VI—Studies on the Onychophora

II—The Feeding, Digestion, Excretion, and Food Storage of *Peripatopsis*

By S. M. Manton, *M.A.*, *Sc.D.*

with

Biochemical Estimations and Analyses

By N. G. Heatley, *M.A.*

(Communicated by W. T. Calman, F.R.S.—Received 20 October, 1936—Read 17 June, 1937)

[Plates 38–40]

Contents

[Published July 29, 1937

Introduction

The material for this work consisted of several hundred specimens of *Peripatopsis* obtained in South Africa in 1933 and brought to England alive. At least six species were present, four being referable to existing descriptions. Two of these, *P. moseleyi* and *P. sedgwicki*, have been kept in captivity in perfect health for three years, and were represented in 1937 by some of the original animals and their sexually mature offspring. A study of their feeding, digestion, and excretion has been made during this period. The animals were killed primarily to obtain their embryos, but the material was used as extensively as possible for other purposes. In another paper a general account will be given of the biology of the South African species of *Peripatopsis*.

Part of this work has been done in collaboration with Mr. N. G. Heatley, who has made a special study of the digestive enzymes of *Peripatopsis* (1936). Mr. Heatley has also carried out the analyses for uric acid and reserve products, and has done the determinations of p_H, the chitin tests, etc., and has provided other data acknowledged in the text.

No previous work has been done on this subject beyond a few records of the type of food eaten by Peripatus. It has been found necessary to describe the structure of most of the organs concerned with feeding and digestion. The various species of *Peripatopsis* used do not differ from one another either structurally or functionally in any material way ; specific references are usually given, but the results may be considered to be generally applicable to the genus, and probably to the group as a whole. A morphological account is given first, including results of work on the living animals which show the movements or functions of the parts and the periodic formation and removal of the peritrophic membrane, etc.

Part of the cost of obtaining material in Africa was defrayed by grants from the Government Grant Committee of the Royal Society. I wish to record my thanks to Dr. Lawrence, Miss Johns, Mr. Saxton, and Dr. Hewitt for their assistance in finding the animals ; to Professor Adamson and Miss Stevens for providing me with laboratory facilities in the University of Cape Town ; to the Union and Castle Mail Steamship Co. for facilities provided on several occasions for transport of *Peripatopsis* at suitable temperatures ; and to Mr. Heatley for placing his specialized knowledge and ingenuity at my service so unreservedly for two years.

Methods

The methods employed in rearing and maintaining *Peripatopsis* in a healthy state will be described in another paper. The animals were provided with sheep's liver every three days and with living and dead woodlice, and they fed also on the microfauna of decaying wood.

The animals were killed with chloroform. For examination of structure, small pieces of tissue rather than whole animals had to be fixed separately in order to obtain good preservation. The usual fixatives were satisfactory for gross structures.

Duboscq Brazil was particularly useful for the alimentary canal, sense organs, etc., but dehydration and embedding had to be done rapidly and be completed within 24 hours of fixing, or excessive hardening of the tissue followed. 10% formalin was used for sections to be cut on a freezing microtome. If an aqueous fixative was to be used on tissue including the body wall, the latter was wetted for a moment with alcohol so that penetration was not unduly delayed. Many stains were employed, but Mallory's triple stain and iron haematoxylin counterstained proved most serviceable.

Injections of living animals were done with an ordinary tuberculin syringe. The body wall is very tough, and a sharp, strong needle is necessary. If no damage is done to the internal organs the animals show little or no reactions after injection.

The chemical methods employed by Mr. HEATLEY which have not been mentioned in the text are as follows :—

For the determination of *glycogen* PFLÜGER's (1904) method was used, the reducing sugar being estimated by the colorimetric method of FOLIN (1927). *Total carbohydrate* was estimated by hydrolysing the tissue direct with 3% hydrochloric acid for four hours in a boiling water-bath, and then determining the reducing power of an aliquot portion after precipitating proteins with tungstic acid.

Fat was estimated by sealing a weighed amount of tissue in a tube with 0·5 ml. of 50% potassium hydroxide and heating in a boiling water-bath for two hours. The contents of the tube were then extracted with four successive amounts of chloroform (no trouble was experienced in these experiments with stable emulsions), and the massed extracts were washed twice with water. The washings were added to the aqueous alkaline solution, which was acidified and extracted with five successive amounts of ether, the combined extracts being washed as before. The choloroform and ether extracts were then evaporated in tared micro-beakers, the weights of the residues giving an approximate indication of the amounts of unsaponifiable matter and of fatty acids respectively. In the text only the sum of these two values has been presented.

The *uric acid* content of the peritrophic membranes recorded in fig. 13 was determined by the colorimetric method of FOLIN (1933). After each excretory mass had been dried over sulphuric acid, it was weighed on a micro-balance, and ground up in a very small mortar with a few drops of 0·06% lithium carbonate, and then transferred, with the help of 10 ml. of the lithium carbonate solution, to a 50 ml. volumetric flask. A drop of toluol was added and the flasks were corked and shaken at intervals for several hours. The cyanide and Folin's reagent were then added, and after 20 minutes the solutions were made up to the marks and compared with the standards in the usual manner.

The qualitative test for small amounts of *urea* was carried out in the following way. A single tablet of Dunning's urease was crushed up in about 1 ml. of water containing a drop of phenol red. Dilute acid or alkali was added till the solution was orange. The solution to be tested was then mixed with a small amount of phenol red on a glass slide, and the colour adjusted to orange by the addition of

acid or alkali. This fluid was sucked up a pyrex tube about 2 mm. in diameter, and was followed by a drop of liquid paraffin. Finally a few cubic millimetres of the urease preparation was sucked up, and the tube was sealed off at both ends. A control was then set up, and both tubes were centrifuged. It was found that if much urea was present the test tube would become pink at once ; 5 gamma of urea will give a pink colour in a few seconds, and 10 c.mm. of a 0·01% solution will give an easily detectable change of colour in less than 15 minutes at room temperature.

The *protein spheres* of the gut were isolated as follows :—The gut was mashed up lightly with distilled water and strained through glass wool. The fluid was then centrifuged at 3500 r.p.m. for 15 minutes ; the fat floated to the top, and the centrifugate, which was used for enzyme experiments, was siphoned off through a bent glass capillary. The residue was stirred up with more distilled water and the tube was again spun at maximum speed for 15 minutes. The spheres were completely separated from the fat at this stage by filling the top of the tube with paraffin wax, and then, after it had solidified, cutting off the bottom part of the tube containing the spheres. The spheres were removed and stirred up with water and allowed to stand for 15 minutes. The suspension, from which large particles had settled out, was carefully drawn off and centrifuged at 2000 r.p.m. for five minutes. The centrifugate was neglected and the residue was stirred up with distilled water and again centrifuged. This operation was repeated four or five times, and the spheres as isolated in this way were apparently unaltered in size, shape, or refractility.

Alimentary System and Pericardial Network

The structure of the alimentary tract has not received so much attention from former authors as have the genital organs, limbs, and integument, and no description or figures exist which illustrate the form of these parts of the body at all adequately. A consideration of these features is necessary before the feeding and digestive mechanisms can be described. Balfour (1883) gives a short and careful description of the buccal cavity, tongue and jaws, pharynx, oesophagus, stomach, and rectum. He describes the jaw endo-skeleton ("jaw lever"), but gives no details of muscles further than defining a category of muscles supplying mouth, pharynx, and jaws. Hewitt (1905) correctly re-describes the structure of the hollow "jaw lever". Moseley (1874) refers to a pair of pharyngeal protractors, and later Schneider (1902) and others have described with care the body musculature of a typical segment, but the head region has received no further attention, and the results of the earlier workers have not been quoted in subsequent monographs and textbooks.

Lips, Buccal Cavity, and Tongue

The mouth is surrounded by the well-known circular *lip*, which has been partially described by Balfour (1883) for *P. capensis*, and has been clearly figured and described in a fairly expanded state by Evans (1901) for *Eoperipatus* and by Kemp

(1914) for *Typhloperipatus*. The lips of *Peripatopsis sedgwicki*, shown in fig. 1 in the expanded and contracted states, may be taken as characteristic of the African

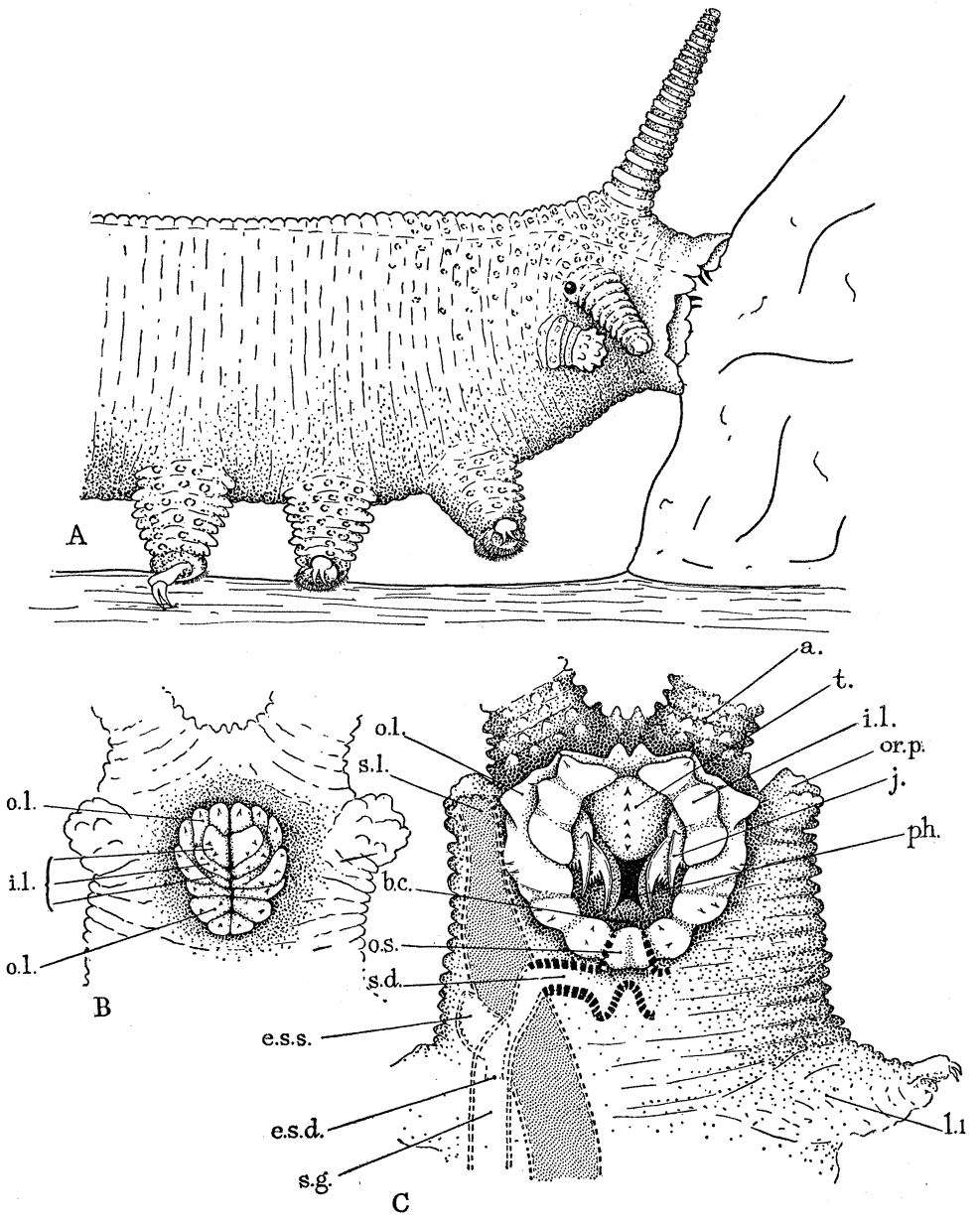


FIG. 1—The oral region of *Peripatopsis sedgwicki*. (A) Side view of an animal feeding on meat ; the inner and outer lips are distended, forming a sucking tube, the tips of the jaw blades being visible in their anterior position. Support of the body on the substratum is secured by walking legs 3 and those behind. Leg 1 is not used for either prehension or support. (B) Ventral view of the mouth with the lip closing the buccal cavity. (C) Ventral view of the mouth region of a larger specimen showing extended outer and inner lips, and the form of the buccal cavity and associated structures. The positions of the salivary and slime glands is shown by dotted lines. The heavy lines on the inner parts of the salivary duct represent the region lined by thick, smooth cuticle similar to that of the buccal cavity.

species of this genus, and probably of the group as a whole. The lips, buccal cavity, tongue, and pharynx are covered with chitin which is thicker than that on the surface of the body, and is quite smooth, whereas that on the rest of the body is raised into numerous secondary papillae or scales corresponding in number with the ectodermal cells below. The outer cuticle (staining black with iron haematoxylin and red with Mallory's stain) is thick and smooth over the oral parts, whereas on the body surface this layer is thin and forms the sculpturing on the scales (figs. 18 and 20, Plate 39, *cu.o.* and *cu.o.s.*). The inner chitin (unstained by iron haematoxylin, blue with Mallory's stain) is also thicker over the oral parts than on the body surface (*cu.i.*). This smooth cuticle can be wetted with aqueous fluids, while that of the body surface cannot. The lip is divided into a continuous outer fold (fig. 1, C, *o.l.*) within which lies a pair of antero-lateral folds (*i.l.*). The lip is raised into lobes bearing a few long spines as seen in the figure. Both inner and outer folds are strongly muscular. The whole lip can be contracted so as to close the buccal cavity completely (fig. 1, B), the lobes of the two folds being closely pressed together, but not invaginated (*o.l.* and *i.l.*). When expanded, the lip can form a short, wide tube projecting from the head (fig. 1, A).

Within the circular lip lies the *buccal cavity*. Into it project laterally the paired jaws and anteriorly the median tongue (fig. 1, C, *j* and *t*). Just behind the tongue the pharynx opening passes upwards and backwards, (*ph.*), and within the posterior edge of the lip opens a median salivary duct (*o.s.*).

The *salivary glands* of *Opisthopatus cinctipes* have been described by DUBOSCQ (1920). The proportions of the parts differ from those shown here for *Peripatopsis* (fig. 1, C). In the latter the end sac duct joins the main duct over leg 1 ; the region of the duct corresponding with DUBOSCQ's " branche descendant muqueuse " does not extend further back than segment 4 (varying with the species) ; and the exit tube, lined by thick chitin (heavy lines in fig. 1, C), opens into the buccal cavity and not into the pharynx (fig. 2).

The *tongue*, lying between the anterior part of the jaw bases, is covered with smooth chitin and cuticle (*see* above). The whole organ is muscular and mobile ; it is shown projecting freely in fig. 1, C, and more retracted in fig. 2, which includes its main antagonistic muscles. A pair of compact bands (*t.r.*) run on either side of the median plane from the anterior part of the tongue and lip backwards to the dorsal body wall just behind the cerebral ganglion (*see also* fig. 3, A) ; and numerous bands of muscles extend from the tongue forwards to the anterior wall of the head just below the antennae (*a.t.*).

Organs of Taste

The spines on the lips and tongue (fig. 1, C) have variously been described as " spines " or " denticles ", possibly implying a masticatory function. FEDOROW (1929, p. 283) describes " Sinnesknospen " scattered over the hinder part of the tongue and provided with small spines which he distinguishes from the sensory spines of the body papillae, and believes to be " Geschmacksorganen ". He also followed the course of paired labial and recurrent nerves from the brain to supply

the lips and throat, and sending small nerves to each sense capsule. HANSTROM (1935) gives a further description of the tongue sense organs.

The good fixation here obtained of adult and embryonic *Peripatopsis* has extended the observations of HANSTROM. The lips and tongue are provided with sense organs which are similar in structure (figs. 20 and 21, Plate 39) ; these consist of a dense connective tissue capsule up to 55 μ in diameter enclosing sensory and other cells, the nuclei being situated mainly around the periphery. The capsule is surmounted by a thick chitinous spine 8–55 μ long which is hollow and filled by extensions of the capsule cells. The spine is blunt and appears to have a terminal opening 2–4 μ in diameter, the protoplasmic contents extending up to the tip. No covering membrane could be detected in sections cut in various planes. The chitin of the spine is of uniform thickness, except at the tip where it becomes thinner ; it is yellowish and does not stain with iron haematoxylin or Mallory's stain, with the exception of a basal ring, which stains darkly with haematoxylin, and red with Mallory's stain, as does the outer layer of body cuticle. No cell boundaries are visible within the capsule. The central protoplasm stains less densely than does the periphery, and shows fibrils or striations extending from the nucleated region up into the core of the spine (figs. 20 and 21, Plate 39, *f.*). Sense cell nuclei cannot be distinguished from supporting cell nuclei by size, shape, or staining reactions. A few nuclei stain darkly, but these are various in shape. Some of the outer elongated nuclei are doubtless those of supporting cells. At the base of each capsule a strand of looser connective tissue ensheathes a nerve and a bundle of tracheae, both of which penetrate the capsule (fig. 22, Plate 39). Nerve fibrils could not be traced far into the capsule owing to their minute size and lack of affinity for dyes. The capsules lie mainly below the ectoderm and project into the subcutaneous connective tissue, and may be so close together that their connective tissue sheaths unite. A median longitudinal row of eleven or more capsules lies along the tongue, and capsules with long or very short spines are numerous over the edges and inner surfaces of the lips and sides of the tongue. About 110 of these sense organs may be present on the oral parts of one adult animal.

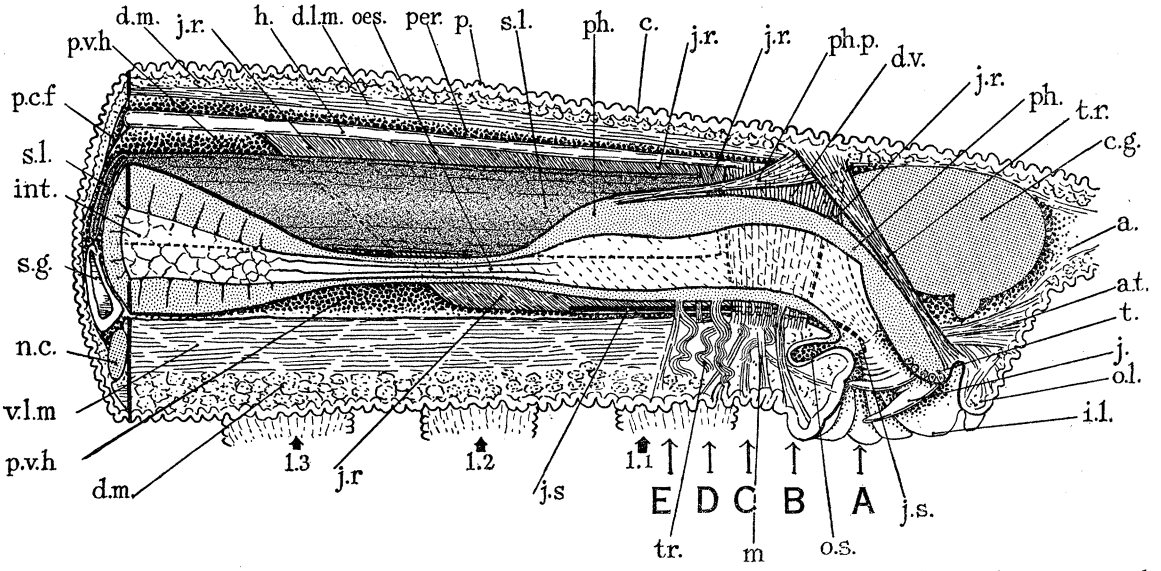
These organs are undoubtedly concerned with taste (*see* p. 437). The protoplasm at the tips of the spines will suffer no desiccation within the buccal cavity as this region is always wet, even when the lips are widely stretched. Although the spines may be longest on the lip edges, which may be dry between meals, the terminal openings are smallest here. Spines with large terminal openings are only found within the permanently wet buccal cavity.

For comparison with these taste organs, the sensory spines of the general body surface and of the antennae are shown in figs. 18 and 19. The capsules associated with these spines are similar to those just described for the mouth, but the striations extending into the spines are fewer and less distinct. On the primary body papillae (fig. 18, Plate 39) only one organ is present, and the ectoderm is so thick terminally that the capsule does not project below it. The spine is closed and its base shows surface sculpturings (*s.*) with similar staining reactions to those of the cuticle on the

secondary spines (*cu.o.s.*). In other genera this basal region of the spine may be much larger. On the antennae three or more organs may be borne by each papilla ; their nerves are large, and the capsules are surrounded mainly by haemocoel, owing to the thinness of the ectoderm and the absence of a thick subcutaneous layer of connective tissue.

The Jaws and their Associated Muscles and Endoskeleton

The jaws, unlike the mandibles or biting gnathobases of most other arthropods, do not bite against each other, their cutting blades being directed backwards (figs. 1, C, and 2). Each jaw, as is well known, consists of two sickle-shaped cutting blades set on a common base ; each pair resembles the paired claws on the walking



FIGS. 2 and 3 illustrate diagrammatically the structure of the main organs and muscles concerned with feeding.

FIG. 2—Longitudinal half of the anterior end of an animal viewed from the cut sagittal surface. The oesophageal-intestinal junction is seldom extended as here shown, and is usually intucked to form a "valve ", as seen in fig. 4. The slime gland, *s.l.*, lies median to the jaw retractor muscle, *j.r.*, posteriorly, but perforates this muscle over the first leg to reach the lateral slime papilla. The jaw endoskeleton is shown as dotted line when viewed through the pharynx wall anteriorly, and posteriorly as a solid line along the lower edge of the jaw retractor muscle *j.s.* The arrows, A–E, represent the levels of transverse sections shown in text, fig. 3.

legs, but is much larger in size. The edges of the four blades are seen in fig. 1, C, and an inner blade and part of the outer can be seen in fig. 2.

The musculature and endoskeleton associated with the jaws are extensive, and have not received fuller attention than that of BALFOUR (1883). Both differ greatly from those of other Arthropods where the mandibles are opposable (*see* p. 454). From the lower posterior border of the inner blade of each jaw a hollow tube of thick chitin passes as a strut into the body, and extends backwards almost to the level

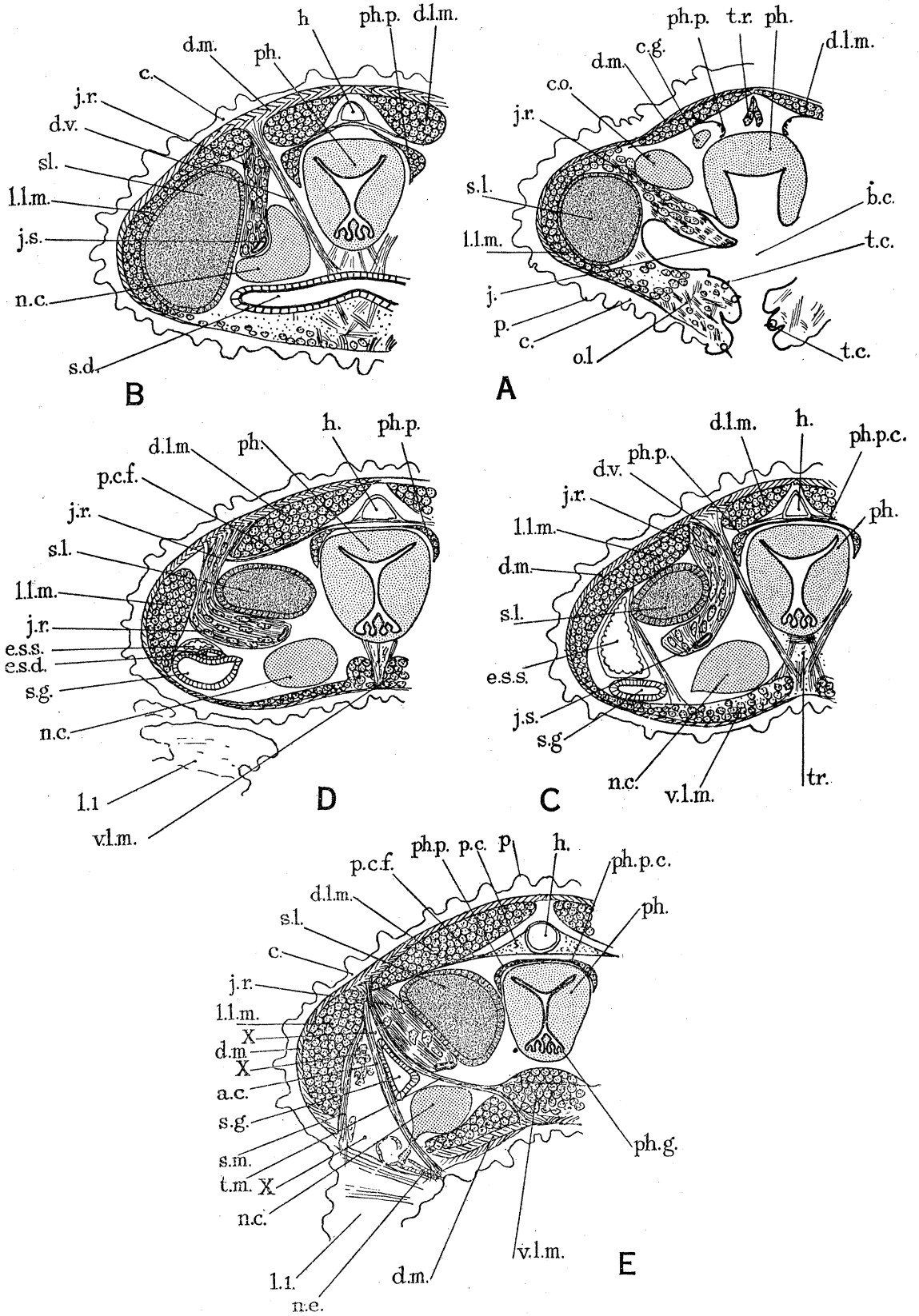


FIG. 3—Transverse sections at levels shown by arrows in fig. 2, illustrating the structures concerned in feeding, the positions of the various parts of the salivary gland, the anterior limit of the heart, the pericardial network, and lateral accumulatory excretory tissue.

of the second walking legs (figs. 2 and 3, *j.s.*) ; it lies laterally just above the nerve cords. On to these chitinous tubes are attached the retractor muscles. The chitin is covered by cubical ectodermal cells, it is shed at ecdysis, and resembles in every way the hollow type of Arthropod endoskeleton which remains attached to the exoskeleton throughout life (MANTON, 1934, pp. 202 and 219). The bulk of the jaw muscles radiate outwards and backwards from the endoskeletal strut, forming a pair of thick sheets which are attached to the dorso-lateral body wall from behind the cerebral ganglion to above the middle of the third walking legs (fig. 3, *j.r.*). These muscular sheets lie outside the circumoesophageal commissures and internal to the slime glands as far back as the first walking legs, but posteriorly they become external to the slime glands (fig. 2 and compare fig. 3, A, B, and C, with D and E). Contraction of these muscles results in the powerful backward cut of the jaw blades.

