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On the Morphology of the Alimentary Canal, Process of Feeding, and Physiology of Digestion of the Nudibranch Mollusc Jorunna tomentosa (Cuvier)

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IV—ON THE MORPHOLOGY OF THE ALIMENTARY CANAL, PROCESS OF FEEDING, AND PHYSIOLOGY OF DIGESTION OF THE NUDIBRANCH MOLLUSC *JORUNNA TOMENTOSA* (CUVIER)

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I—Introduction

Jorunna tomentosa is a nudibranch which feeds on a diet of sponges.

It was thought that an investigation of the anatomy and physiological processes of the gut of such an animal might yield interesting results, by comparison with those gained by investigators of less specialized molluscs. The following account aims at providing a detailed description of the anatomy and histology of the gut, of the mechanism of feeding, and physiology of digestion of *Jorunna tomentosa* which are compared, so far as possible, with similar structures and processes in other molluscs and phyla.

It is with great pleasure that I acknowledge the invaluable help of Professor H. Graham Cannon, F.R.S. I also wish to thank Dr. C. F. A. Pantin for much valuable help and advice. My thanks are also due to Professor L. E. S. Eastham for his continued interest and advice, and to Professor E. A. Spaul for permission to use the Leeds University Laboratory at Robin Hood's Bay, where a portion of the work was carried out.

II—Methods

The anatomy was determined as far as possible by dissection, aided where necessary by reconstruction from serial sections in the transverse, longitudinal and frontal planes.

Various staining and fixing methods were employed for the investigation of the histology. The statements made by other workers concerning the results given by fixatives are confusing. Nicol (1930), working on the gut of *Sabella*, found that Duboscq-Brasil gave excellent results, while chrome-osmic mixtures such as Flemming (with and without acetic) and Champy proved unsatisfactory. Graham (1932), working on the gut of *Patella*, found that exactly the opposite was the case. Both Flemming without acetic (alone, and in the form of Champy-Kull's modification) and Duboscq-Brasil were accordingly used, both proving useful in demonstrating various structures within the cell. It was found that Flemming without acetic proved

most satisfactory in demonstrating cilia, cell inclusions, and nuclear detail, and in differentiating various kinds of cytoplasm, while Duboscq-Brasil proved most satisfactory in demonstrating the presence of intracellular fibrils. Neither GRAHAM's contention that fixation in Duboscq-Brasil refused to differentiate between the various epithelia of the gut, nor NICOL's statement that Flemming without acetic gave unsatisfactory results, were borne out by this investigation.

In addition, Bouin's aqueous fluid, corrosive acetic, and Susa fixatives were used; the first proved relatively valueless, the second useless for demonstrating cell inclusions, but useful in combination with Mallory's triple stain for picking out connective tissue, while the latter proved excellent for fixing cilia, basal granules, and intracellular fibrillae.

After Flemming without acetic, safranin and light green and the Champy-Kull stain (BOLLES LEE, p. 333) were employed to demonstrate nuclear and cytoplasmic detail. For general reconstruction, Delafield's haematoxylin and eosin, Mann's methyl-blue eosin, and Mallory's triple stain were used. Meyer's mucicarmine with orange G as counterstain was used for the demonstration of mucus cells.

III—BIONOMICS

Jorunna is commonly found round our coasts, crawling on the under-side of large slabs of rock at low-water mark. Here it is found rasping away such sponges as *Halichondria*, making visible trails as it passes inwards into the sponge mass.

IV—MORPHOLOGY OF THE ALIMENTARY CANAL

1—*General Anatomy* (fig. 1)

The mouth leads into a buccal cavity (*b.m.*) with very muscular walls and provided with a triturating apparatus. The buccal cavity passes into a simple oesophagus (*oes.*), which runs backwards to open into a sac-like region (*M.G.*) situated roughly in the middle of the animal and receiving the openings of a complex racemose gland, the so-called "digestive gland" or "liver". Dorsally the sac-like region narrows to form the tubular intestine (*int.*), which, running forwards to the anterior end of the body, bends round upon itself and passes backwards to the mid-dorsal anus (*a.*).

For purposes of description the gut may be divided into foregut, midgut, and hindgut.

2—*Detailed Anatomy*

The Foregut (fig. 2)—The buccal mass has an irregular shape, protruding anteriorly at the mouth, while posteriorly it is produced into the radula sac ventrally, and the oesophagus dorsally (figs. 1 and 2, *r.s.* and *oes.*). The walls are thick and muscular, and in addition the whole mass can be rotated in a sagittal plane about the mouth, by the action of extrinsic muscles. The buccal mass is figured and described in the position

of retraction, that is, when the radula sac is pressed against the floor of the body cavity.

The prosbosciform mouth (*m.* fig. 2) is in the form of a hollow cylinder projecting forward from the buccal mass and surrounding an inner cylinder, also forwardly projecting, consisting of three so-called "lips" (Alder and Hancock 1845), which are freely movable and can be protruded through the mouth, as shown in fig. 2. Commencing at the anterior end of this inner tube, the first or outer lips (*o.l.* fig. 2) form a short plicated tube running posteriorly to end in a muscular ring-like thickening, the

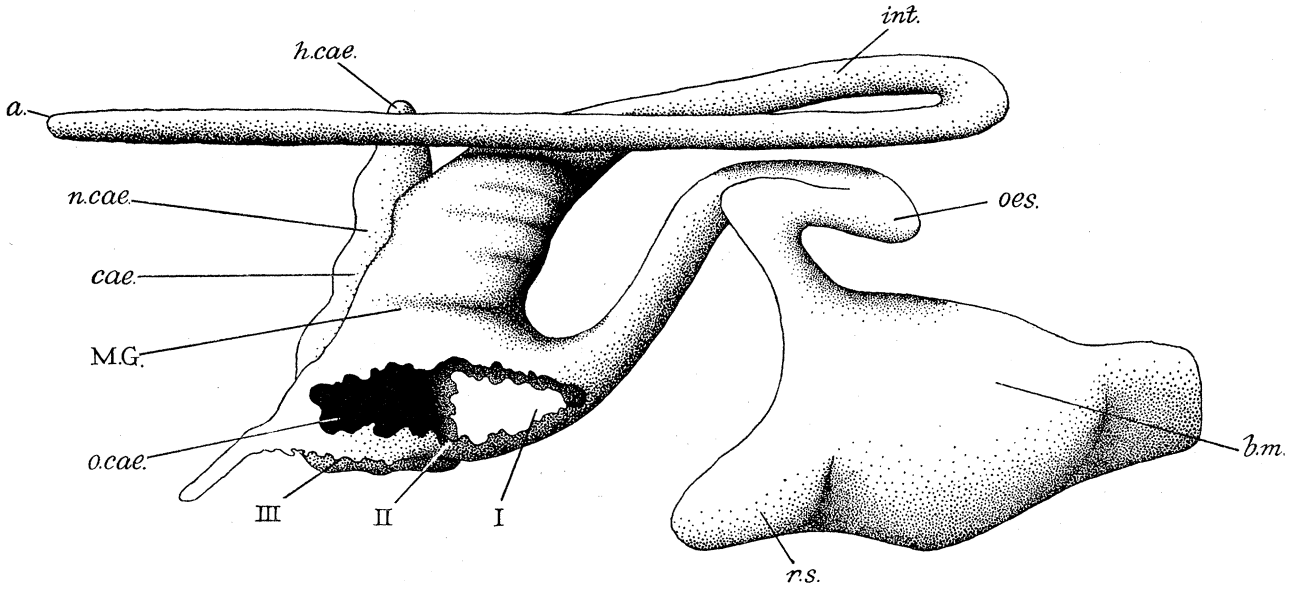

Fig. 1—A reconstruction of the gut viewed from the right side, so as to display the midgut; the digestive gland, which normally surrounds it, has been omitted. *N.B.* This reconstruction was not drawn with numerical accuracy. An endeavour has been made to obtain the correct proportions by correlating transverse, longitudinal and frontal sections. *a.* anus; *b.m.* buccal mass; *cae.* caecum; *h.cae.* head of caecum; *n.cae.* neck of caecum; *int.* intestine; *M.G.* midgut; *o.cae.* opening of caecum into midgut; *oes.* oesophagus; *r.s.* radula sac; *I*, *II* and *III*, openings into digestive gland. × about 15.

"inner lips" (*i.l.* fig. 2), which in turn are succeeded almost immediately by another ring-like thickening, the extremely large and muscular "buccal lips" (*b.l.*). The lips can be protruded through the mouth opening (see "Feeding", § VI).

Behind the buccal lips lies the main cavity of the buccal mass which houses the odontophore (*od.*) with its radula (*rad.*). From the dorsal aspect of this cavity arises the oesophagus, from the ventral aspect the radula sac (*r.s.*).

The odontophore is a muscular tongue-like prominence arising from the floor of the buccal cavity. Its surface, remote from the buccal lip, is deeply grooved down the middle. Applied to this surface, and passing into the groove, is the chitinous ribbon or radula, beset with transverse rows of recurved teeth. The radula thus appears

bilobed: in fig. 2, which is a sagittal section of the buccal mass, one lobe of the radula is seen in surface view. The form of the radula and the number and arrangement of teeth have been described by ALDER and HANCOCK (1845). Only a portion of the radula is exposed, the remainder is housed in the radula sac which forms a conical

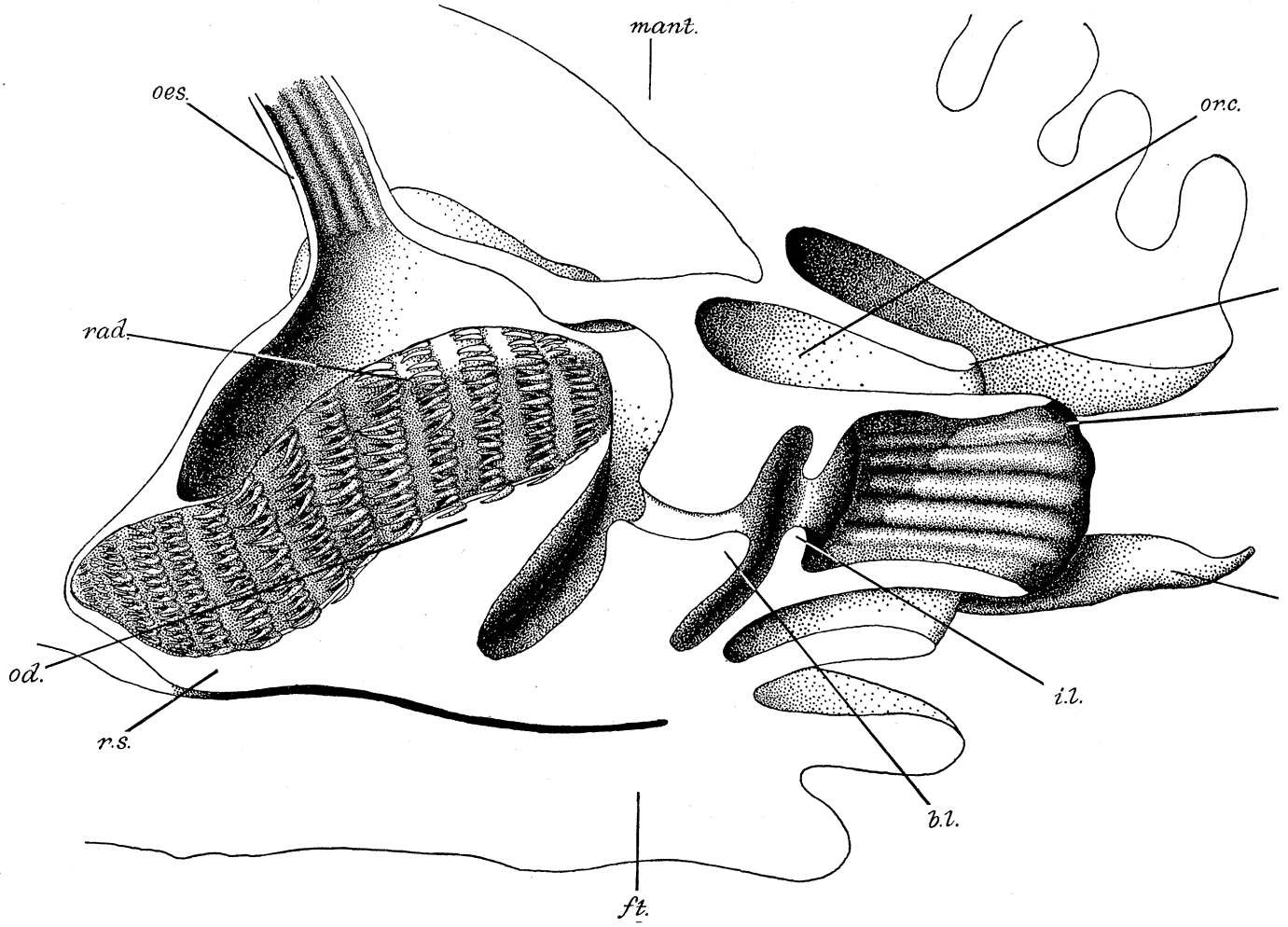

FIG. 2—Sagittal section of the head region of *Jorunna* showing the buccal mass and associated structures. Cut surfaces are left blank. *N.B.* No attempt has been made to show the precise number, and arrangement, of the teeth of the radula. *b.l.* buccal lip; *ft.* foot; *i.l.* inner lip; *m.* mouth; *mant.* mantle; *od.* odontophore; *oes.* oesophagus; *o.l.* outer lip; *or.t.* oral tentacle; *or.c.* oral channel; *rad.* radula; *r.s.* radula sac. × about 30.

projection from the postero-ventral angle of the buccal mass. Partly as a protection against the radula teeth themselves, and partly to provide an effective surface against which the teeth can crush and macerate the food, the walls of the buccal mass, from the inner lips inwards, are covered with a thick cuticle.

The term "oesophagus" is applied to the rest of the foregut by ALDER and HANCOCK. The term is widely used, and is retained here; but it is unfortunate, since it implies a

function comparable with that of the oesophagus of vertebrates, whereas the oesophagus of *Jorunna* has a different physiological significance.

The actual course of the oesophagus varies according to the movements of the buccal mass. Thus when the buccal mass is erected, the oesophagus takes the form of a straight tube, as figured by ALDER and HANCOCK, but when retracted, that is, pulled down, it takes a more or less tortuous course as in fig. 1. The exact course has little significance in this account, and it is best regarded as a straight tube running backwards to open into the sac-like midgut.

Internally the lining of the oesophagus is thrown into pliable longitudinal muscular ridges, the shape of which, and their relations to one another, as well as their relative sizes, varies enormously with the state of distension of the gut with food, and with the tonus of the muscles of the gut wall. Though the ridges may be obliterated in varying numbers and to a varying extent, they return to their original shape when the pressure is removed; usually, examination of the inner surface of the wall of the oesophagus in the living animal reveals the presence of nine distinct longitudinal ridges.

Each of these "primary" ridges is thrown into a series of secondary ridges which run at right angles to the direction of the former, and meeting their fellows from adjacent ridges, form a series of hoops around the lumen of the foregut. These secondary folds occur throughout the oesophagus and, like the primary folds, they are pliable and even more easily obliterated by the gut contents, but return to their original form when the pressure is removed.

The secondary ridges contrast with the primary ridges, first, in being only slight elevations of the surface whereas the primary ridges may be so raised up as almost to block the lumen of the gut, and secondly, in having no significance as food grooves (see p. 201).

Salivary Glands—Neither careful dissection, nor examination of serial sections, revealed the presence of any salivary glands in connexion with the foregut. In this feature *Jorunna* differs from *Archidoris*, which possesses large salivary glands that are figured and described by ALDER and HANCOCK (1845).

The Midgut—The midgut follows the oesophagus and receives the openings of the digestive gland.

The midgut is very difficult to see by dissection, first because it is surrounded by the complex digestive gland, which must be carefully teased away before it can be reached, and secondly because its walls are exceedingly thin and easily damaged. It has therefore been found necessary to reconstruct its anatomy largely from serial sections.

The midgut may best be regarded as a very regular sac (*M.G.* fig. 1) receiving the oesophagus anteriorly and passing dorsally into the tubular hindgut (*int.* fig. 1); its walls are pierced by four openings, one leading into the caecum (*cae.*) and the other three into the surrounding digestive gland. The most anterior of these latter three

openings is ventro-lateral in position, encroaching over about half the left side (I, fig. 1). The second or lateral opening occupies the whole of the right side (II, fig. 1), while the third and posterior opening occupies practically the whole posterior aspect of the midgut (III, fig. 1). In addition a diverticulum or caecum (*cae.*) opens into the left-hand side, its opening (*o.cae.*) occupies the remaining half of this aspect.

