ON THE NERVOUS SYSTEM OF *SACCOGLOSSUS CAMBRENSIS* (ENTEROPNEUSTA)

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A layer of nerve fibres is present almost everywhere at the base of the epidermis. It consists of a very thin basal layer of irregularly arranged fibres, and generally a thicker, more superficial layer of orientated fibres, which forms the main nerve cords and subsidiary systems of smaller through-conducting bundles. In the proboscis there are numerous longitudinal bundles, an anterior nerve ring round the basal periphery and a nerve loop under the pre-oral ciliary organ. The neurocord appears to be a simple conducting tract. In the collar epidermis numerous bundles are formed posteriorly, connecting with the prebranchial nerve ring. In the trunk the size of the longitudinal cords and the distribution of the general plexus is related to the degree of development of the muscles and cilia. The gut is well equipped with nerve fibres anteriorly, where it is particularly muscular.

Practically all the nerve-cell nuclei lie outside the plexus of nerve fibres. They are very numerous and widely distributed. A concentration of bipolar neurones (Hess's cells) occurs at the proboscis tip. Cells regarded as sensory on histological grounds are abundant round the base of the proboscis and in the groove of the ciliary organ. Large unipolar neurones are concentrated in the neurocord, some possessing 'giant' axons, which run posteriorly or anteriorly.

The cilia of the epidermis are the chief agents of locomotion, those of the trunk being capable of synchronized reversal. They are aided by peristaltic contractions of the longitudinal muscles, which are controlled by the main longitudinal nerve cords. Burrowing peristalsis is controlled by the dorsal nerve cord of the proboscis. Some reactions to light, to the presence of fine particles and to adrenaline are described. The proboscis is necessary for spontaneous and varied activity, but the considerable degree of co-ordination shown is not due to any localized centre but to a longitudinal reflex path involving the main nerve cords. Rapid contractions of the anterior end are probably due to the giant axons.

The peculiarities of the neurocord are difficult to interpret, except as a result of degeneration and paedomorphosis. The greater part of the richly nervous epidermis may be compared with the vertebrate neural plate.

Introduction

Van der Horst's (1927–39) review of the Enteropneusta revealed little advance in knowledge of the nervous system since the first excellent accounts of Spengel (1877, 1884, 1893), so the discovery of *Saccoglossus cambrensis* Brambell & Cole (1939*a*) led naturally to the present investigation. During the interruption of the war years Bullock described the functioning (1940) and histology (1945) of the nervous system in several forms, including the system of giant nerve fibres (1944). The present account gives additional details and places a different interpretation on the peculiarities of the Enteropneusta.

Silén (1950) has revived the interesting idea that the Enteropneusta have a coelomic nervous system, like that of echinoderms. This, like some other of Silén's conclusions (p. 329), is questionable (p. 338). The difficult problem of the motor innervation in Enteropneusta will be solved only after a more detailed study of the muscles.

Methods

Many *S. cambrensis* were collected from near Bangor. Some were fixed immediately, after the gut contents had been evacuated; others were partially narcotized by treatment for about an hour with finely powdered menthol. Entire specimens could not be obtained, because the body is very fragile and elongated, and it was found advisable to fix unmutilated portions not less than 1 cm long. Smaller, mutilated fragments became greatly distorted during fixation, for the soft body of an enteropneust depends for the maintenance of its shape on the integrity of its epithelia and their basal membranes. The fixatives used included Bouin in sea water, strong Flemming without acetic acid, and Bodian's fixative no. 2 (5 ml. glacial acetic acid, 5 ml. 40 % formaldehyde and 90 ml. 80 % alcohol). All material was embedded in paraffin wax and sectioned at 5 to 10μ.

The method of silver impregnation devised by Bodian (1936, 1937) was found most useful, the sections being left in protargol from 2 to 10 h at 36° C. This gave good general pictures and stained the nerve fibres. Roger's (1931) technique was less reliable and less clear, but Holmes's method (1942, 1943, 1947) proved as successful as that of Bodian, the best preparations following impregnation for 24 h in 0·002 % silver nitrate solution, +0·1 % pyridine buffered to pH 8·4. Heidenhain's iron haematoxylin showed cytological detail and the muscle fibres; Delafield's and phospho-molybdic acid haematoxylin stained both nerve fibres and supporting fibres. Mallory's triple stain revealed gland cells and the basal membranes of epithelia, but the best stain for basal membranes was light green.

Intra-vitam methylene blue did not give good results. The abundant mucus produced by the soft tissues readily took up the stain but nerve cells did not appear to do so. Hess (1937) and Bullock (1945) both recorded the successful use of this method, but the results obtained with it by Hess were justifiably questioned by Bullock.

Saccoglossus horsti Brambell & Goodhart (1941) was collected from near Lymington-on-Solent for comparison.

Arrangement of the nerve fibres

Spengel (1877) described the neurocord and the general nerve fibre layer of the epidermis, thickened round the proboscis base to form the anterior nerve ring and in the mid-line of the trunk to form the dorsal and the ventral nerve cords. The latter is joined to the neurocord by a nerve ring, which encircles the body just behind the collar and may be termed prebranchial to distinguish it from the anterior nerve ring (figure 1). Little progress in knowledge of the arrangement of the fibres has been made since Spengel's work. Silén (1950) found in the nerve cords a thin layer of irregular fibres adjoining the basal membrane, with a thick layer of longitudinal fibres superimposed upon it, but Bullock (1945) found no stratification in the nerve-fibre layer.

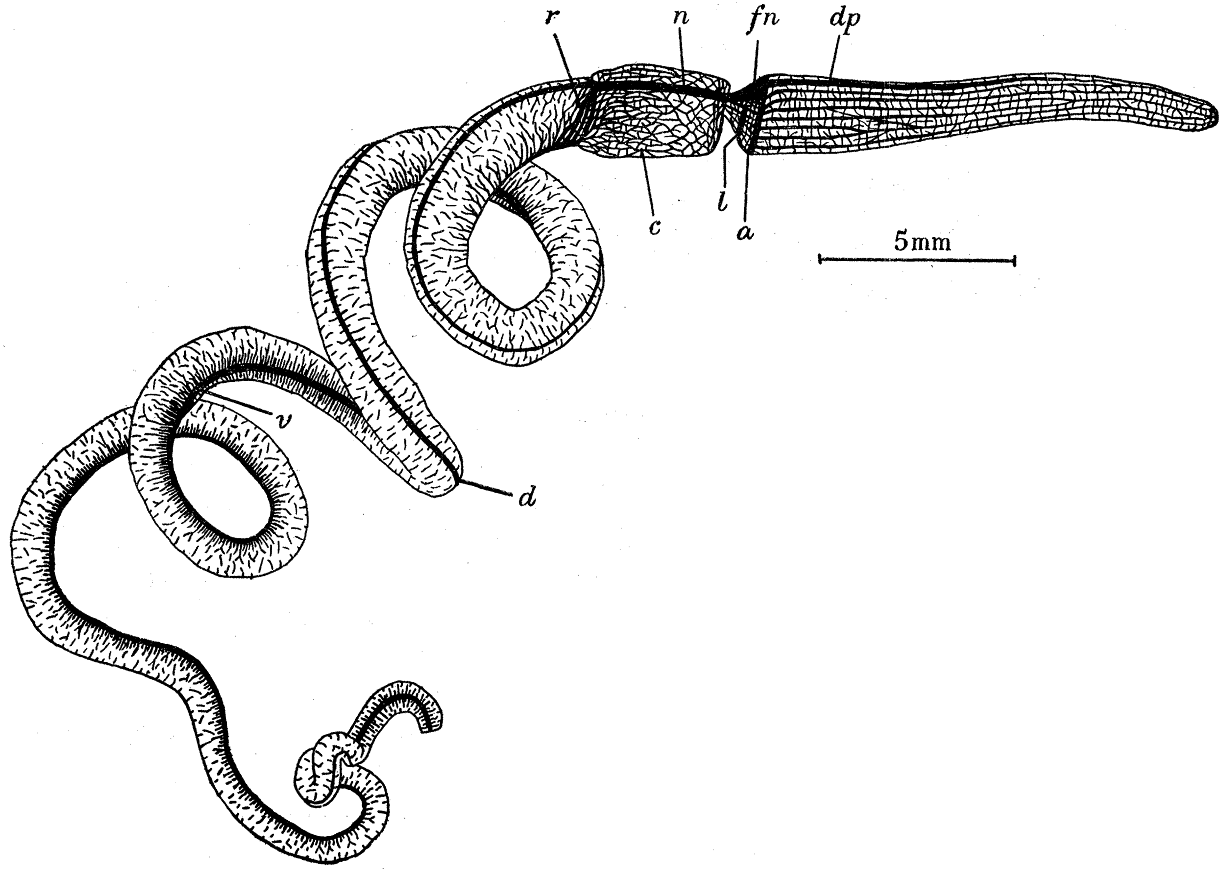

Figure 1. Plan of the nervous system, to indicate diagrammatically the general orientation of nerve fibres and the relative thicknesses of the nerve-fibre layer in different regions of the epidermis. Tracts and bundles of orientated nerve fibres are represented by thicker lines; individual fibres, which are greatly reduced in numbers, are often shown ending blindly, as they could not be followed for more than short distances. *a*, anterior nerve ring; *c*, nerve fibres of the collar epidermis; *d*, dorsal nerve cord of the trunk; *dp*, dorsal nerve cord of the proboscis; *fn*, fan-shaped thickening; *l*, nerve loop of the ciliary organ; *n*, neurocord seen by transparency; *r*, prebranchial nerve ring; *v*, ventral nerve cord.

(*a*) *Proboscis*

A layer of nerve fibres lies deeply in the epidermis throughout the proboscis. It is thick in the basal region (figure 2) and thin anteriorly to this, except for a thicker dorsal nerve cord. Its fibres are for the most part longitudinal or transverse, as in other forms (Koehler

1886; Hess 1937). The longitudinal fibres form bundles which extend throughout the length of the proboscis, becoming larger towards the base, where some project prominently above the surface of the nerve-fibre layer (figures 21 and 22, plate 32). In a large specimen there are about sixty prominent bundles and several hundred smaller ones, the latter anastomosing occasionally, especially towards the anterior end. The transverse fibres are not organized into bundles, and the majority run uninterruptedly through the longitudinal bundles. In moderately extended specimens the layer of transverse fibres and small bundles is of uniform thickness throughout the length of the proboscis. The bundles are particularly distinct in the superficial part of the nerve-fibre layer; next to the basal membrane the fibres are irregularly arranged and bundles are absent.

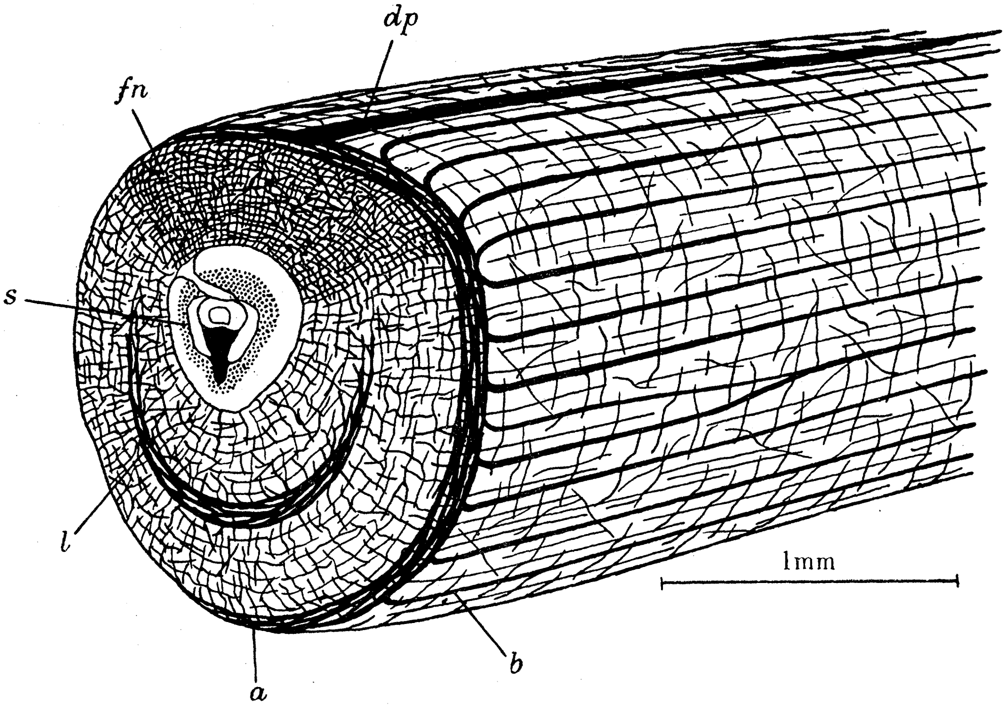

FIGURE 2. Diagrammatical plan, as in figure 1, of the nervous system of the proboscis base, being a postero-dorsal view from the right side, with the proboscis stalk shown in transverse section, nerve fibres elsewhere in surface view. The number of longitudinal bundles has been greatly reduced for the sake of clarity. *a*, anterior nerve ring; *b*, longitudinal bundles; *dp*, dorsal nerve cord of the proboscis; *fn*, fan-shaped thickening; *l*, nerve loop of the ciliary organ; *s*, nerve-fibre layer of proboscis stalk.

The dorsal nerve cord lies under a mid-dorsal groove in the posterior two-thirds of the proboscis. Towards the anterior end it cannot be distinguished from the rest of the nerve-fibre layer. Most of its fibres run longitudinally, but its deepest fibres are irregularly arranged. On each side it connects with the transverse fibres of the general nerve-fibre layer, which do not run uninterruptedly through it as they do through the longitudinal bundles, so that in transverse section the dorsal cord appears purely granular, unmarked by regular cross-striations (figure 3).

The nerve-fibre layer of the proboscis base is especially thick dorsally, for there the thick dorsal nerve tract of the proboscis stalk spreads out fanwise without becoming much thinner in the process (figure 2). Laterally this fan-shaped thickening is ill-defined, and from the middle of its anterior edge springs the dorsal nerve cord of the proboscis. Elsewhere its anterior edge is clearly defined and formed predominantly of circular fibres, which are arranged in bundles. These run round the periphery of the proboscis base (figure 4) and join ventrally to form a complete nerve ring (figure 5). The anterior bundles are particularly well marked (figure 28, plate 33) and anastomose less freely than the others.

