Observations on the Stomach and Digestive Diverticula of the Lamellibranchia

II. The Nuculidae

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SUMMARY

The anatomy and histology of the stomach and digestive diverticula and the physiology of digestion in the Nuculidae have been investigated.

The stomach is relatively large and possesses a well-developed style sac, although a rod-like crystalline style is absent. Sorting areas are present in the dorsal region of the stomach but the ciliary currents are relatively weak and have little effect on the ingested material.

The digestive diverticula consist of three regions, the physiological relations of which have not been fully elucidated. The three regions are: (a) a much-branched system of non-ciliated main ducts, the epithelium of which shows a well-developed brush-border; (b) short, unbranched ciliated secondary ducts; (c) irregularly branched blindly ending tubules. The epithelium of the tubules is divided into two regions, one composed of large cells containing numerous conspicuous granules and the other of less frequent and smaller darkly staining cells. Each darkly staining cell bears a single flagellum.

Separate mucous glands cannot be demonstrated in any part of the stomach or digestive diverticula.

The epithelium of the style sac secretes an amylase and a lipase. Extracts of the digestive diverticula contain an amylase, a lipase, and a protease.

Unlike the majority of lamellibranchs, fluid and solid particles do not appear to enter the digestive diverticula from the stomach and in no part of the gut or of the diverticula is there ever any evidence of intracellular digestion. It is suggested that in the Nuculidae digestion is exclusively extra-cellular and confined to the stomach. The soluble products are absorbed by the epithelium of the stomach and first part of the intestine.

The diverticula of the rest of the Lamellibranchia are compared with those of the Nuculidae. While the Nuculidae are in many respects primitive lamellibranchs, the conclusion is reached that the digestive diverticula present many specialized features possibly correlated with the method of feeding.

INTRODUCTION

It has been suggested by many writers (Stempell, 1898; Heath, 1937; Yonge, 1939) that the protobranchiate molluscs are in many respects the most primitive living lamellibranchs and the structure and physiology of the gut in these animals is therefore of considerable interest. A study of the digestive diverticula of the Anisomyaria and Eulamellibranchia revealed certain features hitherto undescribed (Owen, 1955), and the reason for this present study was to determine whether similar features are present in the Taxodonta. However, variations in the structure and function of these organs both within the Taxodonta and when compared with the Anisomyaria and Eulamellibranchia are considerable, and as a consequence of this it was

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desirable to restrict the initial study to a single family. A number of species belonging to the Nuculidae—including the largest British species, *Nucula sulcata*—occur in the Clyde sea area (Allen, 1954), and since Yonge (1939) concluded that the members of this family are the least specialized of the protobranch molluscs, the choice of family was easily made. While all four species which occur in the Clyde sea area, *N. sulcata*, *M. turgida*, *N. tenuis*, and *N. nucleus*, were examined, the structure and function of the stomach and digestive diverticula are described with particular reference to *N. sulcata* and the minor differences which occur in the other species noted where necessary.

Dissections of the stomach and digestive diverticula were carried out on living specimens and the course of the ciliary currents followed by means of fine carborundum power and colloidal graphite (‘Aquadag’). Bouin’s fluid and a modified Bouin-Duboscq’s fluid (Atkins, 1937) were satisfactory fixatives for general histological purposes. Sections 2–8 μ thick were stained either in Heidenhain’s ‘Azan’ or in Heidenhain’s iron haematoxylin, alcian blue 8GN (replaces alcian blue 8GS, Steedman, 1950), and orange G in clove oil; Mayer’s haemalum was frequently used in place of Heidenhain’s iron haematoxylin. To prevent the growth of mould, the alcian blue contained 0.5% propylene phenoxetol (Owen and Steedman, 1956). Before embedding in ester wax the material was cleared in monochlorisothymol (Steedman, unpublished work).

**The Morphology of the Stomach**

In describing the stomach of *N. sulcata* the nomenclature used by Graham (1949), Owen (1953), and Purchon (1954, 1955) has been followed where possible. The external appearance of the stomach and style sac when viewed from the right and left sides respectively is shown in fig. 1, A and B. As in the majority of the Nuculidae (Yonge, 1939), the structure is divided into a globular dorsal region and an elongated, reddish region extending ventrally into the foot; the dorsal region is further divided into dark green and cream-coloured portions. As both Yonge (1939) and Graham (1949) have already emphasized, the tapering ventral region is homologous with the style sac of other Lamellibranchia, while the dorsal region is the stomach proper. The oesophagus enters the stomach antero-dorsally and a little to the right of the mid-line, while the intestine emerges from the style sac ventrally and passes dorsally posterior to the stomach. As in the Anisomyaria and Eulamellibranchia a well-developed dorsal hood (‘dorsal pouch’ of Graham, 1949) extends on to the left wall of the stomach to end blindly on the left dorsal side near the aperture of the left duct. Three ducts enter the stomach from the diverticula, one on the left and two side by side near the mid-line ventral to the oesophagus. To the left of the oesophageal aperture a small pouch or caecum extends dorsally to end blindly to the right of the mid-dorsal line. A similar pouch on the right side of the oesophageal aperture is formed by the anterior region of the posterior sorting area which is seen through the wall of the stomach and extends over most of the right side.
The drawing of the internal features of the stomach and style sac (fig. 2) is the result of observations on a number of specimens, since no matter how carefully the stomach is opened some part of the internal anatomy is invariably lost or distorted. Extending ventrally from the stomach is the combined style sac and intestinal groove. Along the right anterior wall of the style sac run two ciliated ridges, designated by Graham (1949) in his description of the stomach of *N. hanleyi* as the major and minor typhlosoles, with the intestinal groove between. Ventrally, at the junction of the style sac with the mid-gut, the two typhlosoles give rise to numerous longitudinal ridges which run the length of this portion of the intestine; the intestinal groove is continuous with the grooves between these ridges. On reaching the stomach dorsally the two typhlosoles separate, the major crossing on to the left wall of the stomach to end at the aperture of the left duct, while the minor typhlosole runs posteriorly along the ventral region of the right wall before curving dorsally and anteriorly to end near the oesophageal aperture. It thus forms a U-shaped structure on the right wall of the stomach, the open part of the U being directed anteriorly.
and partially closed by the well-developed fold surrounding the oesophageal aperture. As in *N. hanleyi* (Graham, 1949), the minor typhlosole encloses a regular series of folds and ridges which run dorso-ventrally and extend over almost the entire right-hand wall of the stomach. In *N. sulcata*, a somewhat narrower but similar series of grooves and ridges, the rejection tract, runs

across the anterior floor of the stomach and extends on to the anterior left wall to enter the slit-like aperture of the caecum to the left of the oesophageal opening. In *N. turgida* the rejection tract consists of a single groove, more or less continuous with the intestinal groove of the style sac and extending to the mouth of the caecum, while in *N. tenuis* and *N. nucleus* conditions are similar to those figured for *N. hanleyi* by Graham (1949). In all four species a pouch-like caecum, not shown by Graham in his drawing of the stomach of *N. hanleyi*, is present to the left of the oesophageal aperture; the wall of this caecum is produced into a series of folds and ridges.