In consequence of the four large openings, the midgut walls are very limited in extent, and in sharp contrast with the foregut and hindgut, they are attached by connective tissue to the surrounding liver mass.

The Digestive Gland—The digestive gland is a complex racemose gland formed of a mass of blind, branching tubules separated by strands of connective tissue. It is closely invested by the gonad, parts of which tend to penetrate the connective tissue between the tubules. These tubules are collected into three large channels which open into the midgut by the large openings described above.

The Caecum—The caecum or diverticulum forms a blind sac communicating with the hindgut (fig. 1).

Partly because it is a minute structure, and partly because it is deeply embedded in the tissue of the digestive gland, it proved impossible to investigate its structure by dissection. The following account of its anatomy is based entirely on reconstruction from serial sections cut in the transverse and frontal planes.

The caecum is thin-walled and exceedingly delicate, and its structure is shown in fig. 3. The expanded ventral portion or fundus (*f.*), which has a large opening (*o.cae.* figs. 1 and 3) into the left side of the midgut, passes dorsally into a narrow neck-like region (*n.cae.* figs. 1 and 3) which in turn expands to form the blind head of the caecum (*h.cae.* figs. 1 and 3).

The walls remote from the midgut are thrown into pliable muscular ridges which, like those of the oesophagus, may be temporarily obliterated or distorted by the pressure of contents. There are two series of ridges, a mesial series (*mes.r.*), running dorso-ventrally, and a lateral series on either side (*a.lat.r.* and *p.lat.r.*), running obliquely upwards and inwards from the fundus into the neck region.

The head has thicker and more muscular walls than the rest (see p. 188).

The Hindgut—The hindgut arises from the dorsal aspect of the midgut as a simple dilated tube subcircular in cross-section. It passes dorsally and forwards slightly to the left of the middle line, progressively narrowing as it does so. Passing forward to a point approximately overlying the origin of the oesophagus from the buccal mass (fig. 1), it curves towards the right and, continuing its curvature, passes over to the right-hand side of the body, along which it passes backwards as a simple uniform tube curving gradually towards the median line to reach the anus, which lies amid the gills at the posterior end in the mid-dorsal line.

The first dilated portion of the hindgut is frequently called the "stomach" (Alder and Hancock) and corresponds with the similarly named greatly dilated portion in

Archidoris (Alder and Hancock 1845, and Yonge 1925*b*). The remaining portion which passes back to the anus as a simple uniform tube is frequently called "intestine". For these regions in *Jorunna*, both these names are unsatisfactory, since no enzymes are produced, neither does digestion or absorption occur (as the suggested names would imply). The internal surface, from the point of emergence from the midgut to the anus, is thrown into primary and secondary ridges exactly like those of the oesophagus.

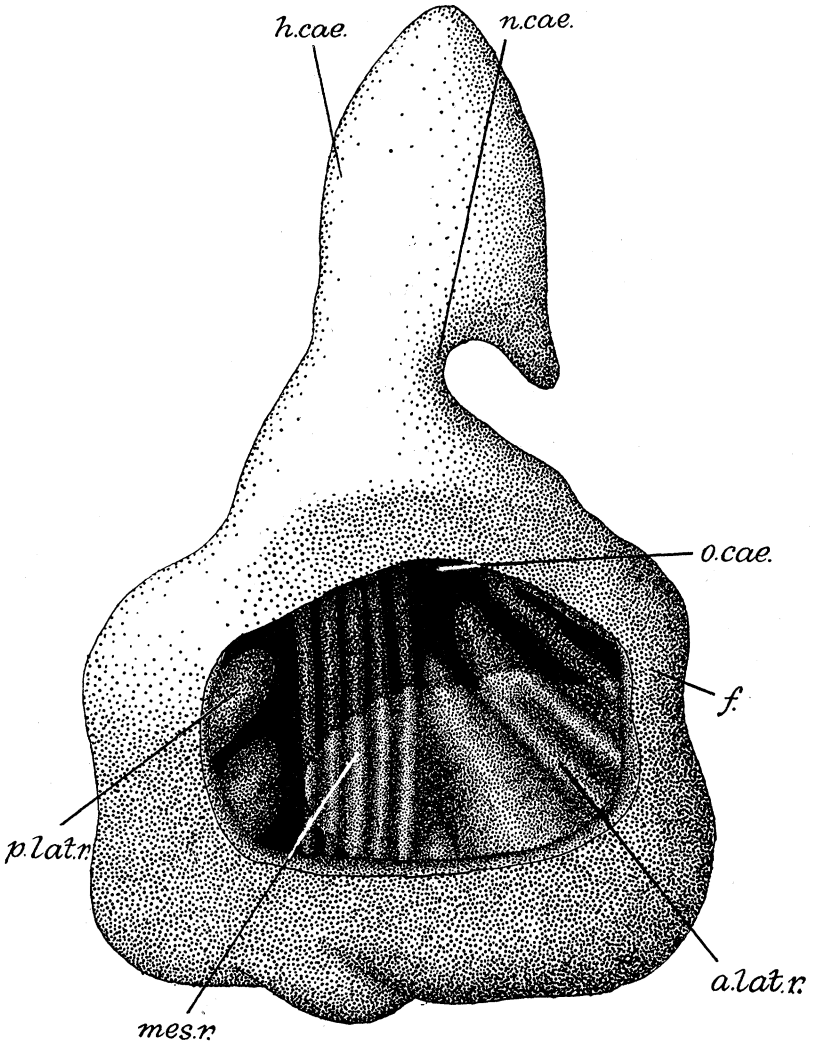

Fig. 3—Reconstruction of caecum, viewed from the right side. *a.lat.r.* anterior lateral ridge; *f.* fundus; *h.cae.* head of caecum; *mes.r.* mesial ridge; *n.cae.* neck region; *o.cae.* opening of caecum into midgut; *p.lat.r.* posterior lateral ridge. × about 52.

V—Histology

1—*The Buccal Mass* (for anatomy see p. 174)

In the following account the histological structure of the buccal cavity proper (i.e. the cavity beyond the inner lips) is described. No attempt is made to describe the

histological structure of the radula sac, which would involve a discussion of the formation of the radula and its teeth, a subject which is beyond the scope of this account.

Details of the formation of the radula in molluscs generally are given by Sollas (1907*a*).

Fig. 4 is a transverse section of the wall of the buccal mass as seen after fixation in Flemming without acetic and stained in safranin and light green.

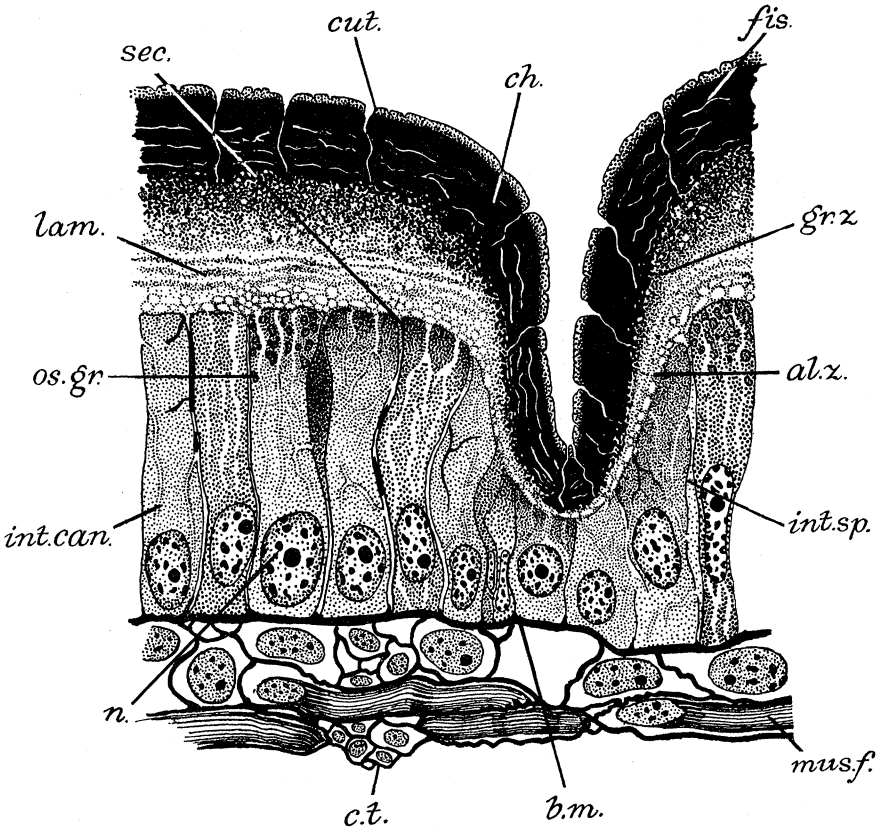

Fig. 4—Transverse section of the wall of the buccal mass. Fixed Flemming without acetic, stained safranin and light green. *al.z.* alveolar zone; *b.m.* basement membrane; *ch.* chitin; *c.t.* connective tissue fibre; *cut.* cuticle; *fis.* fissure in chitin; *gr.z.* granular zone; *int.can.* intracellular canaliculus; *int.sp.* intercellular canaliculus; *lam.* lamina of laminated zone; *mus.f.* muscle fibre; *n.* nucleus; *os.gr.* granules appearing greyish after osmic acid; *sec.* apparent secretion of chitin from intercellular canaliculus. × 1600.

The gut wall is conspicuous here by its possession of a cuticular layer which appears differentiated into zones. These are particularly easily distinguished where the layer is thick, as for example on the buccal lips. It is assumed that this layer is chitinous, since a similar layer found in similar situations in certain other molluscs has been shown to exhibit the same chemical and physical properties as the chitin of arthropods (Sollas 1907*b*; Wester 1910). The zones of the chitin are as follows:

Nearest the epithelium is an alveolar zone (*al.z.*), followed by a laminated region, the laminae (*lam.*) usually staining a pale green (see below). Following this is a granular zone (*gr.z.*), the granules becoming compacted distally to merge into a broad zone of solid chitin (*ch.*), the continuity of which is broken only by vertical and longitudinal fissures. This is followed by a shallow zone (*cut.*) which is more retentive of safranin than the underlying chitin, and, as it forms the outermost layer, may be termed cuticle. The alveolar, laminated and granular zones may be absent.

The reactions of the chitin and cuticle to various stains is shown in Table I.

TABLE I

Stain	Chitin	Cuticle
Meyer's mucicarmine	Does not stain	May appear pinkish or unstained
Orange G	Yellow	Does not stain
Saurë fuchsin	Pink or reddish	Red
Safranin and light green	Green and/or red	Green and/or red
	The cuticle tends to retain safranin more readily than the chitin	
Mallory	Orange and/or blue, and/or red	Orange and/or blue, and/or red
	The zone of chitin nearest the epithelium always stains blue	
Delafield's haematoxylin	Pinkish	Pinkish
Mann's methyl-blue-eosin	Colours varying from blue to red are common to both chitin and cuticle	

The underlying chitogenous epithelium consists of one type of cell only. These are usually columnar, though they vary greatly in shape and dimensions according to the exact region in which they are observed.

Their outstanding feature is the possession of intracellular canaliculi (*int.can.*) which open either at the free surface of the cell, or into intercellular canaliculi (*int.sp.*). Within the cell the canaliculi become subdivided into numerous branches, all of which end blindly. The main course of these branches appears to be parallel to the long axis of the cell.

Usually the intracellular canaliculi and intercellular spaces appear clear, but often they appear to contain a secretion which stains a bright red in safranin, and blue in Mallory. The significance of this secretion is discussed below. Frequently the upper third of the cytoplasm either stains more deeply, or contains numbers of granules (*os.gr.*), which appear greyish after osmic fixation or may take up light green or safranin.

The nuclei (*n.*) are basal, ovoid, and possess a prominent nucleolus and scattered irregular chromatin masses.

The epithelium rests on a basement membrane (*b.m.*) which overlies the main part of the wall of the buccal mass which is thickly beset with connective tissue (*c.t.*), and muscle fibres (*mus.f.*), running in all directions.

The most significant features of the gut wall of this region are the possession of chitin and cuticle, and a very well-developed muscular layer.

The hard surface of the chitin, combined with a certain amount of flexibility, no doubt enhanced by the fissures running through it, together with the powerful

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

musculature, provide an effective combination for the titurating function of the buccal mass.

Fixation in Flemming without acetic demonstrates the zonation of the chitin particularly clearly. After fixation in Duboscq-Brasil and Susa's fluids, however, the chitin appears relatively homogeneous, and the zonation described above is not so clear.

It may be argued, in view of the above, that the zonation is due to bands of precipitate (Liesegang effect), being formed by the action of a particular fixative. That this is not so, is shown by the fact that the zonation always runs parallel to the free surface of the epithelium, and never parallel to the vertical fissures, or in any other plane.

The intracellular canaliculi are particularly clearly demonstrated by fixatives such as Duboscq-Brasil and Susa, which tend to dissolve the cell contents and cause shrinkage. Though the canaliculi may be seen after fixation in Flemming without acetic, their course is more difficult to follow than when fixed in the former fluids.

Discussion—Vitzou (1882) has expressed the view that the chitin of the integument of decapod Crustacea arises by gradual transformation of the outer layers of the cells of the chitogenous epithelium. Thus from each cell a minute block of chitin is produced, one row of such blocks forming a lamina of the fully formed chitin. The blocks become separated off from the cells, and since, as the process goes on, the chitinous blocks produced by adjoining cells do not fuse, the chitin eventually comes to consist of multi-layered cylinders.

A consideration of the histological features of the chitogenous epithelium of the buccal mass of *Jorunna* does not accord with this view, and seems to indicate that the chitin here does not arise by transformation of the outer layers of the epithelial cells, but that it is secreted by these cells.

The particular features affording support to this view are the following:

First, if the chitin consisted of multi-layered cylinders, we would expect to find at least slight evidences of vertical striations running through the chitin. No such striations have been observed.

Secondly, the alveolar layer presents the appearance of being a secretion, as shown particularly by the way in which it dips down into the intercellular canaliculi.

This appearance, coupled with the occasional appearance of a secretion in the intercellular and intracellular canaliculi seems, to indicate that the secretion in question is passed out through the canaliculi. The staining reactions of the secretion in the canaliculi lend support to this view, for though the chitin as a whole stains in an arbitrary fashion (see below), the newest layers of chitin (i.e. those nearest the epithelium) always stain blue in Mallory, as does the secretion in the canaliculi.

This view is in accordance with the views expressed by Bloch (1896), Sollas (1907*a*), and others, concerning the origin of the radula and its teeth. They believe them to be secreted and not to arise by transformation of the formative cells.

The absence of any definite ducts through the chitin, and any associated glands,

renders it clear that the cuticle cannot arise in a manner similar to that described by YONGE (1932*b*) for the decapod Crustacea, and it must therefore arise either by the transformation of the peripheral layers of chitin into a cuticular substance, or in a manner similar to that described for *Rhodnius prolixus* by WIGGLESWORTH (1933), where he suggests that the underlying epithelium secretes the cuticle first, followed by the chitin afterwards. The former suggestion is supported by the fact that the chitin of the radula changes its properties with age. This was shown by SOLLAS (1907*a*, *b*), who demonstrated that the young and old portions of the radula differed in chemical and physical properties, and by PANTIN and ROGERS (1925), who found that the older portion of the radula of *Buccinum undatum*, unlike the younger portion, was amphoteric.

The reaction of chitin and cuticle to stains as shown by a study of the buccal mass of *Jorunna* is not in agreement with the results gained by YONGE in the decapod Crustacea. This affords further support to the idea that the cuticle in *Jorunna* does not arise in a manner similar to that described by YONGE for cuticle of the decapods. YONGE states that the chitin and cuticle *always* stain differently. This was not found to be so in *Jorunna*, as Table I on p. 181 shows. Moreover, Professor CANNON has informed me privately that he has not found this difference of staining reaction between chitin and cuticle to be constantly manifest in the Entomostraca, and has shown me sections of a rare arachnid in which the staining reactions were reversed.

2—*The Oesophagus* (for anatomy see p. 177)

In considering the remainder of the gut from the histological standpoint, it is no longer sufficient to retain the division of the gut into regions such as we have hitherto used, since both the oesophagus and hindgut can be subdivided into two regions which differ appreciably in the characters of their lining epithelium, and on the other hand two such morphologically distinct regions as the midgut and a section of the oesophagus have lining epithelia which are precisely similar.

