Studies on *Ligia oceanica.* Part II. The Processes of Feeding, Digestion and Absorption, with a Description of the Structure of the Foregut.

By

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With 1 Plate and 12 Figures in the Text.

CONTENTS.

1. FOOD AND FEEDING.

The normal food of Ligia is *Fucus vesiculosus*, though other species of Fucus and even Laminaria are eaten. Fine epiphytic algæ which form furry growths upon the surface of the larger seaweeds when growing in places sheltered from the full force of the waves are also eaten extensively. Ligia is not, however, entirely vegetarian in its diet, having definite tendencies towards scavenging and even cannibalism, as observed by other workers who have kept this animal in captivity for any length of time.

The method of feeding is best described as "browsing." The animal clings to its food and cuts off very small portions with its mandibles, passing them through to the œsophagus, whence they arrive at the foregut.

Owing to the animal's dislike of bright light it feeds at night and, since its food is found on the Fucus zone, half-tide is required before it can feed; thus there is only a limited period during every twenty-four hours during which feeding can occur. It is, therefore, necessary that the food taken in should be dealt with as quickly as possible and the animal can be observed feeding voraciously, passing a continuous stream through its alimentary canal, the fæces containing nearly as much undigested as indigestible food.

2. STRUCTURE OF THE GUT.

(*a*) Introductory.

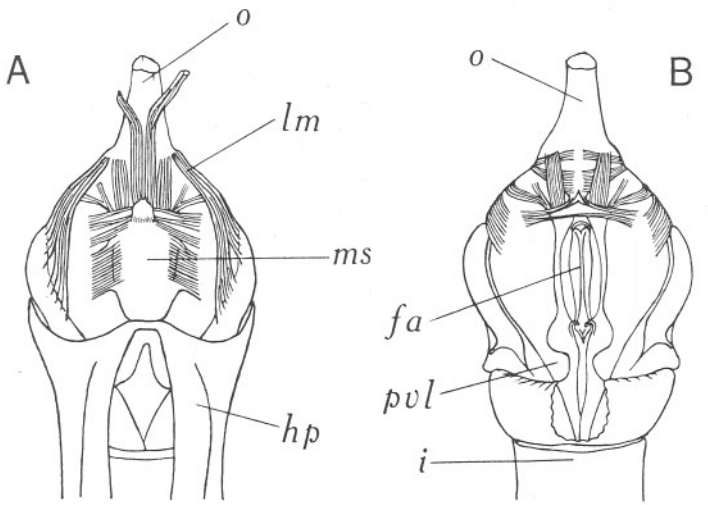
One of the earliest workers on Crustacea to refer to the armature of the foregut, or stomach as it was then called, was Sars (1867). Subsequently Huet (1883), Rosenstadt (1888), Ide (1892), and Schonichen (1898) studied the alimentary canal and its armature in practically every group of Crustacea. Ide concluded that the stomach of the Edriophthalmate orders (Tanaidacea, Isopoda and Amphipoda) was built on the same structural plan as that of the Decapoda, whilst Gelderd (1906) found homologies between the Schizopoda and the Decapoda. Hewitt (1907) described the structure of the gut of Ligia, but ascribed to some parts of the foregut names which are somewhat misleading. Rehorst (1914), in his paper "Der Filtermagen von *Asellus aquaticus*," gives an admirable résumé of the conclusions of previous authors on this subject, as well as giving a detailed description of the foregut of Asellus and assigning functions to its various parts. Barnard (1924) deals with the general shape of the foregut in Isopods, but does not go into detail of structure. Finally, we have detailed accounts of the foregut in Astacus by Jordan (1904), in Homarus by Williams (1907), and in Nephrops by Yonge (1924).

(*b*) The Foregut.

(i) *The External Form and Musculature.*

The main part of the foregut lies in the cephalic and first free thoracic segments, supported by the sternal alæ of the endophragmal skeleton (see Jackson [1926], p. 899, and Lloyd [1908]). Viewed dorsally it is rounded in front, tapering slightly posteriorly, its length being twice its greatest width. The intestine envelops about two-thirds of its posterior portion, only the anterior portion being closed in on all sides, the hinder region consisting of plates which project freely into the intestine.

A general idea of the musculature will be obtained from Text-Figure 1. On the ventral surface are muscles attached to the ventro-lateral walls of the gut and running towards the middle line, where they meet and form a sheath (*m.s.*) to the structure lying immediately above. To the anterior end of the sheath is attached a pair of muscles which can be traced forwards and upwards, separating round the œsophagus and finding their



TEXT-FIG. 1.—A, Foregut and hepato-pancreas from ventral surface showing musculature of foregut. B, same with hepato-pancreas, lateral muscles, muscle sheath, and portion of intestine removed showing some of internal structure of foregut through the ventral wall.

f.a., filter apparatus; *h.p.*, hepato-pancreas; *i.*, intestine; *l.m.*, lateral muscle; *m.s.*, muscle sheath; *o.*, œsophagus; *p.v.l.*, posterior ventral lamella.

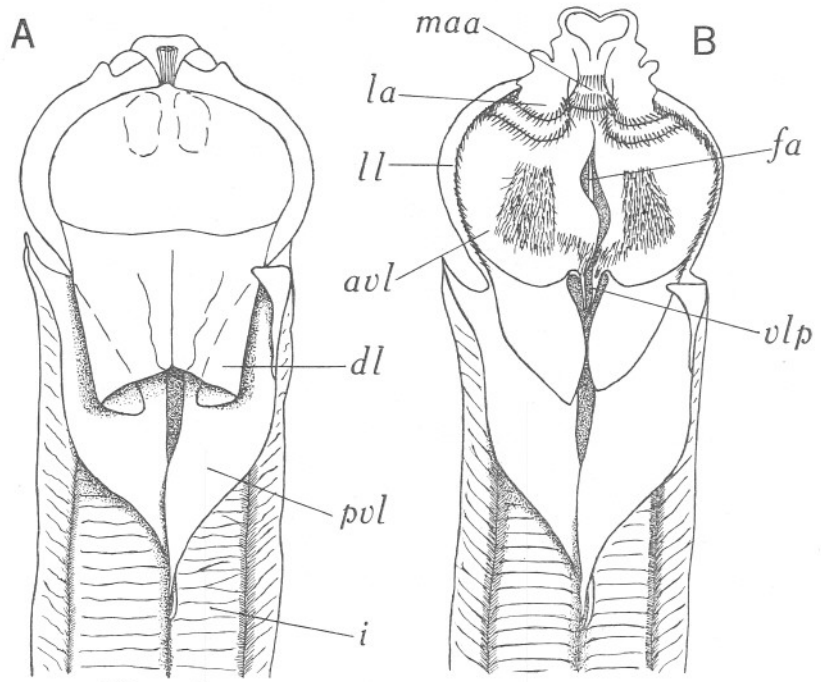
distal attachment dorsal to the antennal fossæ. On removal of these muscles others will be seen attached to the ventral wall of the gut and to the "median tooth" of Hewitt's description.

(ii) *Internal Structure.*

In the description of the foregut that follows the terminology employed by Hewitt has been retained as far as possible, to avoid further confusion. It has been essential to delete the term "tooth," which is very misleading, as are also the terms "cardiac" and "pyloric" when applied to this animal, the use of which suggests a division into two parts comparable with the state of affairs in other Malacostraca. No such division can be observed in the foregut of Ligia. For the term "tooth" the word "ampulla" has been substituted for all cushion-like projections, hollow

and covered with a thin layer of chitin. The term lamella has been retained throughout.

From the anterior regions of the side walls of the foregut arises a pair of large, bilobed projections, the lateral ampullæ (*l.a.*, Text-Figs. 2, 3, 4, 6, and 8), meeting above the opening to the œsophagus. Anterior to these and hidden behind them is a pair of very small antero-lateral ampullæ



TEXT-FIG. 2.—A, Foregut from dorsal surface with intestine cut open along mid-dorsal line. B, same with dorsal lamella removed to show underlying structures.