Other jaw muscles are very much smaller in size. A pair passes forwards from the base of the jaws to the anterior body wall near the antennae, and a third set of fibres arising from the anterior part of the jaws passes transversely across and into the dorsal part of the tongue. These latter muscles alone can be adductor in function, and will be used for adjusting the position of the jaw blades. Unlike the mandibular adductors of other Arthropoda, they have no median endoskeletal tendon, and are pre-oral instead of post-oral in position, and hence can scarcely be comparable.

A further pair of muscles, functionally connected with movement of the jaws, must here be mentioned. The " transverse muscles " (SCHNEIDER, 1902, etc.) form a continuous sheet separating the lateral haemocoelic space from the central perivisceral cavity throughout the trunk region (fig. 3, E, *t.m.*), extending from the first pair of walking legs backwards (fig. 3, D). In the region between the mouth and these legs a pair of thick muscle bands is attached mid-ventrally through the disappearing ventral longitudinal muscle, and passes almost vertically to the dorsal body wall, running between the pharynx and the jaw retractors (figs. 2 and 3, B and C, *d.v.*). These muscles may represent the " transverse muscles " of the oral papilla segment, which have separated from those of posterior segments and shifted their dorsal insertions close to those of the jaw retractors. Contraction of these dorso-ventral muscles will give rigidity to the body just behind the jaws, and will cause the contraction of the jaw retractors to exert a maximum pull on the jaw blades. Any shortening of the body, caused by a swelling in diameter as the retractors contract, will be counteracted by the dorso-ventral muscles, which will thus compensate for the absence of a rigid exoskeleton and assist in giving efficiency to the blades. The latter muscles may also be of importance in assisting in the protraction of the jaws.

The Pharynx, Oesophagus, and Oesophageal Valve

The form of the pharynx has been described by BALFOUR (1883). He followed the course of the dorsal and ventral grooves from the buccal cavity to the oesophagus (*see* fig. 3, B–E), and differentiated intrinsic from extrinsic muscles. The

intrinsic muscles are seen in section in fig. 4, B, the circular fibres are more numerous externally and lie between wedges of radial fibres. The fibrils of the latter pass through the cubical epithelium to the cuticle, but do not stain darkly within these cells. Connective tissue cells and tracheae also lie among the muscle fibres. The extrinsic muscles consist of (1) numerous small ventral muscles which pass, together with the main tracheal supply, to the ventral body wall (fig. 2, *m.* and *tr.*), and (2) a large pair of muscles inserted dorso-laterally at the posterior end of the pharynx, which pass forwards close to the pharynx to end in the dorsal body wall just behind the brain (figs. 2 and 3, A–E, *ph.p.*). These pharyngeal protractors are united above the pharynx and below the pericardial floor by a thin layer of dense connective tissue (fig. 3, C, *ph.p.c.*). These muscles were seen but incorrectly interpreted by MOSELEY (1874). The chitinous lining of the pharynx is very thick and resembles that of the buccal cavity (*see* fig. 4, B, *cu.o.* and *cu.i.*).

The pharynx passes insensibly into the oesophagus at the level of the second legs (figs. 2 and 4, A). The oesophagus is much narrower than the pharynx ; the grooves from the latter continue, but are augmented by many additional longitudinal furrows, all of which are irregular. The walls of the oesophagus consist of inner and outer layers separated by an extensive vascular space. The inner layer is formed by cubical epithelium lined with smooth, thick chitin, and surrounded by a thin layer of dense fibrous connective tissue. The outer layer, or sheath, consists of connective tissue of a less dense nature, formed of cells and loose fibres, and in this is embedded the outer longitudinal and inner circular muscles fig. 4, C). A few strands of connective tissue may pass across the haemocoelic space (which may be almost virtual in sections of animals killed in certain positions), but there is no doubt that this space is always present, as it can invariably be found in sections and dissections, and is particularly clear in late embryos where fibrous connective tissue is hardly developed (fig. 4, D).

The oesophagus passes abruptly into the intestine, which is of much greater diameter (figs. 4, A, and 5), and the chitinous lining of the oesophagus stops at this junction. The connective tissue-muscular sheath of the oesophagus is continuous with that of the intestine, but over the latter the connective tissue becomes dense and fibrous, and the muscle layers cross over, so that the circular fibres are external over the intestine (fig. 5). The connective tissue forms a thick annulus where the transition occurs, but there is no sphincter muscle (fig. 4, A).

The state of contraction of the oesophageal muscles profoundly affects the junction of the oesophagus with the intestine. If the outer muscular layer is longitudinally extended, the haemocoelic space becomes narrow, and the oesophagus forms a tube continuous with the intestine (fig. 2). Conversely, if the muscle layer is longitudinally contracted, it shortens to half the internal length. The inner layer is thereby pleated throughout, and posteriorly it is invaginated into the intestine (figs. 4, A and 5). The haemocoelic space is then wider (*h.s.*) and separates the two layers of the intucked epithelium. This double intucking will be called the *oesophageal valve*. When killed with chloroform, Peripatus contracts, and on

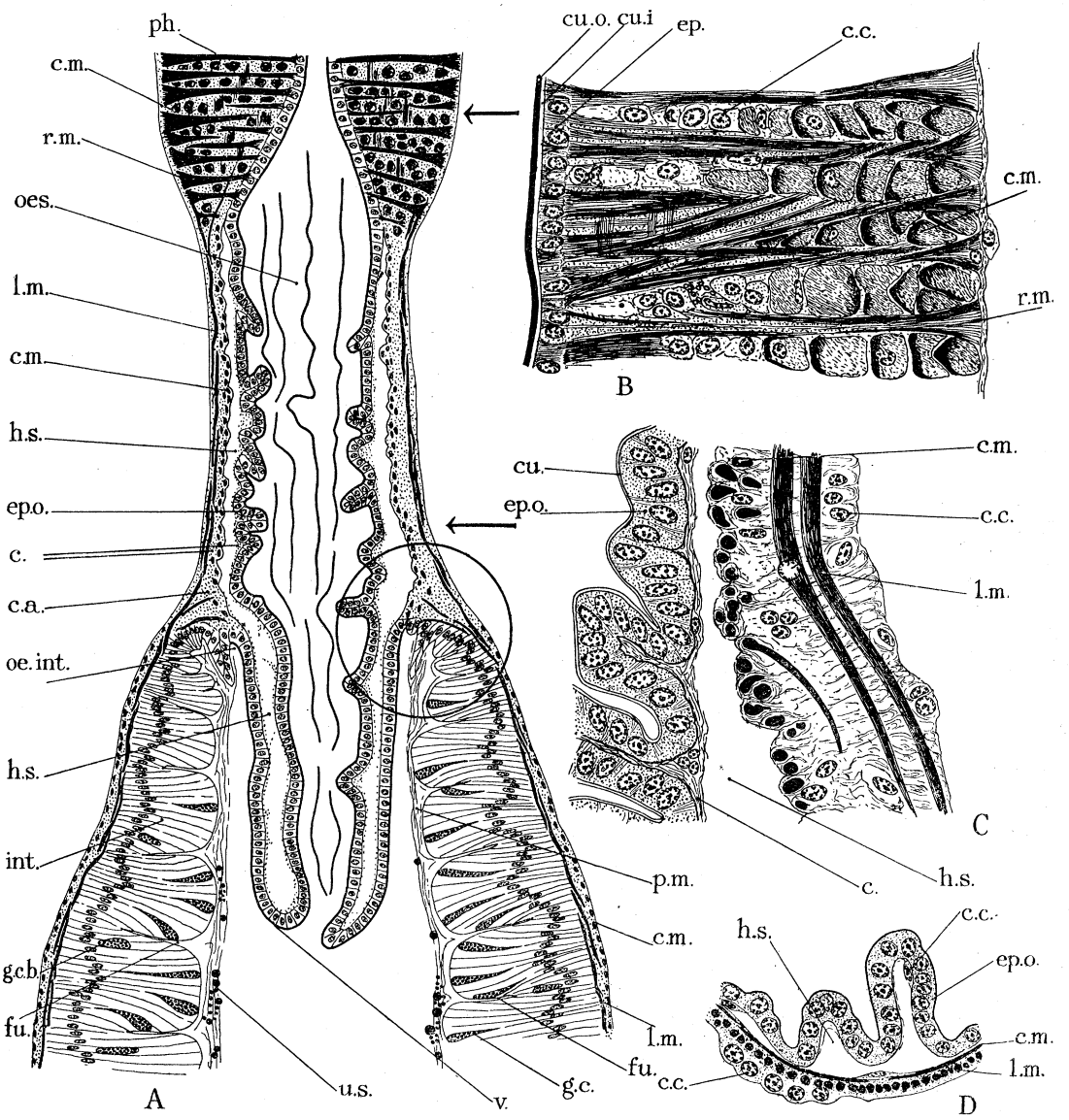


FIG. 4—Figures illustrating the structures of the anterior parts of the alimentary canal. Muscles and chitin are indicated by thick black lines; connective tissue is stippled, and haemocoelic spaces, etc., are white. (A) Diagrammatic L.S. of hinder end of the pharynx, oesophagus, oesophageal valve, and anterior end of the intestine. Cut ridges of chitin are shown in the oesophageal lumen. The peritrophic membrane is just raised from the intestinal epithelium, but has not yet shrunk to form a narrow tube. Crystallization of uric acid has started. The arrows and the circle indicate the positions of the regions shown in greater detail in fig. 4, B and C, and fig. 5. (B) L.S. of pharynx showing details of structure. × 650. (C) L.S. of oesophagus showing details of structure. ×650. (D) T.S. of oesophagus of a late embryo showing the scanty development of connective tissue, the haemocoelic space, and the simple muscle layers. ×650.

dissection or sectioning the valve is usually found well invaginated into the intestine. Probably this is the normal position, as expanded specimens usually show it thus, and only rarely has it been seen completely extended as in fig. 2. The valve is always invaginated in unborn embryos. It should be noted that the surface of the valve against the intestine is quite smooth, the chitin being devoid of any projections. The function and movements of this valve will be considered on pp. 431, 438, and 455.

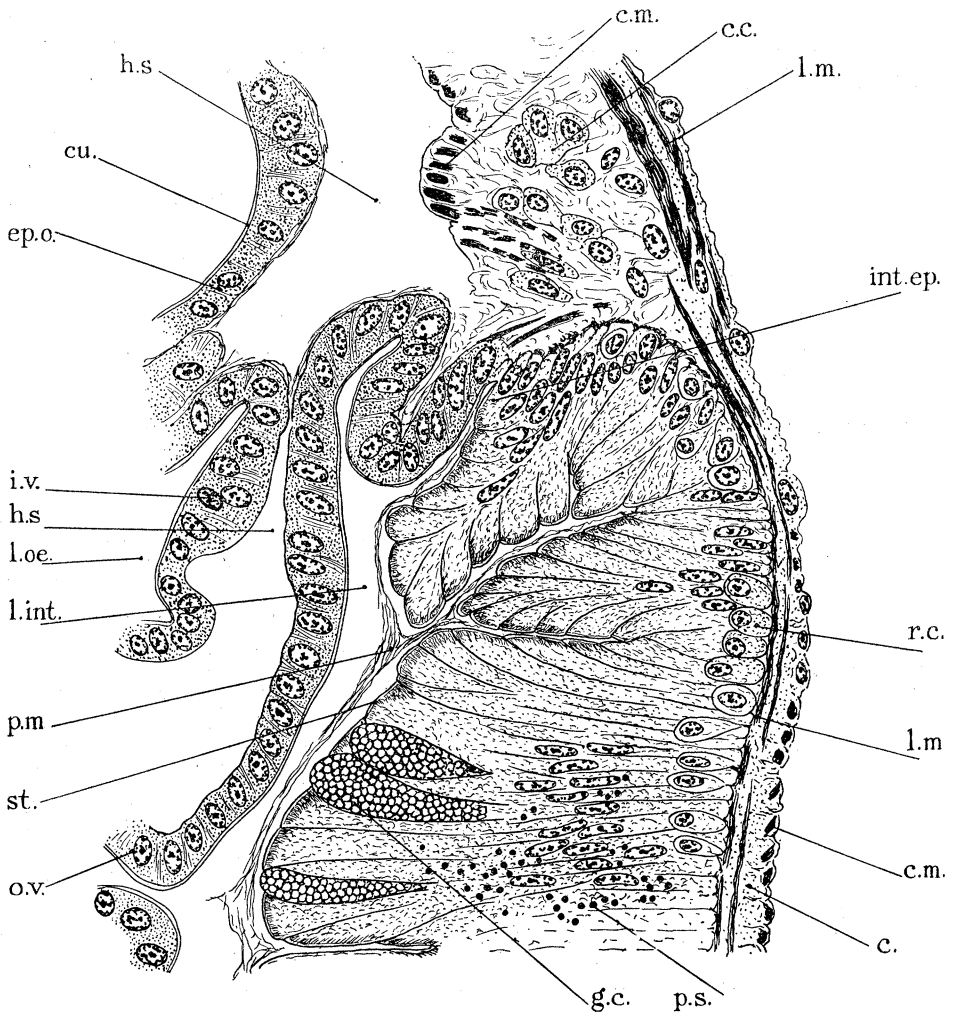


FIG. 5—L.S. of the junction of oesophagus and intestine showing details of structure of the organs and of the oesophageal valve. The position is shown by the circle in fig. 4. ×650.

The Intestine and the Secretory Cycle of the Gland Cells

The plicate *intestine*, which is longer than the body, extends from the level of the second or third walking legs to the posterior end of the animal. Its form has been described by GRUBE (1853) and MOSELEY (1874), and BALFOUR (1883, p. 226) records its cytological structure, but his account is incomplete and not entirely

correct. The intestine is almost uniform throughout. A connective tissue investment, composed of cells and of fibres staining intensely blue with Mallory's stain, lodges the outer circular and inner longitudinal muscles (fig. 4, A, and fig. 15, Plate 38). Between the muscle layers lies a mass of coiled tracheae. In paraffin sections these tracheae are indistinct owing to the penetration of balsam, but in glycerine preparations from a freezing microtome the air remains in the tracheae, and their course can be followed. Their intracellular endings filled with fluid could not be traced here or elsewhere in the body. Within the connective tissue lies a single layer of epithelial cells, the bulk of which is formed by long columnar cells, with scattered gland cells which do not all reach the lumen (fig. 4, A). The epithelium shows fairly regular furrows caused by the varying heights of the columnar cells (*fu.*). The furrows are 0·1–0·25 mm. apart and form a network over the surface; they may be shallow, or descend to half the height of the epithelium.

Columnar epithelial cells are 4–7μ wide, roughly hexagonal in cross-section (fig. 10, C), and are 180–500 μ long with their nuclei usually, but not always, near the basal end. Towards the lumen they are provided with a convex striated border 1–1·5 μ in thickness. This appears to be fairly rigid, and the surface of the intestine shows a regular pavement of convex hexagonal areas. The free ends of the cells occasionally give off cytoplasmic buds (*see* pp. 127 and 432), and contain a variety of inclusions (*see* pp. 439, 440, 445, and 449).

The *gland cells* are far less numerous and are much shorter than the columnar cells. They are absent from the extreme anterior end of the intestine near the oesophageal valve (figs. 4 and 5), they are scarce in the region near the rectum, and are most abundant in the anterior third of the intestine. The gland cells are continually being formed from the regenerative cells (*see* below) in the basal part of the epithelium, and shift towards the lumen (fig. 4, *g.c.b.* and *g.c.*) but do not always reach it (fig. 11, D). They are pear-shaped, with the nucleus, which may be obscured by secretory granules, usually at the basal pointed end. No striated border is present, and the distal rounded or pointed end may project slightly into the intestinal lumen through the peritrophic membrane (figs. 7, A, and 11, and figs. 15 and 17, Plate 38). The gland cells show no reactions to mucicarmine or thionin, and are thus not mucous in function. They all appear to be ferment cells. They do not all stain alike, but the differences indicate various phases of activity rather than differentiation into various types, and all variations can be found in cells either at or below the surface of the epithelium.

The reactions to haematoxylin are indicated in fig. 11, D–F. The whole cell may be packed with black secretory granules, usually slightly larger than the protein inclusions of the columnar cells (*see* below); and in this state stain red with Mallory's stain. The cytoplasm between the granules is almost uncoloured by both stains. In contrast, the cell may appear to contain slightly larger secretory granules which do not stain with iron haematoxylin, and which are bright or pale blue with Mallory; the cytoplasm between the granules stains with haematoxylin

(fig. 11, F, and fig. 17, Plate 35). Both these extreme conditions may be abundant ; they are quite unlike, and all intermediates can be found, some cells showing both types of staining in different parts (fig. 11, E). In animals fixed during or just after feeding, large numbers of discharged gland cells are present ; very few cells show secretory spheres staining blue with Mallory's stain, but plenty are present showing the smaller spheres staining red with Mallory's stain (fig. 11, D). The discharged cells, with clear cell wall and nucleus, are often distended, and are not always situated at the epithelial surface, but may be found well below it even during a meal. Thus their secretion must pass out between the columnar cells and through the peritrophic membrane. Discharged gland cells are few in animals which have not been feeding.

The phases of the secretory activity of the gland cells thus appears to be from the stage seen in figs. 11, D–F. Small secretory granules formed in the young cell (fig. 11, D) swell up and charge their staining reactions (fig. 11, E) to reach the condition in fig. 11, F. The cells wait in this state until their secretions are required, and then pass into the discharged state (fig. 11, G). It is believed that the cells then shrink and pass into the lumen of the intestine. Degenerating nucleated bodies are present in the gut lumen, and are collected and removed by the peritrophic membrane (fig. 7, F). There is no indication that any regeneration of secretory granules occurs or any repeated activity in one gland cell.

Numerous round *regenerative cells* lie between the bases of the columnar cells (fig. 11, A, *r.c.*), and are most abundant in the anterior third of the intestine where gland cells are most numerous, and thus lie in the region where greatest replacement is needed. Mitoses in the intestine have only been seen in these cells ; their descendants become elongated (fig. 11, C, *r.c.* 1) and shift between the columnar cells. These young cells thus replace effete intestinal cells, as in the alimentary canals of many insects, etc.

The Peritrophic Membrane, its Formation and Removal

A *peritrophic membrane* is secreted from the striated border of the columnar cells throughout the whole length of the intestine. It consists of several layers of very thin chitin, staining blue with Mallory's stain and giving positive chitin tests by CAMBELL'S method (1929). The membrane is less than 1μ thick when the chitinous lamellae are close together. The membrane ends at the oesophageal valve and at the rectal junction, and is not continuous with oesophageal or rectal cuticle. That the peritrophic membrane is formed from the whole surface of the intestine and not from a secretion poured out near the oesophageal valve is certain. There are no gland cells at the junction of the valve and intestine, as there are in many insects ; the membrane follows every curve of the intestinal surface, and dips down as a double layer to the bottom of every furrow ; and the processes of formation of the membrane can be followed on all parts of intestinal surface. The whole membrane is shed and replaced approximately once every 24 hours, regardless of the occurrence of

feeding. It becomes raised from the intestinal surface (fig. 6, A), contracts in diameter (fig. 6, B), and lies as a narrow longitudinal tube in the intestine. It remains in this state for about 18 hours and is then evacuated from the anus. A new peritrophic membrane is formed from the intestinal epithelium, and becomes raised from the surface about 6 hours after evacuation of the previous membrane,

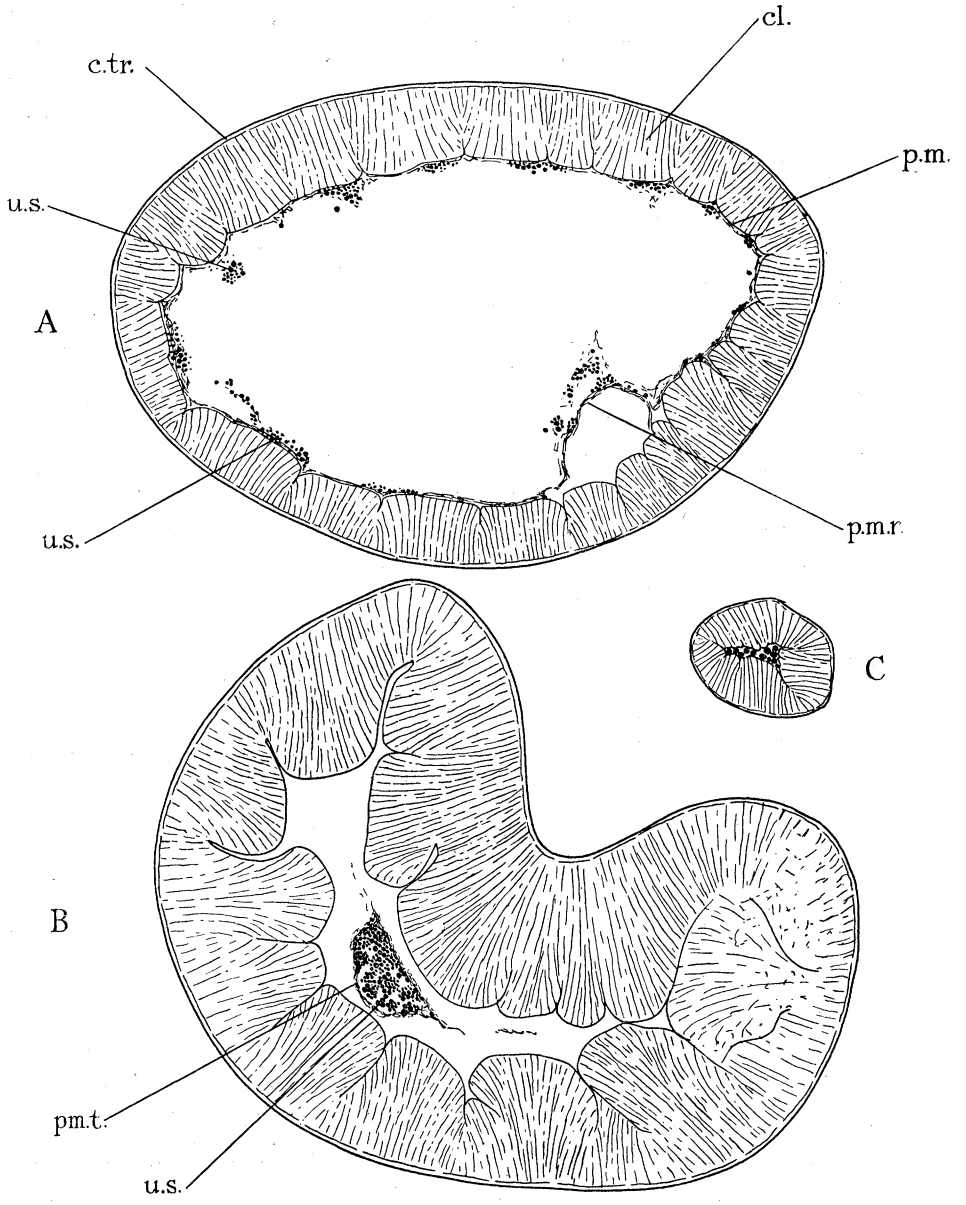


FIG. 6—Diagrammatic transverse sections of the intestine showing the peritrophic membrane. × 50. (A) The membrane is fully formed at the epithelial surface, and uric acid crystallization is complete. The membrane is raised up at *p.m.r.* (B) Section at a later stage showing the peritrophic membrane raised and contracted to form a tube enclosing the uric acid crystals. (C) Section of an embryo showing the uric acid crystals in the intestine, which are larger in size than in most adults.

and rapidly shrinks to form a new longitudinal tube. This contraction of the peritrophic membrane collects the indigestible gut contents, the disintegration products of epitheial cells, and the daily quota of uric acid crystals (*see* p. 445), and other excretory products, and carries them to the exterior when the tube is evacuated (fig. 6).

Formation of the Peritrophic Membrane—The peritrophic membrane is secreted in layers. The process is illustrated in fig. 7. During the 18 hours in which the contracted membrane lies free in the intestinal lumen, the striated border of the intestinal cells in section shows a sharp, almost refringent, outline towards the lumen, as in fig. 7, A, and figs. 15 and 17, Plate 38. This clear line is probably the first chitinous layer of the next peritrophic membrane. Later, distinct layers of chitin become apparent (fig. 7, B). Sometimes they are so closely apposed that they appear as one, or they may in places be separated by uric acid crystals (fig. 7, C, *p.m.* 1, *p.m.* 2, *p.m.* 3). Each layer when raised from the striated border leaves the latter with no firm outer boundary (fig. 7, C, *p.m.* 3, left side, and 7, E). There is no indication that the layers of chitin are formed at the bottom of the striated border and liberated by the wearing away of this border, followed by the formation of a fresh striated border below the chitin, as in Hymenoptera (VON DEHN, 1933).

The raising of the peritrophic membrane in *Peripatopsis* must be a rapid process, as so few individuals show it out of the large number sectioned. It is related in time to the evacuation of the previous membrane, and occurred at from $3\frac{1}{2}$–6 hours after this event in the animals examined at known intervals after evacuation of a peritrophic membrane (Table I). Reference to columns 3, 4, and 6 of the table shows that the raising of a membrane is associated with a conditions of " bubbling " of the epithelial surface. This is shown in fig. 7, D and E. Just before this occurrence, spherical clear regions or swellings of the distal parts of the columnar cells can be seen (fig. 7, B, *b.* 1). These regions are apparent because their contents, other than fluid, stain less darkly than the surrounding cytoplasm. The next condition is one in which few or many bubbles of cytoplasm are partly or completely separated from the epithelium, some retaining connexion with the cytoplasm by a neck passing through the striated border *b.* 2. In all cases of bubbling the striated border shows no rigid external surface and lacks its chitinous covering. The peritrophic membrane lies over the bubbles and may be raised up by them ; it is not far removed in fig. 7, E, while in 7, D, the membrane had shrunk right away into the gut lumen. It has not been possible to ascertain whether this bubbling causes the lifting of the peritrophic membrane or whether it is an accompanying event. It is certainly associated with the lifting, as no bubbling occurs at any other time (*see also* p. 432). Once free from the striated border, the membrane shrinks rapidly, probably in a few minutes, to form the narrow tube lying along the intestine. Its walls are much folded, they are elastic, and shrink again after mechanical stretching.