Therefore it is proposed, while discussing the histological features of the gut, to adopt a scheme based on that used by GRAHAM (1932) in his description of the gut of *Patella*, and to subdivide the oesophagus and hindgut into sections A and B (fig. 5).

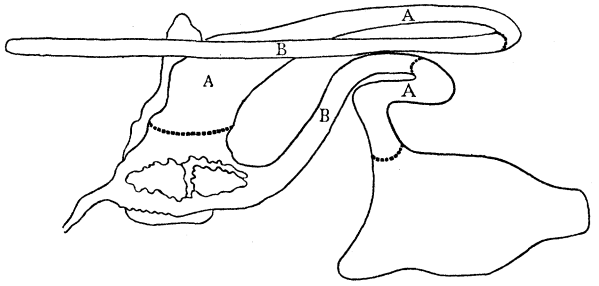

FIG. 5—Diagram showing scheme of subdivision of gut into histological regions. The broken lines indicate the approximate boundaries between the regions.

1—*The Oesophagus, Section A.* The main histological features of section A are shown in fig. 6, which is a transverse section of the gut wall of this region, as seen after fixation in Flemming without acetic and staining in safranin and light green.

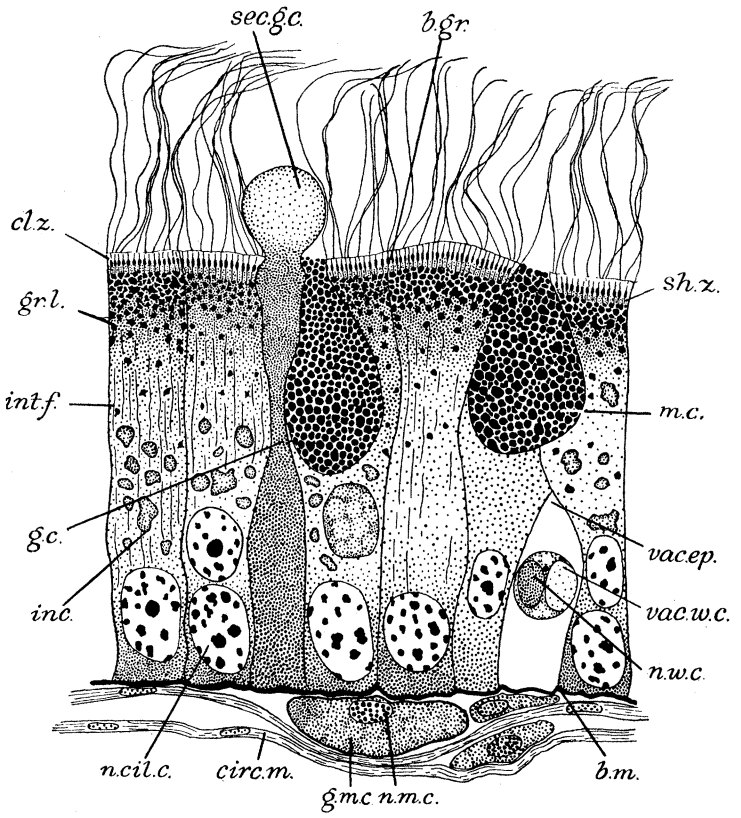

FIG. 6—Portion of epithelium of section A of the oesophagus. Fixed Flemming without acetic, stained safranin and light green. *b.gr.* basal granules; *b.m.* basement membrane; *circ.m.* circular muscle; *cl.z.* clear zone; *g.c.* gland cell; *g.m.c.* giant mucus cell; *gr.l.* layer of irregular granules; *inc.* solid inclusions appearing greyish after osmic fixation; *int.f.* intracellular fibrils; *m.c.* mucus cell in epithelium; *n.cil.c.* nucleus of ciliated cell; *n.m.c.* nucleus of mucus cell of submucosa; *n.w.c.* nucleus of wandering cell; *sec.g.c.* secretion of gland cell; *sh.z.* zone beneath basal granules; *vac.ep.* vacuole in epithelium; *vac.w.c.* vacuole in wandering cell. × 1330.

After osmic fixation the cilia appear well extended and very conspicuous owing to their dark colour. The inclusions (*inc.*) and basal granules (*b.gr.*) are well defined. Mucigen granules appear a dense black. Intracellular fibrillae (*int.f.*) are demonstrated but their course is not easily followed.

The intracellular fibrillae are best demonstrated in these cells by fixation in Duboscq-Brasil, after which their course within the cell is easily followed.

Staining in Meyer's mucicarmine colours the mucus glands (*m.c.* and *g.m.c.*) a deep red or purple, showing that their secretion is quite definitely mucin.

After Mallory's triple stain a shallow zone of cytoplasm (*sh.z.*), immediately below the basal granules of the ciliated cells, takes up a characteristic pale blue colour, while the remainder of

the cytoplasm stains purplish. The corresponding zone, after staining in safranin, appears pinkish, the rest of the cytoplasm takes up little or no stain.

Osmic fixation, by virtue of its less violent action, is by far the best fixative for this epithelium, for what is lost in lack of clearness of the intracellular fibrillae is more than compensated for by the retention and clear differentiation of the cell inclusions. The mucigen granules of the mucus glands, and the irregular bodies of the granulose layer (*gr.l.*) of the ciliated cells, blacken with osmic acid and consequently show very distinctly.

Duboscq-Brasil, on the other hand, though showing basal granules and intracellular fibrillae very clearly, tends to dissolve the cell inclusions and cause retraction of the cilia.

The ciliated cells are most predominant. They are tall and columnar, about 40μ high and 6μ across and separated by distinct boundaries. The nucleus (*n.cil.c.*) is oval, basal, and possesses scattered irregular chromatin masses, a distinct nuclear membrane, and sometimes a nucleolus is visible. Often, as is quite common in similar epithelia, more than one nucleus may be present in a cell.

The mucus cells (*m.c.*) are much less numerous. They are vesicular in form, and appear as a series of small sacs sunk within the epithelium. Their secretion is extruded as a discrete granular goblet. The least predominant are the gland cells (*g.c.*). These are very inconspicuous, particularly if discharged, when they appear to form merely a thickened boundary between two ciliated cells. The cytoplasm may possess a few scattered granules, presumably precursors of the secretion which is extruded as a discrete hyaline globule (*sec.g.c.*).

Throughout the gut wandering cells are common. They may be seen in the lumen of the gut, in the epithelial cells themselves, in intra-epithelial spaces (*vac.ep.*), or in the subjacent tissues. They occasionally present evidence of being amoeboid. The cytoplasm varies enormously in its capacity to take up various stains. After osmic fixation the cytoplasm may refuse to take up stain and appears greyish with coarse blackish granules. In other cases the cytoplasm may show marked avidity for stains like safranin, Altmann's acid fuchsin and Delafield's haematoxylin, and the cells appear merely as deeply staining bodies devoid of any obvious structure. Sometimes the cytoplasm may take up stains such as safranin and light green, or Mallory, differentially. Very frequently the cytoplasm is vacuolated. The nucleus, when visible, may be spherical, oval, or crescentic, with small scattered chromatin masses.

The epithelium rests on a basement membrane (*b.m.*) staining deeply in light green, the fuchsin of Champy-Kull, and the aniline blue of Mallory. This overlies a layer of circular smooth muscles (*circ.m.*). Giant mucus cells (*g.m.c.*), sometimes measuring as much as 32μ across, occur in the submucosa; their cytoplasm gives the characteristic reaction with muci-carmine, and their nuclei (*n.m.c.*) are ovoid, with small scattered chromatin granules.

Staining with Mallory revealed no such structures as the intraepithelial canals described by Mackintosh (1925) in *Crepidula*, Yonge (1926*b*) in *Ostrea*, and Graham (1932) in *Patella*.

2—*The Oesophagus, Section B.* Fig. 7 depicts a transverse section of the gut wall of this region, and was drawn from a preparation that had been fixed in Flemming without acetic and stained in safranin and light green. The epithelium lining this region of the oesophagus contrasts sharply with that lining the preceding section. It exhibits a markedly ragged appearance associated with the great predominance of mucus cells, and due to the irregular masses of secretion (*sec.g.c.*), produced by these cells, and to the spaces left in the epithelium by the discharged goblets of mucin (*dis.g.*).

These goblets appear very conspicuous after such stains as light green or Meyer's mucicarmine, and after fixation in Flemming without acetic they are seen to be loaded with coarse granules of mucigen which blacken with osmic acid. The remainder of the cytoplasm of the mucus cells contains diffusely scattered granules, some of which (*bl.gr.*) are very small, irregular, and blacken with osmic acid, others (*g.gr.*) are larger, more regular, and appear greyish after osmic fixation. Vacuoles of varying sizes are present; the small ones (*s.v.*) are inconspicuous and appear to be devoid of inclusions, but the large ones (*l.v.*) are very conspicuous and contain

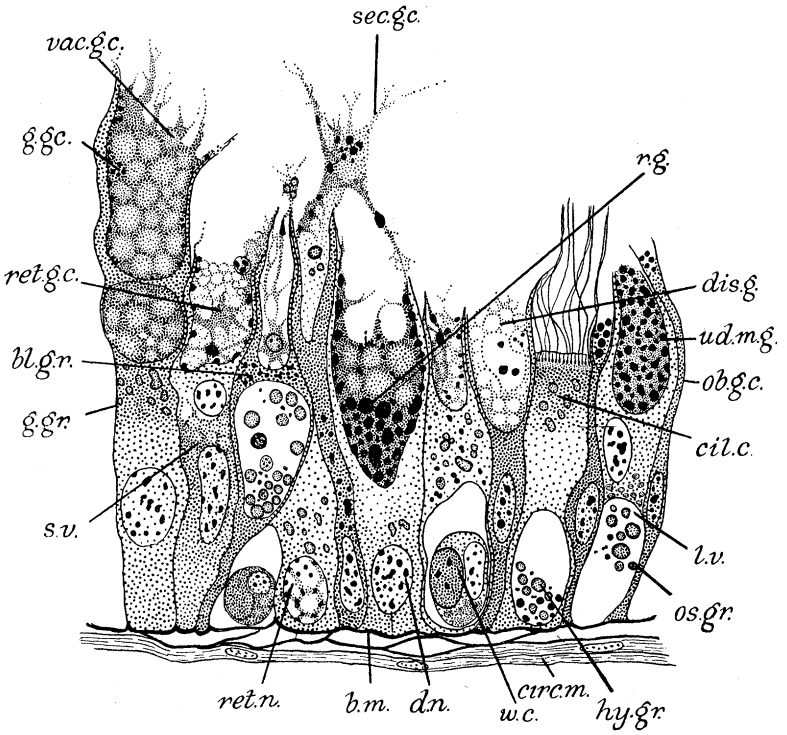

FIG. 7—Portion of a transverse section through the wall of Section B of the oesophagus. Fixed Flemming without acetic; stained safranin and light green. *b.m.* basement membrane; *bl.gr.* irregular granules; *cil.c.* ciliated cell; *circ.m.* circular muscle; *dis.g.* cavity left by discharge of goblet; *d.n.* nucleus with diffuse chromatin granules; *g.gc.* mucigen granules remaining behind in discharged goblet; *g.gr.* granules appearing greyish after osmic fixation; *hy.gr.* hyaline granules; *l.v.* large vacuole; *ob.g.c.* shrunken gland cell; *os.gr.* granules appearing blackish after osmic fixation; *r.g.* undischarged portion of goblet; *ret.g.c.* reticulum remaining in discharged goblet; *ret.n.* nucleus with reticular structure; *s.v.* small vacuole; *sec.g.c.* secretion of goblet cell; *ud.m.g.* undischarged goblet of mucigen; *vac.g.c.* vacuole in goblet; *w.c.* wandering cell. × 1330.

spheroids about 2μ in diameter which vary in their reactions to stains and fixatives, for while some (*hy.gr.*) stain in light green and appear hyaline, others (*os.gr.*) take up safranin and blacken with osmic acid to a varied extent.

The nucleus is ovoid, with a distinct nucleolus staining light red in safranin and Altmann's acid fuchsin; the chromatin may appear in the form of irregular scattered granules (*d.n.*) or in the form of a reticulum (*ret.n.*).

Their secretion consists of the whole, or part of one of the goblets (*ud.m.g.*) described above, but usually owing to the pressure of food in the lumen of the gut, and the action of the ciliated

cells, the mucin goblets become drawn out into irregular strands of secretion (*sec.g.c.*). The discharge of secretion leaves behind a cavity in the cell (*dis.g.*) which contains either a sparse reticulum (*ret.g.c.*) or a denser vacuolated mass of undischarged secretion (*vac.g.c.*) with adherent granules (*g.gc.*). When only a part of a goblet is discharged, the undischarged portion (*r.g.*) remains in the bottom of the cavity.

Cells which have been secreting for a considerable time become much shrunken, and frequently almost obliterated (*ob.g.c.*) by the pressure of the surrounding secreting cells.

Ciliated cells, precisely the same in structure as those described for section A, occur scattered throughout section B. The wandering cells (*w.c.*), though having the same structure and distribution as those described in the preceding section, are here much more numerous.

The tissues of the submucosa are the same as those described for the preceding section.

Compared with the epithelium of the buccal mass, that of the oesophagus is conspicuously different in possessing no hard chitinous covering and a relatively scanty muscular coat. This is correlated with a complete difference of function. The distribution of ciliated and mucus cells in the oesophagus accords excellently with its functioning. In section A, which is nearest the buccal mass, the predominance of ciliated cells is correlated with a need for the rapid transference of small food particles thrust up, on the radula (see p. 198), into the oesophagus. The scattered mucus cells produce a viscid secretion which appears to serve two purposes; first in cementing small food particles into strings, and secondly in acting as a protection for the delicate epithelium, against the numerous projecting sponge spicules which are thrust up with the triturated food on the radula. This latter conclusion is supported by the observation that the amount of mucus secreted seems greater than would be required merely to cement food particles into strands.

By the time the food particles enter into section B of the oesophagus, they are already segregated into food strands, and consequently the chief function of this region is to ensure the stability of these strands by admixture with abundant mucus. Hence the primarily important mucus cells are very predominant.

3—*The Caecum* (for anatomy see p. 178)

Fig. 8 is a transverse section of the wall of the caecum as seen after fixation in Flemming without acetic and staining in safranin and light green.

The epithelium lining the caecum is conspicuous by its large number of ciliated cells, which differ from those previously described, in being smaller, having large nuclei, and very sparse cytoplasmic contents. Owing to the latter feature it is possible to see an extremely well-defined system of intracellular fibrils within the cell.

These cells are fairly regular in outline and are shortly columnar. They have large vacuoles (*vac.*) and the cilia end in distinct basal granules (*b.gr.*), which are prolonged within the cell into intracellular fibrils (*int.f.*).

The basal granules overlie a narrow clear zone of cytoplasm (*cl.z.*), which in turn overlies a deeper zone with irregular solid inclusions (*ir.g.*) appearing greyish or, more rarely, black, after osmic acid.

Deeper in the cell, in the region of the nucleus, regular ovoid inclusions (*ov.b.*), which take up safranin or light green, are present.

The nuclei (*n.cil.c.*) are ovoid or spherical, having a well-marked nuclear membrane and a very prominent nucleolus (*ncl.cil.c.*), which stains a characteristic bright red in safranin.

Between the ciliated cells small gland cells (*g.c.*) are present; these are conspicuous on account of their dense cytoplasm which appears a deep grey or brown after osmic fixation and which may contain a number of small dark granules (*gr.*). The nucleus (*n.g.c.*) never shows a reticular structure, has a distinct nucleolus and small irregular chromatin masses. These cells produce a secretion in the form of discrete globules (*sec.gl.*) staining bright red after safranin. The function of this secretion is discussed later.

