	21	.
1–2	9	66	89	33	.
2–4	33	60	69	48	.
Over 4	13	15	61	69	8
Exposed					
0–1	26	73	81	19	.
1–2	25	28	76	68	16
2–3	17	12	59	77	35
3–5	39	21	56	78	38
Over 5	49	18	39	72	39

the results are shown on the right of Fig. 7. The speed of the digestive cycle is thus controlled by the length of time of submersion and of feeding. The cycle in upper tidal *Lasaea* may be not an intrinsic one, but one imposed merely by the external factor of feeding periodicity. It can be experimentally varied, and an actively absorbing phase of the digestive cells can at any time be produced by experimental feeding. In low-tidal *Lasaea*, and probably in most other lamellibranchs, the relation of the digestive cycle to the tidal rhythm must be much less clear-cut.

Fig. 8 sums up in a diagram the probable course of the digestive cycle of *Lasaea rubra* in relation to the tides. A tidal level was chosen at which there is 9 h exposure and 3 h submersion at normal tides. From Table I food is seen to have left the stomach within the first hour after tidal withdrawal. After 1–2 h faeces lie in the middle intestine, and after 8 h exposure the last remains of faeces are in the rectum or discharged into the pallial cavity. The style is largest while food is in the stomach, drawing in the food string by its

rotation. During exposure it becomes too small to be rotated in its sac, but is probably not often lost altogether. It is rapidly rebuilt during the first hour after the tide returns. The sequence of the four phases of the digestive gland extends throughout the 12 h of the tidal period, and is evidently controlled primarily by the presence of food in the stomach.

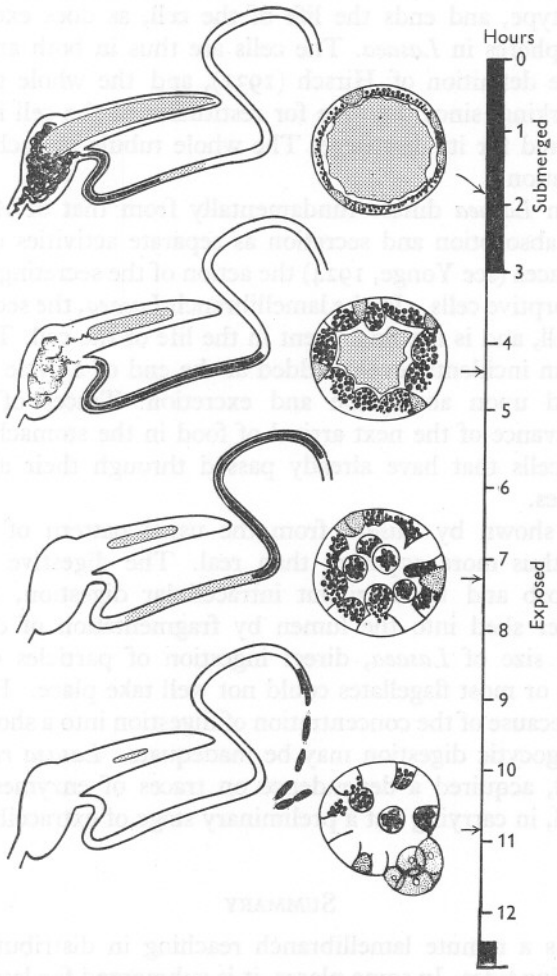

Fig. 8. Diagram showing the relationship between the periodicity of the digestive system, and the submergence or exposure of the animal by the tides. The outline drawings of the stomach, style sac and intestine indicate the size of the crystalline style, and the position of food in the various parts of the gut. The diagrams of digestive tubules, in transverse section, show the following stages: (I) absorption; (II) fully loaded cells, presumably with intracellular digestion in progress; (III) fragmentation, and formation of spherules; (IV) discharged epithelium, with spherules in the lumen, and a developing tubule composed of young cells.

Comparison may be drawn between the cycle of the digestive gland in *Lasaea rubra* and that of the digestive diverticula of the decapod crustacean *Astacus*, which was carefully investigated by Hirsch & Jacobs (1928, 1930). In decapods, the young cells forming the tip of the tubule give rise to two separate types of cell, secretory and absorptive. The secretory cells are under rhythmic control, which is speeded up greatly after feeding. Secretion is of the holocrine type, and ends the life of the cell, as does excretion by the formation of spheres in *Lasaea*. The cells are thus in both animals 'monophasic', by the definition of Hirsch (1931), and the whole gland shows a rhythmical working, since the time for restitution of the cell is greater than the time required for its discharge. The whole tubule in each constitutes a 'field of restitution'.

The cycle in *Lasaea* differs fundamentally from that of *Astacus*, in the occurrence of absorption and secretion as separate activities of one type of cell. In Crustacea (see Yonge, 1924) the action of the secreting cells precedes that of the absorptive cells. In the lamellibranch *Lasaea*, the secretory activity comes last of all, and is the final event in the life of the cell. This is because 'secretion' is an incidental event, added at the end of a cycle that is fundamentally based upon absorption and excretion. Traces of enzymes are provided in advance of the next arrival of food in the stomach, by the fragmentation of cells that have already passed through their absorptive and excretory phases.

Differences shown by *Lasaea* from the usual pattern of lamellibranch digestion are thus more apparent than real. The digestive cell has been shown to absorb and to carry out intracellular digestion, and excretory material is later shed into the lumen by fragmentation of digestive cells. From the cell size of *Lasaea*, direct ingestion of particles of the size of *Phaeodactylum* or most flagellates could not well take place. For this reason too, and also because of the concentration of digestion into a short time during each tide, phagocytic digestion may be inadequate. *Lasaea rubra* has thus, it is suggested, acquired a dependence on traces of enzymes shed by the digestive gland, in carrying out a preliminary stage of extracellular digestion.

Summary

Lasaea rubra is a minute lamellibranch reaching in distribution almost to high-water spring tides. In some places, it is submerged for less than an hour at each tide and a tidal periodicity is thus imposed upon its feeding. The effect of this periodicity is reflected in the mode of action of the gut. The amount and position of food varies regularly with the state of the tide. The crystalline style undergoes a regular cycle, being partly dissolved and becoming vestigial at low tide, and being resecreted at the high tide. A sequence of four phases was established in the digestive gland, by the study of large numbers

of animals, living and after sectioning, and after both natural and artificial feeding. While the animal is feeding, and shortly afterwards, finely divided material is absorbed into the cells of the digestive gland. Intracellular digestion then takes place, with the loaded digestive cell attaining its maximum size. Fragmentation of the digestive cells next occurs, with the discharge of rounded spheres, filled with vacuoles of rejected pigmented material. Finally, just before the tide returns, the digestive gland shows a discharged epithelium, with the digestive cells being replaced by young cells. Evidence is given of the close relation of this cycle to the tides. It is also shown that extracellular digestion of dogfish erythrocytes and phytoplanktonic organisms takes place within the stomach before absorption, and it is suggested that this is brought about by traces of enzymes in the fragmented spheres issuing from the digestive gland.

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