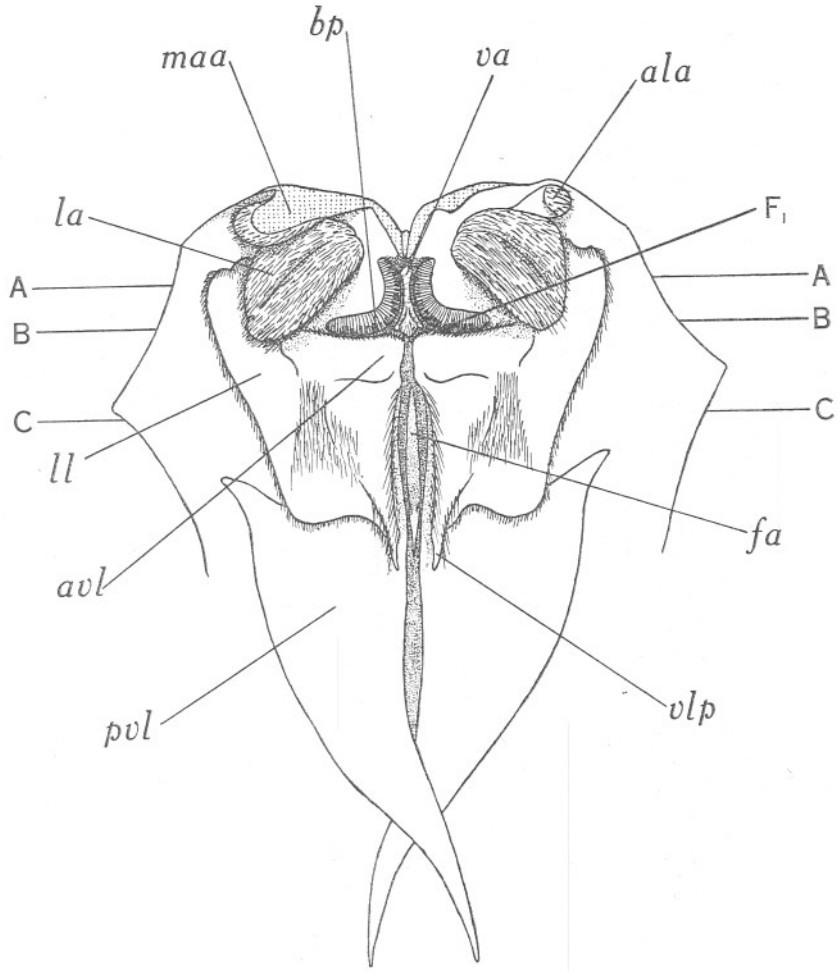
a.v.l., anterior ventral lamella; *d.l.*, dorsal lamella; *f.a.*, filter apparatus; *i.*, intestine; *l.a.*, lateral ampulla; *l.l.*, lateral lamella; *m.a.a.*, median anterior ampulla; *p.v.l.*, posterior ventral lamella; *v.l.p.*, projections from anterior ventral lamellæ.

(*a.l.a.*); in the mid-line of the anterior wall arises a single large median anterior ampulla (*m.a.a.*), while in the floor of the foregut lies a single median projection, the ventral ampulla (*v.a.*). These are all provided with strong, backwardly projecting bristles, and on contraction of the muscles of this region these ampullæ meet and effectively close the entrance to the œsophagus.

From the floor of the foregut and directly behind the ventral ampulla arises a pair of plates, the anterior ventral lamellæ (*a.v.l.*) forming to all appearances the actual floor which here appears to have a median ridge.

This ridge is formed by the approximated inner edges of these plates which rise towards the mid-line, each bearing a pointed projection (*v.l.p.*).

The anterior ventral lamellæ fuse laterally with the lateral lamellæ (*l.l.*), a pair of plates arising from the antero-lateral walls of the chamber,



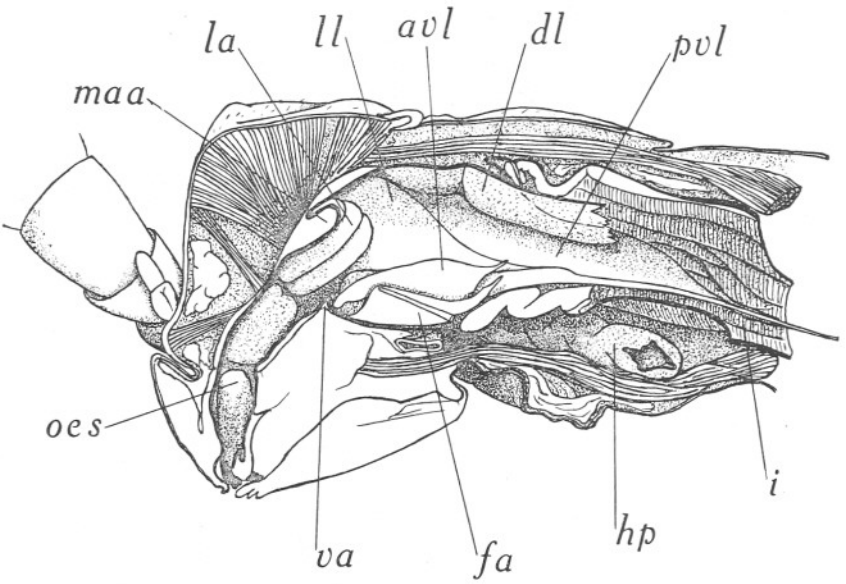
TEXT-FIG. 3.—Foregut opened along mid-line of the dorsal and anterior walls as far as the opening of the œsophagus. The two halves of the dorsal lamella are drawn apart, giving a flattened view of the main structures of the interior. The right half of the median anterior ampulla has been removed to disclose the small antero-lateral ampulla.

AA, BB, and CC indicate the lines through which the sections shown in Fig. 8 are drawn.

a.l.a., antero-lateral ampulla ; *a.v.l.*, anterior ventral lamella ; *b.p.*, bristle plate ; FI., Filter I ; *f.a.*, filter apparatus ; *l.a.*, lateral ampulla ; *l.l.*, lateral lamella ; *m.a.a.*, median anterior ampulla ; *p.v.l.*, posterior ventral lamella ; *v.a.*, ventral ampulla ; *v.l.p.*, projection from anterior ventral lamella.

beneath the lateral ampullæ and proceeding backwards and downwards as a flap attached ventrally. They are provided along the free dorsal edge with strong backwardly projecting bristles. The anterior ventral lamellæ, moreover, fuse posteriorly with a pair of long plates, the posterior ventral lamellæ (*p.v.l.*) which taper gradually to end in the intestine, projecting considerably beyond the apparent limits of the foregut.

If the ventral lamellæ are drawn apart a wedge-shaped projection is seen arising from the true floor of the foregut, widening posteriorly and



TEXT-FIG. 4.—Sagittal section through cephalon and first free thoracic segment, slightly to left of mid-line to include filter apparatus, showing internal structure of the right side. The cut edges of the alimentary canal are heavily outlined in black.

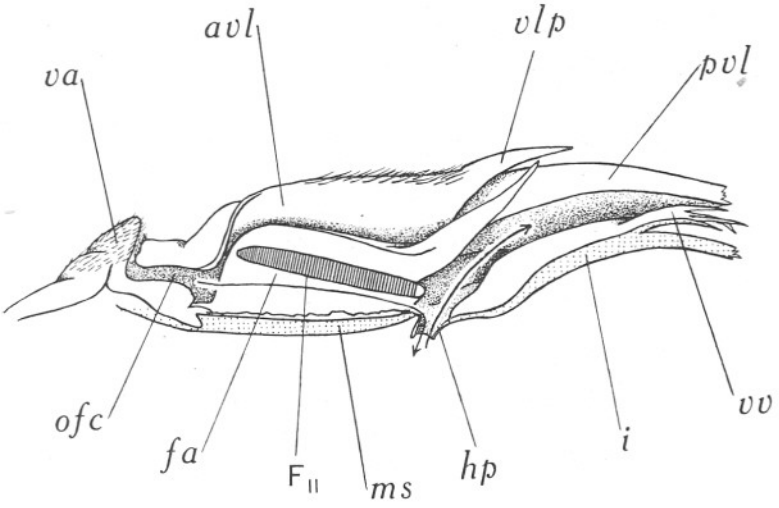
a.v.l., anterior ventral lamella ; *d.l.*, dorsal lamella ; *f.a.*, filter apparatus ; *h.p.*, hepato-pancreas ; *i.*, intestine ; *l.a.*, lateral ampulla ; *l.l.*, lateral lamella ; *m.a.a.*, median anterior ampulla ; *oes.*, œsophagus ; *p.v.l.*, posterior ventral lamella ; *v.a.*, ventral ampulla.

bearing on its postero-dorsal surface a finely pointed projection, similar to those of the ventral lamellæ, between which it is inserted. This structure, for want of a better name, may be called the filter apparatus (*f.a.*). It is, presumably, the "median ventral tooth" of Hewitt. Nothing appears more certain than that it is not a tooth ; a more detailed description of this apparatus appears below.

There remains one more lamellar structure to be mentioned, a simple projection from the dorsal surface of the foregut, arising almost directly over the junction of the anterior and posterior ventral lamellæ and

proceeding backwards to project freely into the lumen of the intestine, the dorsal lamella (*d.l.*). This is, apparently, without setose armature.

On the floor of the foregut and on either side of the elongate ventral ampulla lies a crescentic row of bristles, fused at their bases to form a plate (*b.p.*), but with their distal ends lying against the sides of the ventral ampulla in the mid-line and against the anterior ventral lamellæ behind. Beneath these bristles and on each side of the ventral ampulla is a channel running posteriorly under the anterior ventral lamellæ.



TEXT-FIG. 5.—Section through the foregut, slightly to left of mid-line, showing the filter apparatus and associated structures. The arrows indicate the course of food passing into the hepato-pancreas and the path taken by the secretion when forced out.

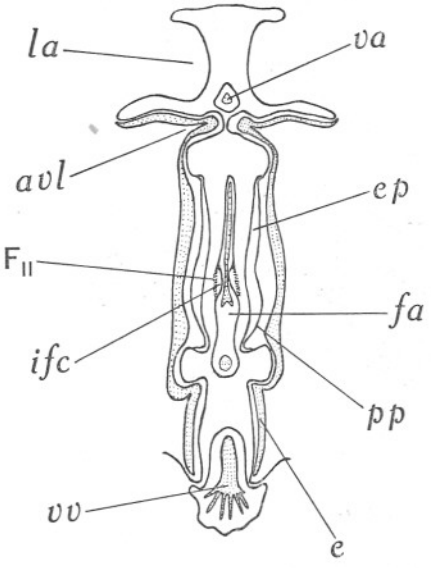
a.v.l., anterior ventral lamella ; *f.a.*, filter apparatus ; FII., Filter II ; *h.p.*, junction of hepato-pancreas with intestine ; *i.*, intestine ; *m.s.*, muscle sheath ; *o.f.c.* outer filter channel ; *p.v.l.*, posterior ventral lamella ; *v.a.*, ventral ampulla, *v.l.p.*, projection from anterior ventral lamella ; *v.v.*, ventral valve.