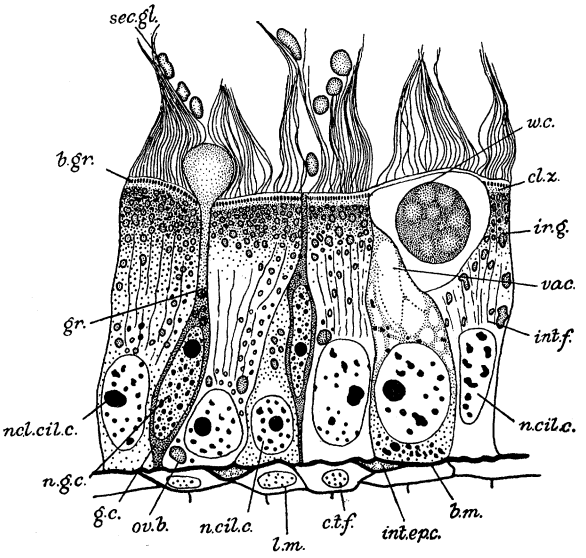

FIG. 8—Portion of epithelium of wall of diverticulum. Fixed Flemming without acetic; stained safranin and light green. *b.gr.* basal granules; *b.m.* basement membrane; *cl.z.* clear zone beneath basal granules; *c.t.f.* connective tissue fibre; *g.c.* gland cell; *gr.* granule in neck of gland cell; *int.ep.c.* structure resembling intra-epithelial canal (see text); *int.f.* intracellular fibril; *ir.g.* solid inclusions appearing greyish after osmic acid; *l.m.* longitudinal muscle; *n.cil.c.* nucleus of ciliated cell; *n.g.c.* nucleus of gland cell; *ncl.cil.c.* nucleolus of ciliated cell; *ov.b.* ovoid inclusion; *sec.gl.* secretion of gland cell; *vac.* vacuole in ciliated cell; *w.c.* wandering cell. × 1500.

Wandering cells (*w.c.*) are common throughout the epithelium.

For the demonstration of cell inclusions, Flemming's fluid proved by far the best fixative.

The intracellular fibrils tend to be hidden by cell inclusions, and hence they are not well demonstrated after fixation in Flemming, but they appear very clearly after fixing in Susa, when they can be traced from the basal granules, through the cell, to a point of insertion on the nuclear membrane.

The structure of the basement tissues yields a valuable clue for determining the method of function of the caecum (see p. 211). The epithelium rests on a conspicuous basement membrane (*b.m.*) which overlies connective tissue permeated by fibres (*c.t.f.*), and in the fundus and neck regions of the caecum, by a few longitudinal (*l.m.*) and circular muscles. In the head region, however, the muscular coat is greatly increased,

and here also, there is a greater development of small canals (*int.ep.c.*) between the bases of the epithelial cells. These canals contain a coagulum in which no structure is recognizable but which stains a bright red in Mallory. Their significance is unknown; they may possibly be a strengthening device, comparable with the intraepithelial canals described by MACKINTOSH (1925) for the style sac of *Crepidula*, by YONGE (1926*b*) for the style sac of *Ostrea*, and by GRAHAM (1932) for the midgut of *Patella*. Such strengthening devices occur where an epithelium is subjected to a considerable stress, e.g. in the style sacs noted above, and here such a function would accord with the well-developed muscular layer of the region, and the suggestion (see p. 212) that the gut wall is here chiefly concerned in moulding and ejecting a faecal bolus.

Certain of the ciliated cells described above present the peculiar appearance shown in fig. 26. Their free ends become expanded into globular masses (*ex.gl.*) which appear finely granular and stain a bright pink in Mallory, contrasting sharply with the rest of the cell, which stains purplish.

The expansions become abstricted as free rounded bodies which pass into the lumen of the caecum and have also been seen in the cavities of the mid- and hindgut.

These bodies cannot be droplets of secretion, since they are seen to retain their definite shape. Their appearance and mode of formation recalls that of the "ballots d'excrétion" described by DARBOUX (1900), and noted later by FORDHAM (1925), which are produced by abstriction from the cells lining the intestinal caeca of *Aphrodite aculeata*. Possibly like them, they form a means of excretion, being ejected from the caecum, together with the faecal masses produced there (see p. 212), and carried into the hindgut.

4—*The Digestive Gland* (for anatomy see p. 178)

Fig. 9 represents a portion of a transverse section of one of the tubules of the digestive gland, drawn from a preparation fixed in Flemming without acetic and stained in safranin and light green.

The epithelium is extremely variable in height, and is composed of cells of varied shapes. These cells appear to be ingestive, since they show large areas either loaded with inclusions or densely packed with vacuoles. This observation is confirmed by the results of iron-saccharate feeding (see p. 208). The free surface of the cells is variable in appearance. Sometimes it is produced into irregular cytoplasmic processes (*cy.p.*) which appear capable of ingesting solid particles. Elsewhere phagocytes may be abstricted from the surface, while at other times the epithelium may possess a striated border, like the cells of the digestive gland of *Patella* (GRAHAM 1932). When living tissue is examined, many of the liver cells may be seen to bear cilia.

As in other regions of the epithelium, Flemming without acetic proved to be by far the best fixative. It does not produce swelling nor dissolution of the cell inclusions, it clearly differentiates various regions of cytoplasm such as the homogeneous (*hom.a.*), and granulose (*gr.a.*) areas seen in fig. 9, and does not tend to cause retraction of the pseudopodial processes of the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

cells. Duboscq-Brasil and Susa, on the other hand, tended to produce distortion of the cells, accompanied by retraction of the pseudopodial processes, and a certain amount of dissolution of the contents.

The cilia observed in living tissue only appeared in limited regions of preparations fixed in Susa, and indications of cilia were never found after fixation in Flemming without acetic. The free border of the cells may appear striated after fixation in Duboscq-Brasil. This appearance is frequently seen in fixed ciliated cells and the rare appearance of cilia in fixed preparations is

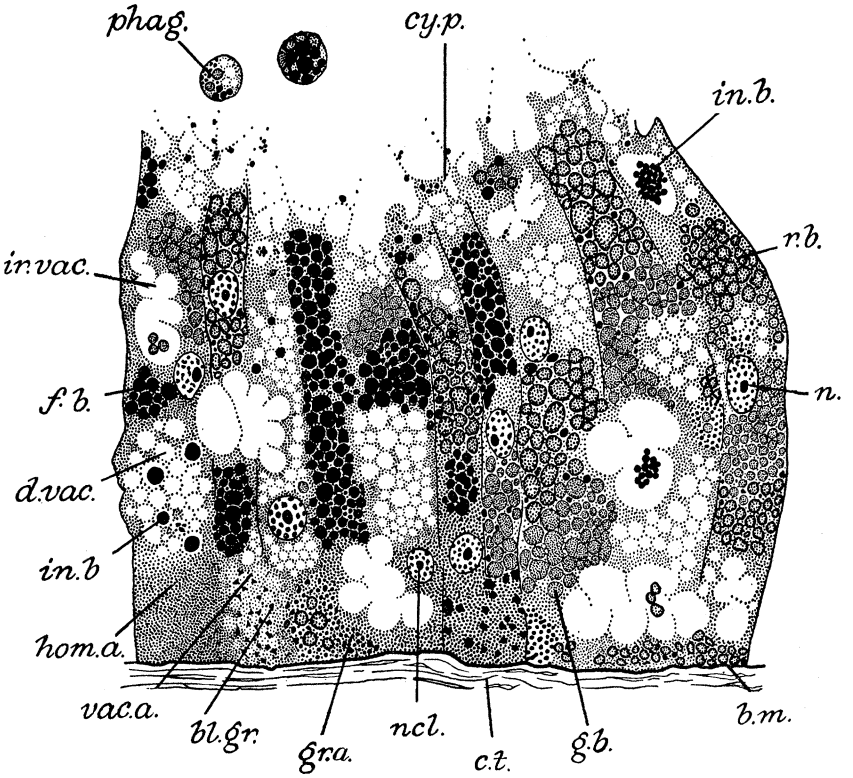

FIG. 9—Portion of epithelium lining tubule of digestive gland. Fixed Flemming without acetic, stained safranin and light green. *b.m.* basement membrane; *bl.gr.* granules, appearing black after osmic, between vacuoles; *c.t.* connective tissue underlying epithelium; *cy.p.* cytoplasmic process; *d.vac.* discrete vacuole; *f.b.* fatty body; *g.b.* solid inclusion staining in light green; *gr.a.* granular area; *hom.a.* homogeneous area; *in.b.* ingested body; *ir.vac.* irregular vacuole; *n.* nucleus; *ncl.* nucleolus; *phag.* phagocyte; *r.b.* inclusion appearing reddish after safranin; *vac.a.* vacuolated area. × 1600.

not surprising when the results gained by other workers are considered. Thus POTTS (1923) states that in *Teredo* the cilia invariably found lining the liver tubules in the living animal, always fall off on fixation; while YONGE (1926*a*), working on the liver of *Nucula* and filibranchs, states that cilia can never be seen in sections of fixed material, though they have been observed in the living animals, and that a striated border to the cells is all that remains after fixation to indicate the former presence of cilia.

Many of the cells have highly vacuolated cytoplasm; the vacuoles may be small and discrete (*d.vac.*) or several such vacuoles may run together to form large irregular vacuoles (*ir.vac.*). The vacuoles frequently contain ingested bodies (*in.b.*), and in living tissue many are seen to contain

a clear refractive liquid, others have brownish contents, and some contain a greenish liquid. Almost every cell contains a large number of inclusions, of any size up to 4μ, many being surrounded by vacuoles.

The inclusions react to stains and fixatives in a very varied manner; some blacken in osmic (*f.b.*) and are presumably of a fatty nature, others take up safranin (*r.b.*), eosin or light green (*g.b.*), while after staining in Mallory some of the inclusions appear red, some blue, some purple, and some green.

Cytoplasmic differentiation is very marked, some areas (*gr.a.*) appearing coarsely granular, others free from granules, while other areas appear deep brown after osmic fixation, and either homogeneous (*hom.a.*), or vacuolated (*vac.a.*), with blackish granules (*bl.gr.*) between the vacuoles.

The nuclei (*n.*) are spherical or ovoid, variable in position, and have a distinct nucleolus (*ncl.*) staining bright red in safranin, and chromatin masses which appear small, scattered and indistinct after fixation in Flemming without acetic, but large and well defined after fixation in Susa.

The epithelium rests on a basement membrane (*b.m.*) overlying connective tissue (*c.t.*), which is devoid of muscle fibres and mucus cells, and which separates adjoining tubules from one another and from the overlying gonad.

The variable appearance of the free surface of the epithelium suggests that a phase change occurs. This suggestion is supported by the following facts.

First, several series of sections have been examined in which no phagocytes whatever are visible in the liver tubules.

Secondly, the extremely infrequent appearance of cilia lining the liver tubules in fixed material, while in the fresh material examined they were constantly present, suggests that the cilia may be readily withdrawn.

Thirdly, the free border of the cells is not always thrown out into irregular cytoplasmic processes, frequently it is a smooth uniform surface.

Similar phase changes have been described by other authors. Potts (1923) working on *Teredo* states with regard to the epithelium lining the liver tubules: "I think that the epithelium passes through phases, and that ciliar retraction is followed by the putting out of pseudopodia and ingestion of wood. In such places there is also a multiplication of the nuclei accompanying assimilation, and separation of uninucleate phagocytic cells." Two other cases of phase change have been described by Miss Greenwood (1888, 1892). The first described occurs in the cells lining the archenteron of *Hydra*, where the cells may retract their cilia and form pseudopodia. The second is cited to occur in certain cells lining the gut of *Lumbricus*, where retraction of cilia is followed by a phase in which ingestion of fat occurs, the cells exhibiting a striated or rodded border.

The Phagocytes. When the tubules of the digestive gland of *Jorunna* are examined microscopically, numbers of free amoeboid cells may usually be observed. These cells have the appearance of phagocytes, many having within them vacuoles containing ingested foreign bodies. They are largely confined to the lumen of the digestive gland tubules, fig. 10, the caecum, and the midgut.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Similar cells were observed in three other nudibranchs, viz. *Goniodoris nodosa* (MONTAGU), *Archidoris britannica* (JOHNSTON), and *Polycera quadrilineata* (O. F. MÜLLER). This is of interest, since YONGE (1926*a*) states that phagocytes are not found in the gut of Gastropoda.

The phagocytes of *Jorunna* were examined in preparations made by teasing out the digestive gland tubules of living specimens, and also in sections of fixed material. Several are shown in figs. 10, 14, 22, 23 and 24.

Flemming without acetic, followed by safranin and light green, gave excellent results, nuclear detail and cytoplasmic inclusions were well preserved, fatty substances were demonstrated, and delicate structures such as small vacuoles were only slightly distorted.

Other fixatives such as Duboscq-Brasil and Susa were not so satisfactory, since they tended to dissolve cell inclusions and to cause distortion of vacuoles, though the former fixative proved useful when used in conjunction with Mallory's triple stain, which does not stain satisfactorily after Flemming without acetic, in picking out the various contents of the vacuoles in distinct colours.

The phagocytes, as seen after fixation, are very variable in size (varying from 5 to 24 μ), and assume various shapes, the most common being spherical.

Each cell (fig. 14) has a single conspicuous ovoid nucleus (*n.*) precisely similar to those described for the digestive gland cells. The cytoplasm is like that of the digestive gland cells. Frequently it is highly vacuolated, the vacuoles containing inclusions which take up safranin or light green with avidity, or fatty substances appearing greyish, deep brown, or black after osmic acid. Fatty granules frequently occur scattered through the cytoplasm. In the living cells the cytoplasm is seen to be colourless and usually hyaline, with a few vacuoles containing oil-like globules.

Often when living tissue is examined, the liver tubules are seen to be infested with ciliates. It was thought at first that these organisms when fixed, or in certain phases when alive, might assume the rounded form described above for the phagocytes. That the cells described above as phagocytes are really so, and not in any way connected with these ciliates, is shown by the following.

No cilia could be seen on the cells in question with the exception of a few isolated instances (see below), where the cells bore irregularly disposed clumps of cilia. In these instances, the cells were easily distinguishable from the ciliates since their motion was irregular, jerky and indeterminate.

Again, as noted above, the nuclei of the cells in question resemble those of the liver cells in every way and, moreover, their size, when measured with a micrometer scale, was seen to vary within exactly the same limits as the nuclei of the liver cells.

The latter observation, in addition to providing evidence distinguishing between the cells described as phagocytes and the ciliates, forms a valuable clue to the origin of these cells, which is supported by further evidence.

First, both digestive gland cells and phagocytes are capable of ingesting solid bodies at their free surface, p. 208.

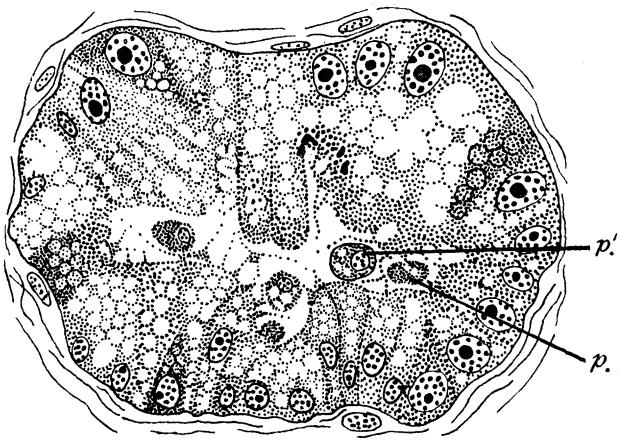

FIG. 10

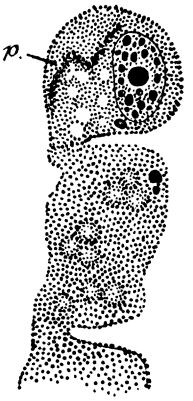

FIG. 13

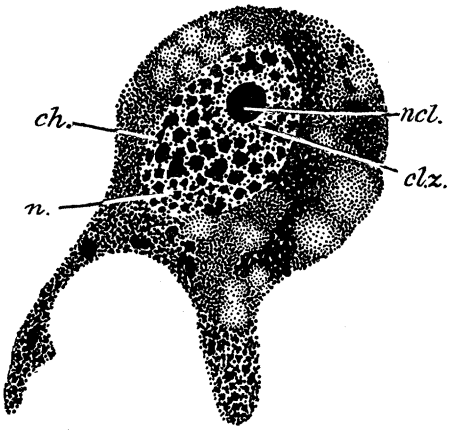

FIG. 14

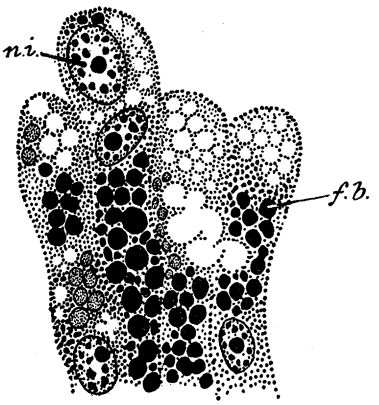

FIG. 11

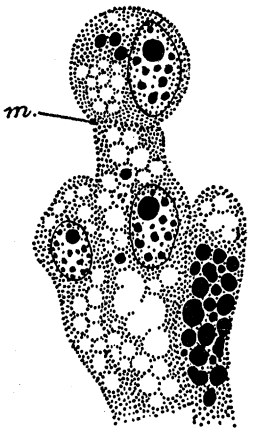

FIG. 12

FIG. 10—Transverse section of tubules of the digestive gland showing phagocytes in the lumen. Fixed Flemming without acetic, stained safranin and light green. × 450.