The removal of the peritrophic membrane occurs normally once a day. Several dozen animals have been kept isolated in glass dishes under suitable conditions, so that the evacuation of peritrophic membranes could be noted. *Peripatopsis* objects to

TABLE I

Reference numbers of animals	Time interval between evacuation of last peritrophic membrane, p. 1, and killing	Condition of animals killed				
		Condition of new peritrophic membranes, p. 2 and p. 3			Presence of " bubbling " at epithelial surface	Crystallization of uric acid at epithelial surface
		Next membrane, p. 2, raised and contracted to form tube enclosing uric acid crystals	Next membrane, p. 2, distinct but not yet raised up from epithelial surface	Membranes, p. 2 and p. 3, thin and developing at epithelial surface		
M.29♀	*60 hours	p. 2 +	—	p. 3 +	—	—
M.23♀	*30 ,,	—	p. 2 +	—	—	complete
M.18♂	24 ,,	p. 2 +	—	p. 3 +	—	—
M.21♂	24 ,,	p. 2 +	—	p. 3 +	—	—
S.26♂	24 ,,	p. 2 +	—	—	+	—
S.17♂	19 ,,	p. 2 +	—	p. 3 +	—	—
M.27♀	18 ,,	p. 2 +	—	—	—	—
M.57♀	16 ,,	p. 2 +	—	p. 3 +	—	—
M.55♀	14 ,,	p. 2 +	—	p. 3 +	—	—
S.19♂	12 ,,	p. 2 +	p. 3 (raised in parts)	p. 3 +	+	—
M.25♀	$10\frac{1}{2}$,,	p. 2 +	—	p. 3 +	—	—
S.40♂	6 ,,	p. 2 +	—	p. 3 +	—	—
M.44♀	6 ,,	p. 2 +	—	p. 3 (very thin)	—	—
M.45♀	6 ,,	—	p. 2 (raised in parts)	—	+	complete
M.60♀	$5\frac{1}{2}$,,	—	p. 2 +	—	—	starting
S.41♀	$4\frac{3}{4}$,,	—	p. 2 (raised in parts)	—	+ (where membrane is raised)	considerable
S.48♂	4 ,,	p. 2 +	—	p. 3 (very thin)	+	—
S.46♂	4 ,,	—	p. 2 (raised in parts)	—	+ (where membrane is raised)	considerable

S.35♂	4 „	—	p. 2 (raised in parts)	—	+ (where membrane is raised)	starting
M.56♀	3½ „	p. 2 +	—	p. 3 (possibly, in places)	—	—
S.16♂	? „	p. 2 (just raised)	—	—	+	—
S.42♀	? „	p. 2 (almost all raised	—	—	+	complete ?
S.59♂	3 „	—	p. 2 (raised in parts	—	+	just starting
S.34♂	1 „	—	—	p. 2 +	— (possibly starting)	—
M.20♂	0 (membrane being evacuated)	—	—	p. 2 +	—	—
S.30♀	membrane about to be evacuated	—	—	p. 2 +	—	—

The letters M and S indicate *Peripatopsis moseleyi* and *P. sedgwicki* respectively.

*Unusually delayed, or with M.23 the membrane may have escaped notice.

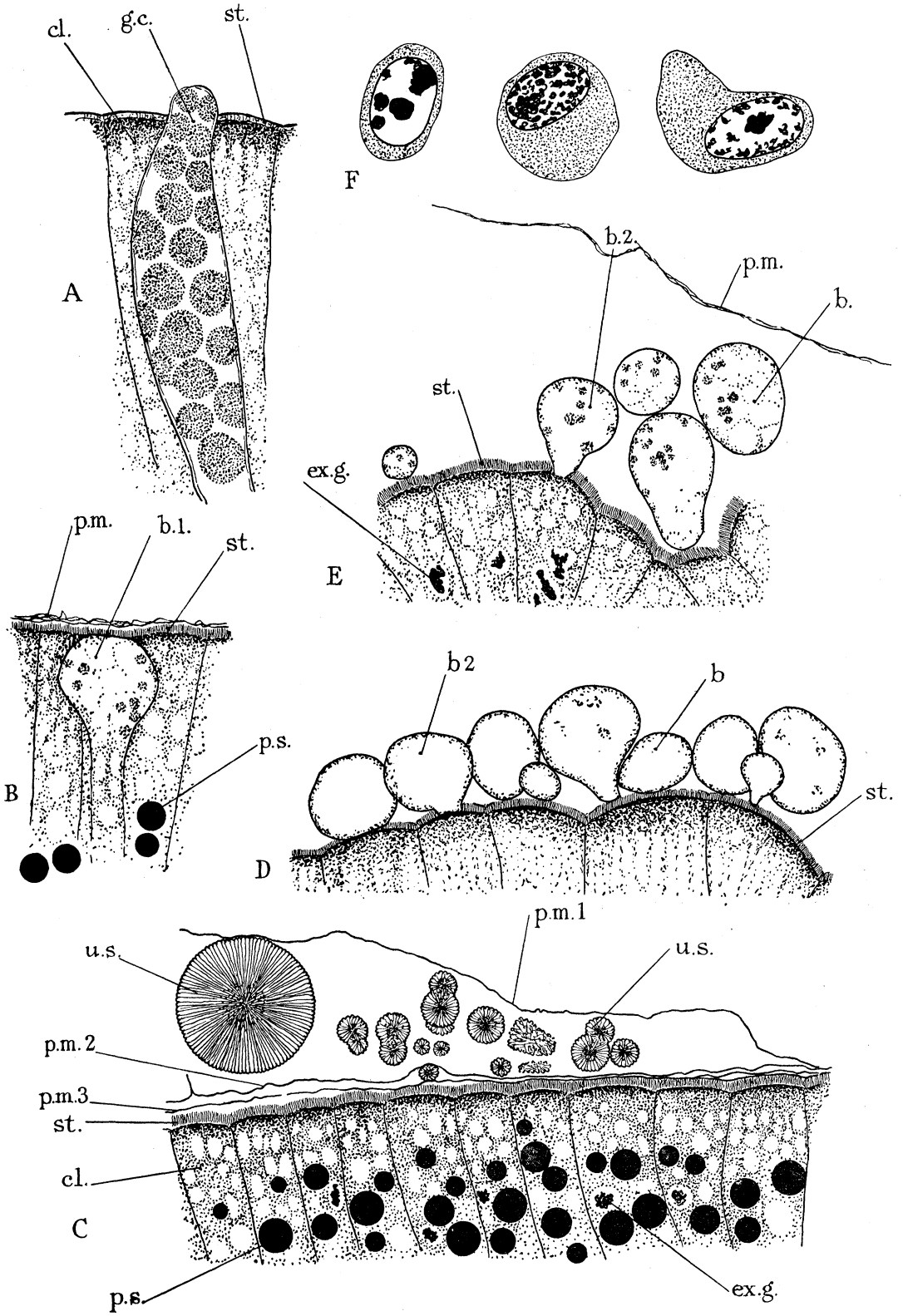


FIG. 7—Sections of the intestine ; only the inner ends of the epithelial cells have been drawn. × 1500. (A) Gland cell projecting byond the striated border and peritrophic membrane into the lumen of the intestine. The peritrophic membrane is not detached from the striated border. (B) The peritrophic membrane becoming free from the striated border, and composed of at least three lamellae. A " bubble " is forming at the distal end of a columnar cell *b*. 1. (C) Part of section shown diagrammatically in fig. 6, A. The membrane is lifting off the striated border, no bubbling is taking place. The uric acid crystals (absent in the animal shown in fig. B) have been formed within and between the several layers of the peritophic membrane. The striated border appears naked below the lifted peritrophic membrane. (D) Animal showing extensive bubbling at the epithelial surface, the bubbles pass through the striated border *b*. 2, and become detached, *b*. The peritrophic membrane has here shrunk away from the epithelium (*see* Table I, S.46). (E) Animal somewhat similar to the last, but showing the raised peritrophic membrane just over the bubbles, and the striated border very clearly lacking the limiting surface membrane seen in fig. 7, A (*see* Table I, S.42). (F) Free epithelial cells in process of disintegration found in the lumen of the intestine within the peritrophic membrane.

living against smooth glass, and frequently refuses to feed, behaves abnormally, and in time becomes pathological under such conditions. However, during the first few days of such isolation almost all individuals regularly evacuated one membrane daily. A record of the behaviour of two individuals during several months' isolation is given in fig. 13. Specimen A refused to feed and ultimately died, while specimen B fed normally and was apparently healthy after 10 weeks' isolation. The daily dry weight of the peritrophic membranes are shown by the vertical columns, and the figures above these columns represent the number of peritrophic membranes evacuated per day. At first both animals usually produced one membrane a day, but later they showed reactions to the conditions and evacuated more than one per day ; specimen B, however, later returned to normal. Most animals which evacuate three or more membranes per day (*see* specimen B) very soon die. Thus more than one membrane a day may be taken to be an abnormal or pathological occurrence.

The oesophageal valve may be of importance in starting the removal of the peritrophic membrane. Muscular movements of the oesophageal sheath (fig. 4, A), acting indirectly via the haemocoelic space between it and the oesophageal epithelium, will cause the intucked valve to move up and down against the anterior walls of the intestine. Although the valve bears no spines (as it does in many insects), such movements may loosen the membrane and push the anterior part backwards. Along the rest of the intestine the free peritrophic membrane is pushed backwards by intestinal contractions, and it collects in a convoluted mass at the hinder end of the intestine for a short time before evacuation through the rectum and anus, a process taking only a few minutes. The membrane is always followed by the evacuation of some fluid containing a number of air bubbles. Some, if not all, of these bubbles have been swallowed through the mouth, and may be seen in the intestinal lumen. The process of swallowing has been seen in narcotized animals, and is mainly caused by pharyngeal peristalsis. These bubbles possibly assist in pushing the mass of the membrane down the intestine.

The periodic formation and lifting of the peritrophic membrane is not related to the feeding, which may be infrequent (*see* p. 435). However, the formation and removal of a membrane may be influenced by the arrival of food in the alimentary canal. When this occurs, a peritrophic membrane, if free, is pushed to the posterior end of the intestine, where it remains until the meal is digested and the next peritrophic membrane is ready for evacuation. This postponement of evacuation of a membrane until a meal is digested is seen in fig. 13, B, on 19 January, 17 February, and 3 March. A new peritrophic membrane is found outside the food and closely pressed to the striated border. The complete secretion of this membrane, not due for perhaps several hours, is hastened, so that food is separated from the epithelium by a well-formed membrane. Probably the epithelium is thus protected from mechanical damage by the food, no mucous glands being present.

The sterility of the intestinal lumen is remarkable. Under conditions of health, bacteria and protozoa are absent. This condition is maintained by the manner

and frequency of peritrophic membrane removal. Parasitic intestinal fungi and protozoa do exist, but they are intracellular, and only their spores pass into the lumen and are removed by the membrane (*see* another paper of this series).

Budding from the Intestinal Epithelium

Budding from the columnar epithelial cells is of two types. One type has already been described above as "*bubbling*" (p. 427), and either causes or accompanies the raising of the peritrophic membrane. Such bubbles have a limiting wall of some sort, but their contents, other than fluid, appear to be scanty, and they do not persist for long (fig. 7, D and E).

Another type of "*budding*" takes place at any time. The buds persist and are removed within the next peritrophic membrane. A columnar cell projects beyond its neighbours into the lumen and loses its striated border, and the projecting portion then separates off. The bud so formed consists of darkly staining cytoplasm similar to that of the columnar cells, and may contain any of the inclusions of the latter, such as excretory granules and spheres and reserve food materials (*see* pp. 440 and 449 and Plate 38, figs. 15 and 16, *bu.*).

The Pericardial Network and Lateral Body Cavity Tissue

A mass of tissue lying on either side of the heart above the pericardial floor is shown in fig. 3, E, *p.c.* The function of this tissue has been variously interpreted. Its component parts were described by GAFFRON (1885), who found two types of cells, small uninucleate bodies 7–14 μ in diameter and large vacuolated multinucleate cells up to 120 μ in length which were of "glandular appearance". He compared the tissue with the fat bodies of other tracheates. SEDGWICK (1888) followed the growth of this tissue, which he called the "*pericardial network*", and suggested that it might be comparable with vertebrate lymphatic tissue as well as having fat body functions. SCHNEIDER (1902) described the two components of this tissue as "lymph" and "lymphoid" cells. The latter correspond with the multinucleates of GAFFRON, and are reported to contain fat and to be granular and darkly staining. SCHNEIDER suggested that they had the appearance of embryonic mesoderm cells, and that they were possibly excretory in function. BRUNTZ (1904) again described this tissue with reference to his injection experiments. He disagreed with GAFFRON, stating that only one type of cell was present, his "nephrocytes à carminate", and he described these cells as attaining a length of 90 μ, and possessing vacuolated cytoplasm including excretory granules and usually three nuclei.

In figs. 23 and 24, Plate 40, is shown the appearance of the pericardial network in transverse section from a late embryo and from an adult respectively. There is no doubt that GAFFRON and SCHNEIDER were right in finding two types of cells, oval multinucleates possessing up to three nuclei (*p.c.l.*), and small uninucleate "nephrocytes" (*see* p. 451) which are round, unless distorted by being closely

packed together (*p.c.s.*). The cells are loosely bound together, leaving vascular spaces of various sizes between them. The adult condition (figs. 24 and 25, Plate 40), which shows a more compact network than the embryo, is permeated by minute spaces (*h.s.p.*) ; and the small cells are most numerous near the pericardial floor. A series of large spaces lies below the network and above the pericardial floor (*p.h.*). These spaces are most clearly understood from longitudinal sections. The pericardial floor, as shown by GAFFRON (1885, fig. 40), is pierced by numerous oval ostia which open into the spaces below the pericardial network (*p.h.*), and communicate via the intercellular gaps in the network to the pericardial cavity above. The pericardial network on either side may cover the whole of the pericardial floor, or only the inner two-thirds where the ostia lie.

The smaller pericardial cells show an exact resemblance to blood leucocytes in size, staining reactions, and the form of the nucleus. In late embryos the nucleus is lobed in both small pericardial cells (*p.c.s.*) and leucocytes (*l.*). Before birth neither type of pericardial cell nor the free leucocytes show granular inclusions. In the adult, on the contrary, an abundance of excretory granules may be present within the *small* pericardial cells. These refringent, yellowish or blackish particles may be so numerous as to fill the small cells, so that when the latter are closely packed their boundaries are not clearly seen (*see also* p. 451). The blackish inclusions only are shown in fig. 25, Plate 40, *ex.g.* Leucocytes may also contain similar refringent granules and inclusions. No such particles are found within the large multinucleate cells in any of the large series of sections variously prepared from animals in different physiological states. The contrary conclusion of BRUNTZ, who claimed to find excretory granules in the large cells, was doubtless due to his fixation which did not show him the distinction between the two types of cells. A very small amount of fat may be found as minute droplets within the small cells in some, but not all, animals showing maximum fat reserves elsewhere (*see* p. 439). Figs. 25 and 26, Plate 40, are from one section of such an animal. Abundant fat lies in the gut epithelium (fig. 26), while it can only just be detected as a few droplets in the uninucleate cells of the pericardial network, and no trace can be found in the multinucleate cells.

Both types of cell occurring in the pericardial network are present in other parts of the body. They are most numerous in the lateral body space :—(1) between the segmental and accessory muscles, near the nephridia, and (2) along the lateral longitudinal muscles, and between the accessory and transverse muscles, mostly above the salivary gland. These positions are marked with "X" in fig. 3, E. This tissue has been examined in well-fixed embryonic and adult material ; but has not been figured as it exactly resembles that of the pericardial network (fig. 24, Plate 40).

The functions of this tissue wherever it occurs is at least twofold. The small uninucleate cells are certainly excretory (*see* experimental evidence, p. 451) and alone may be termed "nephrocytes". The fat store of these cells is very small compared with that of the intestine (*see* p. 439), so that fat storage by this tissue

cannot be a major function. It is probable that leucocytes arise from the small cells which they resemble in structure and properties. The mitosis shown in fig. 23, Plate 40, may be giving rise to a leucocyte ; and many small cells, barely attached to the main mass (especially in late embryos), may separate to form leucocytes. The function of the large multinucleate cells is at present quite obscure.

Feeding Mechanism and Defensive Reactions

There is no precise account of the feeding mechanism of Peripatus, although there are numerous records of its carnivorous habits which have been summarized by Zacher (1933). Bouvier (1902), on the other hand, claims that the group is omnivorous, while Moseley (1874) states that *P. capensis* feeds on decaying wood. The ejection of slime from the oral papillae has been carefully described by Moseley (1874), Kemp (1914), and others ; and although they record the immobilization and death of small animals entangled in the solidified slime, they do not suggest that the ejection of slime is part of the feeding mechanism, or record that the entangled animals were subsequently eaten by Peripatus, and Bouvier (1905) clearly states that the ejection of slime is a defensive reaction. However, Zacher (1933), quoting no authority, lays emphasis on the importance of slime for snaring prey, and a figure has been published elsewhere of a Peripatus " rampant " shooting slime at its prey ! A study of the habits and physiology of individuals of six species of *Peripatopsis* over a period of three years has amply confirmed observations on their carnivorous diet, but in no case have the slime glands been seen to be used in feeding or to contribute towards the obtaining of living prey. Their employment is invariably defensive and not offensive. A contrary conclusion might easily be obtained by misinterpreting the behaviour of the animals.

Slime is ejected from the oral papillae when the animal is startled or molested, or when put in a more intense light, and is directed towards the offending stimulus. Feeding, on the contrary, is a leisurely proceeding, started with no preliminary of slime ejection.

Properties of Slime from the Oral Papillae

The ejected stream of fluid slime instantly becomes sticky on contact with air, forming adhesive beaded threads. It remains extremely sticky for some time, and finally dries to form a transparent and very brittle substance of smaller volume. If the liquid slime is collected from an oral papilla in a glass capillary, it will remain fluid as long as it is not in contact with air.

The properties of the slime were investigated by Mr. Heatley. The solid slime is insoluble in and is unaffected by acetone, ether, alcohol, and chloroform. If, after drying, the slime is placed in water, it becomes opaque, swells, and softens, and becomes sticky like freshly shed slime, and ultimately dissolves to give a non-viscous faintly opalescent solution of p_H 5·2–5·4. On boiling the opalescence increases, but can be made to disappear by adding dilute acid or alkali ; on readjusting the p_H the opalescence reappears.

The slime certainly contains protein, giving positive reactions to Millon's, the xanthoproteic, and sulphur tests. A pink colour with the biuret test indicates a protein of low molecular weight. The aldehyde test for tryptophane was faintly positive, but Molishch's test was negative. A solution of the slime can be hydrolysed to free amino-acids by heating for a few minutes with strong hydrochloric acid.

Of the protein precipitants, tungstic and phosphotungstic acids give a flocculent precipitate with the aqueous solution of slime, but sulphosalicylic acid gives only a faint cloudiness, and uranium acetate has no effect at all.

If the solid slime is heated with 40% sodium hydroxide it quickly turns yellow and loses its plate-like form, rounding up into brownish-yellow globules which rise to the surface of the solution. They are apparently unaffected by further heating ; in the hot solution they are fairly fluid, but on cooling they congeal and take on the consistency of soft cheese. The material is insoluble in ether, but soluble in alcohol and water. When heated alone on a piece of platinum foil, it chars and smells of burning meat.

Lack of material prevented further investigation of this remarkable substance.

Food, Feeding and Drinking

Various observers have stated that Peripatus will eat small arthropods, such as insects, termites, caterpillars, woodlice, etc. In captivity species of *Peripatopsis* have been kept on a diet of sheep's liver, woodlice, and the micro-fauna of decaying wood. These specimens have never been seen to take vegetable food. They will die of starvation if animal food is withheld for a period of three months or more (varying naturally with the initial state of the animals). They never eat the decaying wood in which they live. Remains of wood or other plant material were never found in the alimentary canals of over a hundred individuals examined, and such matter was invariably absent from the excreta. Physiological work, moreover, shows a complete absence of digestive enzymes capable of acting on cellulose, lignin, and such plant materials (HEATLEY, 1936).

The frequency and size of meals taken by *Peripatopsis* varies greatly. When food is abundant and conditions favourable, they may take woodlice or meat every two or three days and perhaps smaller food in addition. When isolated so that their feeding could be observed or deduced, they unfortunately did not behave normally for long, and appear to object to living on a clean glass substratum. They may feed two or three times, but may then refuse food and behave abnormally for unlimited periods. A few individuals did settle down to their unnatural surroundings, and the record of the feeding, excretion, and ecdyses of one such individual is given in fig. 13, B. Feeding occurred about once a week or once a fortnight, and often took place just before ecdysis. An animal feeding continuously for 6 hours may pack the whole intestine with food, and increase in weight by 10%.

An attempt was made to determine whether *Peripatopsis* drinks. The animal is unable to control water loss from the tracheae and general body surface (MANTON and RAMSAY, 1937), and it loses fluid daily on evacuation of each peritrophic

membrane. Fluid may be gained by feeding, but this is usually infrequent. No animal has been seen to drink droplets of water, but they do suck at meat juice offered on filter paper and may scrape up and eat the paper in so doing. They show a strong preference for remaining in contact with damp objects even in a saturated atmosphere. The experiment recorded in fig. 8 indicates that *Peripatopsis* does suck in fluid at the mouth from damp substrata. An individual was isolated in room atmosphere, which is much dryer than its normal environment. It lost water at a slightly decreasing rate until its weight was reduced by 33% in $3\frac{1}{2}$ hours. It was then placed in a saturated atmosphere in which it was alternately prevented from and allowed to (" T ") come in contact with wet cotton wool. In the former environment no increase in weight occurred (the slight decrease was due to evaporation while the

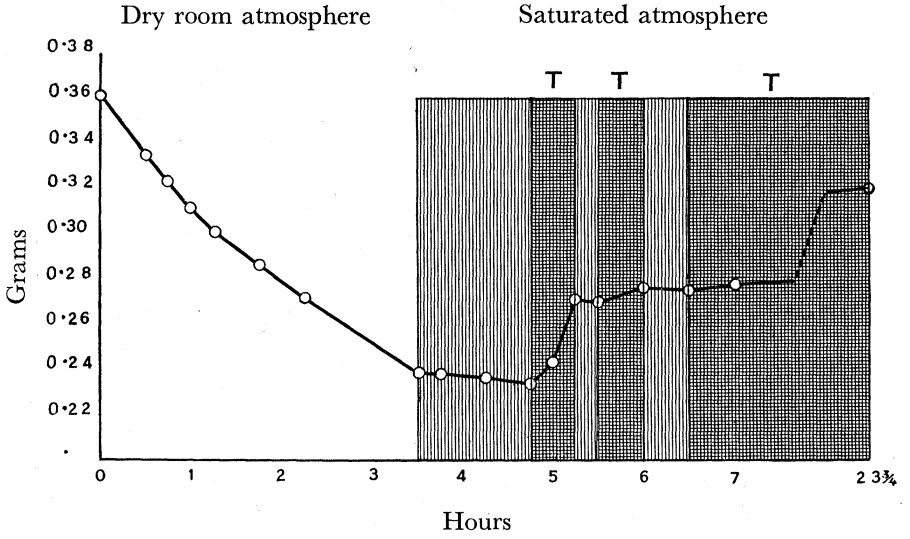


FIG. 8—The occurrence of drinking in *Peripatopsis*; for explanation see text. Only during the periods marked " T " could the animals come in contact with a wet surface.

animal was being weighed). When in contact with the damp cotton wool the weight always increased. The rate of increase was greatest when the water content of the body was lowest, a gain of 10%, 1·6%, and 0·8% occurring during the successive half-hour periods shown in fig. 8. The uptake continued and in $23\frac{3}{4}$ hours 91% of the original weight was restored. As the cuticle is not wetted by water, the increase in weight cannot be due to surface absorption, and must be due to drinking by the suctorial mouth region (*see* below). It is probable that Peripatus habitually sucks fluid from its damp substratum, even if the atmosphere is also damp ; this in accordance with its preference for damp resting places.

The Feeding Mechanism

Feeding takes place at night or in diffuse light. In catching active prey, such as woodlice, neither eyes, prehensile limbs, nor slime ejection is employed. The

antennae are richly provided with sensory hairs (*see* p. 417 and fig. 19, Plate 39), and when walking they are continually used to " feel " the ground in front, either just touching it or held clear. When food is sensed in this way, the animal distends the lips and presses them against the food. If it is meat or a dead animal, the lips and tongue may repeatedly be pressed against the food, so bringing the hollow sensory spines of the oral region (*see* p. 417 and figs. 20 and 21, Plate 39) in contact with the food. The animal may then begin to feed or may walk away. If the food is acceptable the circular lip is distended like a tube and applied to its surface ; the tube formed may be even wider than the animal (fig. 1, A). The lobes of the inner and outer folds fit closely to any surface irregularities of the food, and the junction between lips and food becomes completely closed by moisture from the buccal cavity, as the lip cuticle, unlike that of the body surface, can be wetted with water. Once the lip is so applied, the prey usually cannot escape. The pharynx carries out peristaltic movements causing a powerful lip suction which is strong enough to prevent a woodlouse from walking away. Pharyngeal movements have been directly observed in narcotized animals opened up. There are no strong peristaltic movements of the oesophagus. Neither the oral papillae nor any other limbs are employed to hold the prey.