This will best be seen in Text-Figure 8. It constitutes Filter I. The occlusion of this channel (*o.f.c.*) between the bristle plates and the anterior ventral lamellæ is effected by a row of hairs which project forwards and interlock with those of the bristle plates.

Beneath the anterior ventral lamellæ the channel is divided into two by the filter apparatus (*f.a.*). As shown in Text-Figures 5, 6, and 8, this is wedge-shaped in two directions. It is broader at the base than at its dorsal edge and widens from in front backwards. On either side and parallel with the dorsal edge lies a groove covered with strong bristles attached to the ventral surface and free dorsally. This groove ends blindly anteriorly, opening over the entrance to the hepato-pancreas posteriorly. This is

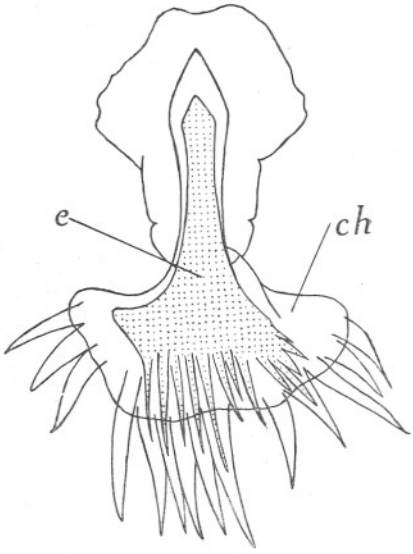
Filter II. Rehorst describes similar structures in the foregut of Asellus and the filters and filter channels of Ligia are directly comparable with those of Asellus, for which reason I have used the names given by him.

Finally, there is a structure, the ventral valve (*v.v.*, Text-Figs. 5, 6, and 7), attached to the ventral wall of the intestine and bearing against the lower surface of the posterior ventral lamellæ. We have now a space stretching from the ventral ampulla anteriorly to the ventral valve



TEXT-FIG. 6.—Semi-diagrammatic horizontal section through the filter apparatus showing its relationship to the surrounding structures.

a.v.l., anterior ventral lamella; *e.*, epithelium; *e.p.*, "elastic pad" of Rehorst; *f.a.*, filter apparatus; FII., Filter II; *i.f.c.*, inner filter channel; *l.a.*, lateral ampulla; *p.p.*, perforated plate; *v.a.*, ventral ampulla; *v.v.*, ventral valve.



TEXT-FIG. 7.—The ventral valve. *e.*, epithelium; *ch.*, chitin.

posteriorly which can be completely closed to any but liquid substances, all the possible entrances being guarded by strong hairs.

The chitinous lining of the anterior ventral lamellæ facing Filter II is composed of a double layer, the outer in contact with the filter being provided with very fine, upwardly projecting hairs, the inner being a plate with many minute holes, the two layers being connected by a network of fine fibres. Behind this inner plate lies the ordinary epithelial tissue, but at a little distance, thus enclosing a second space. This, however, is probably an artifact due to fixation since this space is found throughout

where chitin forms the lining of the foregut. (See Text-Figures 6, 8, and 9.)

Below is a list of the parts of the foregut as named by Hewitt in the first column; the terminology employed in this paper in the second, and that used by Rehorst for Asellus, as far as it is comparable, in the third.

Lateral cardiac tooth.	Lateral ampulla.	Laterale.
Antero-lateral tooth.	Antero-lateral ampulla.	—
Median anterior tooth.	Median anterior ampulla.	—
Ventral cardiac tooth.	Ventral ampulla.	Y-shaped piece.
Ventro-lateral tooth.	Anterior ventral lamella.	Infero-Laterale.
Median ventral tooth.	Filter apparatus.	Infero-Medianum.
Lateral cardiac lamella.	Lateral lamella.	Dorsal branch of the Laterale.
Dorsal lamella.	Dorsal lamella.	Supero-Medianum.
Ventro-lateral pyloric lamella.	Posterior ventral lamella.	Intero-Laterale.

Actually Rehorst includes his Y-shaped piece as part of the Infero-Laterale; he also confines the term "outer filter channel" to that portion of the cavity enclosed between the two ventral lamellæ which lies below Filter II, the whole space being termed the "storeroom."*

(*c*) The Hepato-pancreas.

This consists of three pairs of tubules lying along the alimentary canal which they partially enclose. Their external morphology has been fully described by Hewitt, but a little more must be said about their internal structure. Reference to Plate I, Figures 4 and 5, will show that there are small and large cells, as mentioned by many workers on the histology of this organ in the Isopods. The cell walls are distinct and homogeneous, the contents granular with many small vacuoles, the significance of which cannot be entered into here. Each cell contains a nucleus, and nucleoli are present in most.

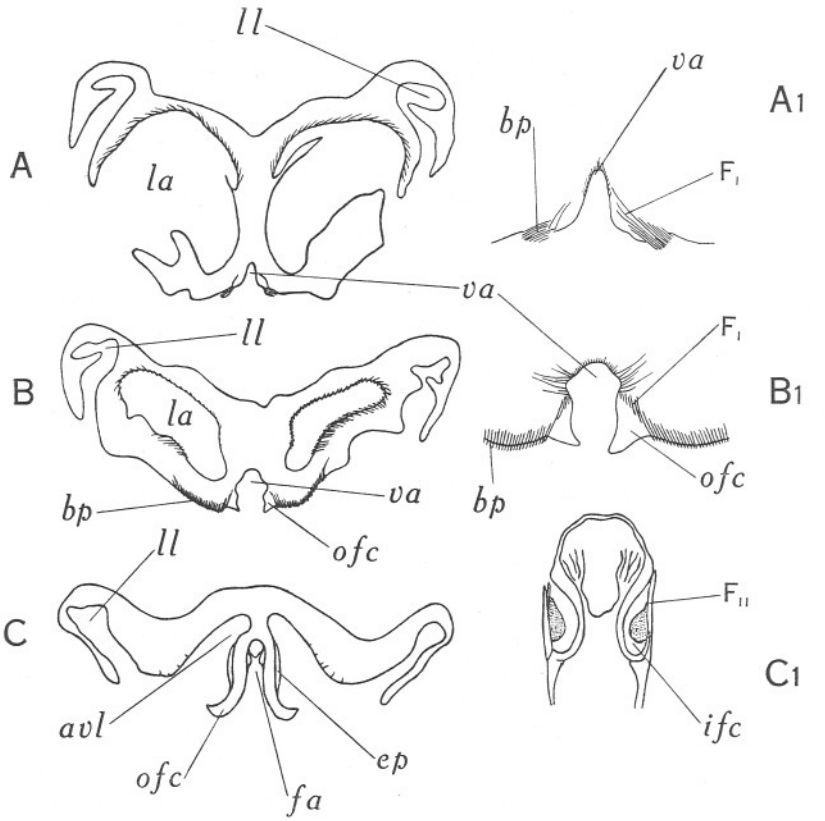
It is the opinion of Nusbaum-Hilarowicz (1920) that the small cells are young stages of the large ones which, as they become mature, break up, liberating their contents into the lumen and thus providing the secretion. In this respect Nusbaum-Hilarowicz supports the view held by Murlin (1902) though Weber (1880) thinks that they are absorbing and secreting cells respectively.

(*d*) The Intestine

As McMurrich (1896) has shown, the portion of the alimentary canal extending from the point of entry of the hepato-pancreatic glands to the

* I wish to thank Dr. H. G. Jackson for details of the technique employed by him in dissecting the head of Ligia.

rectum, usually termed the midgut, is, in Isopods, the anterior portion of the proctodæal invagination and cannot, therefore, be justly termed midgut. This statement is supported by my observations on Ligia in that the whole of this region is lined throughout by chitin. This layer of chitin is, as McMurrich has shown, double, but whereas this author