FIG. 11—First stage in the abstriction of a phagocyte. × 1500.

FIG. 12—Second stage in the abstriction of a phagocyte. × 1350.

FIG. 13—Third stage in the abstriction of a phagocyte. × 1350. All from *Jorunna*. Fixed Flemming without acetic, stained safranin and light green.

FIG. 14—A large phagocyte from the lumen of the midgut, fixed Flemming without acetic, stained safranin and light green. × 2700.

ch. chromatin; *cl.z.* clear zone around nucleolus; *f.b.* fatty body; *m.* membrane (see p. 194); *n.* nucleus; *n.i.* daughter nucleus (see text, p. 194); *ncl.* nucleolus; *p.*, *p'.* phagocytes.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Secondly, when living material is examined, phagocytes bearing irregularly disposed groups of cilia may occasionally be seen. No cilia were ever observed on the cells seen in fixed material. As noted on p. 190, the free border of the digestive gland epithelium may often be seen to bear cilia which tend to disappear on fixation.

These close similarities lead to the conclusion that the phagocytes are derived from the epithelium of the digestive gland. This is confirmed by the appearance of the free border of the epithelium, from which uninucleate cells, identical in appearance with the phagocytes, may occasionally be seen to be abstricted. The stages of the process are shown in figs. 11, 12 and 13, and seem to be as follows:

1 (fig. 11)—A nucleus at the tip of a cell has divided, one of the daughter nuclei (*n.i.*) passes into the slightly swollen apical region.

2 (fig. 12)—The nucleus and the surrounding zone of cytoplasm appears to become separated from the remainder of the cell by a fine membrane (*m.*).

3 (fig. 13)—Separation of the nucleus and surrounding cytoplasm from the tip of the cell as a phagocyte.

Hence the occasional appearance of irregular groups of cilia on phagocytes, which seems at first a very unusual phenomenon, is easily explicable by assuming that the cells are cut off whilst the digestive gland epithelium still bore cilia, and before the change from the ciliated phase, to that in which pseudopodia are put out, and phagocytes are abstricted, was complete.

Potts (1923) has described cells of the digestive gland of *Teredo* as giving rise to similar phagocytes in the same manner.

5—*The Midgut* (for anatomy see p. 177)

The midgut is lined with a very regular epithelium composed of two types of cell.

The most predominant cells are ciliated. They closely resemble those described for the caecum (p. 187).

The other type of cell occurs in very small numbers and is a goblet mucus cell. It is identical with the type described for the oesophagus section B.

The epithelium rests on a basement membrane, below which is a zone of connective tissue permeated by circular, longitudinal, and oblique muscle fibres, and containing large mucus cells. The latter resemble those which occupy a similar position in the oesophagus.

6—*The Hindgut* (for anatomy see p. 178)

Like the oesophagus, the hindgut may be subdivided into sections A and B, on the basis of different histological characters of the lining epithelium.

1—*The Hindgut, Section A.*

Fig. 15 represents a portion of a transverse section of the wall of this region of the hindgut. The fixation and staining was precisely the same as that employed in the preparations figured previously.

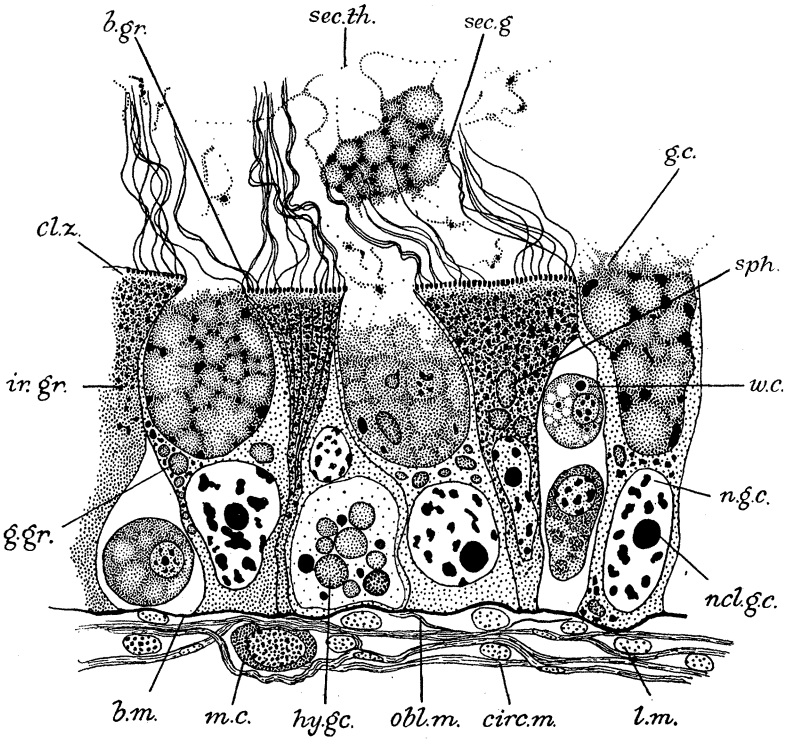

Fig. 15—Portion of a transverse section of the wall of section A of the hindgut. Fixed Flemming without acetic, stained safranin and light green. *b.gr.* basal granules; *b.m.* basement membrane; *circ.m.* circular muscle; *cl.z.* clear zone underlying basal granules; *g.c.* goblet cell; *g.gr.* granules appearing greyish after osmic fixation; *hy.gc.* hyaline granule; *ir.gr.* irregular granules; *l.m.* longitudinal muscle; *m.c.* mucus cell in submucosa; *n.g.c.* nucleus of gland cell; *ncl.g.c.* nucleolus of gland cell; *obl.m.* oblique muscle; *sec.g.*, *sec.th.* secretion of mucus cells (see text); *sph.* spheroid appearing greyish after osmic fixation; *w.c.* wandering cell. × 2000.

The chief feature of the epithelium is the prominence of the goblet mucus cells.

These strongly resemble the corresponding cells of section B of the foregut and need not be described here. Their secretion is discharged as a discrete goblet (*sec.g.*) which becomes more or less whipped out into threads of secretion (*sec.th.*) by the action of cilia of surrounding cells.

The intermingled ciliated cells are irregular in outline and usually shrunken in appearance. The cilia end in basal granules which are not nearly as distinct as in other parts of the gut, and which overlie a narrow strip of cytoplasm (*cl.z.*). This, since it has no obvious inclusions, contrasts strongly with the underlying cytoplasm, which appears denser and is loaded with granules. The cytoplasm of these cells sometimes appears highly vacuolated, sometimes homogeneous and finely granular, but more usually it is seen to contain large numbers of irregular, indistinct granules (*ir.gr.*) and occasionally one or two large spheroids (*sph.*) appearing greyish after osmic. Intracellular fibrils may occasionally be seen running through these cells, and in one or

two cases could be traced to the nuclear membrane. The nucleus is large, basal in position, has a prominent nucleolus and large chromatin granules, not forming a reticulum. Wandering cells (*w.c.*) like those described for other parts of the gut are very numerous here.

2—*The Hindgut, Section B.*

Portion of a transverse section of the gut wall of this region, fixed and stained as before, is shown in fig. 16.

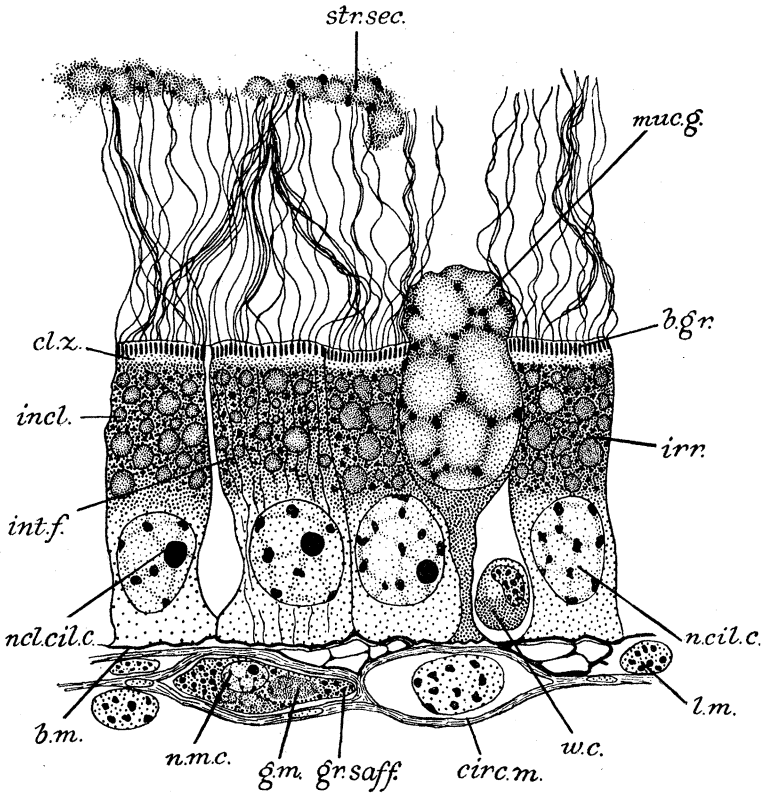

FIG. 16—Portion of transverse section of wall, section B of hindgut. Fixed Flemming without acetic, stained safranin and light green. *g.m.* goblet of mucin in mucus cell of submucosa; *gr.saff.* granules in mucus cell, staining with safranin; *int.f.* intracellular fibrils; *irr.* irregular inclusions; *muc.g.* goblet of mucus; *n.m.c.* nucleus of mucus cell; *str.sec.* string of secretion. Other letters as in previous figures. × 2000.

The dominant feature of this epithelium is its extreme regularity. The cells have a uniform height of about 20 μ and the basal granules (*b.gr.*), clear zone (*cl.z.*), and granular zone, of the ciliated cells, form three very distinct layers, giving the epithelium a markedly characteristic appearance. After staining in safranin and light green, as a result of the even distribution of the granules in the ciliated cells (*irr.* and *incl.*), the upper halves of these cells stain green, while the lower halves appear red.

The epithelium is composed of the same two types of cell as the preceding section, but here, in sharp contrast with section A, it is the ciliated cells which are most numerous.

These cells possess extremely long cilia ending within the cell in very distinct basal granules (*b.gr.*), overlying a strip of clear cytoplasm (*cl.z.*), and which can be seen in certain cells to be

prolonged into intracellular fibrils (*int.f.*). The cytoplasm of the upper half of the cell appears rather denser than that of the lower half, which houses the nucleus. It contains large numbers of regular solid inclusions (*incl.*) which take up light green, with intermingled irregular bodies (*irr.*) appearing dark after osmic acid.

The nucleus (*n.cil.c.*), situated in the less dense cytoplasm of the lower half of the cell, is usually almost spherical and has a very distinct nuclear membrane, a prominent nucleolus (*ncl.cil.c.*), and chromatin granules forming the nodes of a nuclear reticulum.

The mucus cells are precisely similar to those of preceding sections. Their secretion is extruded as a goblet, which becomes whipped out into strands (*str.sec.*) by the cilia of adjoining cells.

Wandering cells (*w.c.*), having precisely the same structure and disposition as in other parts of the gut, occur here, but they are less numerous than in the epithelium of the preceding section A.

The epithelium rests on a basement membrane (*b.m.*) which is prominent after the Champy-Kull stain, when it takes up a deep red colour, and after Mallory when it stains bright blue. Beneath the basement membrane is connective tissue permeated by oblique, longitudinal (*l.m.*), and circular (*circ.m.*), muscle fibres. Large mucus cells (*m.c.*, fig. 15) occur in the connective tissue of the submucosa. These are irregular in outline, and contain large numbers of granules which take up safranin (*gr.saff.*), and one or more goblets of mucin (*g.m.*). Their nuclei (*n.m.c.*) are spherical, having a prominent nucleolus and chromatin in the form of a reticulum.

The hindgut resembles the oesophagus in possessing a lining of ciliated and mucus cells. This histological resemblance is correlated with a functional one, for whereas the oesophagus was concerned with cementing particles into food strands, and transferring the latter to the midgut and liver, the hindgut is concerned with aiding in the cementing of faecal particles to form a compact bolus, and the transference of this bolus to the anus.

It is interesting to note that in section A, where the need for cementing up stray faecal particles ejected from the digestive diverticula (see p. 213) is greatest, the predominance of mucus cells is very marked.

VI—Feeding

Movement of the Radula

The feeding movements can be observed by watching the movement of the mouth in animals creeping upside down on the surface film of the water, or on the glass sides of an aquarium.

During the feeding process, the buccal mass is erected, so that the outer lips, which are depicted in fig. 2 pointing forwards, are pressed closely against the substratum on which the animal is crawling. As the cycle of movement begins, the closely apposed outer lips begin to move apart, thus exposing the inner lips (*i.l.* fig. 2) which protrude between them. The inner lips now begin to part, the process beginning in the middle and spreading outwards. This exposes the buccal lips (*b.l.* fig. 2).

Meanwhile the odontophore (*od.*) is being thrust ventrally, so that it comes to fill

the area enclosed by the buccal lips. As its progress continues, the buccal lips are forced apart until they form merely a narrow rim around the protruded odontophore. As a result of these changes, the radula (*rad.*) has been carried down so that its teeth lie in contact with the substance on which the animal desires to feed. A rapid forward and upward movement of the odontophore now occurs, causing it to be withdrawn into the buccal cavity. Simultaneously, there is a deepening of the median longitudinal groove which separates the two lobes of the radula, so that each of the more lateral rows of teeth are successively exposed.

Since the radula is being drawn upwards and forward, the whole structure is so tilted that all the rows of teeth outside the radula sac are brought to bear on the substratum.

The movement of the radula results in a scraping or rasping process, which tears up the substance across which the radula is drawn, and furthermore results in the pieces so torn being held on the ends of the recurved radula teeth. Consequently torn up shreds of food are constantly being transferred on to the ciliated ridges of the oesophagus each time the radula is carried forwards and upwards into the buccal cavity in completing its stroke.

On the withdrawal of the radula into the buccal cavity, the inner lips close together, and the cycle starts again. By taking the average time occupied by the various phases of the cycle, the following data has been obtained:

Phase 1. Parting of the inner lips	4 sec.
Phase 2. Protrusion of the odontophore	3 sec.
Phase 3. Upward and forward movement of the radula exposing all its functional teeth	4 sec.
Phase 4. Retraction of odontophore	3 sec.
Phase 5. Closing of inner lips	4 sec.

Movement of Particles in the Gut—I. *By Cilia*

Two methods were used for determining the direction of ciliary currents.

In the method of direct observation, fine particles of carmine were projected from a fine pipette on to pieces of excised gut in sea water. The ciliary currents were adduced from the movement of the carmine particles over the internal surface of the gut, observed by a binocular microscope.

The method of determining ciliary currents by the use of carmine particles has several marked disadvantages (see below). It occurred to me that it might be possible to deduce the direction of the beat of the cilia from their appearance in sections of fixed material.

Gray (1930) has shown that a cilium during its forestroke is stretched out straight and, on recovery, bends forwards with the free tip pointing in the direction of its effective beat. Therefore, if in sections of fixed preparations, groups of cilia showed a

constant arrangement, uniformly bent at right angles with their free tips pointing in one direction, then it could be inferred that the cilia were producing a current in the direction of their free tips. This principle was used in determining the ciliary currents inside the gut. Whenever possible, these inferred results were checked against observations in living tissues using the carmine method of direct observation, and the evidence obtained from both methods was correlated and combined. The method of determining ciliary currents by the use of sections was suggested to me by Professor H. Graham Cannon. It is novel and possesses two distinct advantages over the other method.

First, the detection of minute ciliary tracts is rendered much easier. The currents on the sides of the ridges in the oesophagus of *Jorunna* are extremely difficult to detect by the use of carmine particles, partly owing to the smallness of the ridges, and partly because the latter are so closely approximated as to prevent any but the most minute carmine particles penetrating between them.

Secondly, by this method, the cilia-beating surfaces are preserved in their normal spacial and morphological relations to one another. This is impossible when, using the carmine method, it becomes necessary to open out structures in order to put carmine on their internal surfaces. Thus, for example, the tops of the walls of a narrow groove may be normally adjacent, and their cilia may act in co-operation, but the operation of opening up the gut and pinning it out might separate widely these two regions.