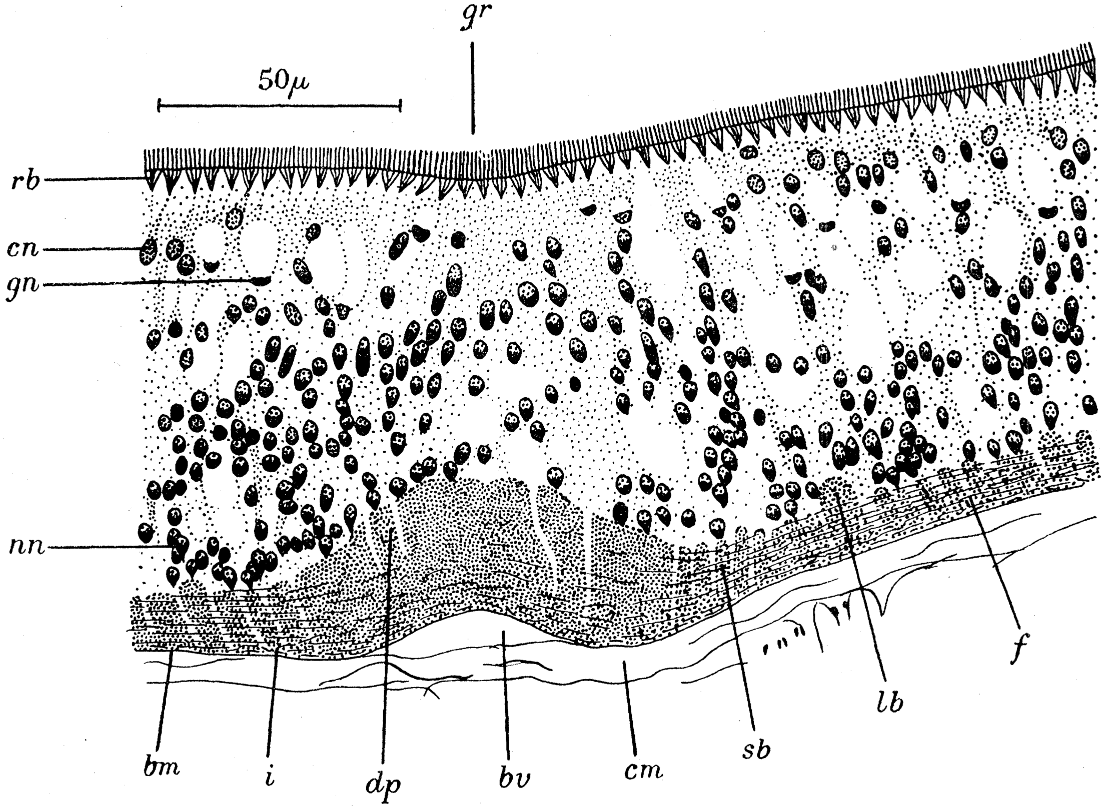

FIGURE 3. Mid-dorsal part of a transverse section through the proboscis. *bm*, basal membrane of epidermis; *bv*, dorsal blood vessel; *cm*, circular muscle fibres; *cn*, nucleus of ciliated cell; *dp*, dorsal nerve cord; *f*, transverse nerve fibres running through longitudinal bundles; *gn*, nucleus of small goblet gland cell; *gr*, dorsal groove; *i*, irregular nerve fibres near basal membrane; *lb*, large longitudinal bundle projecting above nerve-fibre layer; *nn*, nuclei, probably of primitive ganglion cells; *rb*, rootlet bundles of cilia; *sb*, small bundle of longitudinal nerve fibres.

The longitudinal bundles usually curve aside as they approach the nerve ring and enter it obliquely, contributing their fibres directly to the bundles which form it (figures 23, 24 and 25, plate 32). Dorsally and ventrally the bundles turn away from the mid-line, laterally they turn in a dorsal direction. In contrast, the dorsal nerve cord enters the nerve ring at right angles. Thus the cord has its main transverse connexions with the general nerve-fibre layer on each side and not with the anterior nerve ring, whereas the remaining longitudinal bundles join the anterior nerve ring, but are traversed uninterruptedly by the majority of the transverse fibres. The possible significance of this arrangement is referred to later (p. 343).

In the more central area of the proboscis base, which is bounded by the anterior nerve ring, most of the fibres are either radiating or transverse, but many are irregular. Laterally and ventrally the nerve-fibre layer is only about half as thick as it is dorsally, but beneath the groove of the ciliary organ described by Brambell & Cole (1939*b*) there is a nervous thickening, consisting of a few U-shaped bundles which run close together, forming what may be termed the nerve loop of the ciliary organ. Ventrally, this is remarkably distinct from the nerve-fibre layer and superimposed upon it (figure 5 and figure 26, plate 33). Laterally it is thinner and joined to the nerve-fibre layer beneath by fibres running from

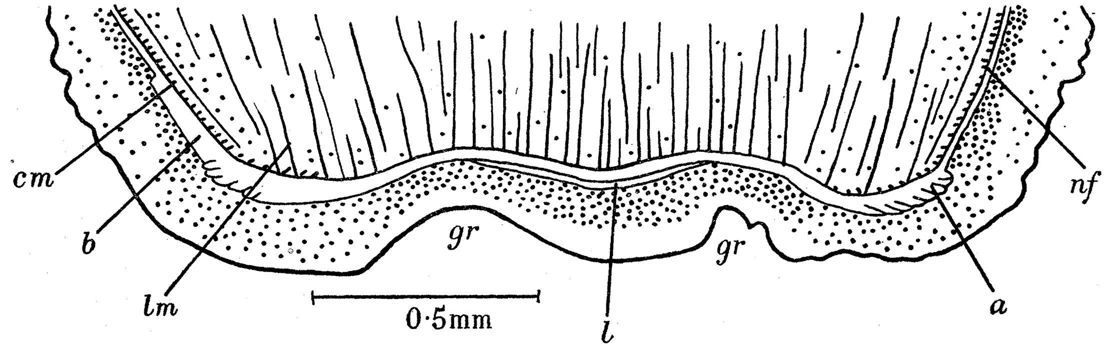

FIGURE 4. Horizontal section through the base of the proboscis, ventral to the proboscis stalk, drawn with the anterior end upwards: dots represent nuclei. *a*, anterior nerve ring; *b*, large bundle of longitudinal fibres entering anterior nerve ring; *cm*, circular muscle fibres immediately inside basal membrane of epidermis; *gr*, groove of the ciliary organ, cut across in two places; *l*, ventral part of the nerve loop of the ciliary organ (this shows the position of the section in relation to the sagittal section in figure 5); *lm*, longitudinal muscle fibres; *nf*, general nerve-fibre layer of proboscis epidermis.

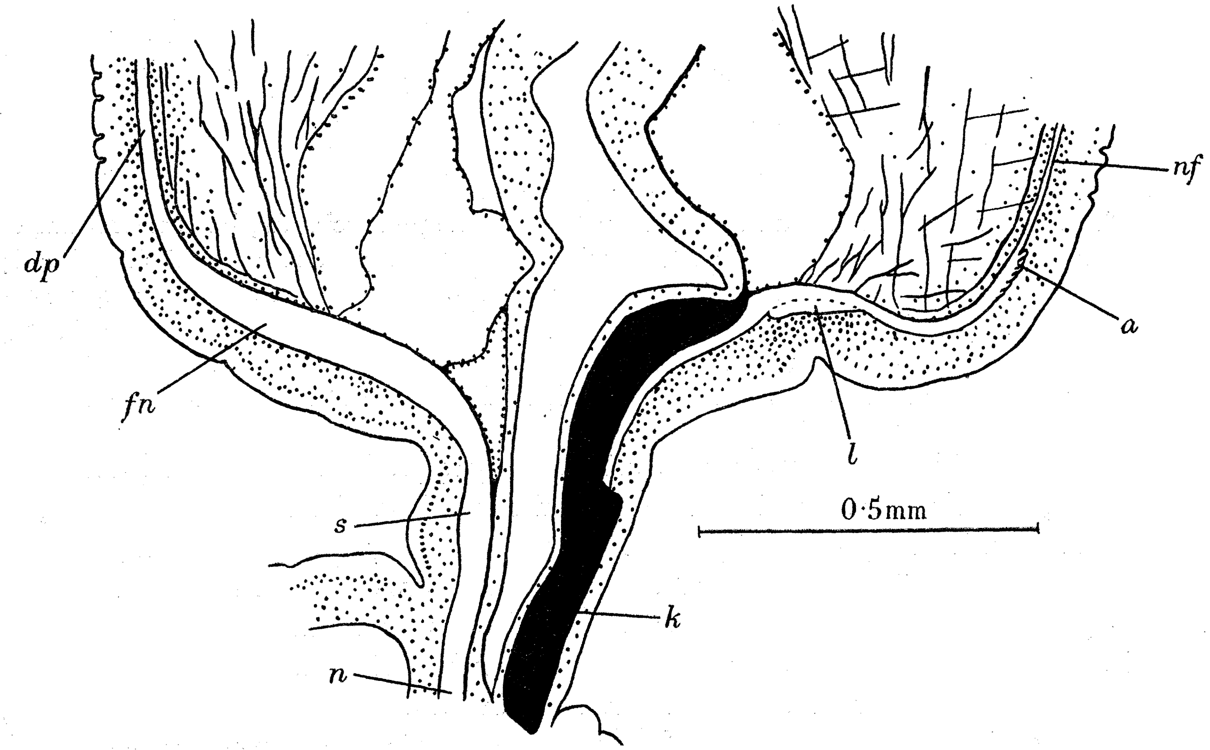

FIGURE 5. Sagittal section through base of proboscis, anterior end upwards. *a*, anterior nerve ring; *dp*, dorsal nerve cord of proboscis; *fn*, fan-shaped thickening of nerve-fibre layer; *k*, keel of proboscis skeleton; *l*, nerve loop of ciliary organ; *n*, neurocord; *nf*, general nerve-fibre layer of proboscis; *s*, nerve-fibre layer of proboscis stalk.

its inner edge. Its dorsal cornua consist of a few superficial fibres, which are only distinguishable because, like the remainder of the nerve loop, they are stained by silver techniques somewhat more darkly than the underlying fibres. The fibres composing the loop follow its semicircular course. They cannot be distinguished beyond its dorsal cornua, having all merged with the nerve-fibre layer beneath.

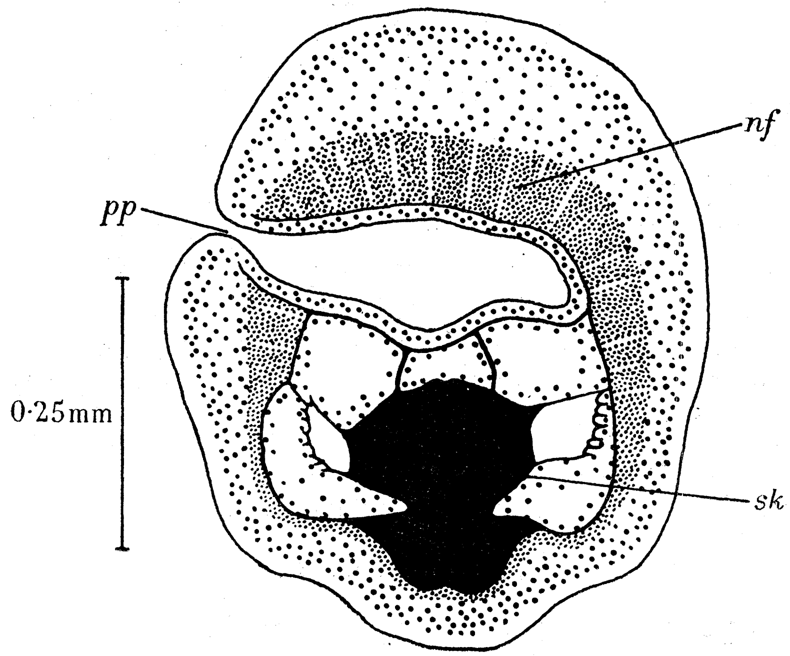

FIGURE 6. Transverse section through proboscis stalk. *nf*, nerve-fibre layer, thick dorsally, thin ventrally; *pp*, proboscis pore, without nerve fibres in its thin epithelium; *sk*, proboscis skeleton, anterior to the keel.

On the ventral side of the keel of the proboscis skeleton nerve fibres are practically absent (figure 5), but elsewhere the nerve-fibre layer of the proboscis stalk is very thick, especially dorsally (figure 6). In surface view it somewhat resembles a network of bundles, arranged for the most part longitudinally, with supporting fibres from the epithelial cells running through the meshes to the basal membrane. The fan-shaped thickening, into which it expands antero-dorsally, occupies a central position in the nervous system (figures 1 and 2) and seems more suitable than any other part of the nervous system for effecting co-ordination, because it is particularly thick and contains a high proportion of irregular fibres. Nevertheless, it is not specially differentiated and did not appear to function as a centre for co-ordination, though it seemed of some importance as a source of initiation of actions (p. 349).

(*b*) *Neurocord*

The neurocord is semicircular in cross-section, with nerve fibres confined to a thick band running along the flat ventral side. The fibres are mainly longitudinal and grouped in ill-defined anastomosing bundles, which are more distinct in the superficial part of the nerve-fibre layer. Ventrally there are no bundles, and next to the perihaemal cavities most fibres are irregular (figure 27, plate 33).

Anteriorly the fibres spread out round the proboscis stalk and a few turn posteriorly inside the buccal cavity, forming a direct but tenuous connexion between the neurocord and the splanchnic nervous system. Other, lateral fibres join the nerve-fibre layer of the collar's anterior lip. Posteriorly the neurocord is continuous with the dorsal nerve cord of the trunk, to which about a third of its fibres contributes. The remaining fibres, which are situated laterally, incline sideways as they approach the posterior neuropore, so that they collect at the sides of the neurocord. At the neuropore the outermost fibres join the nerve-fibre layer of the collar epidermis, but the majority form large dense bundles (figure 37, plate 35) which encircle the body in front of the first pair of gill pores, subdivide and eventually join the anterior end of the ventral nerve cord, to complete the prebranchial nerve ring (figure 7).