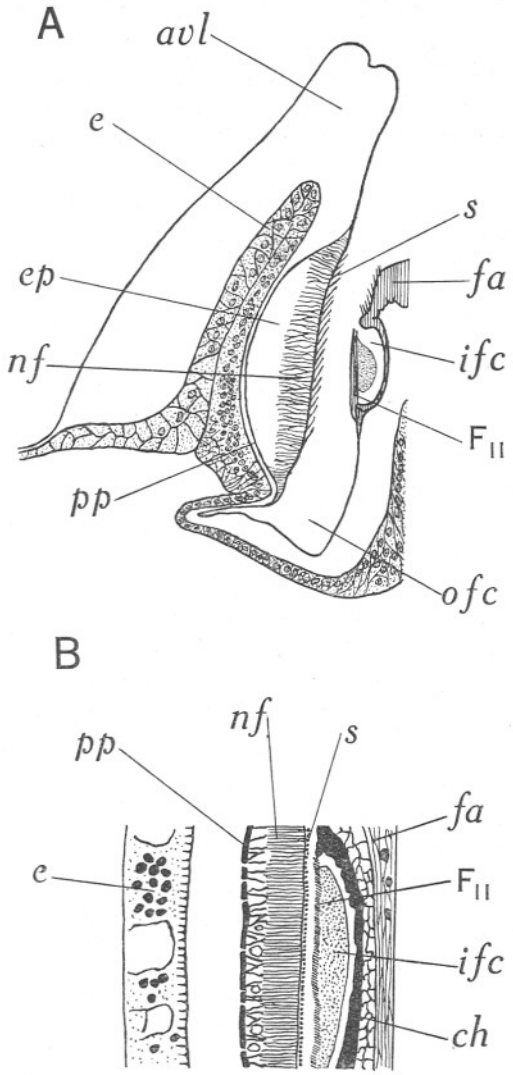


TEXT-FIG. 8.—Semi-diagrammatic transverse section through foregut, A and B passing through bristle plates, ventral ampulla and Filter I ; C, passing through filter apparatus and Filter II, as indicated by AA, BB and CC in Fig. 3. A1, B1 and C1, enlargements of portions of A, B, and C.

a.v.l., anterior ventral lamella ; *b.p.*, bristle plate ; *e.p.*, " elastic pad " of Rehorst ; *f.a.*, filter apparatus ; FI., Filter I ; FII., Filter II ; *i.f.c.*, inner filter channel ; *l.a.*, lateral ampulla ; *l.l.*, lateral lamella ; *o.f.c.*, outer filter channel ; *v.a.*, ventral ampulla.

describes both layers as homogeneous, in Ligia the inner layer appears to be vacuolated (Plate I, Figs. 1, 2, and 3).

Murlin (1902, p. 310) describes the presence of minute pores which perforate the chitinous lining of the lumen of the " midgut " in land Isopods. He does not illustrate these pores though he states that they are easily



TEXT-FIG. 9.—A, semi-diagrammatic representation of transverse section through ventral lamella and filter apparatus, showing relationship between Filter I and "elastic pad" of Rehorst. B, semi-diagrammatic representation of horizontal section through same region, showing network of fibres, perforated plate and space behind plate.

a.v.l., anterior ventral lamella; *ch.*, chitin; *e.*, epithelium; *e.p.*, "elastic pad" of Rehorst; *f.a.*, filter apparatus; FII., Filter II; *i.f.c.*, inner filter channel; *n.f.*, network of fibres; *o.f.c.*, outer filter channel; *p.p.*, perforated plate; *s.*, setæ of "elastic pad."

demonstrated in the fresh intima found in a moulted posterior portion of the animal. Schonichen (1898) also found such pores in the Oniscidæ and Asellidæ. Examination of similar material and also of sections has failed to establish the presence of such pores in Ligia, but as will be seen from Plate I, Figures 2 and 3, the chitin lining the epithelium of the intestine is of a complicated nature.

It is composed of two layers of unequal thickness making up a total of surprising thickness for such a region. The inner layer, comprising about three-quarters of the total thickness, is of a loose spongy texture containing cavities, many of which are permeated by cytoplasm. The outer layer is very thin but apparently homogeneous and quite imperforate.

In the absence of any pores in these layers Ligia is in perfect agreement with McMurrich's description for Armadillidium, Porcellio, Oniscus, and *Idothea robusta*.

The epithelium underlying the chitinous layer is devoid of cell boundaries and forms a syncytium. "Supportive fibres" arise from both this layer and the basement membrane and run towards the opposite sides, representing all that is present of cell walls.

The infoldings of the intima, which project into the syncytial layer, are very regular and viewed from the surface under low magnification give the impression of an epithelium composed of large, very regularly arranged cells, each containing a large nucleus. This impression is heightened by the arrangement of the muscle fibres on the outside which form a network, each strand lying in a groove on the surface. No trace of a typhlosole can be seen in Ligia.

(*e*) The Rectum.

The terminal portion of the alimentary canal is a short region with very much folded walls, well supplied with muscles. It is separated from the intestine by a sphincter. The epithelium in this region is a syncytium continuous with that of the intestine, but much thinner.

3. PHYSIOLOGY OF THE GUT.

The structure and function of what we now term the hepato-pancreas has been investigated in Isopods and other Crustacea by Weber (1880), his work being done largely on Asellus. Murlin (1902) and Nusbaum-Hilarowicz (1920) have dealt similarly with the land Isopods, the latter going into much histological detail, while Patrick (1926) alone has investigated the cells of the hepato-pancreas of Ligia. Gelderd (1906) concluded from his research on the function of the foregut that the "cardiac chamber" primarily masticates the food particles "as an auxiliary to the mouth pieces" and secondarily it "acts as a sieve or filter for the further

retention in the cavity of such particles of food that have not been sufficiently divided." The "pyloric chamber," he says, has the function "of mixing the already masticated food with the ferments of the digestive gland. This is brought about by the action of the spines and hairs upon the pyloric pieces, when the chamber is put into movement by the muscles."

Hewitt (1907), speaking of Ligia, says: "The stomach forms an efficient mill for triturating the miscellaneous substances upon which the animal feeds." Tait (1917), in describing the structure of the foregut of Glyptonotus, says: "The use of the term 'gizzard' (chosen apparently as an improvement on the older and admittedly unsuitable term 'stomach') is in itself misleading, for the name suggests that the function of the organ is to triturate the food. The idea is disposed of by the condition of the ingesta discovered in the midgut of the dissected specimens. When the food had consisted of amphipods these were found, according to size, almost intact or cut into longitudinal blocks of about $\frac{1}{4}$ inch length." He goes on to say: "The cutting had evidently been done by the incisor processes of the mandibles, the length of the blocks corresponded roughly to the reach of these processes from the position of abduction to that of adduction, and the food had evidently been 'bolted' without the occurrence of any further process of comminution in the vestibule." This latter is the term he employs in place of the previous misleading terms. He is further of the opinion that the foregut is "merely a propelling mechanism." While, in my opinion, the above description of the supposed method of feeding of Glyptonotus applies equally well to Ligia, since I have found the contents of the intestine to consist of equal sized particles of algæ, yet it will be shown that the foregut is more than a mechanism for propulsion. I have, moreover, through the kindness of Professor D. M. S. Watson, been able to dissect a specimen of Glyptonotus and find that in main principles the foregut resembles that of Ligia very closely. Hewitt, in using the term "tooth," admits its inadequacy, but still assigns to it a triturating function, which rather nullifies the admission. None of the structures termed by him "teeth" are in any way comparable with the hard chitinous teeth of the Decapods.

Assuming, then, that no further mastication is performed after the food has passed the mouth parts, it will pass up the œsophagus as a "mush" and will enter the foregut between the bristle plates and ventral ampulla below, and the lateral and median anterior ampullæ above. Here, by contraction of the muscles enclosing this part of the vestibule (Text-Fig. 1), to use Tait's term, the liquid portion of the food will be squeezed through Filter I carrying with it only very fine particles. The same motion which compresses the food will tend to force it back into the larger cavity of the foregut, by virtue of the backwardly projecting hairs with which

it will be in contact all round. Imagine now a contraction of the muscles, shown in Text-Figure 1 running forward from the lateral lamellæ, to occur simultaneously with a contraction of the muscle sheath across the ventral, convex surface of the foregut. The result would be the drawing apart of the ventral lamellæ and the pushing up of the filter apparatus. Thus the secretion from the hepato-pancreas would be liberated into the cavity of the vestibule and mixing would also be effected.

Moreover, on the return of the parts to their normal position, a quantity of partly digested food would be caught between each ventral lamella and the filter apparatus, and the liquid portion squeezed through Filter II into the inner filter channel, whence it would pass into the hepato-pancreas along with the liquid forced through Filter I into the outer filter channel.

This process is further aided by the rhythmical contractions of the hepato-pancreatic tubules. These were observed in the living animal, through the transparent ventral surface of a recently moulted specimen, to consist of waves passing forwards along the tubules expelling the contents. On the relaxation of the muscles of the tubules, liquid was observed to return into the lumen. The contraction of the tubules probably coincides with that of the muscle sheath which, as we have seen above, separates the ventral lamellæ and elevates the filter apparatus. Thus is the secretion from the glands passed into the foregut and intestine. With the return of the parts of the foregut to their normal position and the relaxation of the muscles of the tubules, liquid forced through the filters into the filter channels is drawn into the hepato-pancreas. The entrance from the intestine is automatically closed by the action of the ventral valve. Thus the liquid products of digestion may be absorbed in the tubules of the hepato-pancreas. The more solid portions of the food, mixed with the digestive enzymes, will pass into the intestine, where further digestion and absorption will occur.

It will be seen from the above description of the assumed functioning of the foregut that no time is wasted with mastication, the whole principle being one of extracting as quickly as possible the most readily obtainable nourishment. It is suggested that this may be correlated with the limited period during which the animal feeds, as described above, in which case it would be expected also that the enzymes which break down the food would be very powerful, and this will be shown to be the case.