It was found that the fixed tissues gave constant results. Cilia in any region are always found to be fixed in the same position with the sole exception of one particular groove of the gut where a ciliary reversal is indicated (see below).

No cases were found where the cilia were pointing haphazard, except when this appearance was associated with bad fixation, or the presence of abundant food particles, or mucus, in the lumen of the gut, and such cases were not used for determining the direction of currents.

Other instances, in which the beat of the cilia appeared anomalous when determined by this method, all proved to be explicable by some morphological peculiarity of the individual sections. The obliteration of one of the primary ridges of the oesophagus or hindgut causes the cilia on the sides of the ridge, which normally beat up towards the lumen of the gut, to appear as an isolated tract in the wall of the gut, composed of two groups of cilia which beat towards each other without apparent reason.

Critical fixation is absolutely necessary in order to determine ciliary currents in this manner, and though several fixatives were tried, it was not possible to secure critical fixation throughout the entire gut of a single specimen. By combining the evidence of several series, however, it was possible to obtain a complete picture of the ciliation of the whole gut. Duboscq-Brasil, Flemming without acetic and Susa fixatives gave reasonably satisfactory evidence for this method, but the most successful

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

preparations were fixed in a solution of 4 parts absolute alcohol and 1 part glacial acetic acid, for 1 hr., followed by several washes in absolute.

The two methods outlined above, when combined, form an excellent means of determining ciliary currents, for not only does one method serve to check the other, but the currents observed by the carmine particle method can be analysed in detail by the subsequent examination of sections. Thus a current observed by carmine may possibly be the resultant of several component currents acting on one another. Such complex currents are very readily resolved into their components by the method of using sections, which permits the direction of the beat of all the cilia to be determined easily.

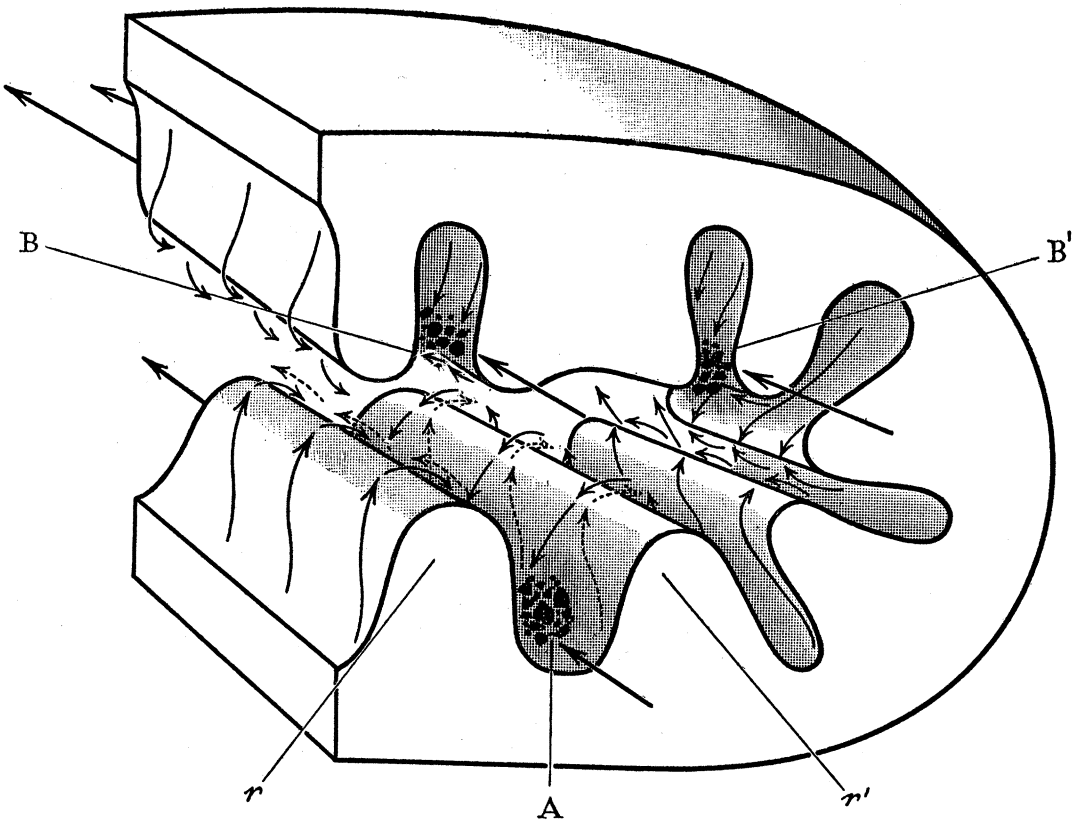

FIG. 17—Diagram showing the ciliary currents in the oesophagus and hindgut. The figure represents a thick slice from the middle of the oesophagus (or hindgut) viewed anteriorly from the right side and slightly from above. In order to give a clearer indication of the ciliary currents, part of the right aspect has been cut away. The secondary ridges are not shown. *A*, *B*, *B'* food tracts with food particles. *r*, *r'*, see text. Dotted arrows indicate ciliary reversal (see text). × about 120.

Transference of Food Particles along the Oesophagus (see fig. 17)—As the odontophore is thrust into the oesophagus at the end of its cycle of movements, it comes into contact with the cilia on the lining ridges, which take up any food particles it may bear.

The majority of particles are taken up by the powerful cilia of the three tracts *A*, *B* and *B'* which beat along the oesophagus into the midgut. Any particles falling on the summit or sides of the ridges *r* and *r'* are swept obliquely downwards into the food tract *A*. The cilia on the summit of the remaining ridges fling particles obliquely backwards into the lumen of the gut, where they are taken up by the tracts *B* and *B'* either directly or after having been taken up by the cilia of a neighbouring ridge.

Particles which fall into any of the grooves, with the exception of that housing the food groove *A*, are carried obliquely backwards to the tops of the surrounding ridges by the cilia lining the sides, and are subsequently projected into the lumen of the gut by the cilia described above.

The secondary ridges (see p. 177) have no real significance in the feeding process and consequently may be disregarded.

The main result of these currents is that the food particles, mixed with mucus, which is secreted by the gut wall throughout the oesophagus (p. 187) and helps to bind the food particles together, pass into the midgut along the three main tracts *A*, *B* and *B'*.

When the animal is feeding actively, the amount of food present in the oesophagus is large enough to cause the food present in the three tracts to coalesce into one large mass of food, which is passed down the oesophagus as a compact bolus.

Neither sorting of food particles according to size, nor rejection of unwanted particles occurs.

The ciliary currents described above are primarily feeding currents and may be observed when the oesophagus contains a large number of particles. When the oesophagus is devoid of food particles, the cilia beating obliquely down the sides of the ridges *r* and *r'* into the food groove *A* reverse the direction of their beat so that they now beat out of the groove and obliquely backwards towards the summit of the ridges on either side. Thus the cilia on the sides of the ridges *r* and *r'* now beat in a similar direction to those on the sides of the remaining ridges, and the food groove *A* ceases to function as such. The ciliary currents now appear to be purely cleaning currents, excess mucus and any food particles which remain in the grooves are carried to the tops of the ridges and transferred to the tracts *B* and *B'* which carry them to the midgut.

The appearance of the cilia on the sides of the groove *A* indicates beyond doubt that the direction of their beat is reversible. Of the series of sections examined, some showed cilia with tips pointing out of the groove, others with cilia which pointed into the groove. In either case the tips of all the cilia lining groove *A* pointed uniformly in one direction or the other, which was constant throughout each series. There was no evidence of ciliary reversal in any of the other grooves.

The nature of the stimulus bringing about reversal has not been determined. It may be that contact between the wall of the oesophagus and masses of food brought up on the radula during the feeding process constitutes the stimulus for reversal. If

this be so, then the stimulus of food entering the oesophagus would cause the cilia in question, which, while the oesophagus was devoid of food, would be beating out of the groove (see above), to reverse the direction of their beat, and thus form an effective food channel.

Similar cases of ciliary reversal have been described before, in particular by YONGE (1930) in *Fungia*, where he describes how contact between food particles and the base of the tentacles brings about a reversal of the cilia on the oral disk.

Movement of Particles in the Midgut (see fig. 18)—As the food particles from the oesophagus enter the midgut, they are taken up by powerful currents circulating through the tubules of the digestive gland. These currents are maintained partly by the cilia lining the openings from the midgut into the digestive gland and partly by the cilia of the digestive gland cells.

Some of the cilia round the openings beat from the midgut into the digestive gland, while others beat in the reverse direction. In every animal examined, the openings of the digestive gland were beset with these two groups of cilia, though the exact disposition of the outward and inwardly beating cilia was found to vary in different individuals.

A similar disposition of cilia around the openings of the digestive gland tubules, maintaining a similar current, has been described by YONGE for lamellibranchs generally (1926*a*).

Owing to the anterior position of opening *I*, the majority of particles leaving the oesophagus enter the digestive gland by the inward current (*ent.c.*).

During the circulation through the digestive gland tubules most of the particles will probably be taken up by phagocytes and by the ingestive cells lining the tubules. Any particles which are not taken up, together with undigested particles which have been rejected by the phagocytes and liver cells, are returned to the midgut by the outward current (*ext.c.*). Here the above particles may be taken up by the spirally ascending current into the hindgut (*H.G.*), or they may be wafted into the caecum (*cae.*), or together with some further food particles passed in from the oesophagus, they may be passed through one of the other openings and circulated through another group of digestive gland tubules. Thus there is set up a mechanism whereby a continuous circulation of food particles through the tubules of the digestive gland is maintained, combined with a mechanism for the rejection of unwanted particles in the caecum (p. 211) and hindgut.

The walls of the midgut are covered with cilia, producing the cleaning currents indicated by the small arrows in fig. 18. These currents are in two groups, the dorsal, which beat directly into the hindgut, and the ventral, which transfer particles to a ventral tract of longitudinally beating cilia. This latter tract, in addition to keeping the floor of the midgut clear of particles and mucus, and hence preventing clogging of the mechanism, ensures that particles which have either fallen out of circulation, or

which on leaving the oesophagus were not taken up by the current (*ent.c.*), are carried to a region where they are likely to meet the powerful currents around the openings of the remaining digestive gland tubules.

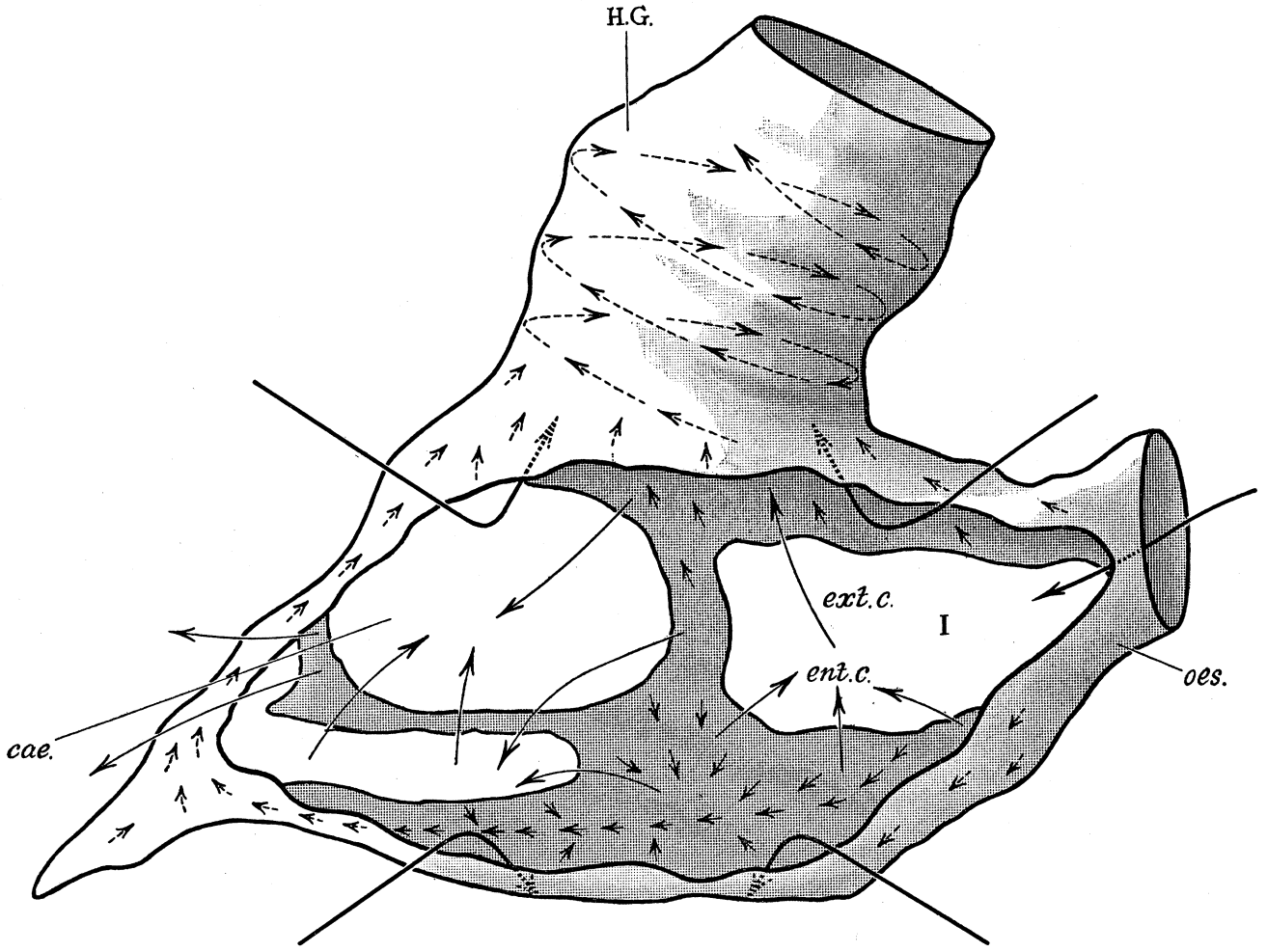

FIG. 18—Diagram showing ciliary currents of the midgut. The midgut is isolated from the caecum, and the surrounding digestive gland, and is viewed from the right side and slightly from above. The dotted arrows are seen by transparency. *cae.* caecum; *ent.c.* current entering digestive gland; *ext.c.* current leaving digestive gland; *H.G.* hindgut; *oes.* oesophagus; *I* left antero-lateral opening to digestive gland. × about 50.

It has not proved possible to discover any system in the midgut whereby the difference in size of the openings to the digestive glands and the peculiar distribution of the cilia about these openings, could have any significance in maintaining a circulation through the tubules of the digestive gland successively, and in a definite rotation.

There is no system ensuring a perfect separation of food and faeces. In the absence of such a system the gut of *Jorunna* differs markedly from that of *Patella* (GRAHAM 1932), where there exists a valve-like thickening of the gut wall ensuring the separation of food and faecal particles.

Transference of Particles along the Hindgut—The ciliation of the hindgut was found to be exactly the same as that of the oesophagus.

Loose faecal particles are mixed with a copious secretion of mucus from the gut wall, and carried down the hindgut in the three tracts *A*, *B* and *B'*. As in the oesophagus, since *A*, *B* and *B'* are close together, the number of particles present is usually large enough to bring about coalescence of the three tracts to form a single bolus of faeces.

Movement of Particles in the Caecum (see fig. 19)—The caecum is ciliated throughout, but its small size and delicate structure, and the fact that it lies deeply embedded in the digestive gland, renders excision of the intact structure impossible. Consequently it is here that the method of studying ciliary action from sections of fixed material has proved most valuable and the following account of the ciliation has been entirely deduced by this method.

The cilia round the opening of the caecum into the midgut beat inwards (heavy arrows, fig. 19), thus drawing all particles in the vicinity of the opening into the caecum. The majority of these particles are swept on to the ridges at the back of the caecum.

The ciliation of these ridges and their complementary grooves is perfectly uniform. The cilia on the summit of each ridge beat along the main axis of the ridge and towards the head of the caecum (*h.*). These may be termed the frontal cilia. The cilia on the sides of the ridges (these may be termed lateral cilia) beat downwards into the grooves, while the cilia lining the base of the grooves (basal cilia) beat along the grooves, and again in the direction of the head of the caecum.

The upper ends of the lateral series of ridges (*lat.r.*) and grooves (*lat.g.*) are closely approximated to the mesial series (*mes.r.* and *mes.g.*), and hence, as a consideration of fig. 19 will show, all particles passing along the lateral series will eventually be taken up by the cilia of the mesial.