A slicing of the food then follows. The jaw blades cut into it from before backwards, and if the prey is motile this action at first takes place entirely within the area enclosed by the lip, the suction of which continues ; thus the prey cannot walk away while a hole is being cut into its exterior. Later the jaws may extend anteriorly beyond the lips, as in fig. 1, A, but the tongue and inner lip on either side fit closely against the moving jaw base so that the suction is maintained. The jaws, like the walking legs, usually work alternately, and the cut is directed first downwards and inwards and then backwards and outwards, so undercutting the tissue at the end of each stroke. The two blades on each jaw show some independence of movement, and in all about 14 cuts may be made in 10 seconds. Soft tissue and thin chitin may be sliced in this way. Longitudinal grooves are cut in meat, and strips are then more or less isolated by undercutting and swallowed in pieces resembling strings of sausages. During this slicing of the food the first walking legs are usually free from the ground, the second may also be unsupported, but the third pair are always firmly attached to the substratum (fig. 1, A). Reference to fig. 2 shows that the jaw endoskeleton and retractor muscles, *j.s.* and *j.r.*, do not extend back beyond the third legs, and thus the body will require support at this point.

Food is rendered available not only by the slicing movements of the jaws but also by the use of salivary juice. A copious secretion is poured into the buccal cavity from the ducts of the salivary glands (figs. 1, C, and 2), and it flows at intervals past the lips and over or into the food. This juice carries out considerable external digestion. It is alternately poured out and sucked back into the buccal cavity, and so the body of an organism such as a woodlouse is completely cleaned out after it has been opened, and an empty skeleton is left with no adherent muscles. The legs may be swallowed whole. The salivary juice enzymes have been studied by

Mr. HEATLEY from extracts prepared from fresh glands dissected from the lateral body space. The juice contains an amylase, a glycogenase, a protease, and a carboxy-polypeptidase. For further details *see* HEATLEY (1936).

DIGESTION AND ABSORPTION OF FOOD

The whole of the digestive processes, other than those performed externally by salivary juice, take place in the lumen of the intestine. Regions are not differentiated, either structurally or functionally. The intestine between meals may contain relatively little fluid, and its lumen may be almost virtual owing to the folding of the walls. From the buccal cavity food is swallowed rapidly into the intestine. Fluid matter probably passes along the pharyngeal and oesophageal grooves by muscular movements of the pharynx, the main lumen of the latter being closed. During the passage of solid matter the pharynx dilates. The oesophageal muscular sheath is probably concerned with controlling the position of the oesophageal valve (fig. 4, A) rather than with direct action on the food (*see* p. 424). The intucked valve will direct food into the intestinal lumen well past the anterior limit of the peritrophic membrane, which will thus not be scraped up. The peritrophic membrane always keeps the food away from direct contact with the epithelial cells (*see* pp. 425 and 431). Digestive juice is secreted from the gland cells (*see* p. 424), and the fluid content of the intestine is increased. The p_H varies between 6 and 8·2 in different animals, but is uniform throughout any one intestine. The following enzymes are present :—invertase, maltase, lipase, esterase, amino- and carboxy-polypeptidases, and dipeptidase. For further details *see* HEATLEY (1936). Digestion is rapid, and indigestible remains, such as empty pieces of cuticle, etc., are passed out enclosed in a contracted peritrophic membrane (*see* p. 425) usually within 18 hours of the commencement of feeding.

Only fluid matter passes through the peritrophic membrane and striated border of the epithelium. The absorption of glycogen can be followed after feeding on sheep's liver, by preparing sections by Best's carmine method of glycogen demonstration. Fig. 10, B, shows the intestine of an animal which has been feeding for two hours before killing. Disorganized liver filled the anterior third of the intestine. The striated border was unstained by Best's carmine, but the distal parts of the cells and their lateral boundaries were very strongly coloured. Such a condition is not found between meals, and must indicate the uptake of glycogen at the surface of the epithelium.

STORAGE OF FOOD MATERIAL

The alimentary canal in *Peripatopsis* has two major functions besides those of digestion and absorption : (1) nitrogenous excretion (*see* p. 445), and (2) the storage of reserve materials. The so-called " fat bodies " of Peripatus referred to in the literature are in reality salivary glands, or pericardial tissue and lateral body cavity cells (*see* p. 432) which are largely excretory in function. The columnar epithelial

cells of the intestine are capable of storing large amounts of fat, carbohydrate, and an extensive protein reserve. These products are used up when food is unavailable, and will maintain the animal for three months or more. When reserve products are abundant the columnar cells are tall (fig. 11, A) and the intestinal wall is thick and usually brown in colour. When reserves are scarce the cells are short (fig. 11, C) and the intestine is thin and pale.

Fat—The extent of the fat storage in the intestine is shown by an analysis (by Mr. HEATLEY) of the lipoids in the intestine and in the body wall (*i.e.*, the rest of the body, excluding the slime glands and genitalia) from a well-fed animal and from one which was healthy but less well provided with food reserves (Table II). In both cases more fat lies in the intestine than elsewhere, and in the well-fed specimen a much greater proportion of the increased lipoids lies in the intestine than in the body wall. These were not extreme examples of either condition.

TABLE II

Condition of animals	Total lipoids in % of wet weight of body wall	Total lipoids in % of wet weight of intestine	Protein reserves
Well fed	1·42	10·23	Abundant
Less well fed	1·04	4·11	Less abundant

Fat is stored in droplets throughout the columnar intestinal cells, and is absent from the connective tissue sheath. The appearance in well-fed and starved individuals is shown in fig. 9, and fig. 26, Plate 40, representing freezing microtome

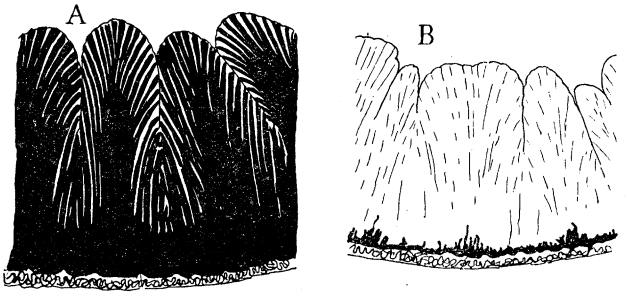


FIG. 9—Sections of intestine fixed in formol, cut on a freezing microtome and stained with Sudan III. Fat is shown in black. (A) Fully fed animal showing abundant fat reserve. (B) Starved animal showing depletion of fat which is present in small amount at the base of the columnar cells.

sections stained with Sudan III. Fat is most abundant in the lower third of the cells, but may extend almost up to the striated border (fig. 26, Plate 40) ; on starvation it persists longest at the base of the cells. Death by starvation usually occurs before the last trace of fat has been removed.

Sections cut on a freezing microtome after neutral formol fixation were stained with a variety of reagents. Nile blue sulphate showed the fatty materials to be unequally distributed through the columnar cells. When fat was abundant, and the whole epithelium orange after Sudan III, it was completely blue with Nile blue sulphate. If the fat was less abundant, Nile blue sulphate showed a pink band at the base of the cells, indicating unsaturated triglycerides, etc., while the rest of the cells were blue, indicating fatty acids, lecithin, etc. (ROMEIS, 1932). The pink band lies in the position where fat persists longest on starvation (fig. 9, B). Its presence in fat rich animals is probably masked by the extensive blue colouring.

There are no localized fat stores in other parts of the body, although a small amount can sometimes be detected in the pericardial nephrocytes and also in cells resembling nephrocytes or leucocytes in appearance, which lie wedged between the muscle fibres of the body wall (*see* p. 433).

The *carbohydrate* stored in the intestine was investigated histologically and chemically. Some rough analyses by Mr. HEATLEY were made of the glycogen content of the body wall and intestine, and also of the free reducing sugar and total carbohydrate of the intestine. In spite of the difficulties in determining the wet weight of such small amounts of tissue, good agreement was obtained between duplicate determinations. Table III shows the percentage of reducing sugar in terms of wet weight of tissue from four animals ; each value represents the average of two or

TABLE III

Animal	Body wall glycogen	Intestine		
		Free glucose	Glycogen	Total carbohydrate
1	—	0·75	—	6·70
2	—	0·35	1·68	3·30
3	0·71	—	1·63	—
4	0·78	—	2·67	—

more determinations. It will be noted that much more glycogen lies in the intestine than in the body wall, and that a small increase in the body wall glycogen is accompanied by a much larger increase in the intestinal glycogen.

The presence of glycogen within the columnar cells of well-fed animals was demonstrated by Langerhans's iodine and Best's carmine methods. Minute particles which take up carmine lie scattered through the cytoplasm in between the reserve protein spheres (*see* below). The glycogen is most abundant at the basal end of the cells and against their lateral boundaries (fig. 10, A and C). No glycogen can be seen within cells of starved or ill-fed animals.

Protein reserves are abundant in well-fed individuals in the form of minute spheres, usually about 4 μ but sometimes up to 7 μ in diameter, which are tightly packed throughout the columnar cells (figs. 11, A, 10, C, and figs. 15 and 17, Plate 38). In the fresh condition they are quite spherical, colourless, and refringent, and show no structure. They are resistant to pressure and are not readily soluble. They

stain strongly with iron haematoxylin, eosin, light green, fuchsin, Mallory's stain, etc., but their reactions are various according to the times of staining. With Mallory's triple stain they usually appear bright red (fig. 15, Plate 38), but by altered timing they will stain red with a blue interior, dark blue with a pale blue interior, or dark blue throughout. With haematoxylin they may either stain darkly, or

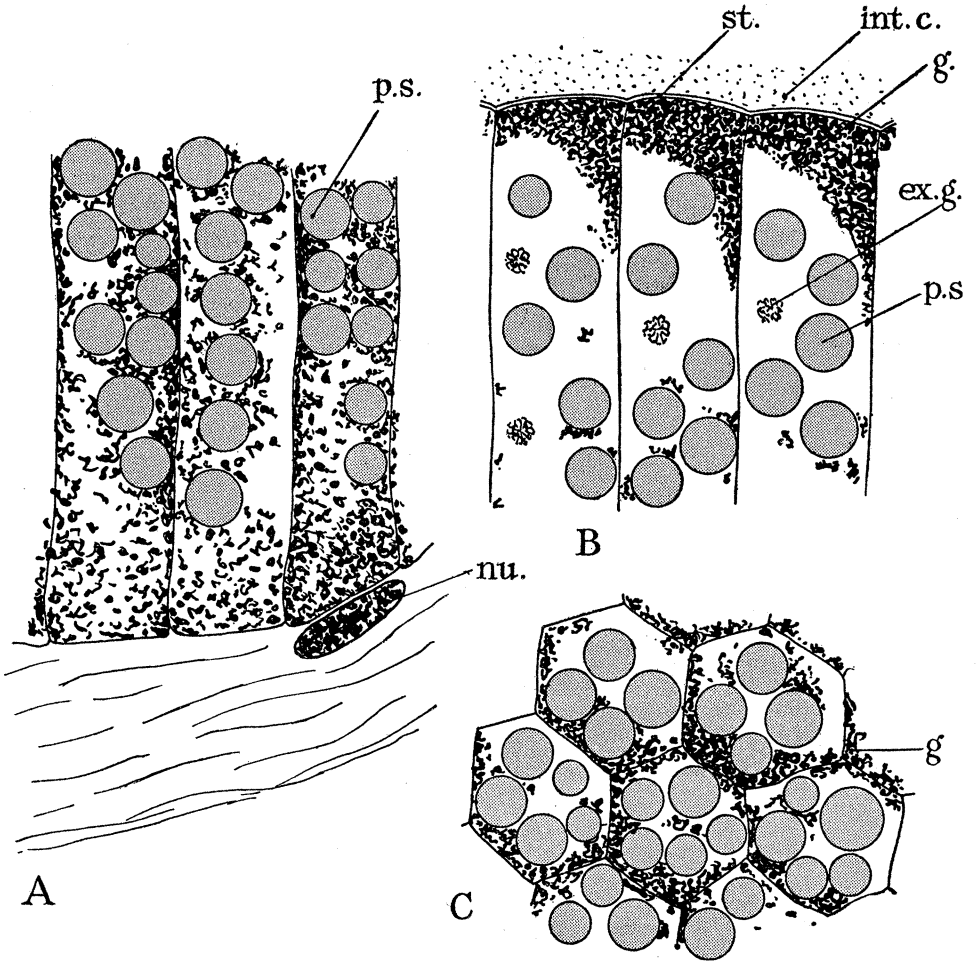


FIG. 10—Sections of intestine prepared by Best's carmine method of glycogen demonstration. The glycogen is shown in black. (A) T.S. of fully fed animal showing abundant glycogen at the base of the columnar cells. The connective tissue sheath is unstained. (B) T.S. of an animal killed after feeding for two hours on sheep's liver. The distal parts of the epithelial cells show glycogen being absorbed through the peritrophic membrane and striated border. (C) Horizontal section through the intestinal epithelium of a fully fed animal showing glycogen reserves near the periphery of the columnar cells and in between the protein spheres.

show a dark central region only. In the latter case the outer parts will counterstain with other dyes. They stain lightly with haemalum.

All perfectly healthy well-fed animals showed abundant protein spheres ; apparently healthy animals, which will feed greedily when newly captured, may show considerable reduction in the number of spheres. On starvation the spheres become

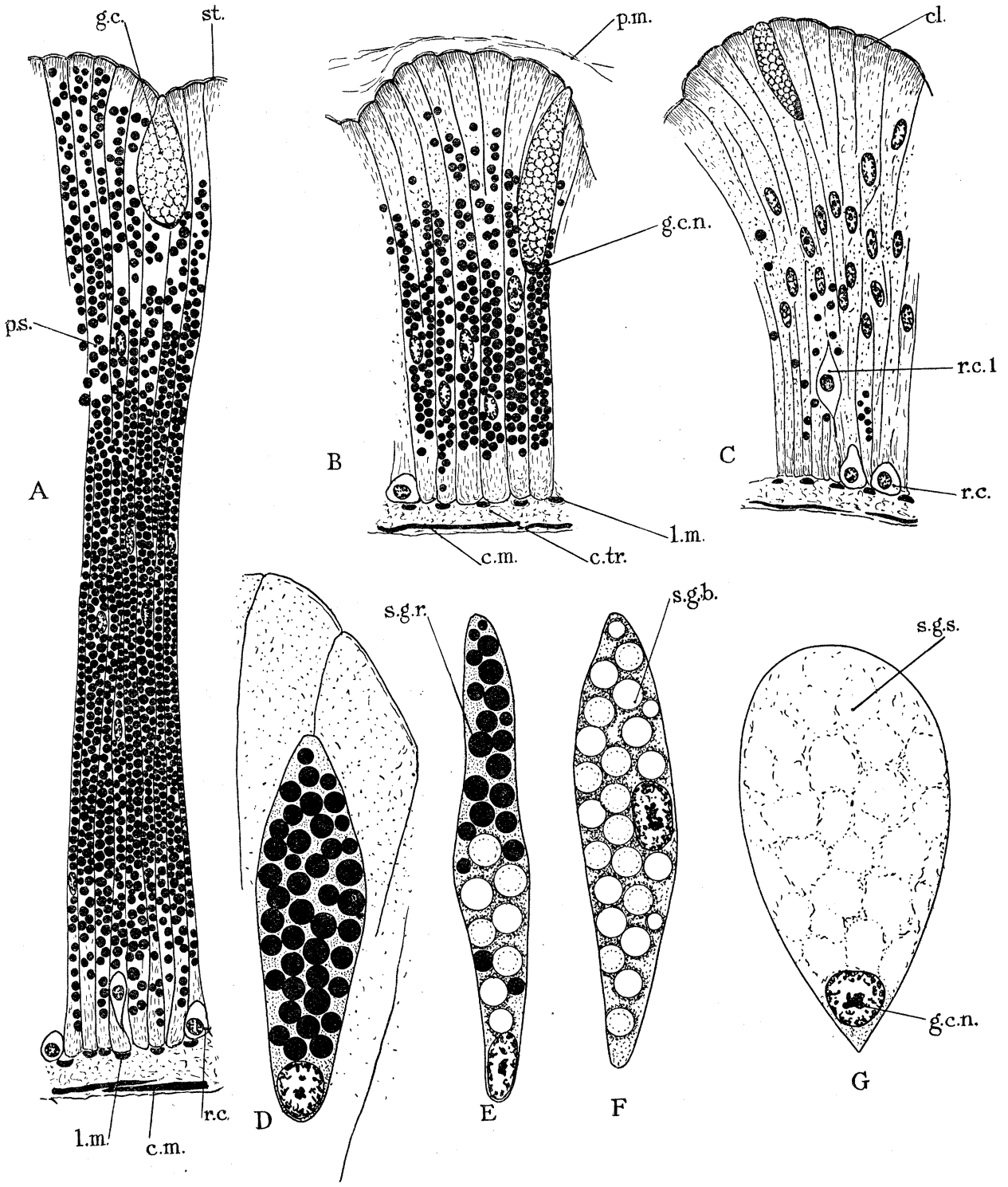


FIG. 11—A–C show the appearance of the intestine containing different amounts of protein reserve, × 310 ; and D–G show the intestinal gland cells in different phases of their secretory cycle, stained with iron haematoxylin. (A) Fully fed specimen, the columnar cells are tall and are packed with protein spheres, *p.s.* (B) Less well-fed specimen, the columnar cells are much shorter and their contained protein spheres are few. (C) Starved specimen, with almost all the protein reserve removed. (D) Young gland cell full of secretory granules staining black with iron haematoxylin (red with Mallory's stain), cytoplasm almost unstained. (E) Gland cell partly as in D, but in the lower region the secretory granules are larger and unstained by iron haematoxylin (blue with Mallory's stain), and the cytoplasm is here darkly stained with iron haematoxylin (red with Mallory's stain). (F) Gland cell completely changed to the condition seen in the lower part of E. (G) Discharged gland cell, empty spaces lie in place of the secretory granules, and a network of cytoplasm remains.

fewer and finally disappear. They persist longest in the lower half of the cells, but vacate the extreme base where most of the fat lies (figs. 11, B, and 9, B). Sick or dying animals show a scarcity or absence of these spheres. In embryos the spheres appear some time before birth ; they are similar in size to those of the parents and are consequently fewer. If the young do not feed soon after birth, their spheres disappear.

It was found possible to isolate the fresh spheres by centrifuging in the manner described on p. 414. They show the following chemical properties :—

When heated alone, the spheres blacken and smell of burning meat. They give positive reactions with the following protein tests :—xanthoproteic, Millon's, Sakaguchi's, sulphur, aldehyde test for tryptophane, and the biuret test.

They are quite insoluble in water, and are apparently unaffected by ether, alcohol, acetone, or glycerol, although the higher refractive index of the latter renders the spheres less conspicuous. They are unaffected by 2% of 10% sodium chloride.

In 2% sodium hydroxide the spheres swell up, becoming less refringent until they apparently dissolve completely. If this solution is neutralized, an amorphous precipitate is obtained ; a similar precipitate is obtained on adding picric, sulphosalicylic, or phosphotungstic acids, uranium acetate, or acid mercuric sulphate. On half saturation with ammonium sulphate a flocculent precipitate is formed, but the protein is not fully precipitated, for the filtrate still gives a faint cloudiness with sulphosalicylic acid. Even with full saturation of ammonium sulphate, the precipitation is incomplete.

In 2% sodium carbonate the spheres swell up, but they do not pass completely into solution as with sodium hydroxide. A certain small percentage of the matter does, however, dissolve.

Acetic or hydrochloric acids produce no change in the size of the spheres, although again some of the material dissolves.

When the spheres are incubated at the appropriate p_H with pepsin and with pancreatin, they are broken down completely, but a good deal of debris consisting of irregular-shaped small granules remains.

Some of the material, which had been dried over sulphuric acid for two days, was found to contain 13·1% nitrogen, as estimated by the micro-Kjeldahl method.

Thus the spheres seem to consist almost entirely of protein, although their solubility and other properties make it difficult to identify the material with any known group of proteins. It is also evident that the spheres are not chemically homogeneous.

The *relative abundance* of the various reserve materials in different individuals is recorded in Table IV. Animals in as different physiological states as possible were killed, and pieces of the intestine were fixed in Duboscq Brazil, Carnoy, and formalin for staining with Mallory, Best's carmine, and Sudan III respectively. Abundant protein spheres (as in fig. 11, A) are accompanied either by abundant glycogen and fat (figs. 9, A, and 10, A) or by a smaller amount of these substances. With

Table IV

Reserve Products in the Columnar Epithelial Cells of the Intestines.

Abundant protein spheres Abundant glycogen Abundant fat	Abundant protein spheres Some glycogen Some fat	Few protein spheres No glycogen Little fat	No protein spheres No glycogen Almost no fat	Few protein spheres Much glycogen distally Some fat	No protein spheres No glycogen Abundant fat
S.19♂ B.24♀ M.25♀ M.27♀	S.28♂ *.39♀ M.44♀	M.20♀ M.23♂ S.40♀ S.41♂	S.26♂ S.34♂ S.35♂ M.36♀ S.42♀	B.37♀ S.46♂	S.38♀ S.45♀ M.50♀
All healthy	All healthy	Two active, and two feeble and dying at ecdysis	Two active; one active and just given birth to young; one dying at ecdysis and birth of young, and one feeble and dying at ecdysis	Just fed on sheep's liver	Pathological state leading to death

The letters S, B, and M indicate *Peripatopsis sedgwicki*, *P. balfouri*, and *P. moseleyi* respectively.

* This is an undescribed species of *Peripatopsis*.

further reduction of reserves, glycogen disappears first, few protein spheres (fig. 17, Plate 38) and a little basal fat remaining (more than in fig. 9, B). When almost all the protein spheres are absent (fig. 11, C), the fat is also almost absent. Animals in the last two conditions may be obviously feeble and unhealthy, and may die at ecdysis, being unable to rid themselves of the cast cuticle.

A pathological fatty condition of the intestine is recorded in the last column of Table IV. It occurs after prolonged captivity and leads to death. Analysis of the tissues of such animals show the intestine to contain the almost incredible figure of over 50% of its wet weight as fat.

Excretion

The removal of nitrogenous waste material in Peripatus has been assumed to take place by means of the nephridia, or segmental excretory organs, which are present in almost every segment of the body. A study of excretion indicates that it occurs in this manner only to a very minor extent. Excretion of nitrogenous material is done mainly by the intestine, uric acid in solid form being removed daily by the raising and evacuation of a peritrophic membrane.

Excretion by the Alimentary Canal

The intestine is the major excretory organ of *Peripatopsis*, and eliminates (1) uric acid, and (2) excretory granules.

Uric acid is formed as compound spherical crystals showing radial components. Their diameter is usually 18–20 μ, although they vary from 5–60 μ (fig. 12, A). Occasionally compound structures may be formed with several centres, such as frequently occur in Mollusca (fig. 12, B) ; and compound masses up to 225 μ in diameter may lie freely in the intestinal lumen of late embryos. Usually the spherical crystals are single and complete ; their size bears no relation to the size of the animal, the largest occurring in unborn embryos (fig. 6, C).

The crystals appear on the inner surface of each peritrophic membrane, or between its various lamellae, just before the membrane is fully raised from the epithelium (figs. 6, A, and 7, C, *u.s.*). The time at which the membrane is raised and the duration of this process has already been described (p. 427). The crystallization must take place rapidly, probably in 1–3 hours. It has not been seen to start earlier than 3 hours after evacuation of the previous peritrophic membrane, and is complete in 6 hours after this event (Table I, last column). Up to 0·2 mg. of uric acid is crystallized daily in the intestine of a full-grown *P. moseleyi* or *P. sedgwicki*, and a smaller amount in smaller species. As soon as the membrane becomes raised from the epithelium, uric acid crystallization stops. The membrane contracts to form a narrow tube, which may be white and opaque with the contained mass of uric acid crystals (fig. 6, B). Here the crystals remain until the tube is evacuated about 18 hours later. In late embryos which are about to free themselves

from the stretched egg membrane, the first peritrophic membrane is evacuated with its contained uric acid crystals, and remains in the oviduct until the birth of the young.

The p_H of the inside of the contracted peritrophic membrane is usually about the same as that of the intestine in any one animal, *i.e.*, about 7·0, but it is sometimes more acid or more alkaline by up to 0·5 of a p_H unit.