Two well-developed folds, with a groove between, enter the dorsal hood.
Diverticula of Lamellibranchia: Nuculidae

The left fold arises on the left wall of the stomach near the aperture of the left duct and runs dorsally, forming the posterior margin of the opening of the caecum, before extending posteriorly to enter the dorsal hood. The right-hand fold is more or less continuous with the minor typhlosole and arises to the right of the oesophageal aperture; it extends posteriorly over the roof of the stomach before curving to the left to enter the dorsal hood. As a result of this disposition of the two folds, the groove between arises from a position dorsal to the oesophageal aperture. Both the folds and the groove run the length of the dorsal hood to terminate at the blind-ending tip.

The remainder of the gastric wall, consisting of the greater part of the left side, the posterior region of the dorsal hood, and a narrow tongue-like projection extending on to the right wall at the junction of the style sac with the stomach, is dark-coloured and non-ciliated, being covered by a layer of cuticle secreted by the underlying epithelium. The cuticle which surrounds the opening of the dorsal hood and extends over the roof of the stomach on to the right dorsal wall is tougher than that covering the remainder of the gastric wall, and can be removed intact. As suggested by Yonge (1939), this region is homologous with the gastric shield of other Lamellibranchia. Anterior to the opening of the dorsal hood the cuticle of the shield is raised to form a prominent tooth-like structure.

The epithelium of the stomach can be divided into three distinct regions: (a) the style sac, (b) the major and minor typhlosoles together with the ciliated regions of the stomach, and (c) the epithelium underlying the cuticle. The wall of the style sac consists of a smooth epithelium, some 50 to 60 μ deep, bearing dense bristle-like cilia, and the distal region of the cells contains numerous small pigment granules. As described by Yonge (1939) this epithelium corresponds in all respects with the style sac epithelium of other lamellibranchs.

The remaining ciliated epithelium is composed of typical columnar ciliated cells of varying heights which produce the grooves and ridges of the stomach wall. Small pigment granules are again present in the distal region of the cells. Although the stomach contents are invariably compacted in a mucus-like substance, no mucous glands are to be found either in the epithelium or lying in the tissue below. Separate mucous glands cannot be demonstrated in any part of the gut.

The epithelium underlying the cuticle is formed of tall slender columnar cells ranging from 100 μ in height to as much as 400 μ under the tooth-like structure of the gastric shield; the height of the tooth above the surface of the gastric shield is almost exclusively due to this increase in height of the underlying epithelium. The cells possess elongated nuclei in the basal third of the cytoplasm and the distal region is frequently packed with spherical pigment granules up to 4 μ in diameter, yellowish green in fresh material and colouring with Sudan black B in wax-embedded sections. They are responsible for the dark green colour of the epithelium underlying the cuticle and as suggested by Yonge (1939) are probably excretory.

Underlying all three types of epithelium is a layer of collagen through which
runs a system of fine anastomosing fibres, 0.5 μ or less in diameter. In fresh preparations these fibres are brown and they retain this colour even after fixation and subsequent wax embedding. As a consequence of this and of their small size they appear black in section and it is difficult to determine their staining properties, but it seems likely that they are reticular fibres serving a skeletal function. External to the layer of collagen is a system of smooth muscle-fibres. Circular fibres predominate at the junction of the style sac with the intestine and form a sphincter muscle. Both stomach and style sac are invested in a loose mesh-work of connective tissue which is frequently packed with small rounded granules 1–2 μ in diameter, staining black with Heidenhain's haematoxylin and red with Azan. They stain intensely with mercuric bromphenol blue (Mazia, Brewer, and Alfert, 1953) and are evidently protein in nature. Similar granules have been described in chitons (Fretter, 1937) and in the gastropod, Carychium (Morton, 1955).

The pattern of ciliary activity within the style sac and stomach is indicated by arrows in fig. 2. The dense bristle-like cilia of the style sac beat in the same direction as in other Lamellibranchia, i.e. a clockwise direction when viewed from above. Along the intestinal groove the ciliary currents are directed ventrally towards the mid-gut while on the major and minor typhlosoles weak ciliary currents are directed dorsally towards the stomach; at the sides of the intestinal groove the cilia beat into the groove.

Over the ridged and grooved area of the right wall of the stomach the ciliary currents are similar to those of the posterior sorting area of other Lamellibranchia. The cilia in the groove beat ventrally and drain into the intestinal groove while those on the crests of the ridges beat anteriorly above and across the grooves. The currents produced by the cilia on the crests are noticeably weaker than those produced by the cilia in the grooves. The ciliary currents over the prominent fold which surrounds the oesophageal aperture are directed dorsally and drain into the dorsal groove. In the groove, and also over both the right and left folds, the ciliary currents are directed towards the dorsal hood in N. sulcata, N. turgida, and N. tenuis. Thus, unlike N. hanleyi (Graham, 1949), there are no ciliary currents beating out of the dorsal hood. Although the grooves and ridges which extend over the anterior floor of the stomach and on to the left wall appear to be an extension of the sorting area of the right wall, the ciliary currents of this region do not exercise any selection of the stomach contents. The cilia both in the grooves and on the ridges are all directed towards the intestinal groove of the style sac. This region is probably homologous with the extension of the intestinal groove across the floor of the stomach in other Lamellibranchia, but to avoid confusion with the intestinal groove of the style sac the term 'rejection tract' is used. A second sorting area is present within the small caecum to the left of the oesophageal aperture, the wall of which also bears a series of folds and grooves. The ciliary currents in the grooves are directed ventrally towards the rejection tract while the cilia on the crests of the folds beat across the grooves and towards the dorsal groove and folds. Again the ciliary currents on the ridges are noticeably weaker than
those in the grooves. Finally, the slit-like entrance to each of the three ducts leading to the digestive diverticula is surrounded by long, fine cilia which beat violently away from the duct and towards the rejection tract.

**The Digestive Diverticula**

As in the majority of lamellibranchs, the digestive diverticula surround the greater part of the stomach and consist of numerous blindly ending tubules which communicate with the stomach by a well-developed system of ducts. In all the species examined, the tubules and ducts are packed loosely together with little connective tissue between them, and the various regions of the diverticula can be readily recognized in dissections of fresh animals (fig. 3).

![Diagram of digestive diverticula](image)

**Fig. 3.** A portion of the digestive diverticula of *N. sulcata* showing the darkly coloured main ducts, the lightly coloured secondary ducts, and the brown, blindly ending tubules.