The arrangement of nerve fibres in the neurocord closely resembles that in the other longitudinal nerve tracts of the body. The vast preponderance of longitudinal fibres suggests that its function is more that of a conducting strand than a centre of nervous activity. As elsewhere it is the deeper fibres alone that by their irregular arrangement can provide alternative nerve paths such as might be suitable for integration, and it is possible that many of the deeper fibres in the neurocord are concerned with the innervation of the adjoining muscles in the perihaemal cavities.

The giant nerve fibres will be dealt with later (p. 335).

(*c*) *Collar epidermis*

In a fully grown specimen the thickness of the nerve-fibre layer of the collar epidermis was about 10μ at the anterior lip and 5μ throughout most of the anterior half of the collar, gradually increasing to 20μ posteriorly. Near the anterior end the fibres are irregularly arranged, but at the anterior lip the majority are transverse (figure 7). Posteriorly longitudinal fibres predominate, some being arranged in bundles of gradually increasing size, with irregular fibres in between. Near the posterior edge of the collar the bundles turn ventrally and join broad bands of fibres, which follow a circular course. Narrow spaces between the bands are crossed by bundles running postero-ventrally. Dorsally, at the posterior edge of the collar, most of the fibres are circular, and the longitudinal bundles which join them diverge from the dorsal mid-line as they approach. The circular bands cross the collar-trunk septum and continue round the trunk to join the ventral nerve cord as part of the prebranchial nerve ring. This constitutes the main nervous pathway between the collar epidermis and the remainder of the body. The connexions with the neurocord and proboscis stalk and with the buccal cavity round the lip of the mouth involve comparatively few fibres, which do not form bundles.

(*d*) *Trunk*

There is a nerve-fibre layer under the greater part of the trunk epidermis, thickened locally to form the prebranchial nerve ring and the dorsal and ventral longitudinal nerve cords. The bundles of nerve fibres from the neurocord and collar epidermis, which form the prebranchial nerve ring (figure 7), spread posteriorly below the anterior gill pores into a layer of fibres, which is particularly thick ventrally and anteriorly, its thickness ranging from 10 to 30μ in a large specimen. The epidermis of this region is distinctively brick-red, and is thickened as though it were an expansion of the broad slightly raised ridge formed by the epidermis of the ventral cord (figure 10). Histologically it differs from this epidermis, containing numerous large cells of a characteristic type. Its basal membrane is thickened locally to form a number of narrow ribs, which run round the body from immediately below the region of the gill pores to the ventral mid-line. These are not folds but solid thickenings, which are narrow antero-posteriorly but project prominently into the epidermis. Associated with each rib there is a bundle of nerve fibres, which stands out prominently because of the rib beneath it. Each bundle encircles the body with its rib until it joins the ventral nerve cord. Between the bundles the nerve fibres are less regularly orientated, but the majority follow a circular course. One effect of the ribs is probably to strengthen the epithelium against transverse stresses, but it is unlikely that they are required to protect their associated bundles of nerve fibres against stretching, as other parts of the nervous system, for instance, the longitudinal nerve cords, have no such protection and are subjected during life to great changes in length. More probably they serve to anchor the bundles to the basal membrane, so that they will not be torn away by contraction of the strong longitudinal muscles, which are attached to the inside of the membrane.

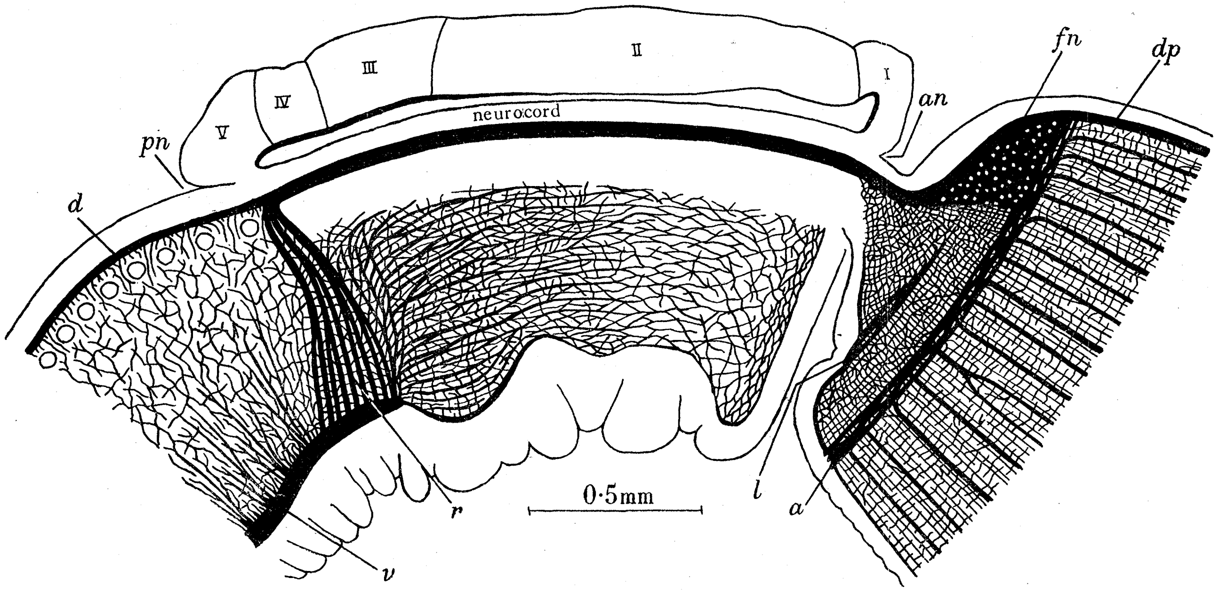

FIGURE 7. Plan of the nervous system of the collar and adjacent regions. The dorsal half of the collar is seen in sagittal section, to show the neurocord and the thickness of the epidermal nerve-fibre layer in successive zones of the collar epidermis. The remainder is in surface view to show the arrangement of fibres in the epidermal nerve-fibre layer. Bundles of orientated nerve fibres, represented by thicker lines, are greatly reduced in number for the sake of clarity. *a*, anterior nerve ring; *an*, anterior neuropore; *d*, dorsal nerve cord of trunk; *dp*, dorsal nerve cord of proboscis; *fn*, fan-shaped thickening of the nerve-fibre layer on the dorsal part of the posterior surface of the proboscis; *l*, nerve loop underlying groove of ciliary organ; *pn*, posterior neuropore; *r*, prebranchial nerve ring; *v*, ventral nerve cord of trunk; I–V, zones of collar epidermis.

The dorsal and ventral nerve cords, which extend throughout the length of the trunk, lie under slightly raised ridges of thickened epithelium (figure 10), but when the trunk contracts the ventral muscle bands push out the body wall on each side of the ventral cord, which comes to lie in a V-shaped depression of the basal membrane (figure 8). Both cords are formed of numerous longitudinal fibres, superimposed upon a thin basal layer of fibres, which run for the most part transversely or irregularly and are stained particularly darkly by the Bodian method. This thin layer is continuous on each side with the general nerve-fibre layer of the adjoining epidermis (figures 8 and 9). Silén (1950) described a similar condition in *Glossobalanus marginatus*, and gave a photograph showing the dorsal cord superimposed upon the basal plexus. Probably the dark staining of this plexus, together with the presence of artefacts within it and of indistinctly stained giant nerve fibres adjoining it, misled him into the belief that ganglion cells are common next to the basal membrane in the nerve cords (p. 329).

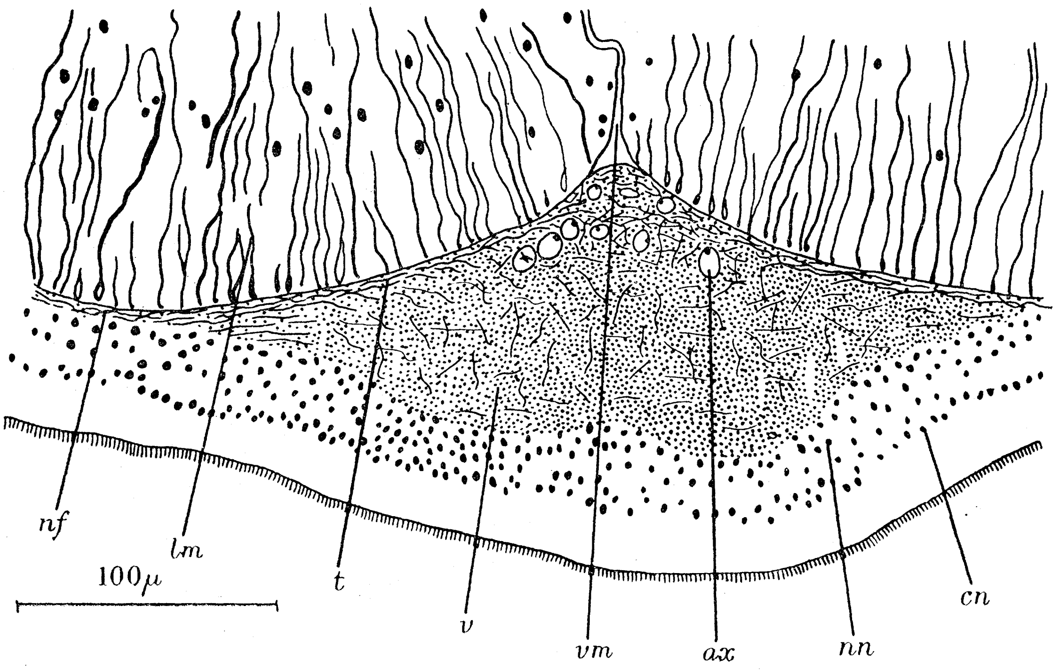

FIGURE 8. Transverse section through the ventral nerve cord in the hepatic region. *ax*, a giant axon, one of nine which appear in this section; *cn*, nuclei of ciliated cells, most of which lie superficially in the epidermis; *lm*, muscle fibres; *nf*, nerve-fibre layer of trunk epidermis, in which most fibres are transverse; *nn*, nuclei which probably belong to primitive ganglion cells; *t*, transverse fibres which lie under the nerve cord and are continuous with those of the neighbouring epidermis; they are more darkly stained by the Bodian method than *v*, the longitudinal fibres of the nerve cord; *vm*, ventral mesentery.

The ventral cord is much larger than the dorsal, the difference in size being exaggerated in fixed material by greater longitudinal contraction of the ventral surface. Even after allowance for this has been made it is only in the anterior part of the branchial region that the dorsal cord is as much as half the size of the ventral. Posteriorly the size of the dorsal cord decreases rapidly *pari passu* with a decrease in the dorsal musculature, but it remains distinct, extending almost to the surface of the epidermis in places where this is thin. To judge from the figures of Spengel (1893) and the review of Bullock (1945), it is the rule in Enteropneusta for the ventral cord to be larger than the dorsal behind the branchial

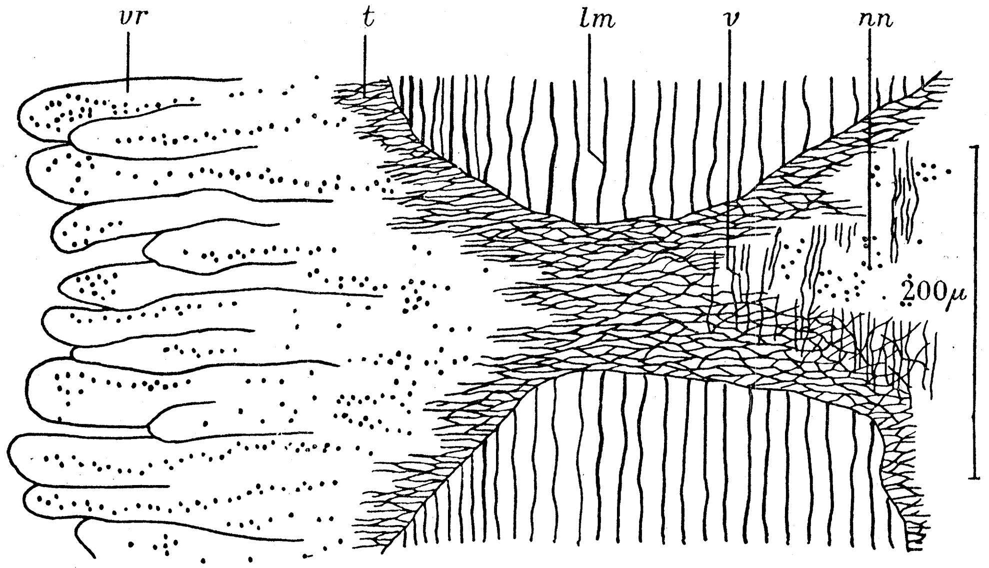

FIGURE 9. Part of a section which is tangential to the nerve-fibre layer at the edge of the ventral nerve cord in the hepatic region. *lm*, muscle fibres; *nn*, nuclei of nerve-cord epithelium; *t*, nerve-fibre layer consisting mostly of transverse fibres, which form ill-defined bundles under the ventro-lateral ridges of the epidermis; *v*, longitudinal fibres of the nerve cord; *vr*, one of the ventro-lateral ridges of the epidermis, seen in surface view in figure 10.