Thus the large particles are taken up by the frontal cilia and carried upwards on the tops of the ridges to the head of the caecum. The particles which are fine enough to pass into the grooves between the ridges, are swept down to the base of the grooves by the lateral cilia, and thence carried to the head of the caecum by the basal cilia.

Particles taken up by the cilia on the dorsal side of the opening of the caecum into the midgut are carried directly to the head of the caecum by the cilia lining the inner wall (*i.w.*). Particles passing into the opening laterally are caught up by the cilia lining the inner wall of the fundus (*f.*), and carried on to the lateral series of ridges and grooves, while particles passing in by the ventral side of the opening are carried by the cilia lining the base of the caecum on to the bottom of both series of ridges and grooves.

As a result, all particles which enter the opening from the midgut are carried up to the head of the caecum. The mass of particles which accumulates there is rotated

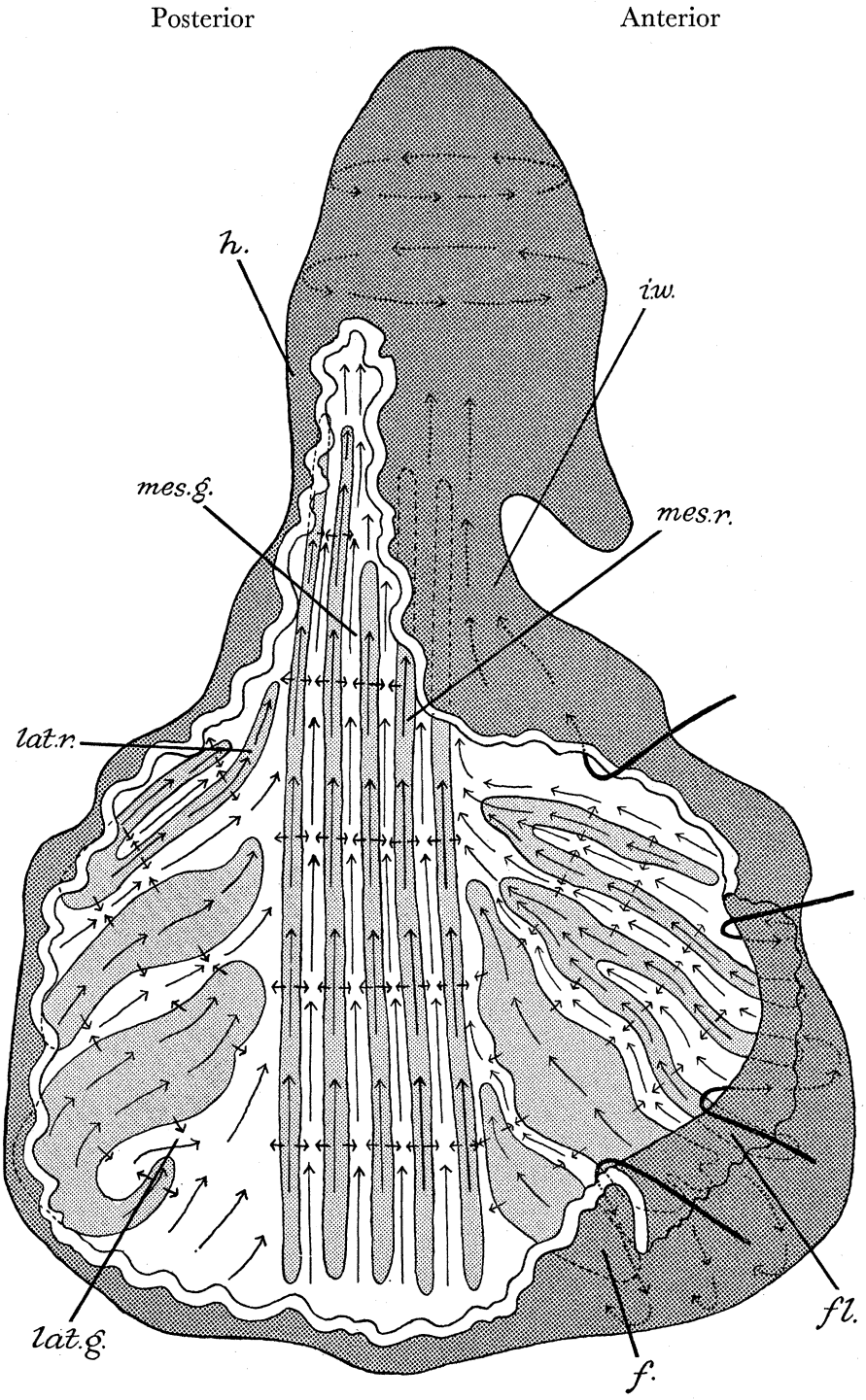

FIG. 19—Diagram showing ciliation of the caecum. The caecum is viewed from the midgut. Part of the wall, in the vicinity of the opening to the midgut, has been cut away so as to show the course of the ridges inside. The dotted arrows are seen by transparency. *f.* fundus of caecum; *fl.* flange formed by piece of wall of midgut; *h.* head of caecum; *i.w.* inner wall; *lat.g.* lateral groove; *lat.r.* lateral ridge; *mes.g.* mesial groove; *mes.r.* mesial ridge. × about 70.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

by the action of the cilia which line the walls. The importance of this is discussed later. Evidence fully substantiating these deductions was obtained from a consideration of the peculiar distribution of particles inside the caecum.

Coarse particles are always found aggregated in small masses on the tips of the ridges, while fine particles are found within the grooves or in the act of descending into them. The only region where fine or coarse particles accumulate is in the head of the caecum, where they form a compact bolus.

Thus all particles entering the caecum must be passing up to the head along the tops of the ridges and in the grooves. All the series of sections examined gave perfectly consistent evidence for these deductions.

Movement of Particles in the Gut—II. *By Muscular Contraction of the Walls*

The oesophagus and hindgut of *Jorunna* lie relatively free within the body cavity, the only attachment to surrounding organs is by thin mesenteries of connective tissue. This fact has considerable significance, since it admits of the possibility of peristalsis, or some similar muscular movement of the gut wall, playing a part in the transference of particles.

The possible existence of such a mechanism is confirmed by the fact that if the oesophagus, or hindgut, be removed by dissection and examined in sea water, they undergo vigorous writhing movements due to muscular contraction of their walls. In addition, occasional waves of contraction, possibly comparable to the peristaltic contraction of the gut of vertebrates, may be observed in the excised pieces of gut. It appears that the movements are under nervous control since they may be accentuated by pricking the gut wall with some such sharp instrument as a dissecting needle, though whether these movements exhibit the characteristics of the true peristalsis of the vertebrate intestine, and whether therefore they should be termed bona fide "peristaltic movements", is a matter for further investigation. It is interesting to note that Yonge (1925*b*) has observed what he describes as "peristaltic action" in the gut of *Archidoris britannica*, and similar movements in the oesophagus of the carnivorous Septibranchs, *Poromya* and *Cuspidaria* (1928).

The Effect of Temperature on the Transference of Food along the Gut

Several specimens of *Jorunna* which had been starved for several days and which had ceased to pass faeces—thus showing that the gut was clear of food—were fed on powdered carmine, by injecting a suspension of carmine in sea water into the buccal cavity by means of a fine pipette. Care was taken not to force the injection down the gut, but only into the buccal cavity.

Groups of four specimens so treated were placed in baths of fresh sea water at known constant temperatures, and the time required for the first appearance of the

carmine-coloured faeces was noted. The average time required in each of the groups of four specimens are given below:

Temp. 18° C.: faeces appeared 8·5 hr. after feeding;
Temp. 21° C.: faeces appeared 7 hr. after feeding;
Temp. 23° C.: faeces appeared 6·5 hr. after feeding;
Temp. 25° C.: gut movement ceased, animals died.

Hence food passes most rapidly through the gut at 23° C.

The various temperatures probably affect the rate of movement of the gut wall, the rate of movement of the cilia, and the rate of digestion in the digestive gland, but to different extents.

VII—The Enzymes

The enzymes present were determined by dissecting out the appropriate regions of the living gut, and, after freeing them as far as possible from surrounding tissues, grinding them up in clean freshly scalded utensils, with clean dry sand and distilled water. The extract thus obtained was filtered several times, and then disinfected by the addition of a few drops of toluol to prevent bacterial action. In order to obtain a strong extract, a considerable number of animals were treated in this way, and as little water as possible was used.

The extracts were tested for proteolytic, lipolytic and amylolytic enzymes by the usual methods.* The experiments were carried out at 30° C. for 20 or 48 hr. Toluol was added to prevent bacterial changes during the period of digestion. Active extracts were obtained from the liver only. This extract contained two proteases (acting in acidic and alkaline media respectively), lipase and possibly amylase. No enzymes were found in the extracts of the buccal cavity, oesophagus, midgut or hindgut. The absence of enzymes from the midgut indicates that the liver enzymes are intracellular.

VIII—Ingestion

In order to ascertain the region of the gut where ingestion occurs, specimens were fed with substances that could easily be traced during their passage through the gut.

A number of animals were fed on neutral olive oil stained with Nile-blue sulphate, others with fish blood, and others with iron saccharate. In each case the substances were introduced into the gut from a fine pipette inserted into the buccal cavity, care

* The following substrates were used: for proteases, Congo Red fibrin, acid casein (at *p*H 1·2), and alkaline casein (at *p*H 8·5); for amylases, starch, sucrose and gum arabic; for lipase, phenol-red milk. In order to detect digestion, the casein solutions were subjected to precipitation tests (Cole 1928, pp. 250 and 260), and the incubated carbohydrate substrates were tested for reducing sugars by Benedict's method.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

being taken not to force the substances along the gut more than was absolutely unavoidable.

The specimens fed on fish blood and iron saccharate were fixed after known successive intervals, the former in Flemming without acetic, the latter, by YONGE's modification of the usual method after iron saccharate feeding, viz. in a mixture of equal parts of 5% ammonium sulphide in 95% alcohol, and Bouin's fluid. Sections were cut at 10 μ.

The digestive glands of specimens fed on olive oil were removed, teased out and examined in the living state. The stained olive oil was easily traceable and furthermore the process of digestion could be followed, since the neutral oil stains red in Nile-blue sulphate while fatty acids stain blue.

Sections of specimens fed on fish blood were stained in safranin and light green, after which the blood corpuscles were easily traceable owing to their avidity for safranin.

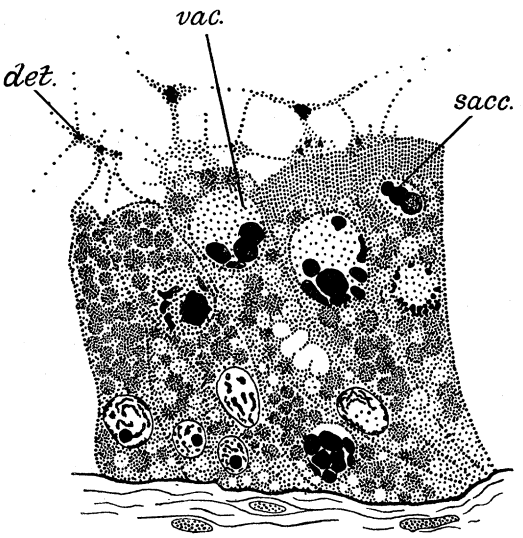

FIG. 20—Portion of transverse section of digestive gland epithelium of a specimen fixed 9 hr. after feeding with iron saccharate. Fixed as described in text. Stained in eosin. *det.* particles of detritus, possibly extruded from underlying cells; *vac.* large vacuole containing particles of iron saccharate; *sacc.* ingested particles of iron saccharate. × 750.

Sections of specimens fed on iron saccharate were immersed for a few minutes in a 10% solution of potassium ferrocyanide and then in dilute hydrochloric acid. This treatment converts the iron into readily detectable Prussian blue. The sections were counter-stained in eosin.

It was found that iron saccharate and blood corpuscles were ingested exclusively by the digestive gland cells, and the phagocytes abstricted from them (fig. 21).

The appearance of the digestive gland epithelium 9 hr. after feeding with iron saccharate is shown in fig. 20. The iron saccharate appeared in the form of large well-defined masses (*sacc.*), ingested within spacious discrete vacuoles (*vac.*).

The mode of absorption of the iron, viz. in the form of large well-defined masses inside large vacuoles, is strongly indicative of intracellular digestion (YONGE 1926*a*), and differs from the true absorption described by HIRSCH (1924) in the salivary glands of *Murex trunculus*, where the iron appears in a diffuse state ("diffuse stadium") until about 24 hr. after feeding. This evidence is fully supported by the mode of absorption of blood corpuscles which were seen to be ingested in large vacuoles (*vac.* fig. 23) in both liver cells and phagocytes.

In sections of a specimen fixed 8 hr. after feeding, successive stages in the process of digestion can readily be observed. Three stages are shown in figs. 22, 23 and 24. Fig. 22 shows a blood corpuscle (*cpl.*) in the process of being ingested by a phagocyte which is just flowing round it. Fig. 23 depicts a later stage. The corpuscle is enclosed in a large vacuole and is beginning to show marked signs of erosin. The envelope is becoming irregular, while the contents are being converted into oil-like droplets (*d.*). The nucleus shows obvious signs of degeneration. Fig. 24 shows the final stage, the corpuscle has been completely resolved into oil-like droplets (*dp.*), which show a marked avidity for safranin, while the cytoplasm appears blackish after the osmic fixation owing to the presence of abundant fatty substances.

Further confirmation is afforded by the presence of the digestive-gland cells and phagocytes (fig. 25) of specimens fed on olive oil stained with Nile-blue sulphate. In the digestive gland of individuals examined 5 hr. after feeding, the oil was seen to be ingested within large vacuoles and was clearly undergoing digestion as shown by the change in colour of the Nile-blue sulphate. Vacuoles stained in colours varying from the pink of the neutral oil to the blue of the fatty acids were seen.

The results of the above experiments are in complete accordance with the evidence gained by a study of the enzymes which were seen to be exclusively intracellular, and with the histological structure of the digestive gland described on p. 189, where it was shown that secreting cells are absent from the gland.

The indigestible remainder of the food is expelled from the vacuoles of the digestive gland cells and phagocytes at the free cell surface. Frequently, masses of detritus can be seen clinging to the cells as if in process of being extruded (fig. 20).

Discussion—The evidence afforded by feeding experiments indicates conclusively that digestion occurs only in the digestive gland. This is in complete accordance with the results gained from a study of the enzymes, which were found only in the digestive gland.

The absence of enzymes from the midgut (which communicates freely with the digestive gland), indicates that no extracellular enzymes are produced by the digestive gland cells. This was supported by the feeding experiments with fish blood which showed that the corpuscles underwent no erosion whilst they remained in the cavity of the midgut. Digestion was only seen to occur *inside* the phagocytes and cells lining the digestive gland.

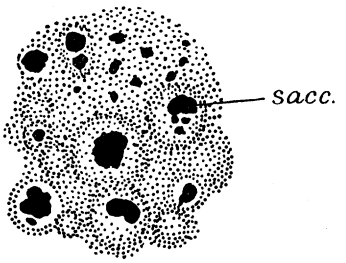

FIG. 21

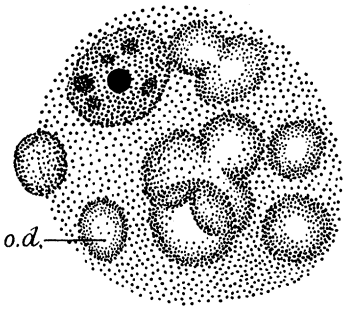

FIG. 25

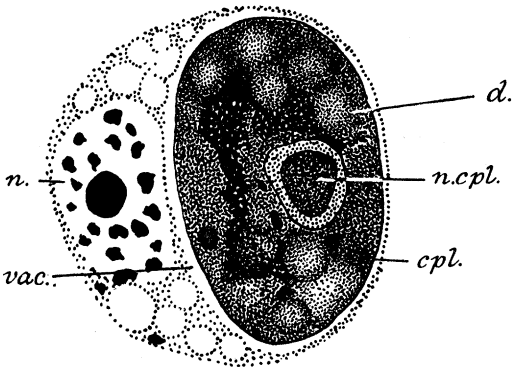

FIG. 23

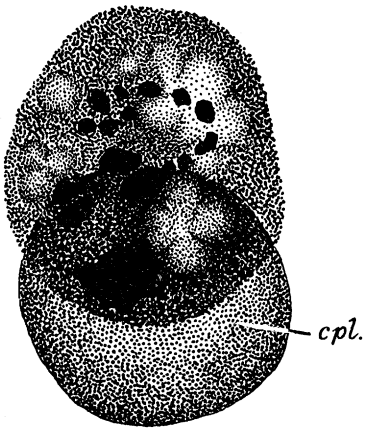

FIG. 22

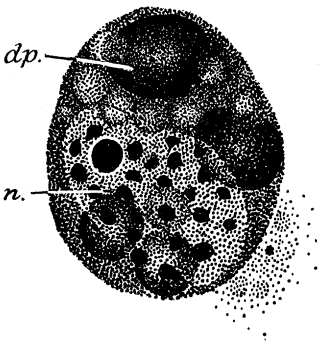

FIG. 24

FIG. 21—Phagocyte with ingested iron. From digestive gland of a specimen which had been fixed 24 hr. after feeding on iron saccharate. Fixed and stained as described in text. × 3000.