While the main ducts which communicate with the stomach are a dark olive green and branch repeatedly, the short secondary ducts to which they give rise are light yellow or cream-colour. They terminate in blindly ending tubules which are brownish and have the form of irregularly branched elongate tubes with numerous saccular outgrowths. The layer of collagen and the system of reticular fibres underlying the epithelium of the stomach is continued beneath the epithelia of the main and secondary ducts and the tubules, but they are progressively less well developed as they approach the blindly ending tubules. Occasional fine muscle fibres are also present in all three regions, but they are never as well developed as in the corresponding regions of the diverticula of the Anisomyaria and Eulamellibranchia (Owen, 1955).

**The main ducts**

Both Heath (1937) and Yonge (1939) describe the Nuculidae as possessing only two ducts from the diverticula into the stomach, but Graham (1949)
found that in all the specimens of *N. hanleyi* examined by him there were three ducts, two on the right and one on the left. In *N. sulcata*, *N. tenuis*, *N. turgida*, and *N. nucleus*, three ducts enter the stomach. One opens on the left wall ventral to the gastric shield and serves the left mass of the diverticula, while the remaining two enter the stomach side by side ventral to the oesophageal opening and slightly to the left of the mid-line (figs. 1 and 2). Of these two ducts, the left one serves the median anterior mass of the diverticula while the right duct, which bends posteriorly immediately on leaving the stomach, serves the right mass.

The ciliated epithelium of the stomach projects only a short distance into the main ducts and is replaced by a non-ciliated epithelium ranging from 20 to 40 μ in height, present throughout the main ducts (fig. 4). It consists of only one type of cell with spherical or slightly oval nuclei and at the free margin a well-developed brush-border which extends 8–10 μ into the lumen of the duct. In fresh preparations of the diverticula squeezed out under a coverslip this epithelium is easily disrupted, and so prominent is the brush border that each cell has the appearance of a miniature shaving brush. In dissections of fresh material, the internal surface of the main ducts possesses a bright bluish-green iridescence probably due to the reflexion of light from the surface of the brush-border. In the region above the nuclei occur numerous pigment granules, their size varying from 5 μ to less than 0.5 μ. In fresh material the granules are yellowish green and give the olive green appearance which the main ducts present in the living animal and which contrasts with

![Diagram of epithelium](image)

**Fig. 4.** *N. sulcata*, the epithelium of the main ducts of the digestive diverticula during the 'extrusion' phase.
the much lighter shade of the secondary ducts where the pigment granules are smaller and fewer. The granules are some form of lipo-pigment and colour strongly with Sudan black B even after impregnation with ester wax. They are invariably reduced in size after Bouin or alcoholic fixation, while after Lewitsky's fluid (Flemming without acetic) or Helly's fluid their appearance is very similar to that in life. While it is difficult to say what part they play in the activities of the cell it is probable that they are excretory since many of the lipo-pigments are formed as by-products of lipid metabolism. Certainly in frozen sections the basal region of the cells is full of lipid material which cannot be demonstrated in wax sections.

![Fig. 5. A transverse section through a secondary duct of the digestive diverticula of N. sulcata.](image)

The cells appear to undergo a secretory or excretory cycle, since the appearance of the epithelium varies considerably although more or less constant in any one animal. Fig. 4 illustrates the epithelium during the 'extrusion' phase. Numerous vesicular outpushings, into which the pigment granules are extruded, are being nipped off from the surface of the epithelium. In wax-embedded sections the lumen of the ducts frequently contains numerous vesicles either apparently empty or containing relatively small amounts of granular material that stains with iron haematoxylin. Presumably the staining properties of the granules are quickly lost after extrusion from the epithelium. Alternating with these extrusion phases the free margin of the epithelium is smooth and vesicular outpushings are absent.

The secondary duct

In contrast to the main ducts the epithelium of the secondary ducts is uniformly covered with a dense coat of long cilia, some 20 to 25μ in length, which extend almost to the middle of the lumen (fig. 5). They beat towards the
main ducts with a marked clockwise trend when viewed from the tubules. There are no cilia beating towards the tubules. Again only one kind of cell is present, ranging from 15 to 25μ in height and containing large, slightly oval nuclei, each with a prominent nucleolus, lying in the mid-region of the cytoplasm. Pigment granules are present in the region above the nucleus, but they are smaller and fewer than those in the epithelium of the main ducts.

![Diagram of granule cells and tubules]

**Fig. 6. Different phases of the granule cells lining the tubules of the digestive diverticula of N. sulcata.**

**The tubules**

The blindly ending tubules of the diverticula are round or oval in cross-section and two types of cell may be distinguished. Most numerous are those ranging from 30 to 80μ in height, while the free margin of the cells varies from regular to pseudopodial in outline (fig. 6, A and B). The nuclei are rounded and somewhat ovoid, basal in position, and each with a small nucleolus. Numerous brown, rounded granules, occasionally showing internal granulation, fill most of the cell. Those in the basal region are fairly regular in outline. They range from 2 to 4μ in diameter and stain light blue with Mallory’s. In the middle region the granules are larger, 5–7μ in diameter, fewer in number, and irregular in outline; they are coloured dark blue or yellow after staining with Mallory’s. Staining with haemalum, alcian blue, and orange G in clove oil indicates that the smaller and more numerous basal granules are surrounded by a ‘mucous’ membrane staining with alcian blue while the contents are
stained with haemalum. The larger irregular granules are coloured yellow. In sections treated with PAS (Hotchkiss) the larger granules give a strongly positive reaction which decreases in intensity in the basal granules; control sections treated with diastase gave similar results. The greatest differentiation between the two types of granules is obtained after treatment with Ritter and Oleson's (1950) modification of Hale's (1946) method for acid mucopolysaccharides. The smaller spherical basal granules are coloured blue while the larger irregular granules in the middle region of the cell are red (for convenience the two types of granules will be described as 'spherical' and 'irregular' respectively). Pearse (1953) has, however, criticized the low specificity of Hale's method and the striking results obtained are of little value in determining the nature of these granules. It would appear that the smaller and more numerous spherical granules are surrounded by an acid mucous membrane which is absent from the larger, irregular granules. Beneath the free margin of the cell is a region of dense cytoplasm followed by a small number of vacuoles which either appear empty or occasionally contain an irregular mass of faintly staining material.

The second type of cell found in the tubules is much less common than those already described (fig. 7, a and b). They are found scattered irregularly in small clusters of up to a dozen cells round the lumen of the tubules and are readily distinguished from the larger 'granule' cells by their larger nuclei and more darkly staining cytoplasm. They are narrow at the distal end but broaden out markedly at the base so as to be triangular in outline. The nucleus is basal in position, circular in outline, and in contrast to the nuclei of the granule cells contains a very prominent nucleolus. The greater part of the cell is filled with dense, darkly staining cytoplasm frequently showing traces of intracellular fibres and including a varying number of irregular granular masses.