Rehorst, apparently, assumes the filtering action to be quite passive, at least in Filter I, and to the surfaces which come into contact with the filters and are themselves setose, he assigns primarily the function of cleaning the filters and keeping them free from large particles. This may easily be an additional function in Ligia. Further, he assumes that the space enclosed between the double layer of chitin lining the opposing walls of the ventral lamellæ is filled with liquid, forming an elastic pad

which, pressed against Filter II, would separate the outer filter channel from the space above. He makes no mention of perforations in the inner wall of the pad, possibly they are absent in Asellus, but such a function as he suggests for this pad, besides being superfluous, would be rendered difficult in Ligia unless liquid could be forced in at will. The function of this part remains somewhat obscure.

4. COMPARISON OF THE FOREGUT OF LIGIA WITH THAT OF THE DECAPODS.

As may be seen from the work of Jordan (1904), Williams (1907), and Yonge (1924), the foregut in the Decapods is divided into two parts, the cardiac and the pyloric, to each of which are assigned definite functions.

The cardiac portion is a sac serving mainly for the reception of food and contains the masticatory ossicles which triturate the food and assist in the mixing of the food with the secretion from the hepato-pancreas. The food then passes into the pyloric region whence the liquid portion and fine particles pass through the filter into the hepato-pancreas, the larger particles passing above the press into the midgut.

Whereas the Decapods merely tear their food with their mouth parts and pass it into the foregut for complete mastication and digestion, Ligia, as has been seen, not being provided with a triturating mechanism in the foregut, cuts up its food with the mouth parts and then depends on the great power of its digestive enzymes to complete the process in the foregut and intestine. Both are provided with means of limiting the size of the particles entering the hepato-pancreatic tubules, and both the valves and the plates of the Decapods and the ventral valve and lamellæ of Ligia prevent the blockage by food particles of the exit from the tubules. Further, in the action of the ventral lamellæ working in conjunction with the filter apparatus, there is in Ligia a mechanism comparable with the press of the Decapods.

5. DIGESTION.

(*a*) The pH of the Gut.

In Table I will be found the pH of the different regions of the gut of normally feeding animals, of starved animals (48 hrs.), and of those whose pH had been estimated at different periods after feeding following on a period of starvation of 48 hours or more.

From Table I it will be seen that the pH of the gut varies very slightly from one end to the other, whether the animals are starved or feeding. As a result of feeding after starvation the pH of the intestine

was found to be slightly higher than the contents of the hepato-pancreas and slightly lower than that in the same region of a starved animal. This was the same whether estimated $\frac{1}{2}$ hr. or 4 hrs. after feeding.

For the estimation of pH on the small quantities of liquid found in the gut of this animal the method employed was that described by Wigglesworth (1927, p. 792), and used by him in similar work on the cockroach.

TABLE I.

Showing pH of Gut under Different Conditions.

ANIMALS ALL FEEDING.

No.	Intestine.	Rectum.
1.	6·0	6·2
2.	6·2	5·9
3.	6·7	—
4.	6·3	—
Mean.	6·3	6·05

ANIMALS STARVED FOR 48 HOURS.

No.	Foregut.	Intestine.	Rectum.
1.	—	6·05	6·0
2.	6·3	6·55	6·2
3.	6·3	6·45	6·2
4.	6·4	6·4	6·5
5.	6·3	6·55	6·1
6.	6·1	6·25	5·8
7.	6·25	7·2	6·3
Mean.	6·27	6·49	6·16

STARVED ANIMALS BEFORE AND AFTER FEEDING.

No.	Period of Starvation hours.	Intestine.	Hepato-pancreas.
1.	48	6·4	6·0
2.	60	6·3	6·0

No.	Period of Starvation hours.	Time after feeding hours.	pH of Intestine.
1.	60	$\frac{1}{2}$	6·2
2.	60	4	6·2
Mean.	–	–	6·2

(*b*) The Digestive Enzymes.

The enzymes secreted by the cells of the hepato-pancreas are passed into the gut by rhythmical contractions brought about by the network of muscles on the outside, described by Pump (1914).

An extract was made from the hepato-pancreatic tubules of a large number of animals ground up with silver sand and extracted on ice in distilled water for 48 hours.

This was then filtered and made up to a 10% solution based on the weight of tubules and secretion obtained, the whole being kept under toluol continuously. The pH of this extract was 5·9. Table II shows the result of incubation of various substances with this extract.

TABLE II.

5 c.c. substrate ; 5 c.c. extract ; toluol. Controls boiled. Temperature 30° C.

Presence of glucose determined by Fehling's solution, and of disaccharides by Barfoed's solution. Fatty acids produced by digestion of fat titrated against N/100 NaOH.

— indicates no action ; ± indicates a trace ; + indicates positive action ; ++ indicates extensive action.

Substrate.	Experiment after				Control after			
	24 hrs.	48 hrs.	4 days.	10 days.	24 hrs.	48 hrs.	4 days.	10 days.
Starch 1%	++	++	..	..	±	±	..	..
Glycogen (Saturated)	+	+	..	..	±	±	..	..
Sucrose 5%	++	++	..	..	–	–	..	..
Raffinose 1%	++	++	..	..	–	–	..	..
Inulin 1%	–	–	–	..	–	–	–	..
Maltose 2%	+	+	..	..	–	–	..	..
Lactose 2%	–	–	–	..	–	–	–	..
Cellulose	–	–	–	–	–	–	–	–
Amygdalin 1%	+	+	..	..	–	–	..	..
		CN evov.						
Salicin 1%	+	+	..	..	–	–	..	..
Fibrin	Digestion complete 24 hours. Positive reactions with Millon's Reag. and Br. water.				–	–	–	..
Olive Oil	..	2·15 c.c.	2.0 c.c.	..	..	1·9 c.c.	1·88 c.c.	..

From the above Table it will be seen that most carbohydrates are readily split up, the exceptions being Lactose, Inulin, and Cellulose, while the protease is exceptionally strong, digestion of a large piece of fibrin being complete within 24 hours. A lipase is also present.

In order to discover if any secretion was liberated by the intestine a number of these were taken, split open, and washed carefully in water and ground up as were the tubules of the hepato-pancreas, being extracted in 50% glycerin for 48 hours. Table III shows the result of this experiment, from which it will be seen that after 24 hours glycogen and sucrose both showed a trace of reduction and starch after 48 hours.

TABLE III.

5 c.c. substrate with 5 c.c. extract and toluol; incubated at 30° C.
— indicates no action; ± indicates a trace; + indicates positive action. Tests as before.

Substrate.	Experiment after		Control after	
	24 hours.	48 hours.	24 hours.	48 hours.
Starch 1%	±	+	—	—
Glycogen (Sat)	±	±	±	±
Sucrose 5%	+	+	—	—
Raffinose 1%	—	—	—	—
Inulin 1%	—	—	—	—
Maltose 2%	±	±	—	—
Lactose 2%	—	—	—	—
Cellulose	—	—	—	—
Amygdalin 1%	—	—	—	—
Salicin 1%	—	—	—	—
Fibrin	—	—	—	—
Olive Oil	..	0·35 c.c.	..	0·30 c.c.

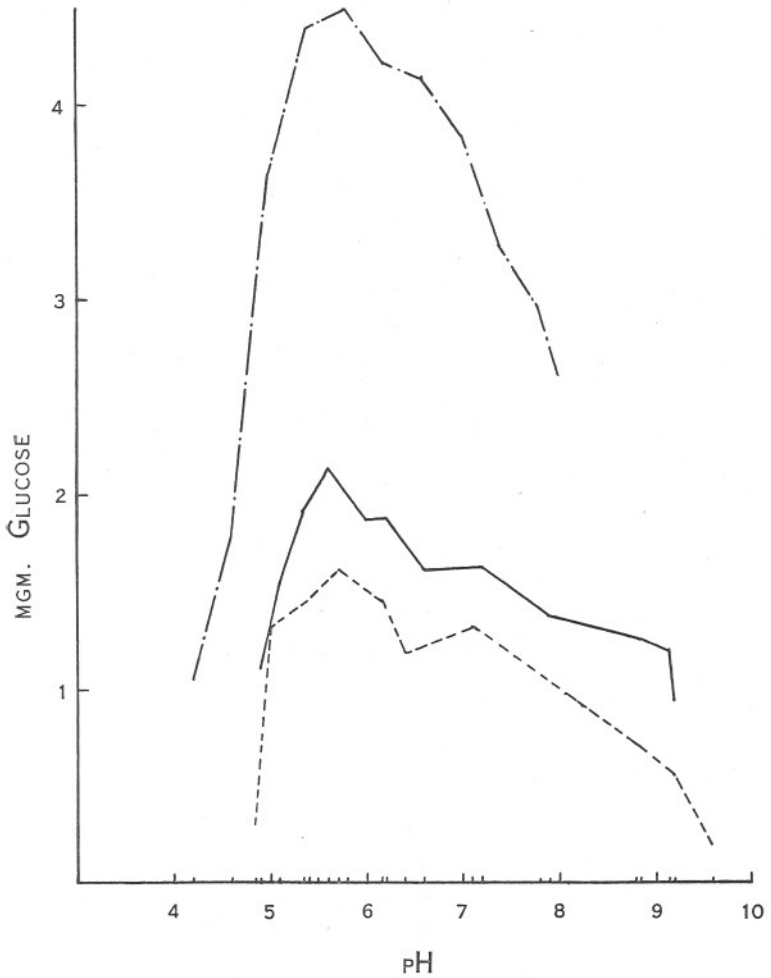
The original sample of glycogen, however, was found to contain a certain amount of reducing sugar, while the trace of reduction in the other two can be explained by the great ease with which they are reduced by the secretion from the hepato-pancreas, combined with the difficulty experienced in washing the intestines completely free from that secretion. It can safely be assumed that there are no enzymes secreted by the epithelium of the intestine which can act on carbohydrates, proteins or fats. Thus the whole of the digestion is performed by the enzymes present in the secretion from the hepato-pancreas.