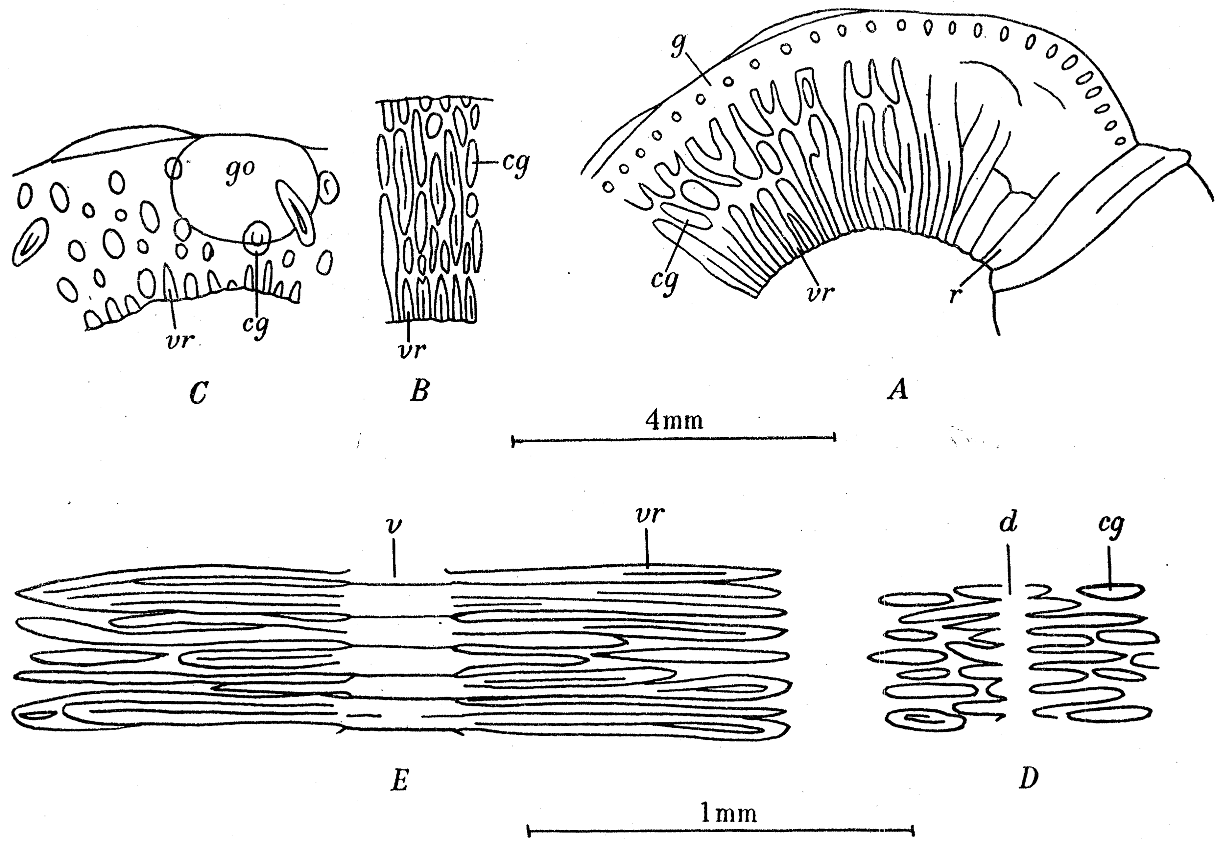

FIGURE 10. Modifications of the trunk epidermis, seen in surface view during life. *A*, anterior end of trunk, from right side; *B*, oesophageal region, lateral view; *C*, hepatic region, lateral view; *D*, the mid-dorsal line, in the oesophageal region; *E*, the ventral surface, in the oesophageal region; *cg*, strongly ciliated, glandular areas of thickened epidermis; *d*, narrow brown ridge overlying the dorsal nerve cord; *g*, thickened epidermis in region of gill pores; *go*, swelling caused by gonad; *r*, rufous patch over prebranchial nerve ring; *v*, thickened epidermis of ventral nerve cord, marked with transverse ridges caused by contraction; *vr*, ventro-lateral ridges, extending transversely from the ventral nerve cord.

region, and for the trunk muscles to be larger ventrally than dorsally. An exception is *Glandiceps,* a genus also remarkable for including forms which have been found swimming in surface waters (Ikeda 1908; Spengel 1909).

The general nerve-fibre layer of the trunk epidermis is better developed anteriorly than posteriorly, and ventrally than dorsally. Its distribution is associated in a general way with that of the muscles beneath the body wall, which are poorly developed dorsally, except in the anterior part of the branchial region. There the dorsal nerve-fibre layer is thicker than elsewhere, small groups of fibres running out transversely from the dorsal nerve cord and forming an irregularly arranged plexus in the epidermis surrounding the gill pores, through which some fibres join the splanchnic nerve fibres of the gill bars.

The nerve-fibre layer is well developed in certain other thickened areas of the trunk epidermis (figure 10). These comprise transverse ventro-lateral ridges on each side of the ventral nerve cord, shorter transverse thickenings on each side of the dorsal nerve cord and small, buff-coloured patches scattered over the lateral and dorsal surfaces of the trunk. The transverse ridges and the buff patches are similar histologically and differ from the neighbouring epidermis in being strongly ciliated, thicker and glandular. A group of nerve fibres runs out from the ventral nerve cord under each transverse ridge (figure 9), and nervous thickenings of irregularly arranged fibres lie under the ciliated patches. Nerve fibres are very sparse in the thin, olive-coloured, non-glandular epithelium which covers the greater part of the trunk, but there are probably nervous links between the dorsal and ventral nerve cords throughout the length of the body.

(*e*) *Splanchnic nervous system*

A nerve-fibre layer lies at the base of the epithelium throughout the buccal cavity. It is thickest anteriorly opposite the oral sphincter, so may well contain motor fibres supplying the splanchnic muscles. The fibres are mostly irregular, but towards the posterior end of the buccal cavity longitudinal fibres predominate and become grouped into somewhat ill-defined bundles. The ventral non-branchial part of the pharynx also has a distinct nerve-fibre layer, which is thickened in the ventral mid-line to form a tract of mostly longitudinal fibres, as in *Glossobalanus marginatus* (Silén 1950). The gut epithelium and the epidermis are in contact in the ventral mid-line throughout the length of the pharynx, so this nerve tract is separated from the ventral nerve cord of the epidermis only by a partition formed from the basal membranes of the two epithelia, but this partition is comparatively thick and apparently imperforate. Nerve fibres are absent from the gill pouches, and sparse or absent in the frontal epithelia of the gill bars, but there are fibres at the base of the lateral epithelia, running along the length of the gill bars and stained darkly, like nerve fibres, by the Bodian method. There is a similar but thicker layer of fibres, mostly circular in their arrangement, in the epithelium of each collar pore. Silén (1950) found nerve fibres in these positions and also in the ciliated epithelium of the proboscis pore of *Glossobalanus marginatus*; the proboscis pore in *Saccoglossus cambrensis* is unciliated and lacks nerve fibres.

The first and second part of the oesophagus are particularly muscular. Experiments on *S. cambrensis* and *S. horsti* showed that the first part executed peristaltic movements on mechanical stimulation of its inner surface. A well-developed layer of nerve fibres, which are mostly circular or longitudinal, is present throughout the first part. A layer of similar thickness composed mainly of longitudinal fibres is present in the second part, but nerve fibres are absent from the shallow epithelia of the grooves, which lie one on each side and run in an antero-dorsal direction to the oesophageal pores.

Behind the second part of the oesophagus the gut is less well provided with nerves and muscles. Its lateral walls are thrown into a series of vertical folds, which are particularly deep in the hepatic region, where they separate adjacent hepatic pouches. Thin circular fibres, which are stained like muscle fibres by iron haematoxylin, lie in the base of the gut epithelium along the ridge of each fold (figure 40, plate 35), and similar longitudinal muscle fibres lie in the splanchnic mesothelium. Fine fibres, stained like nerve fibres by the Bodian method, run along the ridge of each fold amongst the circular muscle fibres (figure 38, plate 35), but otherwise nothing resembling nerve fibres could be distinguished in this region.

Observations on the muscles and cilia of the gut suggested that the splanchnic nervous system serves to co-ordinate the following activities associated with feeding. Dilatation of the mouth allows mucus and sand to be ingested, then the tonus of the circular muscles of the pharynx and second part of the oesophagus, together with the peristaltic movements of the first part of the oesophagus, mould and compact the sand into a food cord, which can easily be propelled along by the cilia and eventually defaecated as a cast. Peristalsis of the gut was not observed except in the first part of the oesophagus, so the fine muscle fibres of the third part of the oesophagus, the hepatic region and the intestine are presumably concerned with maintaining the shape of the vertical folds and resisting the longitudinal stresses produced as the food cord passes through. The cilia in these three posterior regions of the gut are inactive for long periods, during which the food cord is stationary, but occasionally they embark on phases of activity which move it onwards. These phases were not observed to be affected by mutilation and are therefore unlikely to be under nervous control. The lateral cilia of the gill bars, however, were almost invariably immobilized by mutilation of the pharynx, so their activity may well be controlled by the nerve fibres which lie in the lateral epithelia. Silén (1950) found cells which appeared to be sensory in the lateral epithelia of *Glossobalanus*.

(*f*) *The proboscis nervous system in some other Enteropneusta*

The plan of the proboscis nervous system found in *Saccoglossus cambrensis* is not general throughout the Enteropneusta. Most species lack a dorsal nerve cord, though this occurs in *S. ruber*, *S. horsti* (Brambell & Goodhart 1941), *S. serpentinus*, *S. pusillus*, *S. pygmaeus*, *S. otagoensis* and *Glossobalanus ruficollis* (Bullock 1945). An important function of this cord in *Saccoglossus cambrensis* and *S. horsti* is the propagation of burrowing peristalsis (pp. 341, 342) and its wide occurrence in the genus *Saccoglossus* is probably associated with the large size of the proboscis and the actively burrowing habit of most species. Bullock (1945) observed no subsidiary systems of longitudinal or circular bundles in the Enteropneusta he examined, merely noting that the fibres of the proboscis base 'tend to be oriented circularly, forming the anterior nerve ring of some earlier authors'. Spengel (1877, 1893) coined this term, but neither he nor Van der Horst (1927–39) saw in the ring any circular arrangement of the fibres. Material kindly made available by Professor F. W. Rogers Brambell, F.R.S., was therefore examined to see what systems of through-conduction paths occurred in other forms. It was found that bundles of nerve fibres could be distinguished in sections stained with eosin.

Several complete series of vertical, horizontal and transverse sections of *S. horsti* were available, which showed that this species has a proboscis nervous system like that of *S. cambrensis*. The longitudinal bundles were very distinct, and in a single favourable tangential section one could be seen to run for a quarter of the length of the proboscis without branching. *S. inhacensis* Kapelus also has longitudinal bundles in the posterior half of the proboscis and an anterior nerve ring occupying a position similar to that in *S. cambrensis*, but the region of the ciliary organ in the sections of this species was too distorted for critical examination.

S. kowalevskyi Agassiz has prominent longitudinal bundles, and circular bundles which form a slight thickening under the ciliary organ. Brambell & Cole (1939*b*, figure 5, plate III) gave a photograph of the latter showing a large bundle with several smaller ones on each side of it. They do not leap to the eye because they are cut transversely, but they can be followed through the neighbouring sections, for a total distance of about 0·5 mm. No circular bundles could be distinguished elsewhere, so it appears that *S. kowalevskyi* differs from *S. cambrensis* in that the proboscis lacks a dorsal nerve cord and has the entire anterior nerve ring, not merely a detached loop, lying beneath the groove of the ciliary organ.

In *Balanoglossus australiensis* Hill longitudinal bundles occur towards the base of the proboscis, which narrows so rapidly that they become arranged almost radially. Under the ventral part of the ciliary organ there is a thickening with clearly defined anterior and posterior borders, which suggest that it contains a preponderance of circular fibres. If so, it forms with the dorsal thickening of the proboscis stalk an anterior nerve ring, like that of *Saccoglossus kowalevskyi* in lying wholly under the ciliary organ, but bulkier and less distinct. Elsewhere in the proboscis base (which may be defined as that region normally overhung by the anterior edge of the collar, possessing comparatively few epidermal gland cells and a thickened basal membrane) there is a thinner, apparently undifferentiated nerve-fibre layer. Around the basal periphery lies a groove, beneath which the nerve-fibre layer is thinner still.

In *Glossobalanus minutus* Kowalevsky most of the fibres beneath the ventral part of the ciliary organ are transverse, but do not form an appreciable thickening. In *G. hedleyi* Hill a bulky thickening lies ventrally under the ciliary organ, possessing well-defined anterior and posterior borders, which suggest that it contains a preponderance of circular fibres. In *Ptychodera flava* Eschscholtz the epidermis in the postero-ventral region of the proboscis base, immediately anterior to the proboscis skeleton, is thin and appears devoid of nerve fibres; anterior to this the nerve-fibre layer is very thick. This thickening lies under the ciliary organ and its posterior edge is abrupt, so probably most of its fibres are transverse. Anteriorly it becomes gradually thinner. Bullock (1945, figures 5 and 17) showed a similar thickening under the ciliary organ of *P. bahamensis*. There appeared to be no longitudinal bundles in these species of *Glossobalanus* or *Ptychodera*.

These observations suggest that most of the Harrimanidae, and a few of the Ptychoderidae, have longitudinal bundles of nerve fibres in the epidermis of the proboscis, and that most Enteropneusta have an anterior nerve ring, partly or wholly associated with the pre-oral ciliary organ. In some species the ring is not thickened, but can be distinguished from the neighbouring nerve-fibre layer by the preponderance of circular fibres which it contains.

HISTOLOGY OF THE NERVOUS SYSTEM

Spengel (1884, 1893) described the giant nerve cells of the neurocord and suggested that spherical nuclei common in the deeper layers of the epidermis belonged to ganglion cells, which gave off processes into the nerve-fibre layer. Oval nuclei were also common, some belonging to supporting cells, others probably to sensory cells. No later account was more complete or better illustrated until Bullock's descriptions of the giant nerve cells (1944) and epidermal nervous system (1945), but Bullock was probably mistaken in believing that cigar-shaped nuclei, which are often seen in the superficial layers of the epithelium, belong to sensory cells. This view, which Silén (1950) opposed, is not applicable to *Saccoglossus cambrensis*, in which some cigar-shaped nuclei were identified with certainty as belonging to the ciliated cells, the nuclei of which vary in shape according to the state of contraction of the epithelium. Since Bullock was probably mistaken in regarding the cigar-shaped nuclei as belonging to sensory cells, he perhaps overestimated the abundance of sensory cells, of which he found one type, diffusely distributed in the epidermis.

Silén (1950) found a similar but apparently less numerous type of sensory cell in *Glossobalanus marginatus*, capriciously stained by the Bodian method. He presented a new histological picture, in describing nerve cells scattered within the nerve-fibre layer, but particularly common next to the basal membrane. His photographs suggest that he was deceived by artefacts (p. 342), and it seems unlikely that his staining technique had been particularly successful since giant nerve cells, though apparently present, were not clearly stained. In *Saccoglossus cambrensis* these were always clearly stained by the Bodian method. Probably cell bodies rarely occur in the nerve-fibre layer of the Enteropneusta, for if they were common there, their nuclei would almost certainly have been revealed by widely used cytological methods.