In the Eulamellibranchia, flagella are readily observed in fresh preparations of the diverticula moving with a languid, undulating beat within the tubules. On only a few occasions were similar flagella observed in the Nuculidae, yet they are undoubtedly present in all specimens. In the eulamellibranchs, the darkly staining cells of the tubules are flagellated (Owen, 1955), and this is also true of the Nuculidae, but unlike the Eulamellibranchia, each darkly staining cell bears only a single flagellum (fig. 7). As a consequence of this, basal granules and flagella are difficult to observe in section. After fixation with Bouin-Duboscq's fluid and staining with Heidenhain's iron haematoxylin, a single flagellum and basal granule can frequently be seen at the distal ends of the darkly staining cells. Because of the narrow neck-like shape of the upper portion of these cells it was at first difficult to be certain that this appearance was not due to the membranes of the underlying cells. Fortunately, one section passed transversely through the distal region of a clump of darkly staining cells and showed clearly the boundary of each cell with a single basal granule or flagellar shaft in the centre. In previous accounts of the diverticula of Lamellibranchia, the vibratile structures occurring within the tubules have been described as cilia, but unlike the cilia occurring elsewhere in the lamellibranch
body they beat with an undulating or sinusoidal movement rather than a simple flexing or bending motion; and they do not exhibit metachronal rhythm (Owen, 1955). These observations on their mode of beating and distribution (i.e. only one or, at most, a few to a cell) indicate that they may be more correctly termed flagella.

The appearance of both the granule cells and flagellated cells, although more or less constant in any one animal, varies a good deal, suggesting that a phase change occurs. Two extremes of the phase changes of the granule cells are shown in fig. 6, A and B. In fig. 6, B the free margin of the cells is flat and regular in outline and presents a fibrillar appearance similar to that of a poorly developed brush-border. Beneath the free margin is a region of dense cytoplasm followed by one or two large vacuoles either containing an irregular mass of faintly staining material or apparently empty. In fig. 6, A the cells are taller and the distal ends rounded or club-shaped, the free margin no longer presenting a striated appearance. Large spheres some 15 to 20 μ in diameter, which contain numerous granular inclusions similar to those present within the cell, are freely constricted into the lumen. The various phases of the darkly staining cells show fewer differences than do those of the granule cells. As shown in fig. 7, A and B, the different phases suggest that material is built up within the cell and periodically discharged into the lumen of the tubule, aided possibly by the beating of the flagellum. Despite the general similarity in structure of both the granule cells and darkly staining cells to the cells which are found in the tubules of other lamellibranchs and gastropods, it has not been possible to determine their function with any degree of certainty.

A discussion of their possible functions will be left until after the results of the various feeding experiments have been described.

**Reserve Food Material**

Apart from the protein spheres already described, reserve food is stored in the Nuculidae in the form of fat and glycogen. Considerable quantities of fat
occur in the digestive diverticula. Fresh preparations of these organs pressed out under a coverslip show numerous fat droplets. Although fat is present in most of the tissues, gelatine-embedded frozen sections coloured with Sudan IV or Sudan black B show that it is most abundant in the lower third of the cells lining the main ducts and in the basal region of the granule cells of the tubules. In freshly caught animals, fat is abundant throughout the ciliated epithelium of the stomach and the first part of the intestine, but after 9 days' starvation only traces remain at the base of the cells. This short period of starvation has little effect on the amount of fat present in the diverticula. Glycogen is present in considerable quantity in all the tissues of the stomach and digestive diverticula.

**FEEDING EXPERIMENTS**

The absorption of ingested material was tested by feeding the animals on suspensions in sea-water of iron saccharate, colloidal graphite (Aquadag), titanium dioxide, rice starch, and an emulsion of olive oil stained with waxo-line red OS. (Feeding with glycogen was unsuccessful since the relatively short period of starvation which was possible had little effect on the amount of glycogen occurring naturally in the tissues.) Animals were placed in these suspensions and fixed after definite periods; those fed on iron, in 10% formalin buffered to pH 7-7-4 (Lison, 1953), or in a mixture of Bouin’s fluid and ammonium sulphide (Yonge, 1926). Iron was demonstrated by treating the sections for 20 minutes with the following mixture: 25 ml of 5% potassium ferricyanide, 25 ml of 5% potassium ferrocyanide, and 50 ml of 2% hydrochloric acid. Animals fed with colloidal graphite and titanium dioxide were fixed in Bouin’s fluid and stained with haemalum and orange G in clove oil. Starch-fed animals were also fixed in Bouin’s fluid and the sections stained with PAS (Hotchkiss) and with Lugol’s iodine. Animals fed with olive oil were fixed in Baker’s formaldehyde-calcium, and gelatine-embedded frozen sections were employed.

It was at first assumed that digestion would be largely intracellular (Yonge, 1939), and the initial experiments, using colloidal graphite (particle size less than 0-5μ), titanium dioxide (0-5-2μ), and rice starch (4μ), were designed to determine where and how intracellular digestion takes place. All three substances are readily ingested, so that the lumen of the stomach and of the intestine is frequently filled with the material employed. Nevertheless no part of the gut or of the diverticula ever showed any evidence of intracellular digestion. Furthermore, solid particles of the material present in the stomach were never present in the lumina of the ducts or of the tubules. Occasionally some solid material was present just within the entrance to the main ducts, but this may well have resulted from spasmodic muscular contractions during fixation. Caspers (1940) showed that specimens of *Nucula* freely suspended in water could ingest fine particles present in the water and he concluded that *Nucula* is a suspension feeder obtaining its food by means of the respiratory currents. As shown by the present series of feeding experiments, *Nucula* is certainly able
to ingest particles present in suspension in the water but, as demonstrated by Hirasaka (1927) and Yonge (1939), the Nuculidae normally lie beneath the surface of the substrate and use the extensile palp proboscides to collect bottom deposits. Caspers's experiments are therefore of little value in determining the method of feeding of Nucula under natural conditions. As suggested by Jørgensen (1955), the gills of the Nuculidae are so small and the water currents so weak that it is unlikely that suspended material plays a major role in nutrition. The stomach contents of freshly caught animals also support the view that the Nuculidae are essentially deposit feeders, since the bulk of the ingested material consists of relatively large particles incapable of remaining in suspension in the water. Further feeding experiments were therefore carried out, the animals being placed on an adequate quantity of bottom material mixed with small amounts of titanium dioxide and of rice starch. The animals soon buried themselves in the manner described by Yonge (1939), and subsequent examination of the gut contents revealed that they ingested considerable quantities of material, including the titanium dioxide and rice starch. Again there was no evidence of intracellular digestion and solid particles were never present in the digestive diverticula.