(*c*) The Sucroclastic enzymes.

Since algæ form the normal food of this animal, it was thought advisable to investigate the possibility of enzymes being present which would reduce pentosans and cellulose. Accordingly tests were made on Gum Arabic, Agar Agar, and Pectin. No reducing substances were found in any case after 24 hours' incubation at 27° C., using 10% extract.

For the detection of a possible cellulase other tests were applied. A small piece of clean Fucus was placed in the extract and incubated for several days. At the end of that time microscopic examination showed that the contents of only those cells whose walls had been cut through were digested. Further, hand sections were cut and mounted on a slide in the actual secretion from the hepato-pancreas, the cover glass being sealed and the whole left in a warm, constant temperature for several

days, being examined from time to time for dissolution of the cell walls. None, however, was observed. In tests on filter paper and cotton wool incubated for some time with the extract, Fehling's solution failed to demonstrate the presence of any reducing sugar. Thus it will be seen



TEXT-FIG. 10.—Graph showing pH optimum and range of activity for amylase ——; invertase ——.——; glycogenase – – – – –.

that this animal, though its main diet consists of Fucus and other vegetable matter, is quite unable to digest either cellulose or the pentosans contained in such food. Yonge (1927) failed to find a cellulase in extracts of the wood-boring Isopod *Limnoria lignorum*.

A series of experiments was carried out to determine the pH optima of

the various sucroclastic enzymes. The effect of the change of pH upon the activity of the amylase, invertase and glycogenase is shown in Tables IV, V, and VI respectively, below and in Text-Figure 10.

In these experiments Clark and Lub's Buffer Solutions were used, at first, for providing a known pH. These, however, were found in some cases to be altered by the addition of the extract and did not give true readings. The method then resorted to was that of adding a certain quantity of acid or alkali and/or water to the extract and finding the pH of the resultant mixture.

MacLean's blood sugar method was used throughout in estimating the amount of glucose. Briefly, this is as follows: The proteins are precipitated from the sample by heating with acid sodium sulphate and adding dialysed iron to the hot liquid. The mixture is cooled and filtered and to the filtrate is added an excess of alkaline copper iodide solution. This is then boiled for a definite length of time, during which an amount of copper is reduced equivalent to the amount of glucose present in the sample. The iodine is liberated from the excess alkaline copper iodide by the addition of acid and titrated with sodium thiosulphate in the usual way. The amount of iodine present in the total amount of copper iodide solution added is also determined directly, the difference giving the amount of copper iodide reduced by the glucose, from which the amount of glucose present can be determined.

TABLE IV.

pH Optimum for Amylase.

1% starch solution, 1 c.c. 10% enzyme extract, 1 c.c. Acid or alkali and/or water to 2 c.c.

Incubated for $2\frac{1}{2}$ hours. Temperature, 28° C.

No.	Acid		Alkali		H_2O	pH.	Thio. ·01 N	Glucose
	c.c.	N.	c.c.	N.	c.c.		c.c.	mgm.
1.	1·0	·01	–	–	1·0	4·9	5·43	1·11
2.	·5	·01	–	–	1·5	5·1	4·37	1·53
3.	·3	·01	–	–	1·7	5·35	3·49	1·91
4.	–	–	–	–	2·0	5·65	2·96	2·13
5.	–	–	·25	·01	1·75	6·0	3·58	1·87
6.	–	–	1·0	·01	1·0	6·2	3·58	1·87
7.	–	–	2·0	·01	–	6·6	4·20	1·61
8.	–	–	·3	·1	1·7	7·2	4·16	1·62
9.	–	–	·45	·1	1·55	7·9	4·78	1·37
10.	–	–	1·0	·1	1·0	8·85	5·11	1·25
11.	–	–	1·5	·1	·5	9·15	5·27	1·18
12.	–	–	2·0	·1	–	9·2	5·83	0·94

TABLE V.

pH Optimum for Invertase.

5% sucrose solution, 2·5 c.c. 10% enzyme extract, 2·5 c.c. Buffer solution, 5 c.c.

Incubated for 2 hours. Temperature, 32° C.

No.	pH.	Thio. ·01 N. c.c.	Glucose mgm.
1.	4·2	7·60	1·05
2.	4·6	6·60	1·79
3.	5·0	4·36	3·63
4.	5·4	3·46	4·39
5.	5·8	3·35	4·48
6.	6·2	3·66	4·22
7.	6·6	3·87	4·13
8.	7·0	4·12	3·83
9.	7·4	4·78	3·27
10.	7·8	5·15	2·96
11.	8·0	5·60	2·60

Note.—In the above table the amount of glucose represented is, in some cases, greater than the amount that can be estimated by the amount and strength of reagents advised by MacLean. In estimating glucose in this experiment half the quantity was taken and the result doubled.

TABLE VI.

pH Optimum for Glycogenase.

Saturated solution of Glycogen, 3 c.c. 10% enzyme extract, 1 c.c. Acid or alkali and/or water to 2 c.c.

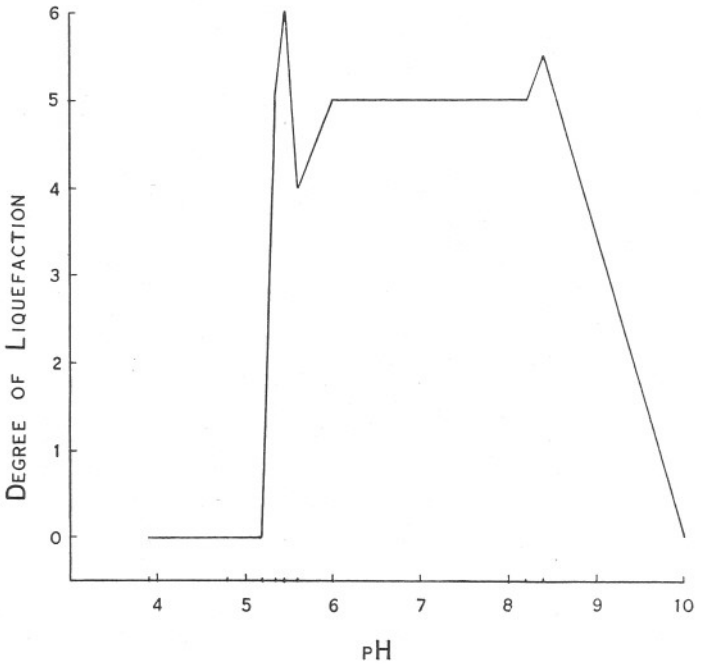
Incubated for 70 minutes. Temperature, 32° C.

No.	Acid		Alkali		H_2O	pH.	Thio. ·01 N. c.c.	Glucose mgm.
	c.c.	N.	c.c.	N.	c.c.			
1.	2·0	·01	–	–	–	4·85	7·16	0·3
2.	1·0	·01	–	–	1·0	5·0	4·97	1·31
3.	·5	·01	–	–	1·5	5·4	4·54	1·46
4.	–	–	–	–	2·0	5·7	4·20	1·61
5.	–	–	1·0	·01	1·0	6·15	4·58	1·45
6.	–	–	2·0	·01	–	6·4	5·25	1·19
7.	–	–	·35	·1	1·65	7·1	5·01	1·32
8.	–	–	·4	·1	1·6	8·8	6·46	0·71
9.	–	–	·6	·1	1·4	9·2	6·86	0·56
10.	–	–	1·0	·1	1·0	9·6+	7·62	0·20

There is a range of activity between pH 5 and pH 7 in the case of all three sucroclastic enzymes, with the optimum lying between pH 5·65 and pH 5·8.

(*d*) The Proteoclastic Enzymes.