The vertical columns in fig. 13 record the daily dry weights of evacuated peritrophic membranes for two animals kept in isolation. The black part of the columns represents their uric acid content determined by analysis (by Mr. HEATLEY). Many animals were so kept and gave similar results. Specimen A reacted unfavourably

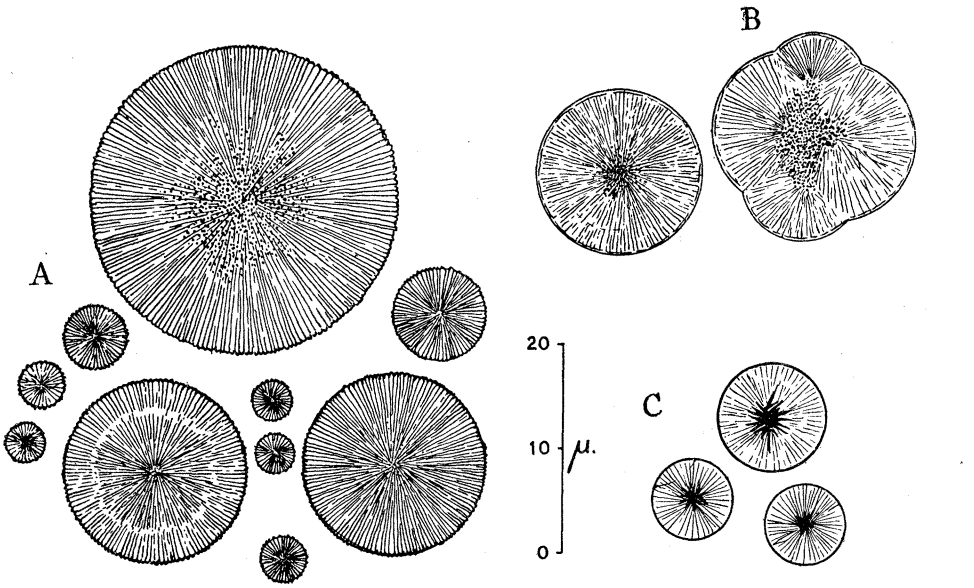
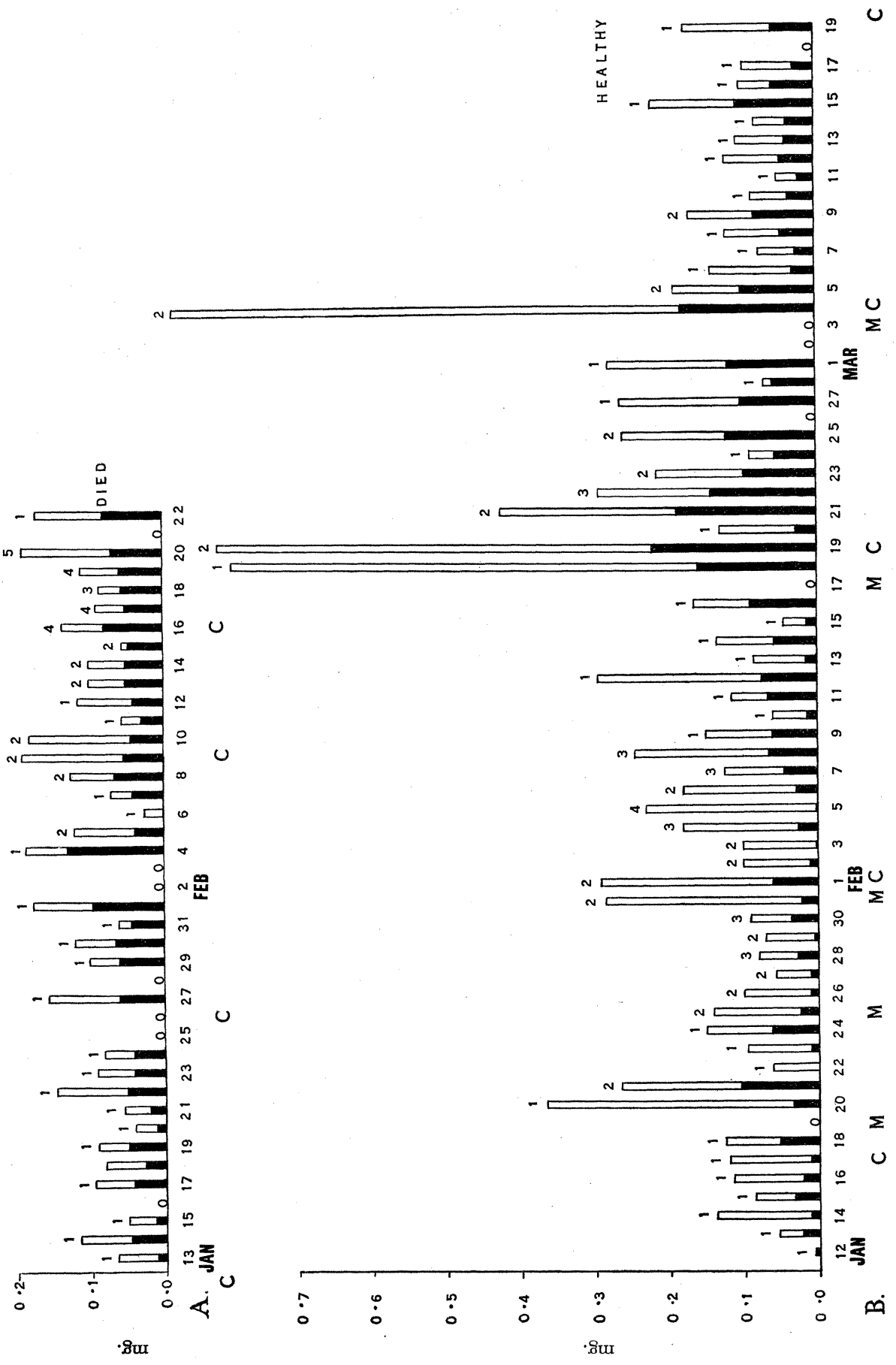


FIG. 12—Diagrams showing the size and shape of uric acid crystals of (A) *Peripatopsis*, (B) *Helix*, and (C) Pigeon. Those of the bug *Rhodnius* are only 3–4 μ in diameter but show radial striations.

to the conditions, did not feed, and became progressively more pathological and finally died, while specimen B fed greedily at the times marked "M", and recovered from its initial reactions and became normal. Both animals were usually inactive, their normal habit. The uric acid excretion is seen to be irregular in amount, even in a healthy animal (fig. 13, B). The meals taken by specimen B were small on 25 and 31 January, but on 17 February and 3 March it ate enormously. The uric acid excreted after meals is not greatly increased. In specimen A, which was a smaller animal than B, the uric acid output was at first about the same as B, but later the animal became pathological and the output was increased. The increase, however, was not proportional to the increased output of peritrophic membranes (normally one per day is evacuated, *see* p. 431). This animal ultimately removed up to five per day (*see* numbers over the columns, fig. 13).



Peripatopsis moseleyi

FIG. 13—The vertical columns record the dry weights of the peritrophic membranes and their contents evacuated daily from two animals kept in isolation. The numbers over the columns represent the numbers of peritrophic membranes evacuated per day, and the black parts of the column indicates the uric acid content of these membranes. Specimen B weighed about 0·285 gm., and specimen A was about $\frac{2}{3}$ that size. The dates of meals taken are marked "M", and the casting of the cuticle, "C". Specimen B was killed just before it would have cast its cuticle. The cast cuticle was eaten in all cases. Both animals behaved normally for about a fortnight and then showed unhealthy reactions to the environment (*see* text); specimen A became progressively more pathological and died, while specimen B recovered and became perfectly normal. Indigestible food remains are responsible for most of the increased weight of the peritrophic membranes evacuated on 18 and 19 February and 4 March.

The uric acid crystals have the following staining and chemical properties :— After fixation with Duboscq Brazil and Mallory staining, they present a blurred outline, due to partial solution, and are otherwise coloured a dark bluish-green (fig. 17, Plate 38, *u.s.*). The staining methods of SCHULTZ (1931) for the histological detection of uric acid did not give as consistent results as with vertebrate material, and as the crystals were in sufficient quantity for chemical examination, analysis by their staining properties was not pursued further.

It was impossible to remove the crystals from the slightly sticky and laminated peritrophic membrane. Chemical tests were therefore done (by Mr. HEATLEY) on whole excreted masses which were taken from non-feeding animals and dried and powdered. Such peritrophic membranes usually contain a small proportion of minute excretory granules and cytoplasmic buds from the endoderm, besides the large uric acid crystals and fluid. Excreta from animals which have not fed are usually white, while after a meal they are brown and bulky and thus easily identified. Positive reactions were obtained for uric acid by Benedict's and the murexide tests, and for ammonia ; and faintly positive results were given for protein by the xanthoproteic and Sakaguchi reactions. A negative test for urea resulted from a micromodification of the standard urease test, which was capable of detecting one gamma of urea. Calcium was present, giving a precipitate with potassium oxalate in acetic acid solution. Chloride was faintly positive, and phosphate and sulphate negative.

A number of the whitest excretory masses (*i.e.*, those containing least material other than uric acid crystals) were dried together and analysed for total nitrogen, uric acid, and ash. The average of three such experiments is given in Table V in terms of the percentage of dry weight of the excreta. Ash A was obtained by calcining the material alone in a platinum boat, and ash B was obtained by moistening the same residue with sulphuric acid and again heating.

TABLE V

Total nitrogen	Uric Acid	Ash A	Ash B
29·25	31·6	6·35	9·05

Owing to the impossibility of separating the crystals from the laminae of the peritrophic membrane, it was not possible to determine what proportions of uric acid were free and in the form of urates. The ash analysis suggests that very little free uric acid is present ; and the p_H of the crystalline masses, and the presence of cations, and relative absence of anions, support this conclusion.

The solubilities of the crystals were determined by sealing up a few in hollow slides, and observing microscopically during periods up to 48 hours or more. They are insoluble in distilled water, and in 2% and concentrated acetic acid. In 10% hydrochloric acid they dissolve in less than an hour if very few crystals are present ; if there are many, some may become transparent after three days, but most remain

unchanged. On solution in hydrochloric acid they leave a ghost-like stroma with a central spot appearing darker than the rest. In 10% ammonia the crystals dissolve in 10–60 minutes ; the central parts vanish first so that a hollow sphere of increasing thinness is formed, and a stroma remains, consisting apparently of a spherical shell the size of the original sphere, and after 24 hours this may also vanish. During solution in ammonia minute rod-like granules (presumably ammonium urate) appear in a few minutes all over the slide. This deposition of uric acid does not occur with hydrochloric acid. On irrigation with 10% sodium hydroxide the crystals vanish instantly, leaving no trace.

The mechanism of the formation of the uric acid crystals has not been elucidated. They are undoubtedly extracellular in origin and rapid in deposition. Uric acid particles are never visible in the living epithelial cells, although on fixation minute irregular particles, of a pale green colour after Mallory staining, may be seen in the columnar cells of a few individuals (7 out of 65 examined). These particles are most numerous in the distal parts of the cells, and they never resemble even the smaller of the free uric acid crystals in structure. An extreme case is shown in fig. 15, Plate 38. Here the green particles are abundant, and are dark in colour like the free spheres. Possibly the abundance here may be abnormal and correlated with a heavy infection of intestinal coccidia, but the animal was healthy and contained plenty of food reserves, and was in a much fitter state than many other captive animals. Uric acid or urates must be present in abundance in solution in the epithelial cells prior to crystallization in the gut lumen, and this uric acid might be precipitated by reagents of fixation, so forming the green particles. However, these cannot be the precursors of the free crystals, as they are unlike them in structure, and have never been observed to pass out of the cells. They may be comparable to the uric acid granules which sometimes occur in kidney tubule cells of Sauropsida (WINTERSTEIN, 1921).

The similarity in size of many uric acid crystals and the " bubbles " from the intestinal cells (*see* p. 427) suggests that possibly these provide an organic basis upon which the crystalline structures form, and the stroma remaining after solution would thus be the remains of this organic basis. No confirmation of this suggestion could be obtained, and as the " bubbles " form outside the peritrophic membrane, and not inside it, it is more likely that the bubbling is concerned with the raising of the membrane rather than with uric acid crystallization.

The intestine is not divided functionally into regions in connexion with uric acid crystallization and circulation of base, as are the Malpighian tubules of *Rhodnius* (WIGGLESWORTH, 1930). Ligaturing of *Peripatopsis* in different regions of the body immediately after evacuation of a peritrophic membrane did not interfere with the deposition of crystals in all parts of the intestine, although the gut lumen was occluded and the haemocoel completely constricted by the ligatures.

Excretory granules, which are not of a uric acid nature, are elaborated within the columnar cells of the intestine. They are of various types and are readily seen in the living state. The inner surface of the intestine may appear various shades of

brown owing to numerous minute particles lying in the distal parts of the columnar cells. In fixed sections they may be scarcely visible, or when large appear as irregular yellowish particles (fig. 7, E, and an extreme condition is seen in fig. 17, Plate 38, *ex.g.*). In the living animal these particles are often found in numbers on the outside of the peritrophic membrane when it is lying free in the intestinal lumen, and brown particles often colour the outside of an evacuated membrane and are present in the fluid passing out with it. Such particles probably leave the columnar cells when the membrane is lifted, as the striated border then shows a naked surface and a fringe-like structure (fig. 7, D, and E).

Another type of intracellular particle which is probably excretory is shown in fig. 16, Plate 38, *ex.g.* It is less frequent in occurrence, larger in size, yellow, and with a smooth, more or less spherical outline. These particles are also found in the lumen of the intestine, but always within the peritrophic membrane and enclosed in a cytoplasmic bud from the epithelial cells (fig. 16, *bu*, Plate 38). It is unlikely that such large particles could leave the columnar cells in a manner other than by inclusion in a bud (*see* p. 427). This budding may be a disintegrative process, as in insects, but it is also a means of removing large intracellular concretions (*see also* p. 457). It may at times also result in the loss of a few reserve protein spheres (fig. 15, Plate 38).

An excretory function of the gut was first suggested by BRUNTZ (1904) when he injected indigo-carmine into the perivisceral haemocoel, and detected this substance 14 hours later as minute crystals in groups within the columnar cells. This experiment was repeated but not confirmed ; however, the available material was too scanty for any definite statement to be made. It is hoped that further work will be done shortly upon the uptake of substances from the haemocoel by the intestine. This organ must have the power of absorbing waste materials of various kinds from the blood and getting rid of them in the above ways.

Excretion by Accumulatory Organs and Blood Filtration

The excretory organs of the Arthropoda may be divided into two groups, those which pass excretory products to the exterior, such as the intestine and nephridia of Peripatus, and those which accumulate excretory products, as do the nephrocytes and uric acid cells of insect fat bodies, etc. *Peripatopsis* is provided with accumulatory organs in the form of small uninucleate nephrocytes in the pericardial network and lateral body space (*see* p. 432 and figs. 23 and 24, Plate 40).

By injecting substances into the body cavity an attempt was made to follow the uptake of waste material by both accumulatory and rejectory excretory organs, and the subsequent removal of such substances by the latter. Such methods have been successfully employed by various workers. On vertebrate material removal of materials by the kidney have been followed by MOLLENDORF (1915 and 1919) using trypan blue, trypan red, and Bayrisch blue ; by GURWITSCH (1902) using toluidin blue, neutral red, methylene blue, etc. ; while KABREHEL (1886) and others

have employed indigo-carmine. On the Arthropoda BRUNTZ (1904) injected ammonia- and indigo-carmine into the haemocoel of a number of types, including Peripatus, and followed the fate of these substances.

BRUNTZ showed that ammonia-carmine injected into Peripatus was absorbed by cells in the pericardial network and along the lateral longitudinal muscles and also at the base of the legs (his description of the cells performing this function is erroneous, *see* p. 432). Such experiments were repeated using fine ground " non-toxic " indian ink in salt solution, such as used for intravitam injection of chick embryos, and kindly supplied to me by Dr. A. F. W. HUGHES. The animals usually stood the injection well. They were killed at different times after injection, and parts were fixed with Duboscq Brazil and sectioned. Ink particles are rapidly taken up by blood leucocytes and by the uninucleate nephrocytes of the pericardial network and lateral regions. In a few hours the ink uptake is so extensive that black patches are formed along the sides of the body, similar to the red patches found by BRUNTZ, and a black layer is formed on the lower side of the pericardial network. The appearance of the network 5 hours after an injection into the ventral perivisceral haemocoel is shown in fig. 24, Plate 40. The nephrocytes are so packed with ink that their normal excretory inclusions and cell boundaries are masked. The large multinucleate cells of all regions show no uptake of ink.

It will be noticed that the nephrocytes close to the pericardial floor have taken up more ink than those near the pericardial cavity (fig. 24, Plate 40). This probably indicates that the normal direction of flow of the blood is from the perivisceral haemocoel, through the ostia of the pericardial floor and into the pericardium, by passing through the pericardial network. Blood and ink from the perivisceral haemocoel must be carried first to the nephrocytes bounding the spaces above the pericardial floor *p.h.* (*see* p. 433), where most of the ink appears to be absorbed, and what remains passes through the intercellular channels, *h.s.p.*, between the large cells, and is absorbed by nephrocytes more dorsally situated. No ink was found in the pericardial cavity or heart, although large quantities were often injected below the pericardial floor. Thus the pericardial network nephrocytes constitute an efficient filter which rids the blood of solid waste matter before it reaches the heart.

It is possible that the lateral nephrocytes also filter the blood reaching the heart by another route. GAFFRON, in his masterly work of 1885, showed that the pericardium communicates with an intramuscular canal system in the body wall by a series of dorsal openings, about 12 per segment, which pass into circular canals lying between the circular and diagonal muscles. These canals can be followed to the lateral haemocoel, and to other smaller spaces which open into the pericardium on either side of the middle line (fig. 23, Plate 40, *o.b.l.*). If the blood flows from the legs and lateral haemocoel, up the sides of the body through this canal system, to reach the pericardium, then it must flow over the lateral nephrocytes, and will be filtered by them to a certain extent. Sections of ventrally injected animals suggest that this is so, as only a little ink finds its way into the intramuscular canal system, while the lateral nephrocytes are saturated. These nephrocytes,

lying almost freely in the haemocoel and in patches on the muscles, do not form a continuous filter across the blood stream as they do over the pericardial floor.

Injection of neutral red into the haemocoel resulted in the rapid uptake of the red colour by the walls of the crural glands, so that red bladders appeared in the limb bases 1–3 hours after injection. No excretion to the exterior could be detected. No colour was picked up by the nephridia.

Excretion by "Nephridia"

Nitrogenous excretion in *Peripatopsis* is accomplished largely by means of uric acid elaborated in the intestine and removed daily (*see* p. 445). The function of the nephridia has been investigated by noting their frequency of discharge and by analysis (by Mr. HEATLEY) of the nephridial fluid naturally evacuated and collected directly from the organs.

The nephridia are usually discharged simultaneously, so that the animal deposits on a non-absorbent surface a double row of droplets, one pair from the exit tubes of each segment. The distance apart of these droplets indicates that they are nephridial in origin and are not deposited by the crural glands. By frequent observation, these droplets can be freshly collected in a glass capillary. About 10 c.mm. may be evacuated by a large *P. moseleyi* or *P. capensis*. The frequency of discharge is not certain, as the only method of determining this is to keep isolated animals in a damp atmosphere on a clean non-absorbent substratum, such as glass, with damp wool above. They frequently do not live well thus, as they prefer damp crevices and each other's company. These conditions are not physiologically harmful, as some individuals accustomed themselves and lived indefinitely. Nearly all animals discharge their nephridia within a few hours of confinement in a glass dish. A second discharge rarely occurs within 24 hours ; sometimes it takes place after 3 or 4 days and often after one or more weeks. Animals discharging at intervals of two or more weeks appeared to be perfectly healthy. It is probable that nephridial discharge is irregular and infrequent.

Another method of collecting nephridial fluid was direct from animals being chloroformed for other purposes. An extreme contraction of body muscles is the first reaction to the vapour, and is followed by relaxation. The contraction causes fluid to appear in droplets at the nephridial exits, where it can be collected with a capillary tube. Only the first few droplets were used from each nephridium, as extreme contraction causes abnormal filtration of blood through the end sac (*see* below).

The natural excretion, and that collected on chloroforming, are both distinctly acid, having a p_H of 4·0–5·0.

If dried, the natural excretion shows feathery sheafs and fans of crystals. These are very soluble, for they dissolve when breathed on. When heated at low temperatures the crystals melt, or dissolve in their water of crystallization. On strongly heating the material remains molten and does not char ; the residue dissolves in water to give a neutral or faintly alkaline solution.

The soluble material in nephridial fluid is small in amount. About 50 natural excretions, made during several weeks from all the nephridia of a number of animals, were collected in the manner described above and dried. The crude weight of the solids was only 1·19 mg. The gross impurities (broken ends of capillaries, etc.) were removed by dissolving the solids in a small amount of distilled water, filtering, and again evaporating to dryness in a desiccator. The weight of the solids was then 0·97 mg. This is a great contrast to the large daily excretion of about 0·2 mg. of uric acid from the intestine of one animal. Part of the nephridial solids were dissolved in water and tested as follows :—

The p_H was 3·7–4·0 as determined colorimetrically with brom-phenol blue.

Chloride, sulphate, and phosphate were present.

A micro-modification of the urease test, sensitive to one gamma of urea, was always negative. A strong positive reaction for ammonia was shown by FEIGL's (1933) test, and no uric acid could be detected.

Only faint traces of protein could be detected by the xanthoproteic reaction (the fluid volume was kept as low as possible, and observed end on in a capillary tube, and compared with a control), and no precipitate could be detected with picric or sulphosalicylic acids.

Traces of calcium were detected by the reaction to potassium oxalate in acetic acid solution, and the delicate Titan yellow test (Kolthoff) for magnesium gave only a very faint reaction.

A very approximate estimation of the amount of ammonia present in an aliquot portion of the solids was made by the method of STANFORD (1923). Two values of 7·2% and 6·1% ammonia were obtained. These results are considered on p. 459.

The mechanism of urine formation has been studied in these specimens of *Peripatopsis* by PICKEN (1936). He found the hydrostatic pressure of the body fluid to be *c.* 10 cm. H_2O, and concludes that filtration is a possible factor in urine formation. It has been noted that an increase in internal pressure caused by either extreme muscular contraction of the body wall (*see* above), or by injection of fluids into the haemocoel, results in formation and extrusion of an abnormal amount of fluid from all the nephridia. That this increase is due to filtration, and not to rupture of the organ so that blood pours out, is probable, because a lesion sometimes is certainly produced, and results in a huge discharge of blood containing leucocytes from a single nephridial orifice. The end sac is probably the site of such filtration, as it is very thin-walled and is surrounded by haemocoel. The behaviour of the salivary glands under abnormal hydrostatic pressure supports this suggestion. Under such conditions fluid, which is devoid of enzymes, passes out from the salivary duct in a volume greatly exceeding that of the lumen of the thick-walled gland. The salivary gland end-sac is exactly similar to those of the nephridia, and as the organ is thick-walled elsewhere, it is probable that filtration occurs through the end-sac walls.

Dr. PICKEN measured the colloidal osmotic pressure and vapour pressure of both blood and urine. In 7 out of 9 cases the urine was hypotonic to the blood. Any

difference in vapour pressure of blood and urine indicates a difference in concentration of these fluids, and secretory work must be done by the nephridia to account for this. He concludes that secretion and resorption almost certainly occur in the formation of urine (for further discussion *see* p. 459).

Discussion

The Feeding Mechanism

The feeding mechanism of the Onychophora appears to rank as a peculiarly specialized and unique feature of the group, as it differs in both structure and use of the parts concerned from all other groups of Arthropoda. It has formerly been appreciated that the jaws are unique among arthropods in that the two blades of each represent the enlarged terminal claws of the walking legs set on a very short base, and that biting is thus performed with the tips and not with the bases of the limbs. The whole musculature and the movements of the jaws, and the absence of a post-mandibular adductor muscle and tendon, contrasts absolutely with the Myriapoda, Crustacea, and Insecta. It is probable that terrestrial feeding organs in the Onychophora have developed from annelid-like ancestors in complete independence of other arthropodan groups, and in an entirely different manner. The efficiency of the lips, pharynx, and jaws in securing and feeding on active prey is remarkable, since neither prehensile limbs nor slime ejection is employed in food capture. The antagonistic arrangement of muscles, which will maintain body shape and give a powerful pull at localized points, despite the absence of a rigid skeleton, is noteworthy.

The structure of the sensory spines of Peripatus has been examined by Balfour (1883), Gaffron (1885), Schneider (1902), and more recently by Hanstrom (1935), but the only worker who appears to have obtained good fixation of these organs is Dubosc̨q (1920), who followed the development of the spines of the major surface papillae. He believes the sensory cells to be bipolar, but he could not detect the distal part of the sensory cells penetrating the interior of the spine, as represented by Schneider. He concludes that the spines on the primary papillae differ in no essential points from sensory spines of other Arthropoda.

The taste organs of the tongue have been partially described by Fedorow (1929) and Hanstrom (1935). The latter paper was published after the observations and figures recorded here were completed. Hanstrom shows microphotographs of a surface sensory spine and of a taste spine in section, but his fixation shows few details. The good fixation here obtained of both spines and capsules supports the view that the sensory cells are bipolar, but as cell limits are not apparent within the capsules although plain elsewhere, and as single nerve fibrils are impossible to follow far, this interpretation cannot be proved. In the taste spines cytoplasmic fibrils running from near the sensory nuclei into the core of the spine are very clear, and there is no doubt that the nerve fibrils entering the capsules extend near to

the sensory nuclei. The taste spines appear to be open distally. In this they represent a unique arthropodan sense organ which will function where the cuticle is wet, and may well be regarded as a peculiar specialization of the Onychophora. The wide distribution of these organs with long or short spines over lips and tongue has not been recorded previously, and their presence doubtless accounts for the habit of the animals of sensing its food with lips and tongue before feeding.

The Peritrophic Membrane

The presence of a peritrophic membrane together with its formation and removal has not been recorded before for the Onychophora; the inner surface of the intestine has even been described as "mit Wimpern versehene" (ZACHER, 1933)! The occurrence and formation of peritrophic membranes in the Arthropoda has been reviewed by VON DEHN (1933). Such a membrane is present in a few Crustacea (*Cyclops*, *Daphnia*), in Myriapoda, and in many Insects. The extreme thinness of the membrane in *Peripatopsis* is probably associated with its rapid removal and replacement in this animal; in a copper underwing caterpillar the same size as *Peripatopsis* the peritrophic membrane is at least 8 μ thick.

The peritrophic membrane in *Peripatopsis* resembles the chitinous lining of the mid-gut of Crustacea and the peritrophic membrane in Insects in being composed of pure chitin, a cuticle covering only the chitin of the fore- and hind-gut and of the body surface. The rapid digestion and absorption of food in *Peripatopsis* is made possible by the absence of cuticle in the intestine, pure chitin being "a simple diffusion membrane" (YONGE, 1936). The cuticle present elsewhere is probably both protective and semi-permeable, as it is in Crustacea. The peritrophic membrane probably has a mechanical function of protecting the intestinal epithelium, as in Insects, there being no mucous glands.

Peritrophic membranes in Arthropoda are formed usually in two ways, either from the whole surface of the mid-gut (many Hymenoptera, *Cyclops*) or from a localized anterior ring aided by an oesophageal valve (Diptera, etc., *see* WIGGLESWORTH, 1930). The oesophageal valve of many Insects bears a great functional resemblance to that of *Peripatopsis*, which is constructed to carry out similar movements by different means. The combined action of the outer muscular sheath and the vascular sinus will give the same movements in *Peripatopsis* as are performed in the Insects by the sphincter muscle, blood sinuses, and longitudinal muscle situated in the valve itself (WIGGLESWORTH, 1933, figs. 1 and 2). The function of the valve in *Peripatopsis* is not that of a press for rolling out the peritrophic membrane, as in some insects, as the membrane is formed throughout the whole intestine, but its movements can loosen and push back the anterior end of the peritrophic membrane and so start its evacuation. Its other function as a funnel in directing food into the intestine without scraping up the membrane may be of importance in other Arthropods as well as in *Peripatopsis*.

The daily removal of the entire peritrophic membrane contrasts with other Arthropoda where it usually wears away posteriorly and is renewed anteriorly.