As a consequence of these results, particular attention was paid to feeding experiments with iron saccharate and olive oil coloured with waxoline red OS. Feeding experiments with iron saccharate appeared at first to confirm the results described by Yonge (1926), but examination of control animals fixed as soon as possible after being caught showed that the various tissues of the digestive system contain considerable amounts of inorganic iron. Furthermore, the bottom deposits ingested by the animal are also rich in inorganic iron, the contents of the lumen of the gut giving a definite blue reaction in sections tested for iron. The main site of iron in the stomach is the pigment granules in the cells underlying the cuticle, while in a few specimens occasional fine granules of iron are also present in the distal regions of the ciliated cells. In the digestive diverticula a faint positive reaction for iron is frequently given by the pigment granules in the epithelium of the main and secondary ducts, and by the inclusions of the darkly staining cells of the tubules. It is, however, the spherical granules in the basal region of the granule cells of the tubules that invariably give the strongest reaction to tests for inorganic iron, the irregular granules being invariably negative.

After relatively short periods of starvation, the pigment granules of both the stomach and digestive diverticula show no trace of iron, while the reaction of the spherical granules in the tubule cells is less marked and frequently negative. Thus before feeding with iron saccharate the animals were kept in filtered sea-water for 48 hours. Iron is ingested in great quantity, filling the stomach and appearing in the first part of the intestine in 6–8 hours, so that the lumen appears as a solid sheet of blue in section. Iron is absorbed by the ciliated epithelium of the stomach and the first part of the intestine, the cells showing a diffuse blue colour. After 24 hours the spherical granules of the tubule cells present a uniform blue colour, but there is never any evidence of
a general absorption of granules of iron throughout the cytoplasm, nor any
diffuse blueness in it. After 48 hours the pigment granules of the darkly
staining cells of the tubules, of the ciliated cells of the secondary ducts, of the
brush-border cells of the main ducts, and of both the ciliated epithelium and
the epithelium underlying the cuticle of the stomach, all give a positive re-
action for iron. The most striking difference from the normal condition is that
shown by the darkly staining cells of the tubules, the cell inclusions giving an
intense blue colour.

Gelatine-embedded frozen sections of animals fed with olive oil coloured
with waxoline red OS show that red droplets of olive oil are readily ingested
and the ciliated epithelium of the stomach and of the first part of the intestine
appears a diffuse faint pink. There is never any evidence of coloured oil in the
lumen and cells of the ducts and tubules of the diverticula.

From these observations it appears that digestion is entirely extracellular
and that the soluble products of digestion are absorbed at the site of digestion.
The role of the digestive diverticula is extremely difficult to evaluate. None
of the feeding experiments provided satisfactory evidence of their being
organs of absorption as in the Anisomyaria and Eulamellibranchia. Animals
kept in filtered sea-water for 48 hours and injected with 0.1 ml of dilute
soluble iron saccharate showed a distribution of iron similar to that shown
after feeding with the substance. There was no diffuse blue colour in the
ciliated epithelium of the stomach but the spherical granules of the large cells
of the tubules and the pigment granules described above all gave a positive
reaction to tests for iron. From the available evidence the digestive diverticula
of the Nuculidae can only be regarded as organs of secretion and excretion. It
is remarkable that while in all other lamellibranchs the digestive diverticula
function as organs of absorption, they do not do so in the Nuculidae. Even
allowing for the fact that the substances used in the feeding experiments
are not in any way related to the natural food of the animals, and that the
techniques employed may have failed to demonstrate low concentrations of
soluble iron, it is difficult to appreciate how fluid could enter and circulate
within the digestive diverticula of the Nuculidae. Cilia surrounding the open-
ings of the main ducts into the stomach beat actively into the stomach, while
within the diverticula, cilia are restricted to the short secondary ducts and
beat away from the tubules. In such finely divided diverticula possessing an
extensive duct system, it is unlikely that muscular activity could effect an
efficient interchange of material between the stomach and the tubules, and it
would almost certainly result in some solid particles entering the diverticula.
This was never observed.

**Enzymes**

The only recorded investigation of the digestive enzymes of the gut of the
Nuculidae is that given by Yonge (1939) for *N. turgida*. The stomach contents
were tested for amylolytic activity with negative results. In this present work,
and at the suggestion of Professor C. M. Yonge, the nature of the enzymes
of the digestive diverticula and of the style sac of *N. sulcata* were investigated and compared with the composition of the stomach juice. For each set of experiments the enzyme extracts were prepared in the same way throughout the course of the work, viz.:

**Stomach juice.** The right valve, labial palps, and ctenidium were removed and the foot slit open to reveal the style sac. Removal of the kidney tissue exposed the right posterior wall of the stomach which was then pierced and the contents removed with a fine pipette. In this way the stomach contents were obtained without damage to the tubules of the digestive diverticula. The pH of the stomach fluid lies between 5.7 and 5.9. The stomach contents of 30 animals were diluted with distilled water and centrifuged at 3,500 r.p.m. for 30 minutes; the clear centrifugate was used as the enzyme extract.

**Style sac.** Unlike the majority of Lamellibranchia the style sac of *N. sulcata* is easily removed free from surrounding tissue. The style sacs of 30 animals were rinsed a number of times with distilled water to remove any traces of the stomach contents and ground up in a small glass mortar with clean sand and distilled water (for protease experiments 25% glycerol). The mixture was centrifuged at 3,500 r.p.m. for 30 minutes and the clear centrifugate used as the enzyme extract.

**Digestive diverticula.** As with the style sac, extracts of the diverticula were made by grinding the tissue with clean sand and centrifuging the brown liquid at 3,500 r.p.m. for 30 minutes. The debris was thrown down and the fat formed a cake on the top of the yellow centrifugate used as the enzyme extract.

The activity of the enzymes was determined at 23°C, mixtures of the extract and substrate being buffered with McIlvaine's solutions. Boiled controls were rigidly set up and care was taken to exclude saliva from the tubes; bacterial action was prevented by the addition of toluene. Reducing sugars were estimated by the method of Hagedorn and Jensen (as modified by Boyland, 1928), while protein digestion was investigated by the gelatine plate method of Gates (1927) as modified by Pickford and Dorris (1934), and by the Sorensen formol-tritration method, the formaldehyde being added after neutralization to phenolphthalein. Qualitative tests for lipase were made by mixing the extracts with an emulsion of olive oil and subsequently staining a small sample with Nile blue sulphate (George, 1952). The duration of the amylase experiments, with 1% soluble starch as the substrate, was 32 hours and the extracts from all three sources, the digestive diverticula, the style sac, and the stomach juice, gave positive results. The optimum pH for the activity of the enzyme from the style sac is about 6.2, that of the stomach about 6.0. The pH optimum for the amylase of the digestive diverticula lies between 4.3 and 4.5 with a small but definite increase in activity on the less acid side between 6.1 and 6.4. While the amylase of the style sac is undoubtedly secreted into the stomach cavity, the contribution of the digestive diverticula to the stomach juice is less certain. The data obtained from experiments on extracts of the digestive diverticula seem to indicate the occurrence of two enzymes, one having a distinct optimum at pH 4.4 and the other an indistinct one at pH 6.2.
The determination of pH optima is not a very safe means for the identification of enzymes, but a comparison of the pH optima of extracts of the digestive diverticula with the stomach juice suggests that the main amylase occurring in the diverticula is not discharged into the stomach but acts intracellularly, while the second and weaker amylase, having a pH optimum between 6.1 and 6.4, is secreted into the stomach. It is interesting to note that the optimum pH of the amylase of the diverticula is lower than that usually recorded for animal amylase (pH 5.5–7.0), although B-amylase, traces of which occur in the crop juice of *Helix pomatia* (Anker and Vonk, 1946), has an optimum pH of about 4.5.