These are very powerful, so much so that it was necessary to reduce the proportion of extract to substrate very considerably in order to prevent the digest from acting too quickly. Table VII and Text Figure 11



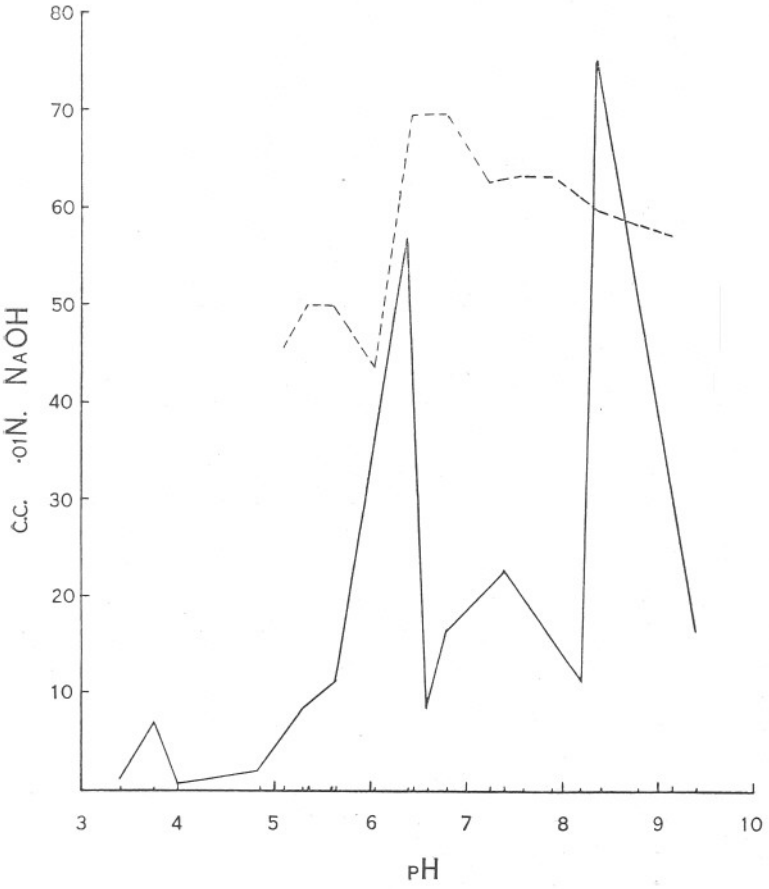
Text-Fig. 11.—Graph showing activity of protease, with change in pH, on the liquefaction of gelatine.

represent the results of an experiment to determine the effect of change in hydrogen ion concentration on the liquefaction of gelatine, i.e. the conversion of proteins to soluble polypeptides.

In this Table the degree of liquefaction is expressed by the figures of an arbitrary scale used by Yonge (1926a) who quotes Dernby, as follows :—

0 Completely solid.
1 Solid, but small pieces may be torn off by strong shaking.
2 Solid, but the surface moves somewhat when the tubes are shaken.
3 Soft.
4 Half liquid.
5 Almost liquid.
6 Entirely liquid.

From these it will be seen that the range of activity extends from pH 5·35 to pH 5·85, with an optimum about pH 5·45 and a second at pH 8·4. To determine the quantities of amino acids produced these were estimated by titration with alkali after treatment of the digest with 90% alcohol. This proved an unsatisfactory method owing to the precipitation of proteins which masked the end point. Sørensen's formaldehyde titration was employed with more success on digests with blood fibrin. The results of this experiment are given in Table VIII and Text-Figure 12. Two optima were again found, the first at pH 6·4 and the second at pH 8·4.



TEXT-FIG. 12.—Graph showing optimum pH and range of activity for protease on blood fibrin ——; and on peptone – – – – – –.

TABLE VII.

pH Optimum for the Protease by the Liquefaction of Gelatine.

10% gelatine solution, 5 c.c. and 1 c.c. of the following mixture: 0·5% enzyme extract in sea-water, 5 c.c., acid or alkali and/or water to 5 c.c. Temperature, 32° C.

No.	Acid		Alkali		H_2O	pH	Degree of liquefaction after given number of hours.							
	c.c.	N.	c.c.	N.	c.c.		1	2	3	4	4½	5	5½	6
1.	5·0	·1	–	–	–	3·9	0	0	0	0	0	0	0	0
2.	2·5	·1	–	–	2·5	4·8	0	0	0	0	0	0	0	0
3.	5·0	·01	–	–	–	5·2	0	0	0	0	0	0	0	0
4.	–	–	–	–	5·0	5·35	0	0	1	1	2	4	4–5	5
5.	–	–	2·5	·01	2·5	5·45	0	1	1–2	2–3	4	5	5–6	6
6.	–	–	1·0	·1	4·0	5·6	0	0	1	1–2	2	3	3	4
7.	–	–	3·0	·1	2·0	6·0	0	1	1	2	3	3	3–4	5
8.	–	–	4·0	·1	1·0	8·2	0	1	1–2	2	3	4	4–5	5
9.	–	–	5·0	·1	–	8·4	0	1	1–2	3	3–4	4	4–5	5–6
10.	–	–	1·0	1·0	4·0	10·0	0	0	0	0	0	0	0	0

TABLE VIII.

pH Optimum for the Protease.

1% enzyme extract in sea-water, 5 c.c. Acid or alkali and/or water to 5 c.c. Blood fibrin, 0·2 gm. Incubated for 5 days. Temperature, 30° C.

No.	Acid		Alkali		H_2O	pH	NaOH ·01N
	c.c.	N.	c.c.	N.	c.c.		c.c.
1.	5·0	·01	–	–	–	3·4	1·25
2.	3·0	·01	–	–	2·0	3·75	6·9
3.	2·0	·01	–	–	3·0	4·0	0·6
4.	1·5	·01	–	–	3·5	4·85	2·05
5.	1·0	·01	–	–	4·0	5·3	8·4
6.	·75	·01	–	–	4·25	5·65	11·3
7.	·5	·01	–	–	4·5	6·0	32·5
8.	–	–	–	–	5·0	6·4	56·6
9.	–	–	·5	·01	4·5	6·6	8·5
10.	–	–	·75	·01	4·25	6·8	16·15
11.	–	–	1·0	·01	4·0	7·4	22·6
12.	–	–	1·25	·01	3·75	8·2	11·25
13.	–	–	1·5	·01	3·5	8·4	75·0
14.	–	–	5·0	·01	–	9·4	16·4

In order to determine whether the two optima were present when a polypeptide was used as a substrate the following experiment was carried out.

A 10% solution of peptone was used as the substrate and the optimum range of activity determined for the production of amino acids. Table IX and Text-Figure 12 show the result of this experiment; it will be noticed that while the range of activity extends from pH 5·6 to pH 8·8 there is now only one optimum point situated at pH 6·8, the higher optimum at pH 8·4 having been eliminated.

TABLE IX.

pH Optimum for the Protease.

1% enzyme extract in sea-water, 5 c.c. Acid or alkali and/or water to 5 c.c. 10% solution of peptone, 3 c.c. Incubated for 22 hours. Temperature, 29°–30° C.

No.	Acid		Alkali		H_2O	pH	NaOH ·01N
	c.c.	N.	c.c.	N.	c.c.		c.c.
1.	5·0	·01	–	–	–	5·1	45·8
2.	–	–	–	–	5·0	5·35	50·0
3.	–	–	5·0	·01	–	5·6	50·0
4.	–	–	1·0	·1	4·0	6·05	43·8
5.	–	–	2·0	·1	3·0	6·45	69·6
6.	–	–	3·0	·1	2·0	6·8	69·7
7.	–	–	4·0	·1	1·0	7·25	62·7
8.	–	–	4·5	·1	·5	7·6	63·3
9.	–	–	5·0	·1	–	7·9	63·2
10.	–	–	·4	1·0	4·6	8·4	59·6
11.	–	–	·5	1·0	4·5	8·8	58·2
12.	–	–	·6	1·0	4·4	9·15	57·1

In view of the fact that the pH of the gut lies between 5·8 and 6·7, the higher optimum cannot be of use to the animal during digestion. It is probable, however, that it may be an autolytic enzyme such as Shinoda (1928) found in Astacus, and this supposition is strengthened by the absence of this optimum when peptone was used as a substrate. Shinoda also found differences between the optimum pH for the working of the protease on different substrates such as have been recorded above.

6. ABSORPTION.

(*a*) Introductory.

This subject has been one of great controversy amongst the workers on the physiology of digestion in Isopods for many years. An excellent résumé of the work of previous authors is provided by Nusbaum-Hilarowicz (1917) in a preliminary summary of his work, and at greater length in the introduction to his paper of 1920.

As mentioned above in the description of the intestine, McMurrich (1896) showed that the so-called midgut is lined throughout by chitin and thus, according to him, cannot absorb, but can serve only for the storage of food, whereas Murlin showed this cuticle to be porous and demonstrated the absorption of fat and certain albuminous substances. This latter author did not discuss the question of absorption in the hepato-pancreas, though he dealt with the structure of its epithelium and with its function of secretion.

(*b*) Methods and Results.

In the investigation of the absorption of food in Ligia, the method used was that frequently employed by workers of recent years. Iron, in the colloidal form of ferrum lacticum or ferrum oxydatum saccharatum, in this case the latter, was fed to the animals under investigation. After a given period the gut and hepato-pancreas were dissected out and fixed, the usual fixative employed being a 5% solution of ammonium sulphide in 95% alcohol. In this case Yonge's modification (1926b, p. 709) of mixing the ammonium sulphide in alcohol with an equal volume of Bouin's fixative, just before use, was employed with satisfactory results. The sections were treated for 10 minutes with a 10% aqueous solution of potassium ferrocyanidè, followed by a few minutes in a dilute solution of HCl, a bright Prussian blue colouration resulting wherever the colloidal iron had been absorbed. The sections were counter-stained with alum carmine.

By means of this treatment it was possible to demonstrate absorption throughout the whole length of the epithelium of the intestine and also in that of the hepato-pancreas, as shown in Plate I, Figures 1–5.

In the latter organ the granules found usually at the bases of the cells, termed zymogen granules by many authors, appear to act as centres round which the absorbed material aggregates.