This feature may be regarded as a specialization correlated with excretion, as it provides the mechanism for the removal of the uric acid crystals.

The Intestine

The *variety of major functions performed by the intestine* of *Peripatopsis* is unusual; yet this organ, capable of dealing with (1) digestion, (2) excretion of nitrogenous waste, and (3) storage of reserve food sufficient to maintain life for three months, shows a complete absence of organ development and of histological specializations correlated with these important functions. In other Arthropoda the alimentary canal frequently shows regional differentiation, diverticula, and histological specializations concerned with digestion; Malpighian tubules concerned with nitrogenous excretion; and a fat body for food storage and other functions. In *Peripatopsis* only two types of cells make up the uniform gut, and the only special adaptation seems to be the daily removal of the peritrophic membrane (*see* above).

That *food storage* is done mainly by the intestine is certain. The large multinucleate cells of the pericardial network and lateral haemocoel may be comparable to insect oenocytes, but they do not store fat or solid inclusions, and their function is unknown.

Reserves in most animal groups occur within the cell as fats, albuminoids, and occasionally glycogen. Protein crystals, crystalloids, and "albuminoid spheres" have been described in the fat bodies and intestinal cells of insects (*see* SCHNEIDER, 1928, UVAROV, 1928, etc.), and BERLESE (1899) figures protein spheres of various sizes within the liver cells of *Tegenaria*, and other arachnids, which disappear on starvation. These may be comparable to the protein spheres in the intestine of *Peripatopsis*, but do not approach the latter in abundance.

It was noted that the output of uric acid after a meal was not much enhanced (p. 446). It is therefore probable that the food protein is stored as such, and that deamination takes place before its use, and not, as in carnivorous vertebrates, before storage.

STRAUS (1911) has shown that glycogen in insects may be of the greatest importance as a reserve substance. The high glycogen content of bee larvae he believes to be due to the high carbohydrate content of their food. In *Peripatopsis* it might be supposed that the glycogen content of the liver fed to them might be responsible for the extensive store of this substance in the gut, but this reserve can also be found in animals from the wild. Unlike the bee, glycogen is here laid down on a carnivorous and not on a vegetarian diet.

On starvation the complete utilization of the glycogen reserves before the fat in *Peripatopsis* resembles what occurs in many other vertebrate and invertebrate animals. The disappearance of the protein spheres before the fat may have a parallel in the disintegration of albuminoids, leaving the fat during development of the imago in some insects (SCHMIEDER, (1928).

The large amount of fat in the food reserves of *Peripatopsis* may be corellated with the need to conserve water. The combustion of fat provides an animal with more

than double the amount of metabolic water produced by the combustion of protein or carbohydrate, " and there is a strong emphasis on the oxidation of fat in animals living under conditions of acute water shortage " (BALDWIN, 1937).

The *digestion* of animal food by salivary and intestinal juice is very efficient and rapid in *Peripatopsis*. External digestion by salivary juice makes the feeding mechanism possible on fairly large and hard food. In many specialized insects and other arthropods (LENGERKEN, 1924) there is an elaborate mechanism for the extrusion of salivary juice which is absent in *Peripatopsis*. The digestive enzymes of salivary and intestinal juice are, as usual, directly correlated with the type of food eaten. They are suited to a carnivorous diet, the large molecules being broken down by the salivary juice and the small by intestinal enzymes. For further discussion, *see* HEATLEY (1936).

Excretion by the Intestine

The excretory activity of the intestine (1) by means of particles formed within the columnar cells and (2) by the daily formation of uric acid crystals in the lumen is remarkable. The former method is also found in other arthropods ; excretion through the walls of the mesenteron is reported for some insects (SNODGRASS, 1935), and this method appears to be of greater importance in some myriapods. Sections of intestines of *Lithobius*, kindly lent to me by Miss VINCENT, show two states as indicated in fig. 14. The columnar epithelial cells in " A " show numerous brown inclusions distally, while in " B " these particles have all passed into the lumen, which is packed with cytoplasmic buds containing all the inclusions. This condition seems to be an exaggerated form of events occurring in *Peripatopsis*.

Excretion by means of *uric acid* is a method commonly employed by terrestrial animals which need to conserve water. The Onychophora can now be placed with the terrestrial Gastropoda (NEEDHAM, 1935), Insecta, Diplopoda, and Sauropsida which exhibit this type of metabolism. In *Peripatopsis*, as in members of the other groups, the water lost at evacuation of the solid excreta probably is not more than is required for the mechanical passage of such matter from the body ; but *Peripatopsis* lacks any special mechanism for absorption of water from the excretory mass just before it leaves the body, as is provided by the cloaca of birds and the rectal glands of insects, and so the excretory masses are far less dry than those of insects and many birds.

The association between uricotelic metabolism and development within a cleidoic egg among terrestrial animals has been stressed by BALDWIN (1937). *Peripatopsis* is viviparous, but there is reason to suppose that oviparity characterized the Onychophora of the past. It is interesting to note that the embryo of *Peripatopsis*, unlike those of the mammals, is not dependent on its mother for the removal of waste materials (except in early stages), since uric acid is stored in solid form and eliminated just before birth ; in this *Peripatopsis* resembles the Sauropsida in which uric acid accumulates in the allantois and is separated at hatching.

The mechanism whereby urates are deposited are not fully known in terrestrial animals. Respecting *Peripatopsis*, nothing can be advanced beyond noting the

absence of zones of secretion and reabsorption of base, such as are seen in the Malpighian tubules of the insect *Rhodnius*. This may indicate that the mechanism in the Onychophora is unlike that of the insect. The rapidity of the crystallization of uric acid, mainly as urates, in *Peripatopsis* resembles its deposition in the kidney duct of the Sauropsida (WINTERSTEIN, 1921).

Only in the Gastropoda are the uric acid crystals formed intracellularly, and compound spheres are here more abundant than in *Peripatopsis* and other groups

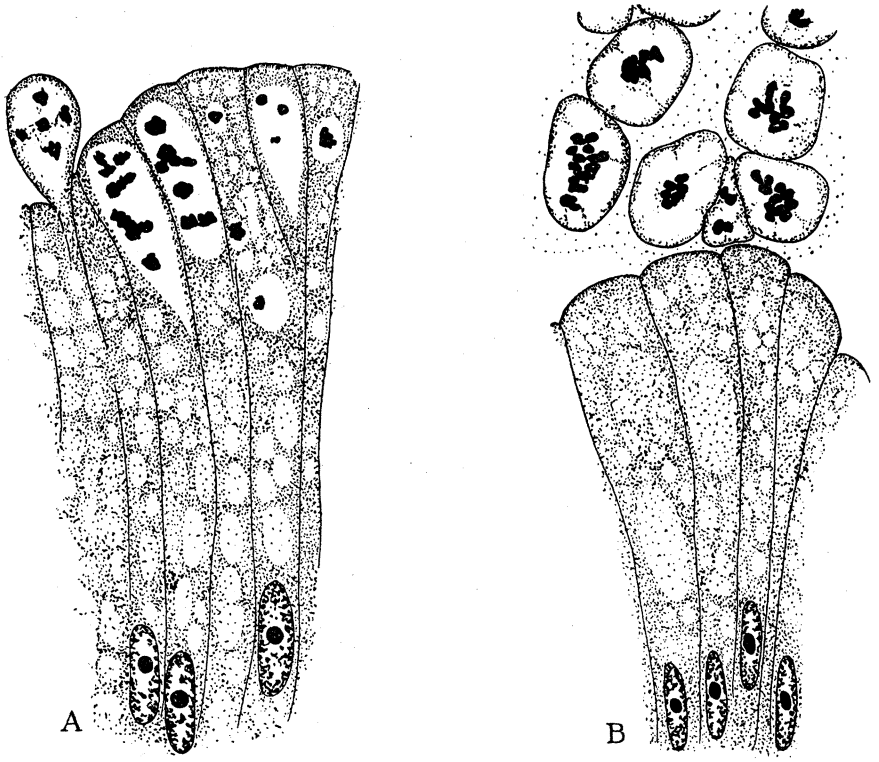


FIG. 14—Sections of the inner ends of the intestinal epithelial cells of *Lithobius* showing two states of the cells. (A) shows numerous distal yellowish or brownish inclusions in the columnar cells, and in parts (as on left side) budding from the surface seems to be starting. (B) appears to represent a later stage of the process starting in A. Budding has ceased, the columnar cells are devoid of inclusions ; these have all passed into the buds, which fill the lumen of the intestine. (Compare with budding in *Peripatopsis*, Plate 38.)

where the crystals are formed freely in a duct lumen. The size of the crystals in *Peripatopsis* is independent of the size of the animal, and greatly exceeds those of other groups (fig. 12). The solubilities of the crystals differ from those of the Sauropsida (MEISSNER, 1868) and *Rhodnius* (WIGGLESWORTH, 1930) in that they do not dissolve in either dilute or concentrated acetic acid. Their reactions to ammonia and caustic soda are the same as described by the above authors, but after treatment with hydrochloric acid no separation of uric acid crystals occurs after the initial rapid solution, as takes place in *Rhodnius*, Reptiles, and Birds.

The difference between *Peripatopsis* and other Arthropoda in the size and properties of their uric acid crystals, and in the methods of formation and removal of these crystals, indicate an independent acquisition of this type of metabolism by the ancestral Onychophora.

Nephridia or Segmental Excretory Organs

The presence of a long series of well-developed nephridia, structurally resembling the segmental excretory organs of other Arthropoda, may seem remarkable in view of the fact that they are unimportant in removing nitrogenous waste, the intestine having taken on this function. The only nitrogenous matter which could be detected in the nephridial fluid is ammonia, and it is noteworthy that this is also the chief excretory product of annelid nephridia (DELAUNAY, 1931). Nephridia, however, also regulate both salt and water content of the body. " The observation that the urine of *Peripatopsis* is hypotonic to the blood is at first sight surprising since *Peripatopsis* is a terrestrial animal and might be expected to conserve water rather than salt. On the other hand, *Peripatopsis* empties the nephridia comparatively infrequently . . . , so that as compared with an aquatic organism the amount of water lost in this way is much reduced (*Potamobius* excretes 4% of its weight per day (HERRMANN, 1931) . . . . The hypotonic urine might perhaps be interpreted as a functional vestige surviving from a time when nitrogenous excretion took place through the nephridia, and when, moreover, the retention of salt was an important factor in the control of the internal medium ". PICKEN (1936).

Further, the work of HEATLEY indicates the possibility that the nephridia of *Peripatopsis* are of real importance to the animal in another way. *Peripatopsis* is carnivorous, and therefore there will be an excess of anions over cations to be excreted (sulphates and phosphate from the proteins). The nephridial fluid is always markedly acid, the p_H often being as low as 4·0, and contains 7% by weight of ammonia in the dried solids. A brei of the body wall and nephridia gives a positive test for urease. No urea can be detected in the nephridial fluid, but it is possible that it is actually formed and escapes detection (the earthworm was thought to excrete ammonia only until DELAUNAY (1931) with improved technique showed it to excrete urea as well).* Thus the nephridia may have the power of converting neutral urea into alkaline ammonia, and they may be important in the excretion of soluble metabolites, such as phosphate and sulphate ions, these ions being partly neutralized by nephridial ammonia.

Blood Circulation

Indirect evidence on the course of blood circulation indicates that it reaches the pericardium partly by the ostia in the pericardial floor and partly by the intramuscular canal system (*see* p. 451). The former is a most unusual route, and the

* This suggestion is supported by the fact that a brei of that part of the body wall containing the nephridia repeatedly has been found to contain urease. Too much importance, however, must not be attached to this since the dorsal body wall also gives a positive reaction but to a less marked extent.

latter takes the place of the common arthropod channel passing from the outer side of the limb base, up the side of the body external to the pericardial floor. The pericardial floor in *Peripatopsis* is fused to the lateral musculature high up, as occurs in other arthropods intersegmentally, and segmentally where limbs are wanting or reduced (MANTON, 1928, fig. 15). The supply of blood to the median and lateral haemocoelic cavities must be mainly from the anterior end of the heart, which extends above the anterior part of the pharynx, and opens by a ventrally directed valve into the haemocoel just behind the dorsal attachment of the tongue retractors. The pericardial floor here becomes non-existent, and so the heart directly supplies the general body haemocoel. Posteriorly the heart becomes very narrow, and appears closed in the last segment of the body in the species examined. No segmental arteries leave the heart. The absence of an anterior aorta extending forwards from the heart is an unusual feature among arthropods, where an anterior pair of somites grows upwards and backwards to form an anterior aorta in many groups (MANTON, 1928). The efficiency of the filtration of the blood supplying the pericardium is also unusual.

In conclusion, it may be emphasized that the specializations seen in the mechanism and organs associated with feeding, and in the uricotelic metabolism of *Peripatopsis*, indicate the independence of this evolutionary line among arthropodan groups.

SUMMARY

The oral parts are described. The circular lip can be extended as a tube or completely closed.

The lips and tongue are provided with numerous chemo-sensory organs of a unique arthropodan type ; these are used for tasting the food prior to its acceptance or rejection.

The jaws and associated muscles and skeleton, together with the movements which they perform, are all unique among Arthropoda. No post-oral mandibular adductor muscle or tendon is present ; a generalized type of endoskeleton is associated with each jaw ; and the muscular system is partly antagonistic, so that body form is maintained without a rigid exoskeleton, and a maximum pull can be exerted on the jaw.

Part of the oesophagus is tucked in to form an oesophageal valve resembling that of insects in general form and movements, but differing in all the details of organization.

There is no regional differentiation of the intestine. Its component parts, the gland cell secretory cycle, and the wearing out and replacement of the intestinal cells, are described.

A chitinous peritrophic membrane is formed daily from the surface of the whole intestine. The membrane is rapidly raised and shrinks to form a narrow tube lying along the intestine for 18 hours, and is evacuated from the anus. All solid matter is thereby removed from the intestinal lumen.

The intestinal cells show two phenomena :—" bubbling " is associated with the raising of the peritrophic membrane, and " budding " is a disintegrative process by which large intracellular excretory particles are removed.

The " pericardial network " is composed of large multinucleate cells and small uninucleate nephrocytes.

Peripatopsis is carnivorous, taking meals infrequently. Slime ejection is a purely defensive action and is not employed in feeding.

The mechanism for feeding on active prey is unique ; no prehensile limbs are used. The tubular lip fits tightly to the surface of the prey, suction preventing its escape. The jaws, within the lip tube, make an incision, pieces are cut up and swallowed, or salivary juice is ejected into the food and sucked back, so that external digestion is effected.

Digestion and absorption in the intestine is effected within 18 hours. Large molecules are broken down by salivary juice, and final breakdown occurs in the intestine. The enzymes are much more suited to a carnivorous than a vegetarian habit.

Fat, glycogen, and protein are stored in the intestinal wall, and will maintain the animal during starvation for three months.

Excretion occurs in three ways :—

(i) By the intestine. Urates are crystallized daily on the epithelial surface, and are then collected and removed by the peritrophic membrane.

(ii) By accumulatory organs. The nephrocytes of the pericardial network and lateral haemocoel filter the blood as it passes to the pericardium.

(iii) By nephridia. These organs are emptied but rarely. The urine contains very little solid matter. It is acid and possesses at least 7% of ammonia by dry weight.

The properties of the urine and its manner of formation indicate that the nephridia in the past have been important organs for nitrogenous excretion and salt retention but that now they are of importance to the animal in other ways.

The uricotelic metabolism of the Onychophora is an independent adaptation to terrestrial life.

The three main functions of the intestine—digestion, storage of reserve food, and excretion—have led to no structural differentiation of this organ.

Indirect evidence has been obtained on the course of circulation of the blood. The vascular system shows great differences from those of other Arthropoda.

Key to the Lettering of the Figures

a., base of antenna.
a.c., accessory muscle.
a.t., muscles from tongue to anterior body wall.
b., cytoplasmic " bubble " formed from columnar epithelial cells and lying in intestinal lumen.
b. 1, swelling of columnar cell prior to " bubble " formation.
b. 2, " bubble " almost liberated through striated border.
b.c., buccal cavity.
bu., cytoplasmic bud from columnar epithelial cell.

c., fibrous connective tissue.
c.a., connective tissue annulus at junction of oesophageal valve and intestine.
c.c., cellular connective tissue.
c.g., cerebral ganglion.
c.m., circular muscles.
c.n., connective tissue nucleus.
c.o., circumoesophageal commissure.
c.s., connective tissue sheath of sense capsule.
c.tr., fibrous connective tissue, muscle, and tracheal layer.
cl., columnar epithelial cell.
cl.b., base of columnar epithelial cell.
cl.n., nucleus of columnar epithelial cell.
cu., cuticle.
cu. 1, cuticle being shed.
cu.i., inner layer of cuticle (unstained by iron haematoxylin, blue with Mallory's stain).
cu.o., outer layer of cuticle (stained black with iron haematoxylin, red with Mallory's stain).
cu.o.s., sculpturing on surface of scales formed by outer layer of cuticle.
d.l.m., dorsal longitudinal muscle.
d.m., diagonal and circular layers of muscles.
d.v., dorso-ventral muscle in front of leg 1.
e., ectoderm.
e.s.d., duct from salivary gland end-sac to the salivary gland.
e.s.s., end-sac of salivary gland.
ep., epithelium.
ep.o., epithelial lining of oesophagus.
ex.g., excretory granule.
ex.s., yellow excretory spheres.
f., fibrils from sense cells into sensory spine.
f.d., fat droplets from broken cell.
f.g., fat globule.
fu., furrow on inner surface of intestine.
g., glycogen.
g.c., gland cell.
g.c.b., gland cell formed in basal part of epithelium, it will subsequently rise near the surface.
g.c.n., nucleus of gland cell.
h., heart.
h.s., haemocoelic space.
h.s.p., minute haemocoelic space between pericardial network cells, here clogged with indian ink.
i.l., inner lip.
i.v., inner layer of oesophageal valve.
int., intestine.
int.c., contents of intestine.
int.ep., junction of intestinal epithelium with that of oesophageal valve.
j., jaw.
j.r., retractor muscle of jaw.
j.s., endoskeleton of jaw.
l., leucocyte.
l.int., lumen of intestine.
l.oe., lumen of oesophagus.
l.l.m., lateral longitudinal muscles.
l.m., longitudinal muscles.
l. 1, *l.* 2, *l.* 3, first, second, and third walking legs respectively.
m., muscle.
m.b.w., muscles of body wall.
n., nerve to sense capsule.
n.c., nerve cord.
n.e., exit tube of nephridium.
nu., nucleus.
o.b.l., opening of intermuscular blood lacuna into pericardium.
o.e., edge of ostium.
o.l., outer lip.
or.p., oral papilla.
o.s., external opening of salivary duct.
o.s.s., opening of sensory spine.
o.v., outer layer of oesophageal valve.
oe.int., junction of oesophageal and intestinal epithelia.
oes., oesophagus.
or.p., oral papilla.
p., sensory papilla.
p.c., pericardial network cells.
p.c.f., pericardial floor.
p.c.f.m., muscle of pericardial floor.
p.c.l., large multinuclear pericardial cell.
p.c.s., small uninucleate pericardial nephrocyte.
p.h., haemocoelic space connecting ostia of pericardial floor with pericardial cavity.
p.m., peritrophic membrane.
p.m.r., peritrophic membrane raising off epithelium.
p.m.t., tube formed by contracted peritrophic membrane.
p.m. 1, *p.m.* 2, *p.m.* 3, successive layers of peritrophic membrane.
p.s., protein spheres.
p.v.h., median perivisceral haemocoel.
per., pericardial haemocoel.

ph., pharynx.
ph.g., pharyngeal grooves.
ph.p., pharyngeal protractor.
ph.p.c., fibrous connective tissue sheet connecting pharyngeal protractors.
r.c., regenerative cell.
r.c. 1, regenerative cell starting to replace epithelial or gland cell.
r.m., radial muscles.
s., sculpturing on the base of sensory spine similar to that on small scales (*cu.o.s.*).
s.b., chitinous base of sensory spine (stained black with iron haemotoxylin, red with Mallory's stain).
s.c., sense and supporting cells of sense capsule.
s.d., salivary duct.
s.g., salivary gland.
s.g.b., secretory granule staining blue with Mallory's stain, unstained with iron haematoxylin.
s.g.r., secretory granule staining red with Mallory's stain, black with iron haematoxylin.
s.g.s., space left after disappearance of secretory granule after discharge of gland.
s.l., slime gland.
s.m., sagittal muscle.
s.s., cuticle of sensory spine.
s.s. 1, cuticle of sensory spine being shed at ecdysis.
st., striated border of columnar epithelial cell.
t., tongue.
t.c., taste capsule and spine.
t.m., transverse muscle.
t.r., tongue retractor muscle.
tr., tracheae.
u.g., uric acid granules.
u.s., uric acid crystalline spheres.
v., oesophageal valve.
v.l.m., ventral longitudinal muscles.
X., positions of nephrocytes and large multinuclear cells in the lateral body cavity.

References

Baldwin, E. (1937). "An Introduction to Comparative Biochemistry." Cambridge, pp. 1–107.
Balfour, F. M. (1883). 'Quart. J. micr. Sci.,' vol. **23**, pp. 213–259.
Berlese, R. T. (1899). 'Riv. Patol. veg.,' vol. **7**, pp. 226–251.
Bouvier, E. L. (1902). 'Zool. Jb. Suppl.,' vol. **5**, pp. 675–730.
—— (1905). 'Ann. Sci. Nat. b. ser. 9,' vol. **2**, pp. 1–383.
Bruntz, L. (1904). 'Arch. Biol. Paris,' vol. **20**, pp. 217–422.
Campbell, F. L. (1929). 'Ann. Ent. Soc. Amer.,' vol. **22**, pp. 401–426.
Dehn, M. v. (1933). 'Z. Zellforsch.,' vol. **19**, pp. 79–105.
Delaunay, H. (1931). 'Biol. Rev.,' vol. **6**, pp. 265–301.
Duboscq, O. (1920). 'Arch. Zool. exp. gén.,' vol. **59**, pp. 21–24.
Evans, R. (1901) 'Quart. J. micr. Sci.,' vol. **44**, pp. 473–538.
Fedorow, B. (1929). 'Zool. Jb. Abt. 2.,' vol. **50**, pp. 279–332.
Feigl, F. (1933). 'Mikrochemie.,' vol. **13**, pp. 129–138.
Folin, O. (1928). 'J. Biol. Chem.,' vol. **77**, 421–430.
—— (1933). 'J. Biol. Chem.,' vol. **101**, pp. 111–125.
Gaffron, E. (1885). "Beitrage zur Anatomie und Histologie von Peripatus. Schneiders Zoologische Beitrage," Breslau, pp. 145–163.
Grube, E. (1853). 'Arch. Anat. Physiol.,' Leipsig, vol. **20**, pp. 322–360.
Gurwitsch, A. (1902). 'Pflügers Arch.,' vol. **91**, pp. 71–118.
Hanstrom, B. (1935). 'Acta Univ. lund.,' vol. **31**, pp. 3–37.

HEATLEY, N. G. (1936). ' J. Exp. Biol.,' vol. **13**, pp. 329–343.
HEWITT, C. G. (1905). ' Mem. Manch. Litr. Phil, Soc.,' vol. **50**, pp. 1–8.
HERRMANN, (1931). ' Z vergl. Physiol.,' vol. **14**, pp. 479–524.
KABREHEL, G. (1886). ' Wien. med. Jb.,' pp. 385–391.
KEMP, S. (1914). ' Rec. Ind. Mus., Calcutta,' vol. **13**, pp. 471–492.
LENGERKEN, H. (1924). ' Biol. Zbl.,' vol. **44**, pp. 273–295.
MANTON, S. M. (1928). ' Phil. Trans.,' B, vol. **216**, pp. 363–463.
—— (1934). ' Phil. Trans.,' B, vol. **223**, pp. 163–238.
MANTON, S. M., and RAMSAY, J. A. (1937). ' J. Exp. Biol.,' vol. **14** (*in the press*).
MEISSNER, G. (1868). ' Z. rat. Med.,' 3 Reihe, vol. **31**, pp. 144–233.
MÖLLENDORF, W. v. (1915). ' Arb. anat. Inst.,' Weisbaden, vol. **53**, pp. 87–318.
—— (1919). ' S.B. heidelburg. Akad. Wiss. (b).,' vol. **10**, No. 9, pp. 1–24.
MOSELEY, H. N. (1874). ' Phil. Trans.,' vol. **164**, pp. 757–782.
NEEDHAM, J. (1935). " Bio-chem. J.,' vol. **29**, pp. 238–251.
PFLÜGER, E. (1904). ' Pflügers Arch.,' vol. **103**, pp. 169–170.
PICKEN, L. E. R. (1936). ' J. Exp. Biol.,' vol. **13**, pp. 309–328.
ROMEIS, B. (1932). " Taschenbuch der Mikroskopichen Technik," München, pp. 1–801.
SCHMIEDER, R. G. (1928). ' J. Morph.,' vol. **45**, pp. 121–174.
SCHNEIDER, K. C. (1902). " Lehrbuch der Vergleichenden Histologie der Tiere," Jena. Protrotracheata, pp. 443–457, figs. 401–409.
SCHULTZ, A. (1931). ' Virchows Arch.,' vol. **280**, pp. 529–536.
SEDGWICK, A. (1888). ' Quart. J. micr. Sci.,' vol. **28**, pp. 373–396.
SNODGRASS, R. E. (1935). " Principles of Insect Morphology," pp. 1–667.
STANFORD, R. V. (1923). ' Bio-chem. J.,' vol. **17**, pp. 847–850.
STRAUS, J. (1911). ' Z. Biol.,' vol. **56**, pp. 347–397.
UVAROV, B. P. (1928). ' Trans. R. Ent. Soc. Lond.,' pp. 255–343.
WIGGLESWORTH, V. B. (1930). ' Quart. J. micr. Sci.,' vol. **73**, pp. 593–616.
WINTERSTEIN, H. (1901–1921). ' Handbuch der Vergleichenden Physiologie,' Jena, vol. **2**, part II.
YONGE, C. M. (1936). ' Proc. Roy. Soc.,' B, vol. **120**, pp. 15–41.
ZACHER, F. (1933). " Onychophora, in Kükenthal's and Krumbach's, Handbuch der Zoologie," vol. **3**, pp. 79–138.