(*a*) *Proboscis epidermis*

The epidermis of the proboscis consists of crowded cells with nuclei numerous at all levels except near the surface and near the basal membrane. The surface layer is occupied by rootlet bundles associated with the cilia and by many gland-cell bodies, whilst the layer adjoining the basal membrane contains the nerve fibres. The ciliated cells and gland cells have their basal portions constricted to form supporting fibres, which traverse the nerve-fibre layer and are attached to the basal membrane. They have been described by Spengel (1893), Van der Horst (1927–39), Brambell & Cole (1939*b*) and Bullock (1945). The ciliated cells have comparatively large vesicular nuclei, which are usually oval, and the gland cells are of three types: mulberry cells, which are sparse; small goblet cells, which are abundant; and large mucus cells, which are about three times as numerous as the small goblet cells and half as numerous as the ciliated cells. Their nuclei lie at the basal ends of the masses of secretion, those of the mucus cells being slightly smaller and more deeply situated than those of the ciliated cells, and those of the small goblet cells being smaller still and usually lying near the surface.

Since the general cytoplasm is usually unstained the cells are distinguishable mainly by their ciliary rootlets or glandular contents; as nerve cells lack these, only their nuclei and fibres can usually be seen. Thus the giant fibres of the neurocord and their large round nuclei show clearly in silver preparations, but their cell bodies often appear as spaces in the surrounding tissue, from each of which a fibre originates. Numerous rounded nuclei lie deeply in the epidermis, just outside the nerve-fibre layer, and darkly stained iron haematoxylin preparations indicate that many of these belong to primitive ganglion cells, with one or more fine processes (figure 11). Probably most of the deeper nuclei belong to cells of this type, which seem sufficiently numerous to provide cell bodies for most of the fibres in the epidermal nervous system. The fibres usually run parallel to the basal membrane, often far below the level at which the nuclei occur, so they must be relatively very long in spite of their fineness.

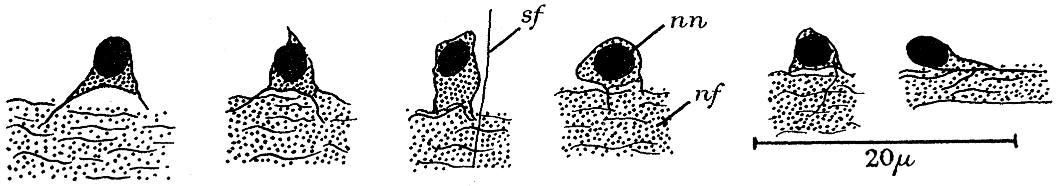

FIGURE 11. Cells lying immediately outside the nerve-fibre layer of the proboscis epidermis. Bouin, Heidenhain's haematoxylin. They appear to be multipolar primitive ganglion cells, contributing fine fibres to *nf*, the nerve-fibre layer; *nn*, nucleus; *sf*, a supporting fibre from a ciliated cell.

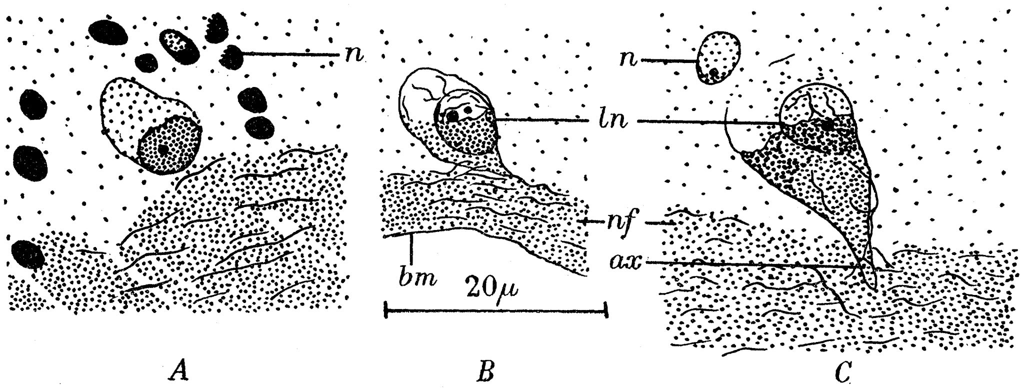

FIGURE 12. Large cells adjoining the nerve-fibre layer. Bodian. *A*, over the anterior nerve ring; *B*, at the tip of the proboscis; *C*, over the posterior end of the dorsal nerve-cord of the proboscis: *ax*, tapering process, probably an axon, which joins the nerve-fibre layer; *bm*, basal membrane of epidermis; *ln*, nucleus of large cell, with conspicuous nucleolus; *n*, nucleus of the size usually found in epidermal cells; *nf*, nerve-fibre layer.

A few large rounded cells were seen next to the nerve-fibre layer of the proboscis, especially towards the posterior end of the dorsal nerve cord and elsewhere in the basal region (figure 12). Single fibres from these joined the nerve-fibre layer, so they are probably unipolar neurones like those of the neurocord (p. 335).

Hess's cells

Hess (1937) described long bipolar neurones in *S. kowalevskyi*; similar cells were seen at the proboscis tip of *S. cambrensis* (figure 13), especially in tangential sections through the epidermis (figures 29 and 30, plate 33), the majority lying immediately outside the nerve-fibre layer. They were from 20 to 40μ long and mostly orientated with the long axis pointing dorso-ventrally, and the nucleus at the dorsal end. In some specimens none could be found. Hess believed that the whole of the nervous system of *S. kowalevskyi* consisted of a plexus of fibres from cells like these, but Bullock (1945) disagreed. In *S. cambrensis* cells of this type were seen occasionally in several parts of the proboscis epidermis, but they were not seen in large numbers except at the tip of the proboscis. In view of the simple arrangement of the proboscis nervous system it is curious to find them so localized.

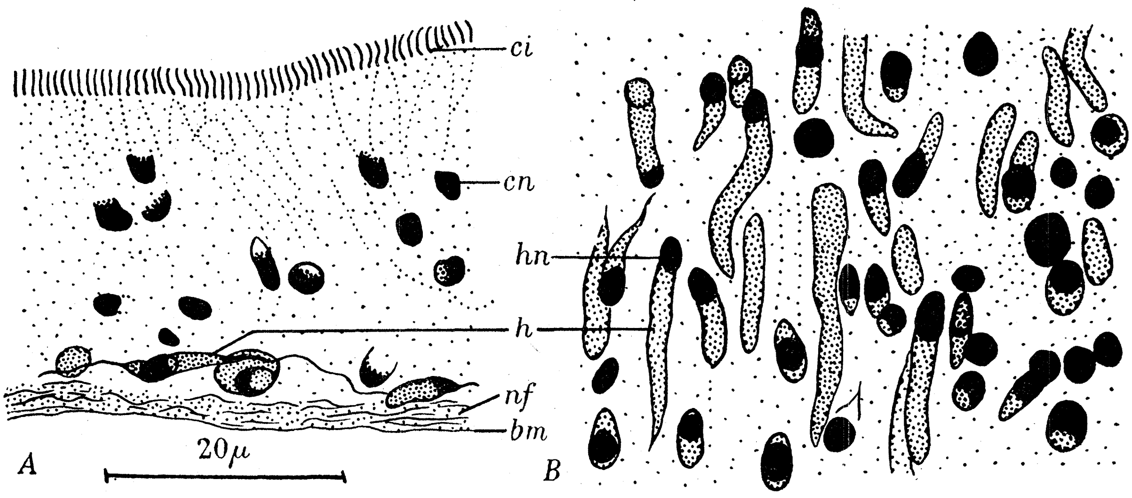

FIGURE 13. *A*, part of a sagittal section through the epidermis of the proboscis tip. Bodian. An elongated cell adjoining the nerve-fibre layer appears to be a bipolar ganglion cell of the type described by Hess; *B*, similar cells from one of the most anterior sections of a transverse series through the proboscis, adjacent to the section shown in figure 29, plate 33. The section passes through the base of the epidermis at the proboscis tip, parallel to and adjoining the nerve-fibre layer. The majority of the cells are orientated dorso-ventrally with the nucleus near the dorsal end. *bm*, basal membrane of epidermis; *ci*, cilia, with indistinctly stained rootlet bundles; *cn*, nucleus of ciliated cell; *h*, one of Hess's cells; *hn*, nucleus of Hess's cell; *nf*, nerve-fibre layer.

The tip of the proboscis arises from the apical region of the larva. Larvae of *S. horsti*, which were obtained without much difficulty, swim for several hours suspended in the water like Tornaria larvae, and possess apical tufts of long cilia, which stand out stiffly or execute slight movements. Sections showed a basi-epithelial concentration of nerve fibres below the apical tuft, as in the apical plate of a Tornaria. The anterior concentration of bipolar neurones may therefore be derived from the ganglion cells of the apical plate, forming in the adult part of a nervous system which is degenerate and simplified. Their apparent absence in certain individuals may be correlated with the fact that some batches of larvae (of *S. horsti*) have the apical tuft much less developed than others. Hess (1938) suggested that his bipolar neurones were photosensitive cells, though Bullock (1945) pointed out that their form and position are those of ganglion cells rather than sensory cells. Removal of the proboscis tip produced no obvious modification of the reactions to light in *Saccoglossus* (p. 346), but Crozier (1917) and Hess (1936) described this region as particularly photosensitive in *Ptychodera bahamensis*, whilst the apical plate of the Tornaria larva includes eyespots.

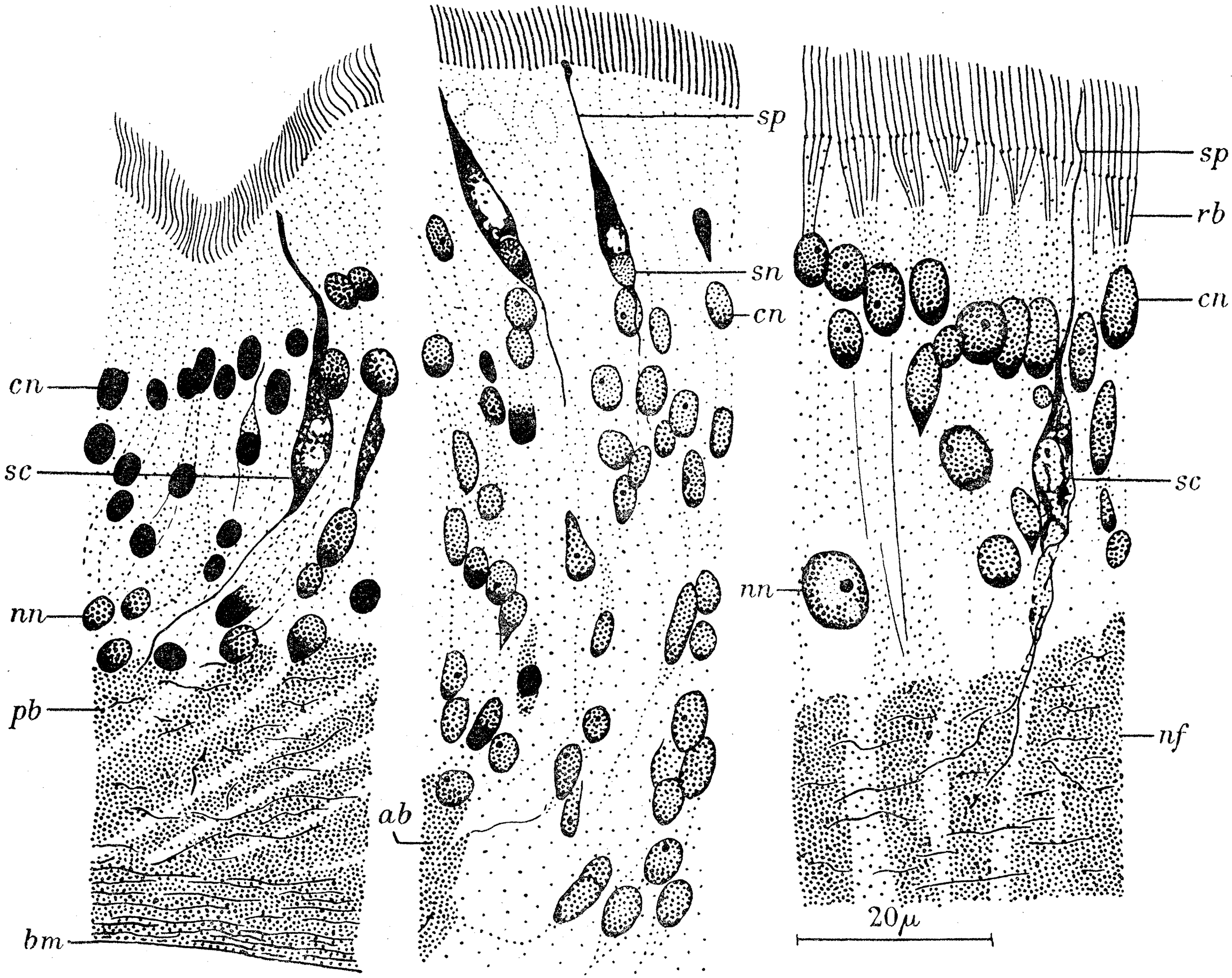

FIGURE 14. Sections perpendicularly through the epidermis of the proboscis base, showing cells which appear to be sensory. Bodian. *ab*, anterior bundle of anterior nerve ring, cut transversely; *bm*, basal membrane of epidermis; *cn*, nucleus of ciliated cell; *nf*, nerve-fibre layer of proboscis stalk, with gaps for the passage of supporting fibres, which were unstained; *nn*, nucleus probably of a primitive ganglion cell; *pb*, posterior bundle of anterior nerve ring, cut transversely; *rb*, ciliary rootlet bundles; *sc*, darkly stained cell, which appears to be sensory and to contribute a fibre to the nerve-fibre layer; *sn*, nucleus of similar cell from periphery of proboscis base; *sp*, process leading to the surface.