A weak protease capable of attacking gelatine and casein was identified in the stomach juice and in extracts of the digestive diverticula; extracts of the style sac gave negative results. The protease in the stomach juice is probably secreted by the digestive diverticula, since both extracts were most active between 5.8 and 6.6. Under the conditions of the experiment sharp optima were not obtained.

Tests for lipase activity were positive in extracts of the digestive diverticula, of the style sac, and of the stomach juice. These results are interesting since George (1952), using a similar method, demonstrated the presence of a lipase in the crystalline styles of members of the Anisomyaria and Eulamellibranchia.

**The Probable Functioning of the Stomach and Digestive Diverticula**

The method of feeding of the Nuculidae has been described by Hirasaka (1927) and Yonge (1939). From the observations given above, the working of the alimentary canal may be summarized as follows. The particulate matter forming the bottom deposits, and including sand grains, living organisms, and organic detritus, is collected by the palp proboscides and ingested in considerable quantity, so that in freshly caught animals the stomach is invariably distended. The presence of a sphincter muscle at the junction of the style sac with the intestine prevents the escape of material from the stomach except by the intestinal groove. Enzymes are secreted into the lumen by the style sac epithelium and by the digestive diverticula, and food and enzymes are thoroughly mixed by the rotating action of the specialized cilia of the style sac. The pressure exerted by the distended wall of the stomach, aided by muscular action, serves to squeeze the soluble products of digestion out of the contained mass and these are absorbed by the lining epithelium. Finally, relaxation of the sphincter muscle allows the compacted faecal mass contained in the style sac to enter the intestine.

The ciliary currents over the major and minor typhlosoles and of the sorting areas of the anterior region of the stomach play only a minor part in the circulation of material within the stomach. The most actively beating cilia in the stomach are those surrounding the slit-like apertures to the ducts of the digestive diverticula. They are of considerable length and presumably serve to drive fluid out of the ducts, while at the same time preventing material from
entering the diverticula. This was frequently demonstrated in sections passing through the junction of the ducts with the stomach. The lumen of the stomach was filled with material, while that of the ducts within the ciliated ridges surrounding the apertures was completely devoid of all solid material other than the various granules derived from the tissues of the digestive diverticula. The ciliary currents over the major and minor typhlosoles, the crests of the folds of the sorting areas, and the various ridges of the stomach are for the most part directed dorsally, while those of the various grooves are directed ventrally and drain into the intestinal groove. In the dissected stomach the dorsally directed currents are very weak and capable of moving only the finest particles. They probably serve to drive fluid dorsally away from the intestine, while those of the grooves direct fine particles into the intestinal groove and so to the intestine.

There seems little doubt that the darkly staining flagellated cells of the tubules are secretory, the undulating motion of the flagella driving the secretion away from the narrow distal regions of the cells, but the function of the granule cells remains obscure. During the fragmentation phase, the cells are tall with the tips rounded and club-shaped (fig. 6, A). Large spheres, containing both spherical and irregular granules, are freely constricted into the lumen and are conveyed out of the tubules by the cilia of the secondary ducts. The majority break down in the lumen of the main ducts to liberate the granules which are found free in the stomach. It is not certain whether the granules disintegrate in the lumen of the stomach, or retain their identity and pass out with the faecal material.

Alternating with the fragmentation phase, the free surface of the granule cells is flat and entire, and shows a striated appearance suggesting a poorly developed brush-border (fig. 6, B). Large clear vacuoles with a slightly granular content occupy the distal region of the cells, while the middle and basal regions are filled with irregular and spherical granules respectively. The general appearance of the cells is very similar to that of the absorptive phase in the corresponding cells of other molluscs, but in the Nuculidae there is never any evidence of absorption. It is possible that in these animals this represents a secretory phase.

**DISCUSSION**

Although the Nuculidae are in certain respects the most primitive living lamellibranchs, the structure of the stomach and digestive diverticula suggests a considerable degree of specialization, probably correlated with what is perhaps the most interesting feature of the alimentary canal in these animals—the complete absence of intracellular digestion.

**The stomach**

As suggested by Morton (1953) the stomach of the primitive mollusc probably consisted of (a) a ciliary sorting area converging on the intestinal groove; (b) an area of cuticle forming the gastric shield; (c) a style sac connecting the stomach with the intestine and lined with strong cilia beating trans-
versely (fig. 8, A). Graham (1949) suggested that the primitive stomach also possessed a caecum into which the major typhlosole extended. A feature of the stomach of the Nuculidae when compared with the primitive mollusc is a general simplification of structure, presumably correlated with the loss of intracellular digestion. This is illustrated by a reduction in importance of that part of the major typhlosole which extends across the floor of the stomach. It has been suggested (Graham, 1949) that in the primitive mollusc the major typhlosole extended across the floor of the stomach to end at the tip of a pouch-like caecum (fig. 8, A). The intestinal groove continued round the tip of the typhlosole and emerged from the caecum to end at the opening of the left duct of the digestive diverticula. Moreover, the typhlosole was probably a flap-like structure which arched over the intestinal groove and isolated the waste products derived from the digestive diverticula from the general circulation of material within the gastric cavity. The flap-like nature of the major typhlosole is well developed in both the Anisomyaria and Eulamellibranchia, and in the latter (fig. 8, B) it extends into the right and left caeca to act as a valve controlling the entry of material into the diverticula (Owen, 1955). These features, which are undoubtedly correlated with the function of the digestive diverticula as organs of intracellular digestion, are not shown by the stomach of the Nuculidae. The extension of the major typhlosole across the floor of the stomach is represented by a poorly developed fold which does not enter the relatively small caecum (fig. 8, C). This trend towards a general reduction in the importance of the major typhlosole and of the caecum also occurs in the Gastropoda (Graham, 1949) and, as in the Nuculidae, is associated with increased extracellular digestion.