Description of Plates

Plate 38

Sections of the intestine of *Peripatopsis* fixed with Duboscq Brazil and stained with Mallory's triple stain. × 1060.

Fig. 15—T.S. showing the distal parts of the epithelium above the arrows, and the bases of the epithelial cells and connective tissue sheath below the arrows. This animal shows an abnormal deposit of uric acid particles (green) within the columnar cells ; this deposit is usually absent, but was found in a less marked degree in 7 out of 65 animals examined. In all other respects the section shows normal features. Protein reserves are numerous (red spheres), but not maximum in abundance ; circular and longitudinal muscles and tracheae lie in the connective tissue sheath.

Fig. 16—Section showing the inner ends of columnar cells containing large rounded yellow inclusions which pass into buds, *bu.*, from these cells. These large yellow inclusions are not frequently present, but are numerous when they do occur. A gland cell projects beyond the striated border.

Fig. 17—Section showing the usual appearance of the inner ends of the epithelial cells, and a newly lifted peritrophic membrane. The irregular yellowish excretory granules are present in most animals, but may be much smaller in size than here shown. The protein spheres are maximum in size, and very abundant below the region here figured. The peritrophic membrane (blue) encloses the uric acid crystals of various sizes, and a bud from the columnar cells.

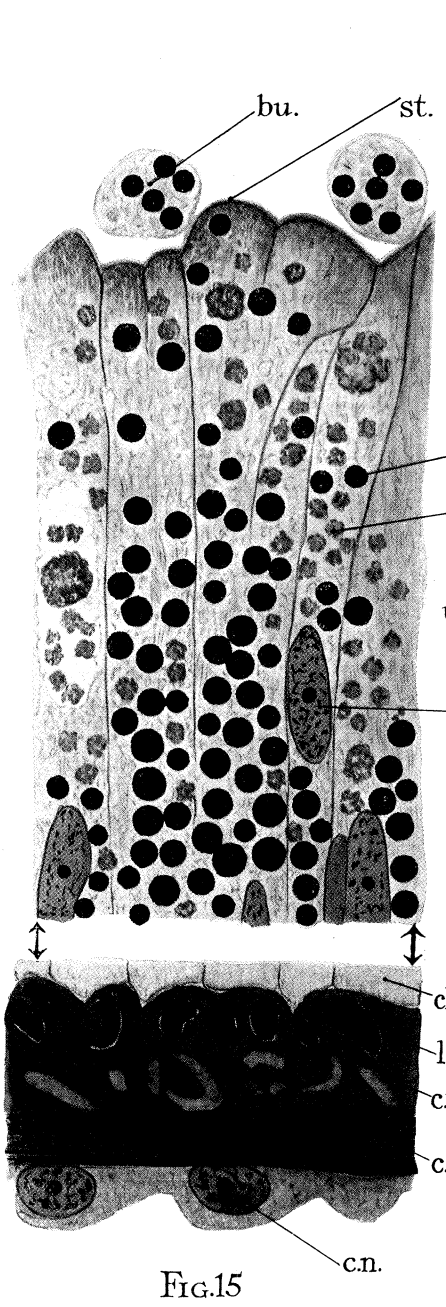


FIG. 15

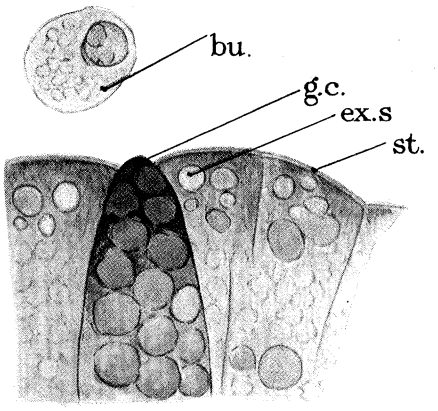
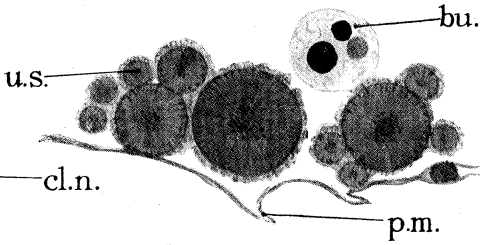


FIG. 16



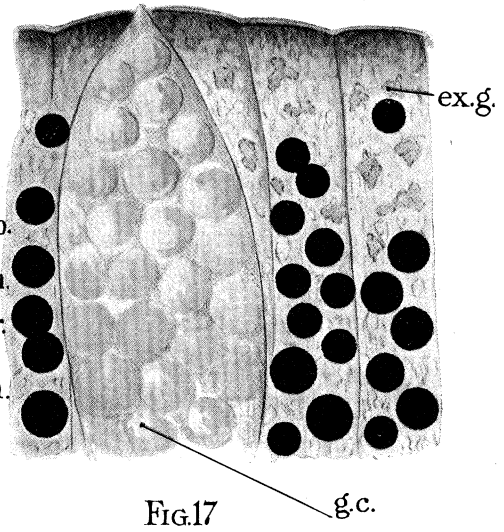


FIG. 17

Plate 39

Fig. 18—Thick freezing microtome section of a surface papilla from the dorsal region with its single sensory spine. The animal is about to cast its cuticle, the old cuticle is free from the body except over the sensory spine where the new and old cuticles are still in contact. The spine is seen in surface view, but is drawn as a transparent object. The sense capsule lies almost entirely within the section, the outer part being cut parasagittally. × 518.

Fig. 19—L.S. of the edge of the antenna showing three sense capsules lodged in one papilla, the section passing through one of the three spines. Unlike the general body surface, the ectoderm is thin everywhere, so that the sense capsules project below the ectoderm. × 518.

Fig. 20—L.S. of large sensory spine and capsule from tongue. The cuticle is about to be shed, and is therefore double. The spine is much broader than in figs. 18 and 19, and appears to be open terminally. Fibrils, *f*, pass from the sensory cells in the capsule up the core of the spine. × 518.

Fig. 21—Another part of the tongue showing much shorter sensory spines with open tips. × 518.

Fig. 22—L.S. of base of sensory capsule on lips showing nerve fibres and tracheae entering the capsule. × 1270.

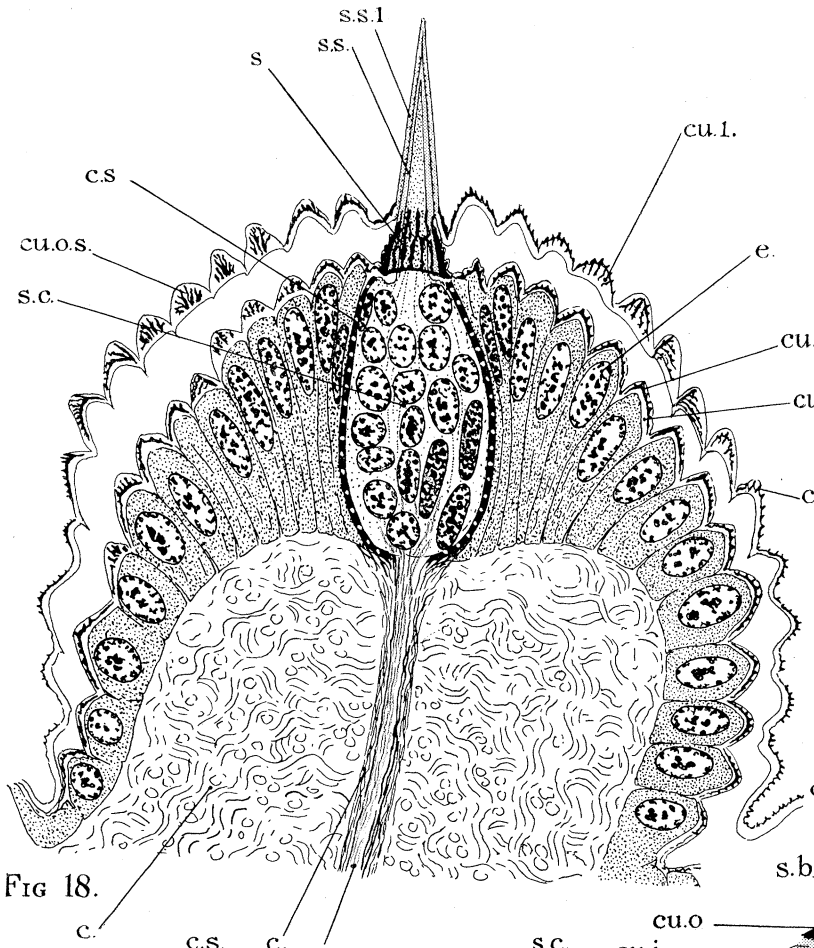


FIG 18.

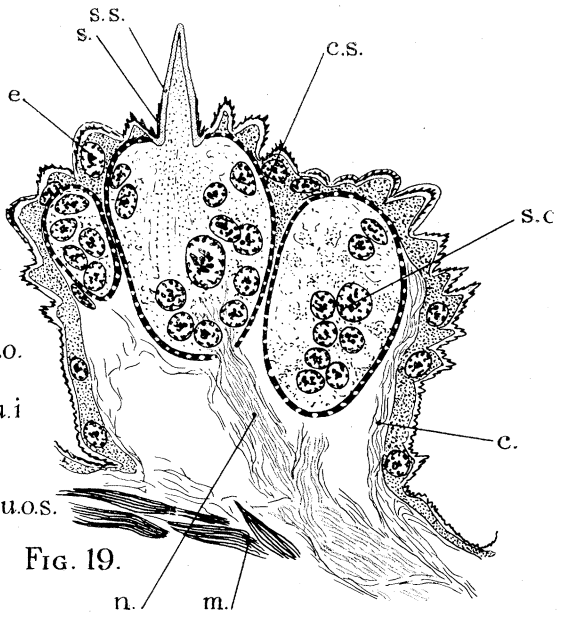


FIG. 19.

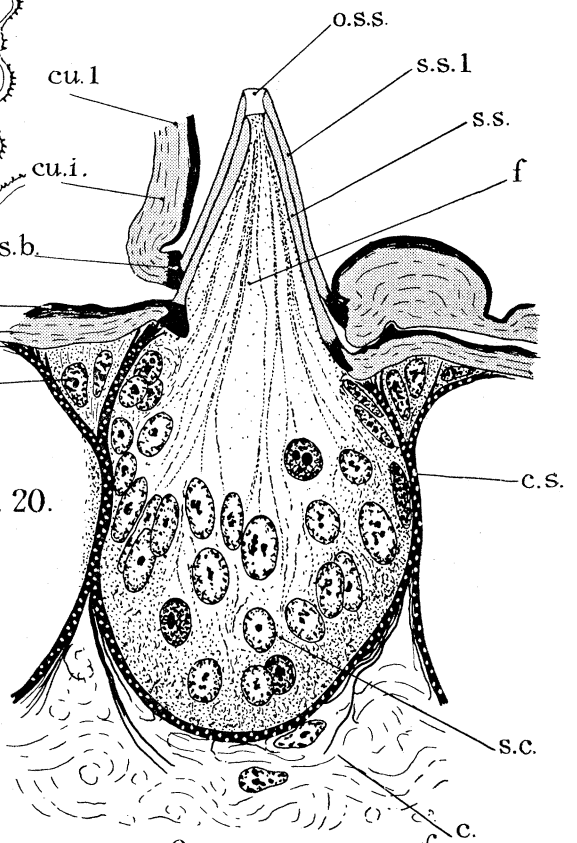


FIG. 20.

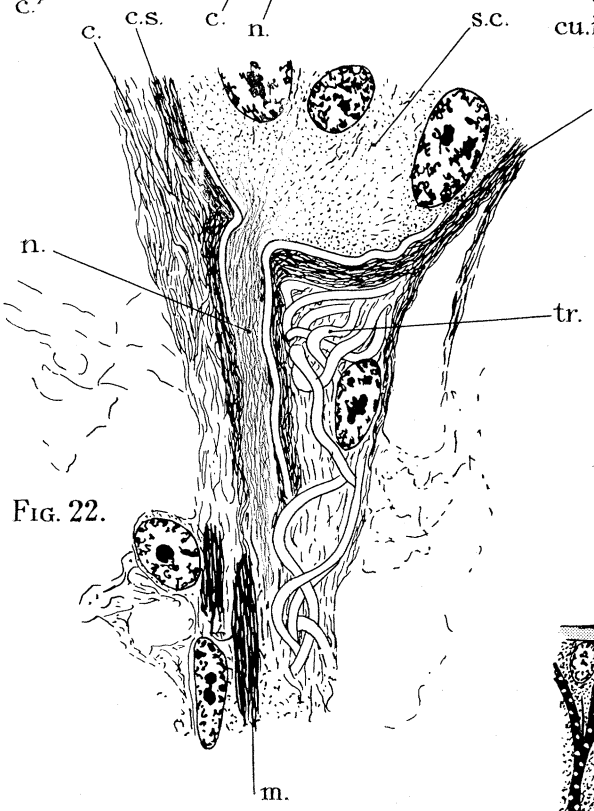


FIG. 22.

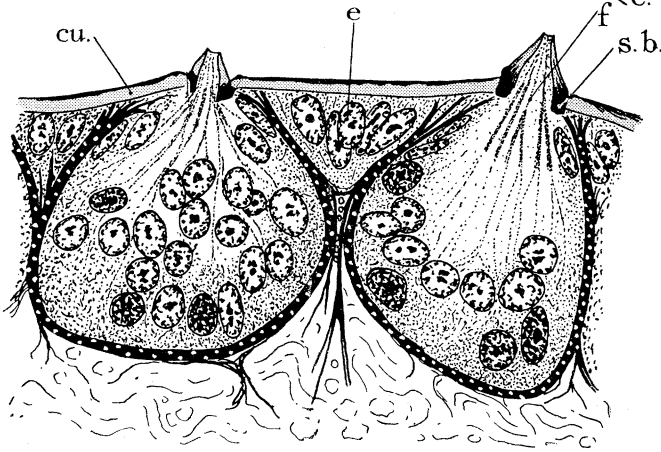


FIG. 21.

Plate 40

Figs. 23–26 illustrating the structure and function of the pericardial network. The position of this tissue is shown in fig. 3, E, *p.c.*

Fig. 23—T.S. of pericardium and associated organs in a late embryo. The ostia in the muscular pericardial floor, which unite pericardial with perivisceral haemocoels, are not shown. The pericardial floor is laterally attached to the muscular body wall. The pericardial network is composed of large multinucleate cells, *p.c.l.*, and small uninucleate nephrocytes, *p.c.s.* The latter have lobed nuclei, as have the leucocytes, *l.* × 280.

Fig. 24—T.S. of the middle region of the pericardial network of *P. moseleyi* 5 hours after injection with non-toxic indian ink ; fixed with Duboscq Brazil. The ink is shown in colour, and has been taken up by the nephrocytes only, and some lies in the small vascular spaces of the network, which is more compact than in the embryo. × 400.

Figs. 25 and 26 are from the same section of *P. balfouri* after alcohol preservation, sectioning on a freezing microtome and staining with Sudan III. (This species is much smaller than *P. moseleyi*, but its cells are the same size.) The animal was fully fed and shows a maximum of food reserves. The colour indicates fat. × 400.

Fig. 25 shows the pericardial network as in B. The nephrocytes show a few minute fat droplets and the normal excretory inclusions, of which only the most opaque blackish ones have been shown, *ex.g.* No fat lies in the multinucleate cells.

Fig. 26 shows the inner $\frac{1}{8}$th of the intestinal epithelium from the same animal as fig. 25 ; on the right a columnar cell has been torn and has liberated fat droplets, *f.d.*, and reserve protein spheres, *p.s.* The fat content of the epithelium extends almost to the striated border. Note the contrast in the amount of fat shown in figs. 25 and 26.

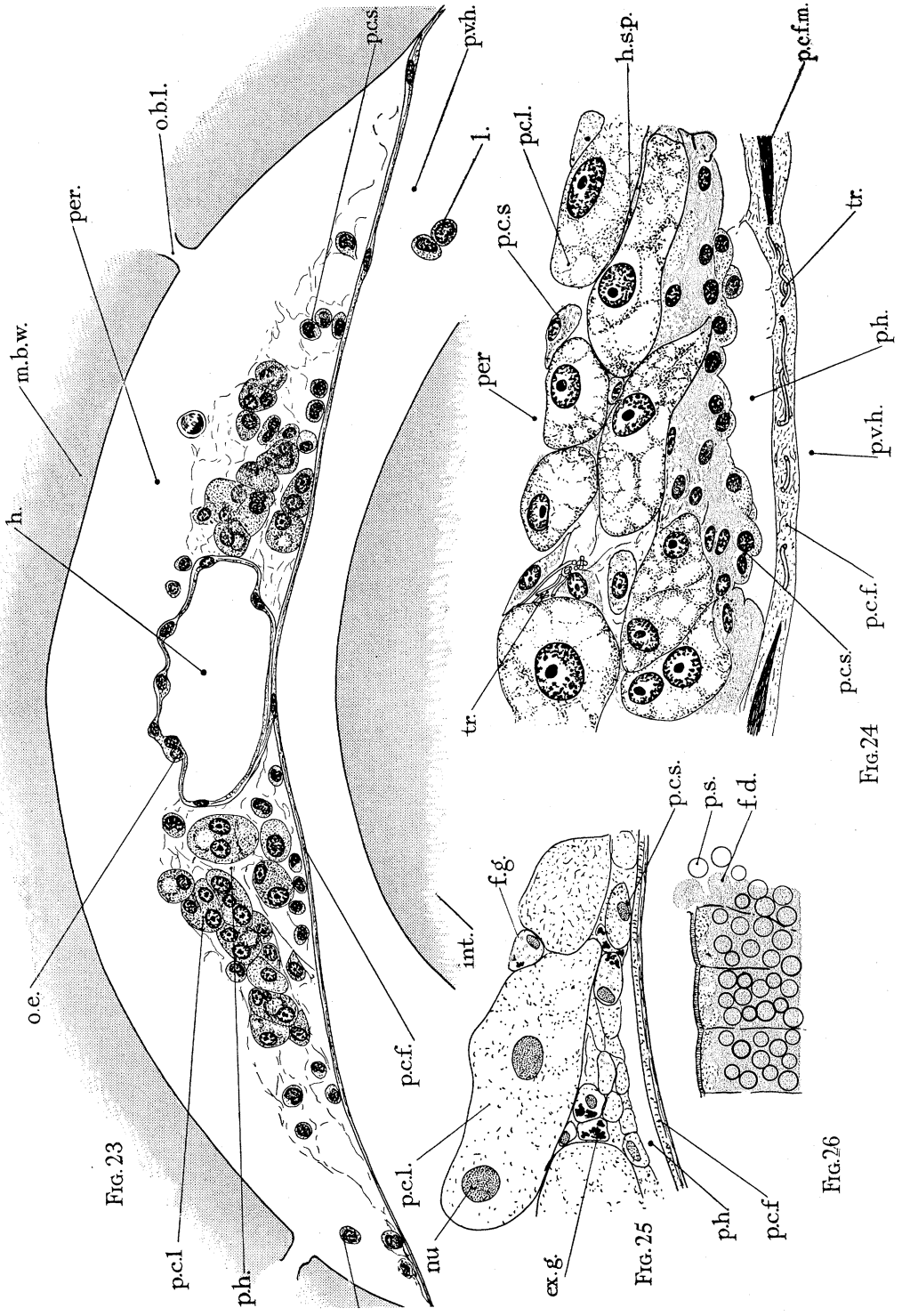


FIG. 23

FIG. 24

FIG. 25

FIG. 26

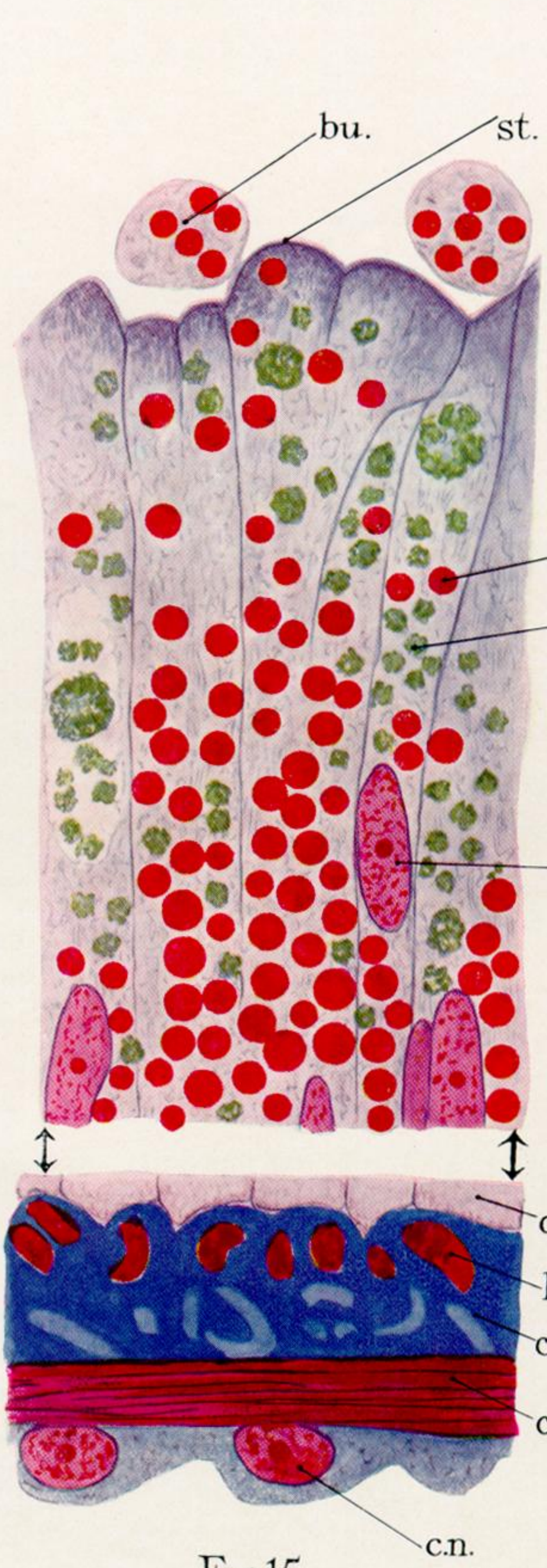


FIG. 15

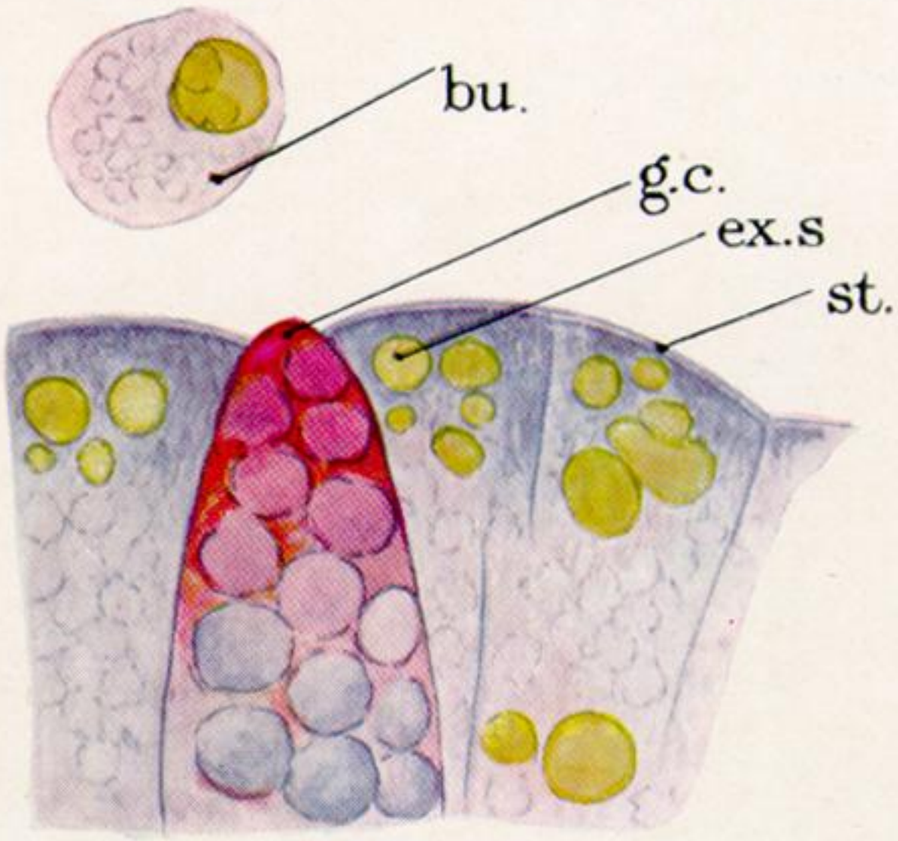
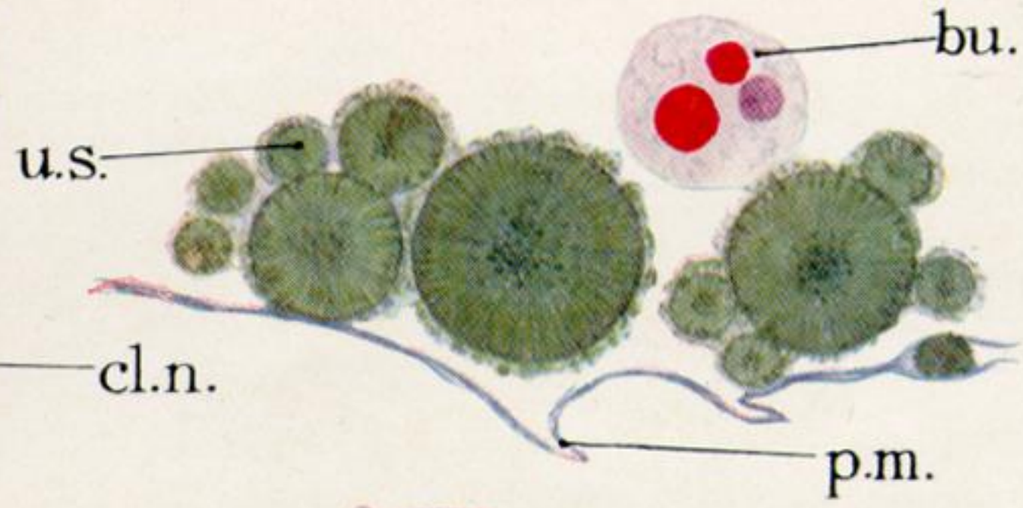


FIG. 16



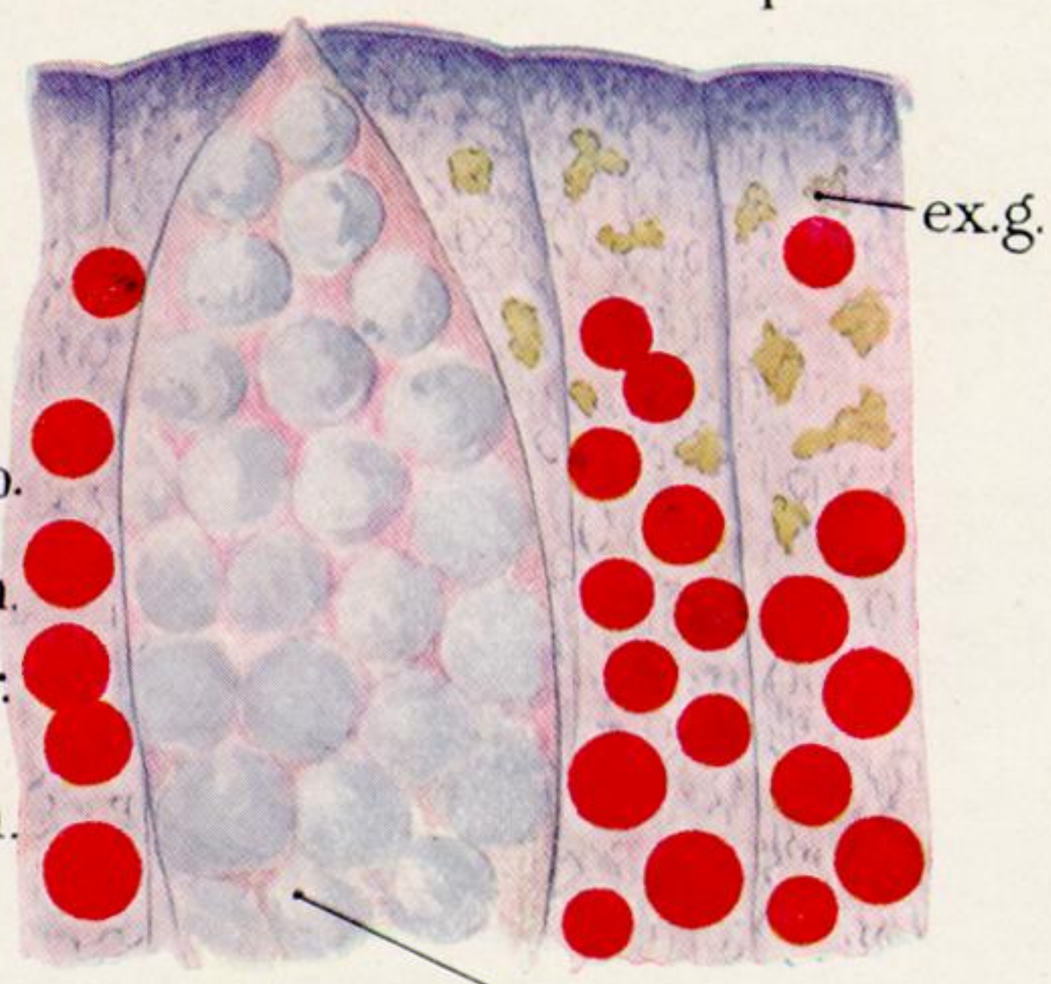


FIG. 17

PLATE 38

Sections of the intestine of *Peripatopsis* fixed with Duboscq Brazil and stained with Mallory's triple stain. × 1060.