Sensory elements

Numerous cells that were stained selectively by silver methods, and could not be demonstrated by any other method used, occur round the periphery of the proboscis base (figure 14 and figures 31 and 32, plate 34). Their nuclei are rounded or oval, smaller than those of the ciliated cells and usually situated nearer the surface. The narrow cell body is prolonged into a distal fibre, which ends amongst the rootlet bundles of the cilia, and an opposite fibre which could occasionally be seen to contribute directly to the nerve-fibre layer. Cells of this type occur sparsely on the proboscis stalk and in still smaller numbers over the whole surface of the proboscis. They are probably sensory because of their form and superficial position; their selective staining by silver methods (in weakly stained sections they stood out particularly clearly against their pale surroundings); their ideal situation for testing the quality of the surrounding sand (since particles embedded in mucus accumulate round the base of the proboscis, before being engulfed during feeding or swept backwards by the collar cilia when not feeding); and lastly, their position opposite the anterior nerve ring, which bounds the important nervous thickening of the proboscis base (p. 321).

In a few preparations made by Holmes's method, darkly stained cells, with the nucleus situated comparatively deeply and with a process leading up to the surface, could be seen in the proboscis epithelium in considerable numbers. These resembled the cells which Bullock (1945) described as sensory, but did not appear so numerous.

Brambell & Cole (1939*b*) suggested that the ciliary organ may have a sensory function, and described cells in the strongly ciliated ridges as having the appearance of sensory cells. These were stained black by iron haematoxylin, but could not be demonstrated with certainty by any other method. Bullock (1945) and Silén (1950) were of the opinion that they are glandular. They have fine distal processes, but these are often associated with gland cells, apparently through the secretion being fixed as discharged. The nerve loop of the ciliary organ (p. 320) lies not under the ridges where they lie, but under the groove between. Its presence is strong evidence of a sensory function, for it is unlikely to be concerned with control of cilia, as the groove is not ciliated particularly strongly, and all the cilia of the organ continue to beat after mutilation, so are probably not under nervous control. In the epithelium adjoining the nerve loop there are many rounded nuclei, of the type which probably belong to primitive ganglion cells (figure 5 and figure 26, plate 33). Nearer the surface there are approximately equal numbers of vesicular nuclei belonging to the ciliated cells, which are often elongated, and smaller denser nuclei, most of which are situated more superficially than those of the ciliated cells. The smaller nuclei belong to narrow cells with processes leading to the surface (figure 26, plate 33), which contain numerous minute granules darkly stained by silver methods or iron haematoxylin. These are probably sensory cells because of their shape, with narrow distal processes and centripetal fibres; their nuclei, which resemble those of the cells from the basal periphery (which are also probably sensory); their dark staining by silver methods; their position opposite the nerve loop of the ciliary organ; and lastly, their ideal situation for functioning as taste-receptors, as observations on the living ciliary organ showed. The strong cilia of the ridges beat into the grooves between, causing a current to flow away from the groove in a direction perpendicular to the proboscis base, thus helping to separate the food-collecting mucus from the epithelium and to carry it towards the mouth. Fine particles were driven into the groove, travelled down the lateral cornua and collected ventrally, whence they were drawn into the mouth with the mucus and sand which was being ingested. Probably the ciliary organ, besides playing a purely mechanical part in the feeding mechanism, functions as an organ of taste, testing the fine particles.

The epithelium of the remainder of the basal region is simple and uniform. Gland cells are very sparse. The nuclei of the ciliated cells occur near the surface, and the numerous other nuclei seem to belong for the most part to primitive ganglion cells.

(*b*) *Neurocord*

Anteriorly and posteriorly the neurocord has a well-developed neural keel, perhaps corresponding to the 'dorsal roots' of Ptychoderidae. In the middle its dorsal surface is in contact with the basal membrane of the epidermis, which separates its tissues from the epidermis throughout the length of the collar. The anterior and posterior neuropores are shallow, the continuous axis canal of some Enteropneusta being represented by small lacunae, which are rare in the middle of the neurocord, but more numerous and larger towards each end, and which may be vesicular or so flattened that the cavities are completely occluded. They are bordered by cells, equipped with the vestigial ciliature noted by Bullock (1945) and with supporting fibres which radiate outwards in all directions and are attached to the basal membrane; these may be compared with the ependyma of vertebrates. Occasionally gland cells occur amongst them, the commonest type being large goblet cells with a homogeneous secretion stained by mucicarmine and haematoxylin, which was sometimes discharged into the lacunae. These mucus cells are abundant round the posterior neuropore, where they discharge to the exterior; cells of the same type are numerous round the gill pores. Mulberry cells occur in the neurocord, but are more rare.

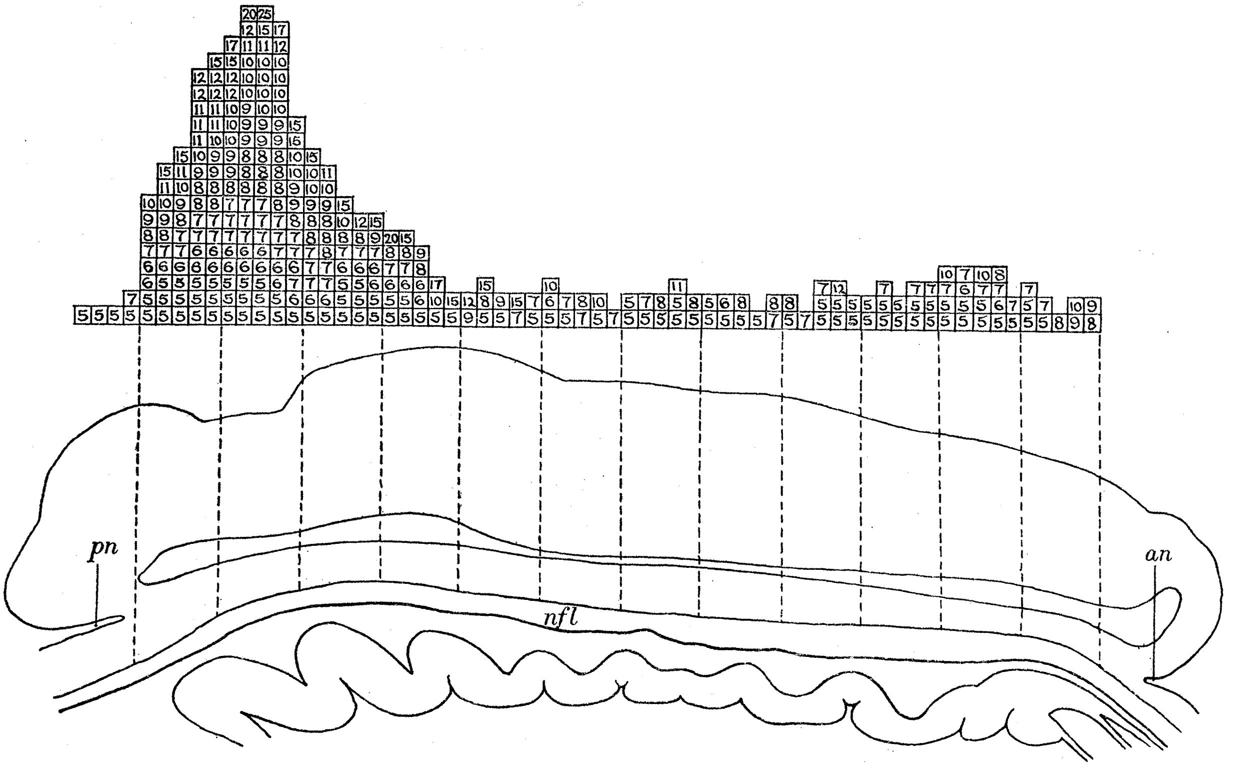

FIGURE 15. The longitudinal distribution of unipolar neurones in the neurocord. Each square, representing a neurone, is placed vertically above the position occupied by the neurone in the neurocord figured in sagittal section below. The antero-posterior diameter of the neurone in micra is given in each square. The cell bodies of the neurones were confined to the cellular part of the neurocord, which lies dorsally to the nerve-fibre layer. The majority lay along the lateral edges of the neurocord, and those of both sides are shown. 'Giant nerve cells' (about 10μ or more in breadth) are sparse, except near the posterior end of the neurocord. A few form a small group near the anterior end. *an*, anterior neuropore; *nfl*, nerve-fibre layer of neurocord; *pn*, posterior neuropore.

The cells most characteristic of the neurocord are unipolar neurones, their nuclei large and rounded with a conspicuous nucleolus, their cell bodies pyriform with no dendrites. Occasionally one was seen with a process connecting it to an adjoining lacuna (figure 35, plate 34), such a cell thus resembling a sensory or epithelial cell. It may be remembered that the early neuroblasts of vertebrates are often of the form of neuro-epithelial cells, and that *Amphioxus* has neuro-sensory cells in the ependyma of the central canal. The neurones occur throughout the length of the neurocord, but are much more numerous and much larger near the posterior end. Their longitudinal distribution in a typical specimen is indicated diagrammatically in figure 15. The smallest, measuring about 5μ across, have nuclei not much larger than those of the ciliated cells, and axons which taper rapidly, enter the nerve fibre layer and become lost amongst the finer fibres.

Giant nerve cells

The unipolar neurones vary in size from about 5 to 25μ, measured antero-posteriorly in parasagittal sections (figure 34, plate 34). Those measuring about 10μ or more have axons which do not taper markedly, but remain much larger than the majority of the nerve fibres; these may conveniently be termed giant nerve cells. They form a small group at the anterior end of the neurocord and a large group at the posterior end, being sparse in between. Occasionally a few occur above the dorsal nerve cord immediately behind the neuropore, but otherwise they are confined to the neurocord (the large neurones observed at the base of the proboscis being without giant axons). The largest observed measured 27μ across its shortest diameter, but in a typical specimen there were only about three or four which measured over 20μ. As in other Enteropneusta (Spengel, 1893; Bullock, 1944), the majority lie near the lateral edges of the neurocord (figure 33, plate 34), in approximately equal numbers on each side. Their axons run deeply into the nerve-fibre layer, then horizontally across the mid-line to the other side, and then more or less longitudinally, keeping near the basal membrane. Those of the anterior group run anteriorly and can be followed in the epidermal nerve-fibre layer almost to the tip of the proboscis. Those of the posterior group run posteriorly in the neurocord, in the dorsal nerve cord of the trunk and, having encircled the trunk in the prebranchial nerve ring, in the ventral nerve cord. Probably all the giant axons of the ventral cord have their cell bodies in the neurocord, for no giant nerve cells have been observed in the ventral epidermis. Had they been present there, their large nuclei would scarcely have escaped observation. The axons in the ventral cord lie near to the basal membrane (figure 8 and figure 36, plate 35), and extend a varying distance posteriorly. In only one specimen were they found in the hepatic region and never in the intestinal region. Typically, as Bullock (1944) observed, they do not extend farther back than the branchio-genital region. Individual axons are difficult to follow, as their course is irregular and their shape and size variable, perhaps because of contraction of the body.

The giant axons often appear as cylinders amongst the finer nerve fibres, their diameter usually lying between 2 and 15μ. There is no stainable connective tissue, myelin or Schwann cell sheath around the cylinder, but merely a thin membrane. The axoplasm is usually stained faintly by the Bodian method, but not by haematoxylin; it often appears to have shrunk away from the membrane. Where axons become narrow their contents are stained deeply by the Bodian method. The axons intertwine, and are closely invested by the fine fibres which surround them. Apart from such contacts no evidence of a synapsis was seen, except in one specimen, in which there was a giant nerve cell embedded deeply in the nerve-fibre layer below the posterior neuropore (figure 37, plate 35). The nerve cell had several large processes associated with it, one of which was evidently the axon. Two of the others appeared to be giant fibres making contact with the cell body at the base of the 'axon hillock'. The end of each fibre was slightly swollen and the bounding membrane of one, although as usual somewhat indistinct, appeared to be intact, suggesting synaptic contact rather than fusion. The contents of the fibres were stained fairly darkly, but that of the cell body with which they made contact was practically unstained.

A careful search through three other complete series of sections and more brief examination of many other specimens failed to reveal another neurone in this position. Typically the nerve cells lie clear of the layer of nerve fibres, which do not make direct contact with cell bodies. This epithelial position of the neurones may possibly be a primitive character, but may equally well be neotenous, since vertebrate neuroblasts develop from neuroepithelial cells. The fact that a cell may occur deep in the nerve-fibre layer as an abnormality, and then appears to receive synaptic connexions from neighbouring axons, would agree with the view that the usual condition is neotenous.

(*c*) *Collar epidermis*

The five zones into which the collar epidermis is divided, as in other Enteropneusta, are outlined in figure 7. The first zone has a comparatively thin epithelium and contains few gland cells (none in its posterior border). Abundant mucus cells are present in the third and fourth zones, and many elongated mulberry cells lie near the surface of the epithelium in the third zone and towards the posterior end of the second zone. The fifth zone contains no mucus cells, but numerous elongated gland cells which are stained darkly by silver methods. The epithelium is ciliated throughout and so thick and complex that the search for nerve cells within it was fruitless, except at the anterior lip, where the only gland cells are elongated mucus cells of a type characteristic of the buccal cavity. Rounded nuclei, which probably belong to primitive ganglion cells, occur there next to the slightly thickened nerve-fibre layer, and more elongated nuclei, belonging to the ciliated cells, lie superficially. Amongst the latter there are smaller, denser nuclei, with narrow cell bodies extending between the ciliary rootlet bundles to the surface, which are probably sensory cells because they resemble those of the proboscis base (p. 332), and because they occur in a region which executes movements suggesting great sensitivity (p. 346). Unlike those of the proboscis base they were not stained selectively by silver methods, so were difficult to distinguish from the ciliated cells. They were seen most clearly in sections which were stained deeply with iron haematoxylin.

The gland cells in the posterior part of the collar epidermis probably assist tube-building (p. 344), and the thickening of the nerve-fibre layer there suggests that they may be under nervous control, but the arrangement of the fibres, connecting chiefly with the ventral cord of the trunk (figure 7), makes it more likely that these are concerned with the through transmission of impulses, perhaps of those co-ordinating peristalsis (p. 343).

(*d*) *Trunk epidermis*

The epithelia of the dorsal and ventral nerve cords are without gland cells, yet contain nuclei which greatly outnumber the ciliary rootlet bundles (except in the much reduced part of the dorsal nerve cord which lies behind the branchial region), so the proportion of ciliated cells must be low. As elsewhere, the nuclei of the ciliated cells are oval and occur near the surface of the epithelium. Cells which appeared to be intercalated between them were occasionally observed in the epithelium of the dorsal nerve cord. They resembled sensory cells in general form, but were not stained selectively by the Bodian method. Bullock (1944) figured similar cells from the dorsal nerve cord immediately posterior to the collar. The majority of the nuclei are rounded and lie deeply in the epithelium next to the nerve cords, apparently belonging to primitive ganglion cells.