FIGS. 22, 23, 24—Successive stages in the digestion of fish blood corpuscles by phagocytes, seen in a specimen fixed 8 hr. after feeding on fish blood. Fixed Flemming without acetic, stained safranin and light green. × 3000.

FIG. 25—A phagocyte from the digestive gland of a specimen which had been teased up 24 hr. after feeding on olive oil. Cleared and mounted in glycerine. × 3000.

cpl. blood corpuscle; *d.* oil-like droplet in corpuscle; *dp.* droplets staining in safranin, derived from digested corpuscles; *n.* nucleus of phagocyte; *n.cpl.* nucleus of blood corpuscle; *o.d.* ingested droplet of olive oil; *sacc.* ingested particles of iron; *vac.* vacuole in phagocyte.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The occurrence of intracellular digestion in the liver is in complete harmony with the results gained by several workers on Mollusca, notably von Bruel (1904) in *Caliphylla* and *Hermaea*, Hirsch (1924) in *Murex*, Peczenik (1925) in *Limnea*, Graham (1932) in *Patella*, Yonge (1931) in *Doris* and Gymnosomatous pteropods, and Hörstadius (1933) in *Pleurobranchea* and *Hermaea*.

The results of the feeding experiments also show beyond all doubt that the amoeboid cells described in the previous sections as phagocytes are really such, and that their function, like that of the digestive-gland cells, is to take up particles of food from the lumen of the gut.

IX—The Faeces

When the faeces are examined microscopically, they are seen to consist of an interlocking mass of sponge spicules and debris, the components of which are largely unrecognizable, rendered consistent by viscid secretions from the walls of the caecum and hindgut. They form brownish, slightly irregular, rod-like pellets which are slightly pliable.

Preparation of the Faeces—Sections of the head of the caecum (see fig. 26) reveal the presence of a large bolus of detritus (*b.d.*) inside the lumen. The bolus is rounded, and consists largely of compacted, unrecognizable debris, among which effete phagocytes (*p.*) and fragments of sponge spicules (*spic.*) may sometimes be seen, and the whole is wrapped in an even layer of secretion (*sec.*), which stains pale blue in Mallory. Judging from the size, shape, and composition of the bolus, there can be little doubt that it is a faecal pellet.

Since the head of the caecum is the *only* region of the gut where such compacted masses of debris are constantly found, and furthermore since all stages in the formation of the faecal mass, from the early accumulation of effete particles to the final stages where a regular compact bolus is formed, may be observed in sections of the head of the caecum, there can be little doubt that it is here that the faecal pellets are prepared.

Bearing in mind the course of the ciliary currents within the caecum discussed on pp. 204–6, the process of formation of the faecal mass may be reconstructed as being thus:

Effete particles, together with mucus, are driven into the caecum, by the cilia around the opening (*o.cae.* figs. 1 and 3) from the midgut. They are then taken up by the cilia lining the walls, and carried directly to the head of the caecum, where, partly in consequence of the pressure of the continual upward current, and partly because of the pressure exerted by the walls, which in this region are provided with strengthening devices (see p. 189), and a well-developed muscular coat (*circ.m.* fig. 26), the loose particles become compacted into a bolus. Meanwhile the whole mass is surrounded by viscid secretion (*sec.*) derived from surrounding gland cells (*g.c.*), and at the

same time it is being rotated by the action of the cilia lining the wall of this region. This motion has the effect of rubbing the bolus against the walls of the caecum and thus smoothing it out in the manner of a potter's wheel, converting it into a smooth and regular faecal pellet.

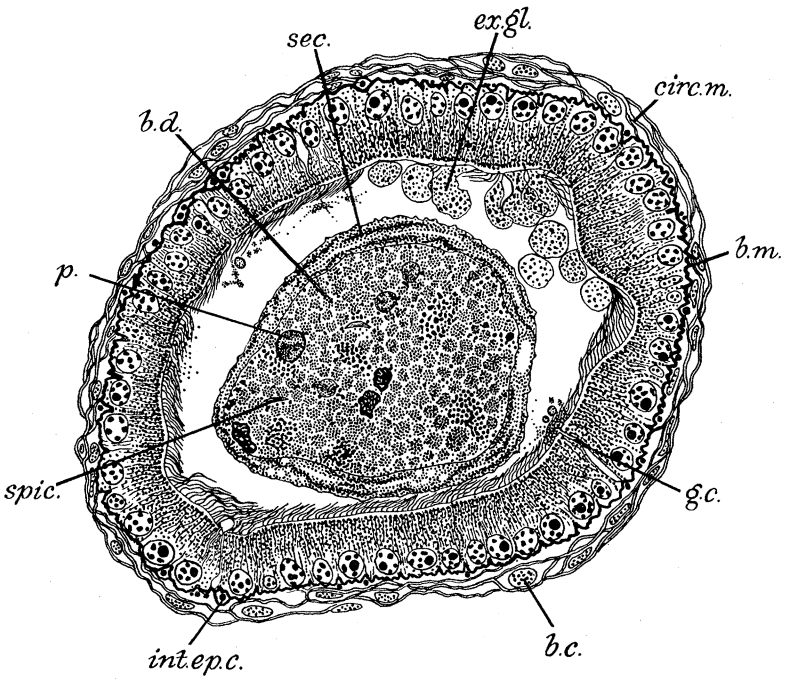

FIG. 26—Transverse section of head region of caecum, showing a faecal bolus in process of formation. From a preparation fixed in Duboscq-Brasil and stained in Mallory. *b.c.* blood cell; *b.d.* faecal bolus; *b.m.* basement membrane of epithelium; *circ.m.* circular muscle band; *ex.gl.* globule of excreta (see p. 189); *g.c.* gland cell producing viscid secretion; *int.ep.c.* structure resembling intraepithelial canal (see p. 197); *p.* phagocyte; *sec.* layer of secretion; *spic.* sponge spicule embedded in faeces. × 387.

The means by which the faecal masses leave the caecum could not be determined. There is no evidence for the occurrence of a ciliary reversal in the caecum, and it therefore seems most probable that the faecal mass is forced out of the caecum and into the midgut by muscular contraction of the head region, possibly by a peristaltic-like movement, induced periodically by the pressure of faecal matter against the walls of the caecum head. The presence of a well-developed layer of circular muscles (*circ.m.*) in the wall of this region of the caecum supports this conjecture.

The faecal bolus thus ejected, would be carried away by the powerful, spirally ascending currents at the beginning of the hindgut (see p. 202).

Since the length of the faecal masses ejected by living animals was usually far in excess of that ever observed for a faecal bolus contained in the caecum, probably several such boli may become joined together during their passage down the hindgut. It is probable also that faecal masses produced in the caecum are supplemented during

their passage down the hindgut by faecal particles that have been carried directly from the midgut, the whole being bound together by mucoid secretion derived from the abundant goblet cells (see p. 195) in the hindgut epithelium.

The form of the faeces recalls that of the faecal mass of *Patella* described by GRAHAM (1932), where the faecal particles are welded into a central consistent rod surrounded by peripheral layers of cement. The similarity is correlated with a similar need on the part of both animals.

In *Patella*, which remains attached to rocks for long periods, it is important that no disintegration of faeces occurs in the vicinity of the anus, resulting in fouling of the mantle cavity (GRAHAM 1932).

In *Jorunna*, where the anus lies within the circlet of branchiae, it is equally essential that no disintegration of faeces occurs.

The fact that the mechanism for the preparation of faeces in *Jorunna* has not the complexity of that of *Patella*, where the whole of the elaborate midgut appears to be largely concerned with preparation of faeces, is possibly correlated with a different mode of life. *Jorunna* is comparatively active, and slight disintegration of the faeces would be of no consequence, since the naked gills are continually bathed by currents of clean water; and the movement of the animal will tend to shake off any loose faecal masses that may remain clinging to the gills. *Patella*, on the other hand, remains in one situation for considerable periods, and thus the chances of fouling of the mantle are considerably greater, hence the more elaborate mechanism for preparation of the faeces.

X—General Discussion

It is remarkable that the alimentary canal of so specialized a mollusc as *Jorunna* has retained such a simple form. The only morphological specialization lies in the secondary detorsion that has occurred, resulting in the hindgut taking up the form of a U. Detorsion is a characteristic feature of all nudibranchs.

The radula is clearly adapted to the peculiar diet of sponge, which is soft, yet contains large numbers of spicules, in that it possesses a large number of stout, simple teeth; there are no central teeth, and there is little differentiation into laterals and marginals. Feeding on sponges necessitates taking into the gut large numbers of spicules. Since these are forcibly thrust into the oesophagus by the radula, the delicate oesophageal epithelium is rendered liable to injury. As a protection, the food is embedded in particularly large quantities of mucus secreted by the extremely abundant goblet cells in the oesophageal wall. Possibly, the process of coating the faeces with viscid secretion (see above) is to some extent a similarly protective measure.

The presence in the gut of large numbers of phagocytes, which have been shown to ingest food particles, indicates the importance of intracellular digestion in *Jorunna*. Their occurrence is noteworthy since YONGE (1926*a*) states that phagocytes are absent in gastropods.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Since the openings from the midgut into the digestive gland are large, the phagocytes cannot form a special provision for ingesting larger particles than those which can enter the digestive gland, as YONGE (1926*a*, 1931) suggests is the case in the lamellibranchs. Moreover, feeding experiments showed that all particles, whether large or small, were ingested indiscriminately by both phagocytes and digestive gland cells. The ultimate fate of the phagocytes was not determined. If they wander back to rejoin the digestive gland epithelium, and thus transfer the products of digestion of the food particles taken up, to the digestive gland cells, then it may be suggested that the significance of the phagocytes lies merely in the fact that they form a convenient means of extending the ingestive surface of the digestive gland into the lumen of the midgut. The phagocytes are not comparable with those described by YONGE and other workers in the gut of lamellibranchs, since they are confined to the lumen of the digestive gland and midgut, and not in any way connected with the blood system, whereas the phagocytes of lamellibranchs wander freely in and out of the lumen of the gut and through all the tissues, and are essentially blood cells.

The investigation of the enzymes, though regrettably incomplete, is sufficient to show that in accordance with the specialized diet, the enzymes of *Jorunna* are different from those of typical herbivorous gastropods, such as *Crepidula* or *Aplysia*, on one hand, and from those of typically carnivorous forms, such as *Murex* or *Natica*, on the other.

As would be expected in a carnivore, a powerful protease is present, though it is *intra*cellular. In this latter particular *Jorunna* resembles the lamellibranchs, including the herbivorous forms such as *Mya* (YONGE 1923), *Ostrea* (YONGE 1926*b*), *Ensis* (GRAHAM 1931), as well as the carnivorous septibranchs *Poromya* and *Cuspidaria* (YONGE 1928), and the herbivorous gastropods which possess a crystalline style, such as *Pterocera* (YONGE 1931 and 1932*a*).

It is remarkable that the enzymes are exclusively intracellular, and correlated with this is the existence of large openings between the midgut and digestive gland, which allow large particles to be ingested directly by the digestive gland cells.

The degree of adaptation of the enzymes to the diet recalls that of the highly specialized lamellibranchs *Poromya* and *Cuspidaria*, which is described by YONGE (1928). In both cases the animals feed on large pieces of animal tissue, which they digest with the aid of a powerful *intra*cellular protease.

In the presence of a powerful protease these animals resemble the typical carnivorous gastropods, but it is an important distinction that the protease of *Jorunna* like that of *Poromya* and *Cuspidaria* is intracellular, whereas that of the carnivorous gastropods is extracellular. The difference is possibly correlated with a different phylogenetic history.

The resemblance is probably due to convergence, both forms adapting themselves to a diet of meat, but whereas the carnivorous gastropods apparently had no immediate herbivorous ancestors, and therefore no limitations were placed on their

diet, *Poromya* and *Cuspidaria*, although able to strengthen the weak protease of their herbivorous ancestors, were unable to evolve an *extra*cellular, form an *intra*cellular, enzyme (Yonge 1928). Possibly, the occurrence of an intracellular protease in *Jorunna* admits of a similar explanation.

Thus the digestive processes of *Jorunna*, like those of *Poromya* and *Cuspidaria*, may possibly be an adaptation of the predominantly carbohydrate digesting processes of a herbivorous ancestor for a carnivorous diet.

A similar change of diet in two groups with Molluscan organization would be expected to have similar physiological effects, and this is exactly what we find, the protease in each case being strengthened.

It is interesting to note the effect of a similar diet on groups of animals with different organization. *Sabella* among the annelids and a tunicate such as *Ciona* are ciliary feeders, feeding on the same fine suspensions of particles as the lamellibranchs, and yet the enzymes of the two former differ distinctly from those of the latter in being entirely *extra*cellular (Nicol 1930; Berril 1929; and Yonge 1925*a*). Hence we must conclude that similarity of food, and similarity of the means whereby it is obtained, are not sufficient to bring about similar digestive processes. Obviously some other factor must be involved, and very probably this factor lies in the difference of organization of the three groups Polychaeta, Tunicata and Mollusca.

XI—Summary

1—The anatomy and histology of the alimentary canal of the sponge-eating nudibranch mollusc *Jorunna tomentosa* are described.

2—The mouth leads into the buccal cavity, which is provided with three pairs of lips, and houses the odontophore. It is followed by a simple oesophagus which expands to form the sac-like midgut.

3—Into the midgut open the caecum and complex racemose digestive gland.

4—The U-shaped hindgut arises from the dorsal aspect of the midgut. Its peculiar shape is due to the secondary detorsion so characteristic of nudibranchs.

5—In accordance with its triturating function, the buccal cavity is lined by a hard cuticular layer, the staining properties and mode of origin of which are discussed.

6—The oesophagus, midgut and hindgut, are lined by ciliated and mucus cells, the relative proportions of which are to some extent correlated with the particular function of each region. In addition, the oesophageal epithelium houses a few gland cells of unknown function.

7—The caecum is lined by ciliated cells, and small gland cells which produce a secretion aiding the cementing of effete particles into a compact faecal bolus. Certain of the ciliated cells cut off their outer ends as rounded bodies which lie free in the caecum. The significance of this is discussed.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

8—Lining the digestive gland are large cells which ingest food particles. Their free ends may occasionally bear cilia or abstrict phagocytes.

9—Numerous phagocytes, the mode of origin of which is described, lie inside the lumen of the midgut, digestive gland and caecum.

10—Wandering cells are common in the lumen, epithelium and submucosa of the gut.

11—The submucosa consists of connective tissue within which are large mucus cells, and longitudinal, circular and oblique muscle fibres.

12—Feeding, and the action of the radula are described.

13—Food is transferred along the gut by means of cilia, possibly aided by muscular contraction of the gut wall. The complete elucidation of ciliary currents was only rendered possible by the use of an entirely new method.

14—Particles are carried down the oesophagus in three main tracts. On entering the midgut, they are taken up by the cilia lining the openings of the digestive gland, and circulated through the tubules. Effete particles are returned to the midgut and either taken directly into the hindgut, or wafted into the caecum, where they are compacted into a faecal bolus. There is no food-sorting mechanism.

15—The effect of temperature on the passage of food along the gut is described.

16—Enzymes are produced only by the digestive gland. They are exclusively intracellular, and digest fats and proteins. It is uncertain whether any carbohydrate is digested.

17—Feeding experiments indicate that the phagocytes and digestive gland cells perform the function of ingestion.

18—The formation of compact faeces is described, and their significance is discussed.

19—The considerable morphological and physiological adaptation of the gut to the peculiar diet of sponge is discussed, and the results of the investigation are viewed in the light of some recent work on the alimentary systems of other Molluscs and phyla. The peculiar nature of the enzymes is regarded as a possible indication of an herbivorous ancestor.

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