FIG. 15—T.S. showing the distal parts of the epithelium above the arrows, and the bases of the epithelial cells and connective tissue sheath below the arrows. This animal shows an abnormal deposit of uric acid particles (green) within the columnar cells ; this deposit is usually absent, but was found in a less marked degree in 7 out of 65 animals examined. In all other respects the section shows normal features. Protein reserves are numerous (red spheres), but not maximum in abundance ; circular and longitudinal muscles and tracheae lie in the connective tissue sheath.

FIG. 16—Section showing the inner ends of columnar cells containing large rounded yellow inclusions which pass into buds, *bu.*, from these cells. These large yellow inclusions are not frequently present, but are numerous when they do occur. A gland cell projects beyond the striated border.

FIG. 17—Section showing the usual appearance of the inner ends of the epithelial cells, and a newly lifted peritrophic membrane. The irregular yellowish excretory granules are present in most animals, but may be much smaller in size than here shown. The protein spheres are maximum in size, and very abundant below the region here figured. The peritrophic membrane (blue) encloses the uric acid crystals of various sizes, and a bud from the columnar cells.

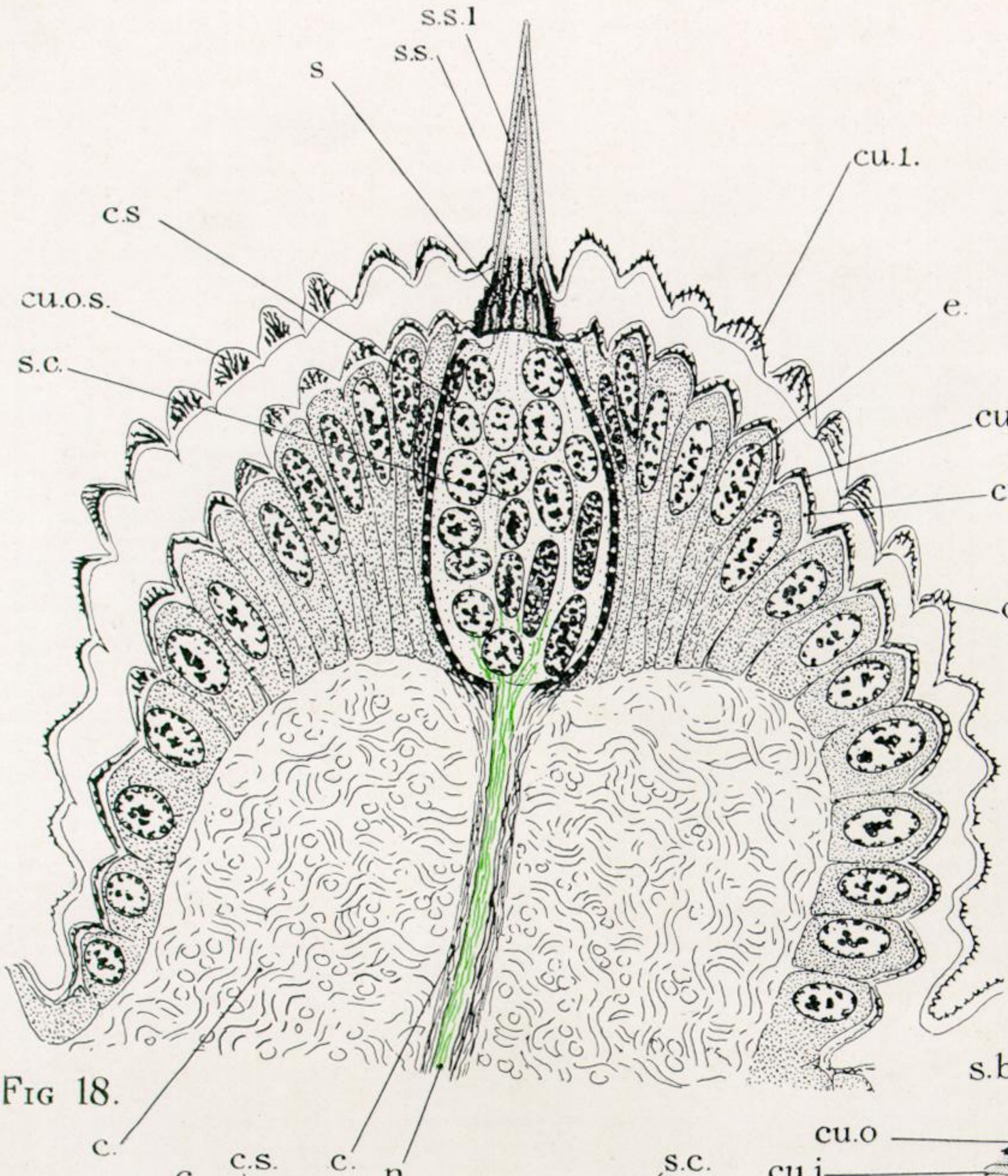


Fig 18.

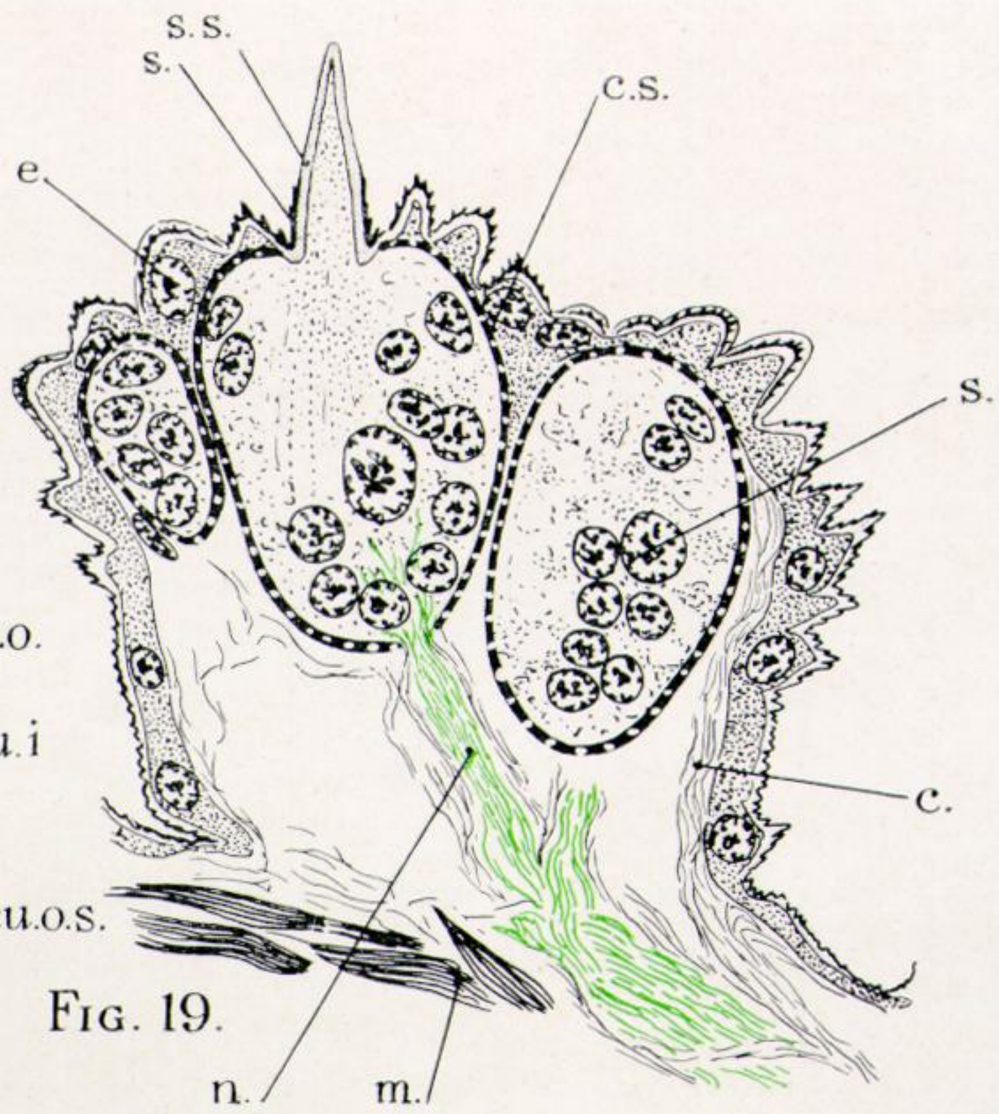


Fig. 19.

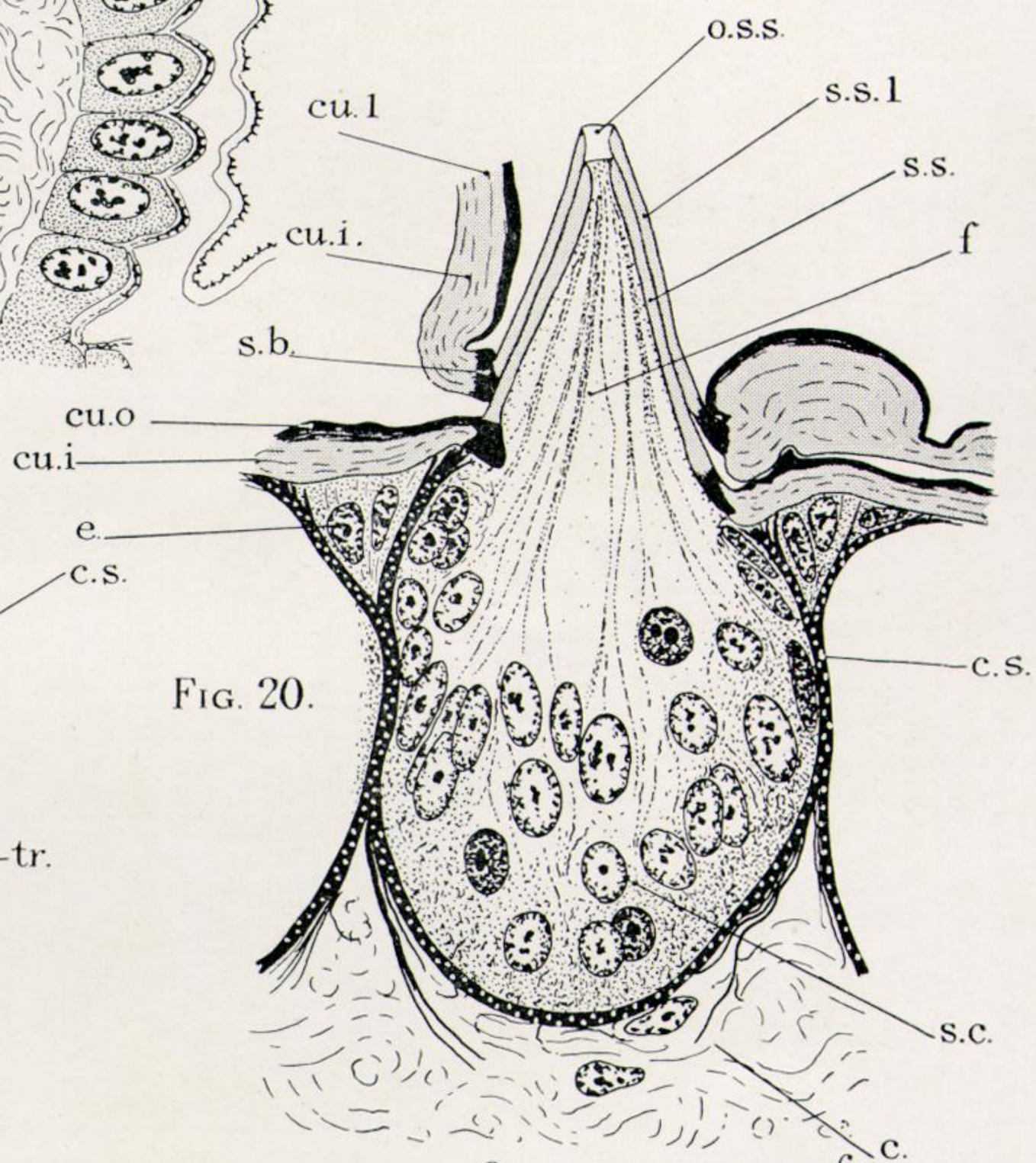


Fig. 20.

Fig. 21.

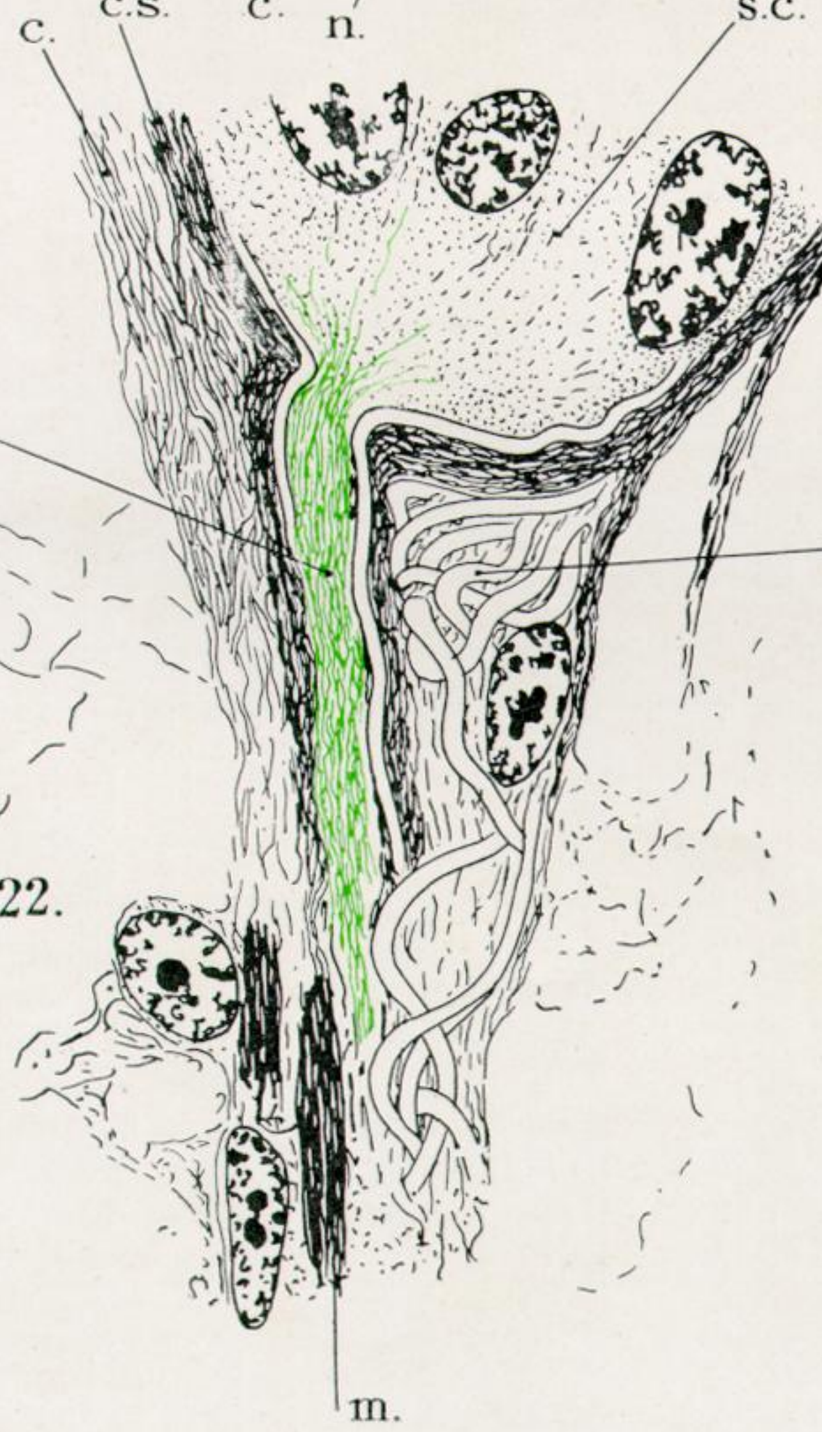


Fig. 22.

Plate 39

Fig. 18—Thick freezing microtome section of a surface papilla from the dorsal region with its single sensory spine. The animal is about to cast its cuticle, the old cuticle is free from the body except over the sensory spine where the new and old cuticles are still in contact. The spine is seen in surface view, but is drawn as a transparent object. The sense capsule lies almost entirely within the section, the outer part being cut parasagittally. × 518.

Fig. 19—L.S. of the edge of the antenna showing three sense capsules lodged in one papilla, the section passing through one of the three spines. Unlike the general body surface, the ectoderm is thin everywhere, so that the sense capsules project below the ectoderm. × 518.

Fig. 20—L.S. of large sensory spine and capsule from tongue. The cuticle is about to be shed, and is therefore double. The spine is much broader than in figs. 18 and 19, and appears to be open terminally. Fibrils, *f*, pass from the sensory cells in the capsule up the core of the spine. × 518.

Fig. 21—Another part of the tongue showing much shorter sensory spines with open tips. × 518.

Fig. 22—L.S. of base of sensory capsule on lips showing nerve fibres and tracheae entering the capsule. × 1270.

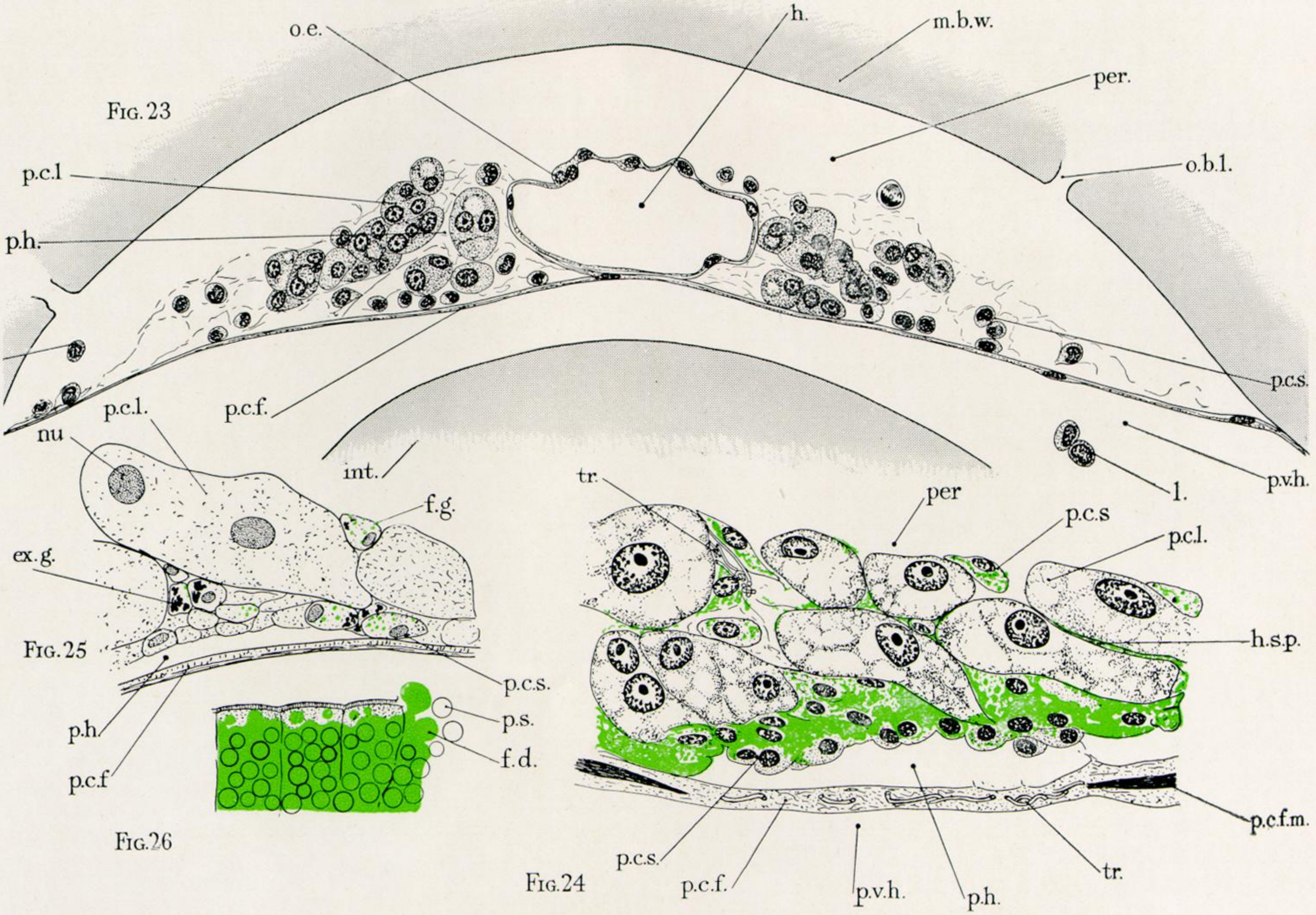


FIG. 23

FIG. 25

FIG. 26

FIG. 24

PLATE 40

FIGS. 23–26 illustrating the structure and function of the pericardial network. The position of this tissue is shown in fig. 3, E, *p.c.*

FIG. 23—T.S. of pericardium and associated organs in a late embryo. The ostia in the muscular pericardial floor, which unite pericardial with perivisceral haemocoels, are not shown. The pericardial floor is laterally attached to the muscular body wall. The pericardial network is composed of large multinucleate cells, *p.c.l.*, and small uninucleate nephrocytes, *p.c.s.* The latter have lobed nuclei, as have the leucocytes, *l.* × 280.

FIG. 24—T.S. of the middle region of the pericardial network of *P. moseleyi* 5 hours after injection with non-toxic indian ink ; fixed with Duboscq Brazil. The ink is shown in colour, and has been taken up by the nephrocytes only, and some lies in the small vascular spaces of the network, which is more compact than in the embryo. × 400.

FIGS. 25 and 26 are from the same section of *P. balfouri* after alcohol preservation, sectioning on a freezing microtome and staining with Sudan III. (This species is much smaller than *P. moseleyi*, but its cells are the same size.) The animal was fully fed and shows a maximum of food reserves. The colour indicates fat. × 400.

FIG. 25 shows the pericardial network as in B. The nephrocytes show a few minute fat droplets and the normal excretory inclusions, of which only the most opaque blackish ones have been shown, *ex.g.* No fat lies in the multinucleate cells.

FIG. 26 shows the inner $\frac{1}{8}$th of the intestinal epithelium from the same animal as fig. 25 ; on the right a columnar cell has been torn and has liberated fat droplets, *f.d.*, and reserve protein spheres, *p.s.* The fat content of the epithelium extends almost to the striated border. Note the contrast in the amount of fat shown in figs. 25 and 26.