The epithelium of the prebranchial nerve ring is crowded with large cells, which are lightly stained by silver methods and are apparently glandular. Its thickness is almost as great as that of the collar, and nervous elements could not be distinguished within it. The gill pores pierce longitudinal ridges (figure 10), which lie one on each side of the dorsal cord and are full of mucus cells. These were not sufficiently well preserved in paraffin sections for critical examination. In the ventro-lateral transverse ridges and other areas of thickened epidermis, which are well provided with cilia, mucus cells and nerve fibres (p. 326 and figure 10), it appeared that most of the more superficial nuclei belong to the ciliated cells, and most of the deeper to primitive ganglion cells. In the hepatic region these thickened areas are small and sparse, and the greater part of the epidermis is thin, with very few nerve fibres and comparatively few nuclei, which appeared to belong to unciliated cubical epithelial cells. In the intestinal region the thickened areas again become numerous and larger.

THE PROBLEM OF THE MOTOR NERVE ENDINGS

Bateson (1885, 1886) described and figured nerve fibres from the epidermal nervous system that passed through the basal membrane and became lost amongst the muscles, but Spengel (1893) could not find such fibres and pointed out that the basal membrane appeared to form a complete barrier between the epidermis and the muscles. He found associated with the dorsal blood vessel of *Glossobalanus sarniensis* four tracts of tissue which he termed the sympathetic nervous system, but these are not general in the Enteropneusta (Bullock, 1945). Brambell & Goodhart (1941) described another peculiar tract of tissue, lying under the ventral blood vessel in the proboscis of *Saccoglossus horsti*, as possibly a neuromotor organ. Professor Brambell kindly showed me his preparations of this structure, which consists of a cord of closely packed cells stretching the whole length of the proboscis. The cells are associated with the outer circular muscle fibres, and seemed to be for the most part cell bodies belonging to these fibres, concentrated in the ventral mid-line. This does not occur in *S. cambrensis*.

Van der Horst (1927–39) suggested that the fine reticular fibres, which lie amongst the muscle fibres of Enteropneusta and have usually been regarded as connective tissue, might belong to nerve cells forming a coelomic nervous system. Hess (1937) placed a similar interpretation upon them. Silén (1950) described a plexus of fibres, with nerve-cell bodies, on each side of the ventral mesentery in the trunk. He showed clearly, in diagrams, his conception of this system, but his photographs do not support his account convincingly. Presumably he regarded the fibres as nerve fibres because they were stained darkly by the Bodian method, but this method often stains a variety of structures, including cytoplasmic precipitation networks and muscle fibres and their bounding membranes. In *S. cambrensis* it often stained the extremely fine muscle fibres which run dorso-ventrally in contact with the coelomic surfaces of the ventral and dorsal mesenteries. Silén did not mention identifying these in his preparations; perhaps he misinterpreted them as nervous tissue. Bodian preparations of *Saccoglossus*, picking out the cell membranes of the muscle cells, showed that most of these cells consist of muscle fibres and large bodies of undifferentiated cytoplasm. Their form varies in different regions, those which are smaller and adjoin the basal membrane somewhat resembling the muscle cells of Nematoda (this type is indicated by letters *l* and *m* in figure 40, plate 35). The majority of the nuclei lie separated from the fibres and are surrounded by undifferentiated cytoplasm, which was almost unstained, but contained a delicate network of fibrils. In some regions, particularly amongst the deeper muscles of the proboscis where cell membranes appeared to be absent, the nuclei and the cytoplasmic fibrils resembled a separate tissue, distinct from the muscle fibres. Probably the multipolar cells described by Hess (1937) and the reticular cells termed connective tissue by other authors were in reality nuclei and undifferentiated cytoplasm of the muscle cells.

Bullock (1945) found no reason to believe that there were nerve cells amongst the muscles. He considered that the muscles are innervated by individual fibres from the epidermal nervous system, and he observed many such fibres turn inwards and appear to penetrate the basal membrane.

Experiments on *S. cambrensis* showed that peristaltic movements of the larger muscles are co-ordinated by the epidermal nervous system, so impulses must get through the basal membrane by some means, though it is an homogeneous sheet, quite unlike the fibrillar network found in echinoderms (Smith 1937). It consists of two layers, the outer formed by the epidermis and the inner by the coelomic epithelium or the muscle cells derived from it. Spaces between the two layers form the vessels of the blood-vascular system, but otherwise they are fused, often indistinguishably. The basal membrane is greatly thickened locally to form such structures as the proboscis skeleton and the supports for the gill bars, but in most parts of the body its thinness approaches the limits of visibility, so it is difficult to obtain evidence of nerve fibres traversing it.

In certain areas where the basal membrane is thicker, fibres can be seen within it. At the base of the proboscis there occur between the two lamellae fine fibres belonging to the chondroid tissue, which were stained like nerve fibres by silver methods (figure 26, plate 33), and, in some sections, appeared to communicate with the nerve-fibre layer in front of the keel of the proboscis skeleton and with the muscles towards the periphery of the proboscis base. The majority of the fibres, however, extend deeply into the chondroid tissue and link the pockets of cells which are contained in it. It seems that they must be concerned with the nutrition of these cells, unless it is supposed that the deposition of skeletal material may be under nervous control. Silver methods revealed other fibres traversing the blood sinuses in the posterior regions of the gut, especially on the ridges which separate adjacent hepatic pouches (figure 39, plate 35). They appeared to link the fine muscle fibres of the mesothelium (figure 40, plate 35) with the fibres at the base of the endothelium (p. 327), some of which were stained like nerve fibres (figure 38, plate 35), but they may well be supporting trabeculae which prevent the two lamellae, separated by the extensive blood sinuses, from being torn apart. In other places where the basal membrane is thickened or contains blood vessels no fibres were observed to cross it.

The outer circular muscles of the proboscis adhere to the basal membrane throughout their length. Since the thinness of the membrane approaches that of a cell wall, they may possibly be innervated by the nerve fibres which are closely apposed to its outer surface, but which may not necessarily penetrate it. The majority of the muscle fibres, however, are in contact with the basal membrane only through their terminal branches, whilst the retractor muscles of the proboscis skeleton are attached to thickenings and septa which have no nerve-fibre layer associated with them. It therefore seems highly probable that some of the fine fibres amongst the muscles are nerve fibres which have traversed the basal membrane. No concentrated tracts of motor nerves were seen going through the membrane, but single fibres often appeared to do so. Nerve fibres are generally thinner than 1μ, and single ones are very difficult to follow or identify.

ON THE FUNCTIONING OF THE NERVOUS SYSTEM

Although Crozier (1915, 1917) had noticed that progression of peristaltic waves along the trunk of *Ptychodera* depended on the continuity of the dorsal and ventral nerve cords, Van der Horst (1932), considering that the epidermal nervous system of Enteropneusta resembles the ectoneural system of Asteroidea, referred to it as probably entirely sensory. The ectoneural system is now regarded as not only sensory but also as an association plexus, forming a system of nerve centres (Smith 1945), and the epidermal nervous system of *Saccoglossus* differs from it, in that the through-conduction paths are superficial whilst the deeper fibres of the nerve-fibre layer are irregular, whereas in the radial cords of *Marthasterias* the deeper layers are organized into bundles and the superficial fibres are irregular (Smith 1937). The radial cords are probably composed of association fibres, together with sensory fibres derived from the numerous sense cells in the ambulacral grooves, whilst the reversal of the arrangement of fibres in *Saccoglossus* suggests that the predominant functions here are association and motor, the superficial bundles providing through-conduction paths, the deeper irregular fibres being concerned with integration and the distribution of motor impulses to the muscles. Such a contrast might be expected, since *Saccoglossus* leads a sheltered life, which is probably governed largely by internal rhythms. The distribution of the nerve fibres, both in the gut and body wall, broadly corresponds to the distribution of muscles and cilia (pp. 324 to 327), which further indicates that this epithelial system contains a high proportion of association and motor fibres. As the main nervous system it probably includes in its functions autonomous activity, integration and the distribution of motor impulses, as well as the reception of stimuli.

S. cambrensis is unfavourable for quantitative work, being small, with tissues too soft for the attachment of levers; but the following qualitative observations and experiments throw some light on the functioning of its nervous system. They refer to specimens kept in shallow dishes in the laboratory, unless otherwise stated. *S. horsti* was also observed in dishes and in glass jars containing mud from the natural habitat. Burrows were made against the glass, which was shaded with opaque covers. After removing the covers behaviour within the burrows could be observed.

(*a*) *Peristaltic locomotion*

Burrowing

Strong light, maltreatment or adrenaline induced burrowing fairly readily. The tip of the proboscis was thrust out, becoming thinner in the process; then it swelled and was withdrawn slightly. The contraction which caused the withdrawal travelled posteriorly as a peristaltic bulge, of which both the anterior and posterior edges were equally distinct. Meanwhile the tip was thrust out and withdrawn again, initiating the next wave of contraction (figure 16*A*). The bulges moved at a speed of about 1 mm per second and with a frequency of about twelve per minute. In an actively burrowing specimen, when one bulge reached the base of the proboscis, the next was half-way and a third was starting at the tip. They did not travel beyond the base of the proboscis, so the collar and trunk play no part in burrowing, as in *S. pusillus* (Ritter 1902). When in the sand the bulges must tend to remain anchored while the proboscis moves forward. Many nemertines, *Cephalothrix* and *Tubulanus* for instance, burrow in a similar way.

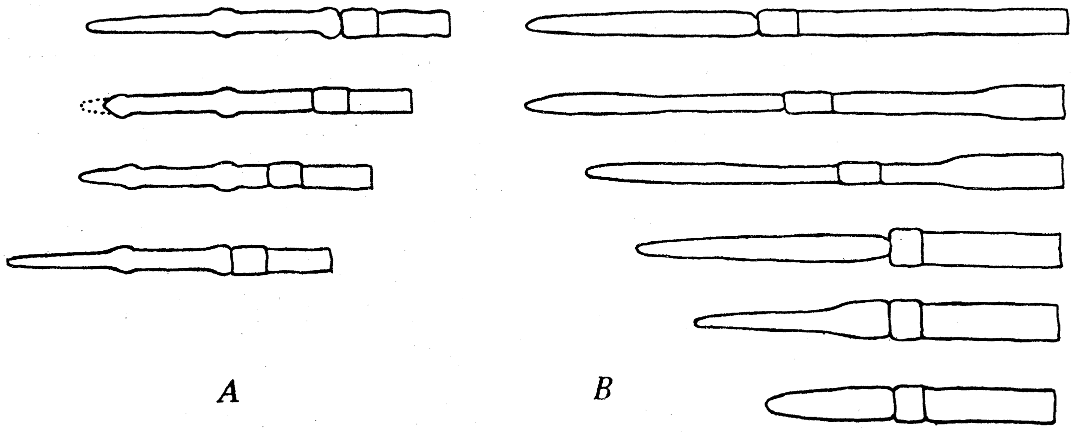

FIGURE 16. Diagrams showing the proboscis, collar and anterior end of the trunk in successive phases of peristaltic locomotion. *A*, burrowing, showing the tip of the proboscis alternately thrust out and withdrawn, forming bulges which travel steadily backwards and, being more or less anchored in the sand, tend to move the proboscis forwards, whilst the trunk follows passively. *B*, retreat, showing firstly the worm at rest; then a contraction appearing posteriorly, while the anterior part of the trunk relaxes and is pulled backwards, and the posterior end of the proboscis elongates actively; then the wave of contraction travelling forwards until the whole body has contracted.

Small pieces of the proboscis carried out burrowing movements like those of the intact organ, so the waves of contraction are not dependent for their initiation on any restricted centre in the proboscis tip, base or elsewhere. When a proboscis was cut across, except for a small bridge of tissue containing the undamaged dorsal nerve cord, the waves passed the cut regularly and uninterruptedly (figure 17*A*), but when the dorsal nerve cord was picked out at one point by needles the waves stopped there, though the neighbouring tissues had been little damaged (figure 17*B*). Waves soon appeared posteriorly to the cut, but these constituted a separate series, for their frequency was invariably less than that of the anterior waves. Often the arrival of a wave at the cut coincided with the appearance of one of the posterior series, producing the illusion of a single wave travelling uninterruptedly, but a time lag soon appeared at the cut, and became progressively longer until waves appearing there did so half-way between the arrival of consecutive waves of the anterior series. Then the two series began to come into step again.

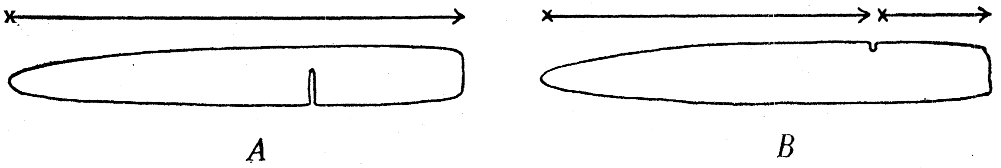

FIGURE 17. The propagation of burrowing waves along the proboscis. Bulges are initiated opposite the points marked X and travel posteriorly as indicated by the arrows. *A*, peristalsis is uninterrupted by a cut through the ventral half of the proboscis. *B*, peristalsis is interrupted by a lesion of the dorsal nerve-cord: an independent series of waves starts behind the lesion.