As in the Anisomyaria and Eulamellibranchia, the stomach of the Nuculidae possesses extensive sorting areas, a feature normally associated with intracellular digestion. In the Nuculidae, however, the ciliary currents over these regions are so weak as to have little effect on the relatively coarse material present in the stomach. It has been suggested that the stomach of the Nuculidae is capable of extensive trituration of the ingested particles, which may attain a diameter of over 100 μ. A comparison of the contents of the stomach with those of the intestine shows an increase in the amount of finely divided material present in the latter, but the maximum particle-size is the same in both. The most striking difference in the contents of the intestine when compared with those of the stomach, is the increase in the number of more or less complete but empty diatom tests and protozoan skeletons. This suggests that while some mechanical trituration occurs in the stomach, enzymes are also present and, as already suggested, the weak ciliary currents of the sorting areas may serve to separate from the soluble products of digestion the fine particles which escape from the central mucous mass.

The style sac

An interesting feature of the stomach of the Nuculidae is the secretion of enzymes by the epithelium of the style sac although a firm rod-like crystalline
Fig. 8. Diagrammatic representations of the stomachs of A, the primitive mollusc (after Graham, 1949); B, a eulamellibranch; C, Nucula.
Diverticula of Lamellibranchia: Nuculidae

style is never developed. Much has been written upon the crystalline style in the Mollusca, but the above observation is of sufficient interest to warrant further discussion on this subject. Most workers are agreed (see Yonge, 1939; Graham, 1949; Morton, 1952) that the relatively loose mucus-bound mass filling the style sac in the Nuculidae is homologous with the firmer rod-like structure of the Anisomyaria and Eulamellibranchia. This is supported by the similarity of the style sac epithelium in all three groups. Yonge (1939), Graham (1949), and Morton (1952) all consider the style sac of the primitive mollusc and its contained protostyle to have been concerned in the first place with purely mechanical functions, and the presence of enzymes a specialized character which subsequently evolved independently in both the Gastropoda and Lamellibranchia. Morton (1952) defines the protostyle as not containing any enzymes.

In a comparison of the various members of the protobranchiate mollusca, Yonge (1939) concluded that the Nuculidae are the least specialized and possibly represent the most primitive living Lamellibranchia. It is of interest, therefore, to compare the conditions now known to exist in the Nuculidae with those which are thought to characterize the primitive protostyle. In both, the style sac serves to consolidate faecal material and aids in the formation of a faecal rod which is periodically released into the intestine by the relaxation of a sphincter muscle. The stiff, bristle-like cilia of the epithelium of the style sac rotate the contained mass and so mix the contents of the stomach. These appear to be the only features common to both the Nuculidae and the primitive mollusc as defined by Morton (1952). The main difference between the style sac of the Nuculidae and that of the primitive mollusc is the secretion in the Nuculidae of amylolytic and lipolytic enzymes by the style sac epithelium. In view of this difference, it is interesting to speculate how far conditions in the Nuculidae may be regarded as primitive. Morton's (1952) definition of the protostyle as not containing any enzymes is based on the absence of enzymes from the protostyle of primitive gastropods and the condition then thought to exist in the Nuculidae. Whether enzymes are absent from the corresponding region of the gut of the Loricata is not known. Thus, the suggestion that the primitive protostyle did not contain enzymes is now based solely on conditions existing in the primitive gastropods. Nevertheless, until the Loricata and, if possible, primitive members of the archaeogastropods are re-investigated, this must be accepted although it is difficult to visualize the same region of the gut evolving independently the ability to secrete enzymes in both the Gastropoda and the Lamellibranchia.

The well-developed style sac of the Nuculidae suggests that the absence of a firm rod-like crystalline style may be a secondary condition, possibly related to the presence of an extracellular protease (Yonge, 1939), but this is unlikely. The Nuculidae feed by means of the labial palps and in particular the palp proboscides, the ctenidia serving only a minor role in the collection of food (Hirasaka, 1927). Thus members of the Nuculidae are essentially detritus feeders and consequently ingest, together with organic 'food', large quantities
of inorganic material. Enzymes are secreted into the lumen of the stomach and mixed with the contained mass by the rotating action of the cilia of the style sac and the soluble products of digestion are squeezed out of the faecal material. Such a method of extraction would be difficult, if not impossible, in a stomach containing a firm rod-like crystalline style. Furthermore, the remaining faecal mass, which constitutes the bulk of the ingested material, is periodically released into the intestine, the style sac forming a through passageway between the stomach and intestine (fig. 8, A and C). In the Anisomyaria and Eulamellibranchia, the firm rod-like crystalline style is no longer faecal and all material entering the intestine does so by way of the intestinal groove alone. This feature is probably correlated with the finely divided nature of the food and is an essential preliminary to the development of a firm, non-faecal crystalline style (fig. 8, B). Yonge (1939) suggested that the development of labial palps as exemplified by the Nuculidae illustrates the transition from the primitive mollusc, having the mouth in contact with the substrate and feeding with the aid of the radula, to the suspension-feeding Anisomyaria and Eulamellibranchia where the greatly enlarged and complex ctenidia remove particulate material from the inhalant current. It follows that the Nuculidae have not evolved from suspension-feeding ancestors and it is unlikely that they ever possessed a firm crystalline style, the failure to develop such a style being correlated with the method of feeding and the functioning of the stomach. The relatively large size of the stomach and style sac is presumably an adaptation for the ingestion of large quantities of bottom material.

The digestive diverticula

Although much work has been done on the digestive diverticula of the Lamellibranchia, attention has been largely restricted to the structure and function of the blindly ending tubules. It is not surprising, therefore, that the considerable morphological differences which exist between the diverticula of the Nuculidae and those of the Anisomyaria and Eulamellibranchia have been overlooked, since they are most marked in the structure of the ducts leading from the tubules to the stomach (fig. 9, C and D). In the Anisomyaria and Eulamellibranchia (fig. 9, C), the main ducts are ciliated with the ciliated cells restricted to a well-defined gutter; the short secondary ducts are non-ciliated and the lumen surrounded by a brush-border epithelium (Owen, 1955). In the Nuculidae, on the other hand (fig. 9, D), it is the main ducts which are non-ciliated while the cells lining the secondary ducts possess well-developed long cilia which extend more or less to the middle of the lumen.

Despite the different results obtained from feeding experiments, the general appearance of the tubules of the Nuculidae is very similar to that of the Anisomyaria and Eulamellibranchia. In all three groups the epithelium is divided into two regions, one composed of numerous lightly staining cells and the other of smaller and less frequent darkly staining, flagellated cells. Yonge (1926) concluded that the epithelium was composed of one type of cell only, the darkly staining cells being nests of young cells which by dividing
replace the older lightly staining cells. This does not appear to be the case in the Nuculidae. Intermediate stages between the two types were never observed, and in these bivalves the darkly staining cells are probably secretory and excretory. In the Nuculidae the appearance of the contents of the granule cells differs markedly in sections from those of the corresponding cells in the Anisomyaria and Eulamellibranchia. In the latter after most fixatives and stains, these cells normally present an extremely vacuolated appearance, while feeding experiments with iron saccharate, colloidal graphite, and titanium dioxide demonstrate convincingly their absorptive function and their ability to ingest solid particles; the ingested material is 'concentrated' in the vacuoles (Owen, 1955). In the Nuculidae, on the other hand, the middle and basal regions of these cells are filled with densely staining granules. There is no ingestion of solid particles nor any evidence of absorption, the appearance of iron in the granules being probably due to transport in the blood after absorption by the stomach epithelium. Moreover, it is difficult to visualize how an interchange of fluid between the lumen of the stomach and of the blindly ending tubules could take place. Cilia are restricted to the relatively short and unbranched secondary ducts and beat away from the tubules, the longer and much-branched main ducts being non-ciliated with their slit-like openings into the stomach surrounded by long cilia beating actively into the stomach (fig. 9, D).