In the experience of most workers who have employed this technique to demonstrate absorption, the colouration due to the iron tends to be concentrated in localised areas, somewhat as in Figures 4 and 5 which illustrate absorption in the hepato-pancreas. In the intestine the absorbed material was distributed much more diffusely, dense in patches as shown in Figures 1–3, but the greater mass of absorbed material (not illustrated) appeared a much paler blue with denser centres, gradually fading away towards the periphery of each region of absorption.

This effect was met with by Steudel (1912) when investigating absorption in the insects and is well illustrated by him. He describes the appearance in the following words: "Unterhalb des Kernes erscheint vielfach das Plasma selbst (ob nur scheinbar oder in Wirklichkeit, ist zweifelhaft) diffus blau gefärbt. Im diffusen Plasma liegen die Eisenkörnchen in allen

Grössen, teils deutliche Flecken, teils Punkte, teils kaum als solche zu unterscheidende feinste Pünktchen, die einen allmählichen Ubergang zu der erwähnten diffus bläulichen Grundfärbung bilden."

It is interesting to note that, though the chitinous lining of the intestine in Ligia is not perforated, yet absorption occurs very largely in this region of the alimentary canal. This can best be seen in Figures 2 and 3, Plate I, in which the absorbed iron is shown in actual transit through the chitinous layer.

One is struck by the resemblance between the relationship of this layer to the underlying epithelium and that of the peritrophic membrane to the absorptive epithelium in insects.

Steudel considers that the peritrophic membrane should be regarded as an organ of protection and compares it thus with the filter of Astacus. He states, moreover, that it allows only dissolved food to come into contact with the epithelium. The chitinous membrane in Ligia appears to function in an exactly similar way and to be analogous to the peritrophic membrane of the insects.

One further point of interest might be noted. McMurrich (1896, p. 93) remarks on the vacuoles "appearing as more or less extensive blisters of the epithelium" which he noticed on the inner surface of the epithelium of the "midgut" in the Isopods he examined. This peculiar condition was met with to a very great extent in Ligia after feeding, suggesting that it is associated with the absorptive processes. Its true significance is yet to be discovered.

7. SUMMARY.

1. Ligia is an omnivore, though normally feeding on Fucus. Portions of food are cut off by the mandibles and passed into the œsophagus. Its feeding period is limited.

2. The foregut is provided with a number of ampullæ and lamellæ which are furnished with spines and bristles and are so arranged as to form a filter for the separation of the liquid portion of the food from the solid particles.

3. A second filter is found in the floor of the foregut protecting the entrance to the hepato-pancreas.

4. A ventral valve is present preventing the entry into the hepato-pancreas of solid food from the intestine.

5. The hepato-pancreas consists of three pairs of tubules provided with a muscular network for producing contractions. The epithelium possesses discrete cells.

6. The intestine extends from the point of entry of the hepato-pancreas

into the foregut, to the rectum. Its epithelium is a syncytium lined throughout by a homogeneous layer of chitin. No typhlosole is present.

7. The rectal epithelium is syncytial, chitin lined and muscular.

8. Food entering the foregut is subjected to pressure which forces liquid through a filter, whence it passes into the hepato-pancreas.

9. The solid food is passed back, mixed with secretion from the hepato-pancreas and further pressed to expel liquid which passes through a second filter into the hepato-pancreas.

10. This is further aided by the tubules of the hepato-pancreas which force the secretion into the lumen of the gut and withdraw liquid by rhythmical contraction and relaxation.

11. The functioning of the foregut and associated organs appears to be designed for quick extraction of nutriment from the food.

12. The pH of the foregut averages 6·3 ; that of the hepato-pancreas, 6·0 ; of the intestine, 6·5 ; and of the rectum, 6·2.

13. The digestive enzymes are secreted by the hepato-pancreas ; none is secreted by the intestine.

14. The enzymes act readily on most carbohydrates, proteins and fats.

15. No cellulase is present, nor any enzyme which will act on pentosans.

16. The range of activity of the sucroclastic enzymes lies between pH 5 and pH 7 ; the optimum occurs between pH 5·65 and pH 5·8.

17. The proteoclastic enzymes are more powerful than the sucroclastic.

18. The range of activity for the liquefaction of gelatine lies between pH 5·35 and pH 8·5, with optima about pH 5·45 and pH 8·4.

19. Digestion of blood fibrin proceeds most rapidly at pH 6·4 and pH 8·4.

20. The range of activity for the digestion of peptone lies between pH 5·6 and pH 8·8 ; the optimum condition of pH being at 6·4. No second optimum was found.

21. The existence of an optimum at pH 8·4 probably indicates the presence of an autolytic enzyme.

22. Absorption was demonstrated by feeding with ferrum oxydatum saccharatum, and occurs in the epithelium of both the hepato-pancreas and intestine.

23. The appearance of the absorbed iron in the intestine was diffuse and resembled that described by Steudel in insects.

24. An analogy is suggested to exist between the chitinous membrane lining the epithelium of the intestine of Ligia and the peritrophic membrane lining the same region in the insects.

I wish to thank Dr. C. M. Yonge for his help throughout the course of this investigation and for reading the manuscript before publication.

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EXPLANATION OF PLATE.

FIG. 1.—Portion of syncytial epithelium of anterior region of intestine in longitudinal section, showing region of absorption demonstrated by iron saccharate. 24 hours after feeding. × 400.

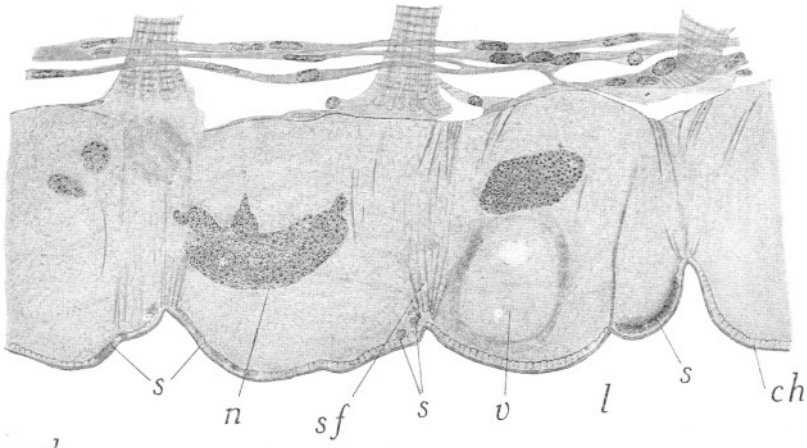
FIGS. 2 AND 3.—Portions of Fig. 1 enlarged to show absorption of the saccharate through the chitin. × 815.

FIG. 4.—T.S. through hepato-pancreatic tubule, showing absorption of iron saccharate. × 100.

FIG. 5.—Cell from epithelium of hepato-pancreas, demonstrating absorption of saccharate. × 425.

ABBREVIATIONS USED IN PLATE.

ch., chitin ; *l.*, lumen ; *n.*, nucleus ; *nl.*, nucleolus ; *s.*, iron saccharate ; *s.f.*, "supportive fibres" ; *v.*, vacuole.



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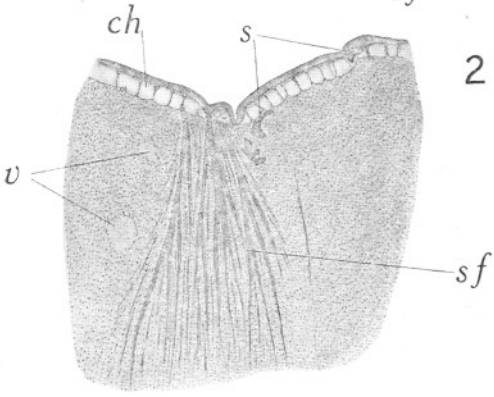

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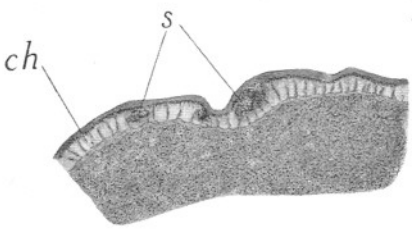

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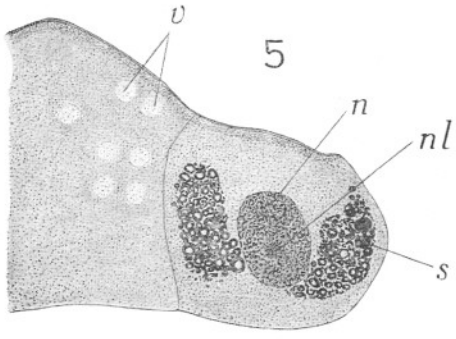

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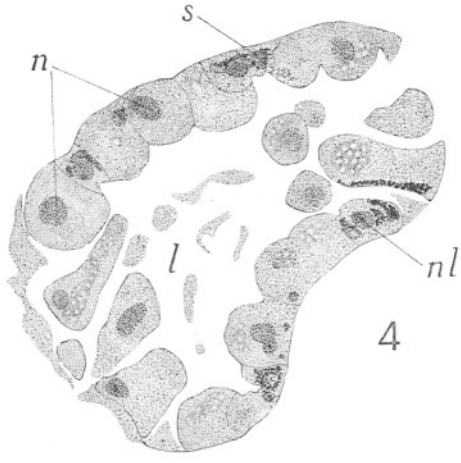

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