In the Lamellibranchia, the structure and functioning of the digestive diverticula are correlated with the nature of the ingested food. The primitive mollusc presumably possessed a well-developed head, and fed with the aid of the radula on living algae and possibly detritus, and on the available evidence digestion must have been in part intracellular. Despite the triturating action of the radula, the ingested food almost certainly included relatively large fragments, and material present in the stomach was probably pumped from and into large, simple, sac-like diverticula by muscular activity, there being practically no duct system (fig. 9, A). Within the diverticula, solid particles were ingested by the cells for the completion of digestion intracellularly in a manner similar to that which takes place in the wide diverticula of the Nuculanidae (Yonge, 1939). The structure and mode of functioning of the digestive diverticula in the larval oyster are similar. Particles are drawn into the diverticula and returned to the stomach by the rhythmic expansion and contraction of the diverticula. Each diverticulum is simple and sac-like and opens more or less directly into the stomach (Millar, 1955). In the septibranchs, Cuspidaria and Poromya (Yonge, 1928), relatively large particles enter the tubules of the diverticula probably as a result of pumping movements by the muscular stomach, but again the ducts are exceptionally short and the tubules relatively large, simple sac-like structures (fig. 9, B).

Intracellular digestion is also retained by the Anisomyaria and Eulamellibranchia, but the area of the ingesting cells is greatly increased in the majority of adults by the development of numerous small tubules served by an extensive and much-branched duct system (fig. 9, C). Muscular activity plays little
part in the circulation of material, and is probably restricted to the extrusion of waste material from the tubules into the main ducts. The main circulation of fluid and particles within the diverticula is maintained by the specialized tracts of cilia of the main ducts (Owen, 1955). The majority of the Anisomyaria and Eulamellibranchia are suspension feeders, and the ciliary mechanisms of the stomach and ducts of the diverticula are admirably adapted for dealing rapidly and continuously with numerous fine particles. Thus muscular pumping movements as the sole means of controlling the passage of material from and into the diverticula appear to function satisfactorily only in those lamellibranchs in which the duct system is poorly developed and the tubules relatively simple sac-like structures opening more or less directly into the stomach. The diverticula of the Nuculidae also possess an extensive and much-branched system of ducts and tubules (fig. 9, D), and it is unlikely that muscular activity could result in an efficient interchange of fluid. Muscle-

FIG. 9. Types of digestive diverticula present in the Lamellibranchia, shown diagrammatically. A, hypothetical primitive condition and also many larval lamellibranchs; B, septibranchs, some eulamellibranchs, and the wide diverticula of the Nuculanidae; C. Anisomyaria and most Eulamellibranchia; D. Nuculidae. Double-headed arrows represent movement resulting from muscular activity; single-headed arrows represent ciliary currents.
fibres round the ducts and tubules are poorly developed and pumping movements by the stomach would almost certainly result in solid particles, in addition to fluid, entering the diverticula. This was never observed.

Thus, although the general appearance of the digestive diverticula of the Nuculidae is similar to those of the Anisomyaria and Eulamellibranchia, they are not organs of intracellular digestion or even of absorption. They are excretory and secretory in nature, and specialized tracts of cilia controlling a two-way flow of fluid and particles are not developed (fig. 9, d). Digestion is entirely extracellular and the soluble products are absorbed by the epithelium of the stomach and the first part of the intestine. This loss of the primitive method of intracellular digestion is presumably correlated with the method of feeding. The Nuculidae are detritus-feeders and the ingested material includes both large particles and a high proportion of inorganic material. Under such conditions the retention of intracellular digestion would result in considerable waste, since the cells of the digestive diverticula would ingest relatively large quantities of inorganic material. (That the cells of the diverticula of other lamellibranchs will ingest inorganic particles is demonstrated by feeding experiments with titanium dioxide (Owen, 1955) and the observation of Yonge (1939) on Yoldia that the wide diverticula 'were almost black in colour owing to the presence in the cells and in the lumina of numerous particles of black sand'.) This loss of intracellular digestion and adoption of extracellular digestion, although surprising in primitive members of the Mollusca, is not unusual, a similar change having taken place on a number of occasions in the Gastropoda. It is remarkable, however, that, as in the Cephalopoda, the digestive diverticula have also lost their absorptive function. While the Nuculidae are in many respects primitive lamellibranchs and in their method of feeding occupy an intermediate position between the radula-feeding primitive mollusc and the suspension-feeding Anisomyaria and Eulamellibranchia, the structure and physiology of the gut present many specialized features. It is suggested that this specialization is correlated with the retention of the labial palps as feeding organs and the ingestion of large quantities of inorganic material with the food.

The ciliation of the tubules

A minor problem presented by the digestive diverticula of the Mollusca, at least in the Loricata, Gastropoda, and Lamellibranchia, is the ciliation of the tubules. From observations on fresh preparations of the diverticula of various lamellibranchs, Potts (1923) described cilia beating with a rather languid motion within the tubules. He was unable to demonstrate their presence in sections of fixed material and concluded that the cilia were retractile. Similar observations on the presence of cilia within the tubules in fresh preparations of the diverticula of a variety of chitons, gastropods, and lamellibranchs have been made by many workers, but in all cases cilia were not seen in sections of fixed material. There have been various explanations: that the cilia are retractile, that they drop off on fixation, that the tubule cells
pass through phases and that the striated appearance frequently presented by them represents the remains of cilia. In the Nuculidae, Anisomyaria, and Eulamellibranchia, the cilia (termed in this paper 'flagella') are borne by the darkly staining cells of the tubules. In the Eulamellibranchia there are a small number of flagella to each cell and consequently they are relatively easily seen in sections of fixed material (Owen, 1955). In the Nuculidae they are more difficult to observe both in fresh material and in sections, there being but a single flagellum to each cell (fig. 6). Flagella are also borne by the darkly staining cells of the Anisomyaria. The number per cell is not known, although it is certainly less than in the Eulamellibranchia and there may be only one per cell as in the Nuculidae. It seems likely that flagella are always present in the tubules of chitons, gastropods, and lamellibranchs, and the difficulty experienced in demonstrating them in sections of fixed material is due to their distribution, e.g. one per cell as in the Nuculidae, rather than to their being retractile or cast off as a result of fixation.

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