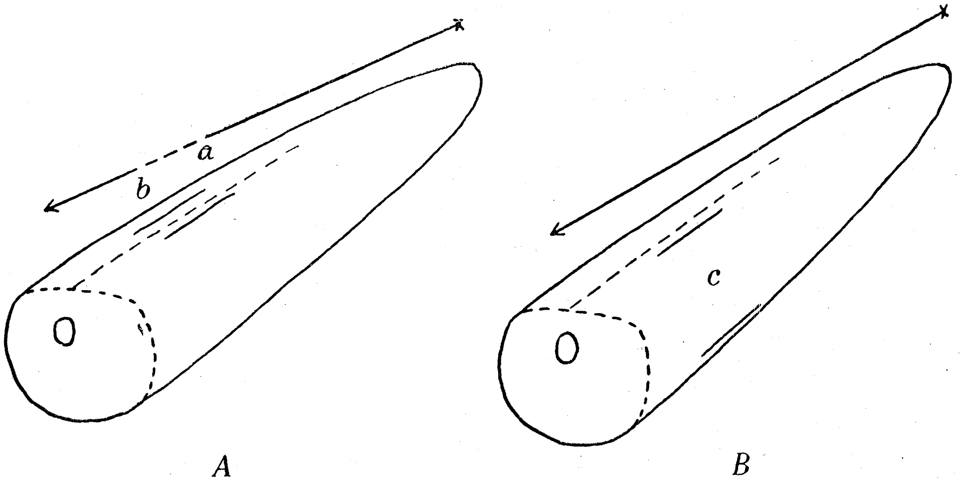

FIGURE 18. As in figure 17, except that *A*, normal peristalsis is interrupted by parallel cuts on either side of the dorsal nerve cord: the bulges disappear at *a*, but reappear at *b* after a short, constant interval. *B*, peristalsis is unaffected by other longitudinal cuts and the area *c* contracts normally.

Evidently the dorsal nerve cord of the proboscis possesses a polarity, which enables burrowing movements to begin at the anterior end of any separate section of it. It is also wholly responsible for the longitudinal propagation of the peristaltic waves. Its connexions with the transverse nerve fibres of the proboscis (p. 318) suggest that these play a part in distributing motor impulses from it. In a series of experiments a cut about 5 mm long was made through the epidermis parallel to the dorsal nerve cord and close beside it. After recovery from this operation burrowing was normal, but when similar cuts were made on both sides close to the dorsal nerve cord, as in figure 18*A*, the region opposite the cuts did not form bulges. Each bulge disappeared at *a* and reappeared at *b* after an interval of a few seconds, which corresponded to the time it would have taken to travel from *a* to *b* in an intact proboscis.

Then two longitudinal cuts were made, one in the ventral surface and one close to the dorsal nerve cord (figure 18*B*). The proboscis burrowed normally after this, the muscles under the area between the cuts contributing to the waves of contraction. When cuts were made on both sides of the cord but not so close to it as in figure 18*A*, burrowing was again normal. Apparently enough impulses to produce a normal peristaltic bulge can get from the cord through the basal membrane on one side of the proboscis alone, or in a limited area immediately adjoining the cord.

In *Saccoglossus horsti* burrowing movements were often confined to the anterior end of the proboscis. The tip was alternately thrust out and withdrawn; peristaltic waves were sometimes indistinguishable and never very distinct. Even when the whole of the comparatively short proboscis was taking part in the movements, these were simple, rhythmical extensions and contractions, not local bulges, but as the anterior end of the proboscis was withdrawn, it became thicker, and as it was thrust out again this ill-defined thickening travelled posteriorly, often as far as the base. After the dorsal nerve cord in the posterior half of the proboscis had been cut, burrowing sometimes went on normally, only the anterior part of the proboscis taking part in it. Sometimes the proboscis anterior to the cut remained relaxed, whilst the posterior portion extended and contracted in typical burrowing movements. Sometimes both portions, anterior and posterior to the cut, performed independent burrowing extensions and contractions, but co-ordinated burrowing of the whole proboscis was never observed. Extensive lesions of the remainder of the proboscis epidermis, which left the dorsal nerve cord intact, had no permanent effect on normal burrowing.

S. horsti is a comparatively poor subject for experiments on burrowing, since the peristaltic swellings are ill defined. Ritter's (1902) account of *S. pusillus* suggests that they are ill defined in this species too. Bullock (1940), who studied mainly *S. pusillus* and *Balanoglossus occidentalis*, described the proboscis as lacking polarity, for when one was divided transversely he observed no difference in behaviour between the two cut faces. This contrasts markedly with *Saccoglossus cambrensis*, for in this species small fragments of the proboscis could easily be orientated by observing the direction of burrowing peristalsis or retreat peristalsis, the movements of which were characteristic. Even in *S. horsti* pieces of the proboscis were occasionally orientated thus, for the extensions which preceded the burrowing contractions appeared earlier at the anterior end. The extensions are vigorous movements, in which the outer circular muscle fibres probably do not act alone, as Van der Horst (1927–39) believed, but are aided by radial muscle fibres. In Ptychoderidae the proboscis is smaller and the proboscis nervous system probably less differentiated than it is in *Saccoglossus*, so it is not surprising that Bullock failed to find any nervous differentiation or polarity in the proboscis of *Balanoglossus occidentalis*, though in *B. hydrocephalus* burrowing contractions start at the tip of the proboscis and travel posteriorly (Van der Horst 1940), and it seems very unlikely that the proboscis of *Saccoglossus pusillus* lacks polarity.

Retreat peristalsis

In vigorous specimens of *S. cambrensis* this was a complex movement. The proboscis elongated posteriorly and swelled anteriorly (when in the burrow the swollen part is probably anchored, whilst the elongation of the base helps to push the collar region backwards). At the same time a wave of contraction started at the posterior end of the trunk and travelled anteriorly throughout the trunk, collar and proboscis. The anterior part of the proboscis relaxed and became thin as the wave approached; then it was drawn back and contracted in its turn (figure 16*B*).

The region behind the wave did not relax until the movement was complete, so the body became shortened. The muscles did not move the posterior end backwards. The movements were repeated only a few times, in contrast with the long-sustained activity of burrowing, because the body was completely shortened after a few repetitions. The waves travelled faster than the burrowing waves of the proboscis, and also differed from these in that no retreat wave started until the preceding one had reached the anterior end. Their usual frequency was about three waves per minute. As each wave of contraction progressed the trunk anterior to it elongated slightly, which is remarkable, because there appear to be no extensor muscles in the trunk of *Saccoglossus*. Probably the longitudinal muscles of the trunk retain a state of tonus which is relaxed in front of the retreat wave, allowing an elongation which is perhaps caused by some elastic property of the tissues. Separate pieces from all parts of the body performed retreat peristalsis when stimulated, so initiation of the movement does not depend on any restricted nerve centre.

S. horsti performed similar movements in its burrows. The region posterior to the wave of contraction remained anchored, and the shortening of the body pulled the relaxed anterior region backwards rapidly.

Retreat waves did not travel past lesions of the ventral nerve cord, so this seems responsible for propagating them throughout the trunk; cutting the dorsal nerve cord did not interfere with their movements. The fibres in the posterior half of the collar (figure 7) are suitably arranged for transmitting them in turn to the retractor muscles of the proboscis skeleton, if individual fibres traverse the coelom to these muscles. A cut through the dorsal half of the collar, which severed the neurocord, prevented the waves from spreading into the proboscis, and a cut in the epidermis completely round the proboscis also stopped them, indicating that their progress there depends on the epidermal nervous system. Such cuts involved damage to deeper tissues, but this does not appear to affect the progress of the waves, for a cut through most of the proboscis, including the dorsal nerve cord, failed to delay them, provided a bridge of epidermis was left intact.

It is rather remarkable that the slow propagation of peristalsis should require through-conduction paths, such as the ventral nerve cord and, during burrowing, the dorsal nerve cord of the proboscis. If retreat peristalsis of the proboscis requires such paths, the numerous longitudinal bundles probably provide them; the anterior nerve ring, being intimately connected with the bundles, could distribute the impulses to them synchronously. The dorsal nerve cord and the transverse fibres appear to constitute another part of the proboscis nervous system, with somewhat different functions (p. 341), but the fibres of the two parts are closely interwoven and extensive damage to either can be compensated for and followed by normal movements.

Forward peristalsis of the trunk

When *S. horsti* moved over a mud surface by ciliary action the posterior end was dragged along, as its cilia appeared comparatively weak, and the body became rather elongated. At intervals a wave of contraction appeared immediately behind the collar and travelled posteriorly to the end of the trunk, at a speed similar to that of a retreat wave. The muscles relaxed soon after the wave had passed, but the trunk was temporarily shortened and pulled forward by each wave. Within the winding burrows it was pulled forwards comparatively rapidly, often moving about 4 cm in 4 s. This movement was not readily induced in *S. cambrensis* kept in glass dishes, perhaps because specimens were rarely very elongated under such conditions. Occasionally stimulation of the posterior end induced it, even in small pieces of the trunk, which indicates that there is no centre necessary for its initiation.

(*b*) *Ciliary movement*

Ritter (1902) and Bullock (1940, 1945) found that locomotion in *S. pusillus* was effected mainly by the cilia. Those of the proboscis beat steadily and posteriorly, but those of the trunk showed reversal and inhibition, involving large or small areas and suggesting to Bullock some degree of nervous control.

In *S. cambrensis* and *S. horsti* the cilia are primarily responsible for movement within the burrow. Those of the proboscis beat posteriorly and were never observed to reverse, but their beat varied in effectiveness, for mucus and particles moving down the proboscis often paused whilst muscular movements were being performed, perhaps because the cilia had lost contact with the mucus. The cilia of small pieces of proboscis, observed under the microscope, beat steadily and posteriorly.

Particles were carried rapidly over the anterior surface of the collar, comprising the first and second epidermal zones (p. 336 and figure 7), indicating that the cilia there beat posteriorly even when specimens were at rest. During forward locomotion the cilia of the remainder of the collar and of the trunk also beat posteriorly, so that specimens moved forwards within a thin, transparent sheath of mucus. At other times the cilia of the posterior half of the collar and the adjoining trunk seemed comparatively inactive, for mucus collected in thick rings round the collar's posterior edge. Bullock (1940) observed in *S. pusillus* a tendency for sand grains to accumulate round the collar, which he associated with tube-building; and Barrington (1941) described particles collecting at the hinder end of the collar in *Glossobalanus minutus* before being pushed posteriorly by the accumulation of other particles in front.

Saccoglossus horsti crawled over mud surfaces with the ventral surface downwards, so that the strong cilia of the ventro-lateral ridges were in contact with the substratum. These are the main locomotor cilia of the trunk. During forward progression they beat posteriorly, but on disturbance specimens contracted, reversed the beat of their ventro-lateral cilia synchronously throughout the length of the trunk, and carried out retreat peristalsis. The entire body, somewhat shortened, then moved backwards under the action of the cilia. Later forward movement would be resumed. Whenever peristalsis occurred, the movement of the peristaltic waves was in the same direction as the effective beat of the ventro-lateral cilia.

Small pieces of the trunk also moved about, showing the direction in which these cilia were beating. At infrequent intervals they stopped and reversed, apparently spontaneously; mechanical stimulation of the leading end was often followed by reversal.

The other trunk cilia were studied by watching their effect on small particles in the water. Those of the glandular patches on the sides of the trunk usually beat ventrally, which may explain how the trunk becomes orientated with the ventral surface in contact with the substratum, for in any other position some of the lateral cilia are in contact and, beating ventrally, would cause rotation. Often they beat otherwise. During forward progression they appeared to join the ventro-lateral cilia in beating posteriorly. In a piece of the intestinal region they beat dorsally, and in a piece of the oesophageal region, those of the lateral glandular patches beat ventrally, whilst those of the dorsal patches beat towards the dorsal mid-line. In a piece which comprised the greater part of the branchial and oesophageal regions, the directions in which the cilia beat were simultaneously forwards along the epithelium of the dorsal nerve cord and gill-pore ridges, ventral on the sides of the body and backwards along the ventro-lateral ridges. In intact animals, which were too active to be observed easily under the microscope, the trunk cilia appeared better co-ordinated, so the variety of behaviour observed in separate pieces may well have been due to damage.

The synchronous reversals of the ventro-lateral cilia and their co-ordination with peristalsis strongly suggest that they are under nervous control. The bundles of nerve fibres, which run out from the ventral nerve cord under each ventro-lateral ridge, probably transmit impulses through which they are co-ordinated. Each glandular patch is also provided with a well-developed nerve-fibre layer, whilst nerve fibres are sparse in the epidermis between the patches, which is practically devoid of cilia.

(*c*) *Reactions to light*

Assheton (1908) noted that *S. serpentinus* kept in aquaria protruded the proboscis only at night, and Crozier (1917) found that *Ptychodera bahamensis* became more active on illumination and crept away from the light. The neurocord was unnecessary for orientation and the tip of the proboscis was particularly sensitive; fragments from the hind end withdrew posteriorly. Exhaustion or anaesthesia destroyed the sensitivity to light but not to mechanical or chemical stimuli (Crozier 1915). Hess (1936) confirmed Crozier's accounts and suggested that the proboscis base contained a nerve centre controlling the normal reaction to light, for a proboscis without the base was slow to orientate. Later he turned to *Saccoglossus kowalevskyi* (1938), in which he had described the nervous system as a plexus of bipolar neurones (1937), similar to those common at the tip of the proboscis in some specimens of *S. cambrensis* (p. 330). He found evidence that these cells were photoreceptors, but his description and conclusions were justifiably questioned by Bullock (1940, 1945). Bullock found *S. pusillus* to be negatively phototropic, a response which was abolished by light anaesthesia but which returned after immersion in fresh sea water.

S. cambrensis in dishes usually responded to a sudden bright light by contraction of the proboscis, followed by retreat movements or burrowing. All the body appeared photosensitive, for separate pieces of the proboscis or trunk contracted on illumination.

The reaction time of the proboscis, from the beginning of exposure to bright light to the beginning of the rapid contraction which followed, varied from 1 to 4 s, covering a similar range in intact specimens, separate proboscides and proboscides lacking the base, so the response is probably local and independent of any nerve centre in the proboscis base or neurocord. A contracting proboscis usually became flexed. If it was lying on its side the flexure was generally ventral, regardless of the direction of the light, as in most preserved specimens; but if it was lying dorsal side uppermost, bright unilateral illumination was usually followed by an initial lateral flexure, which was always away from the source of light.

An isolated proboscis kept illuminated from one side usually became bent with the convex side nearest to the light. Removal of the base and tip did not modify this reaction. Intact specimens showed increased activity of all kinds. The proboscis was occasionally waved about, but usually pointed away from the light. The trunk remained coiled and responded only by peristaltic activity.

Eleven specimens from *S. horsti*, which were put in a large enamel dish about 4 m from a window, all orientated their proboscides and moved by ciliary action away from the window. Some turned temporarily to one side or the other, but a majority were always orientated. When a light brighter than that from the window was brought near the opposite side of the dish, they turned back towards the window. When the light was removed, all re-orientated and within half an hour had collected at the end of the dish remote from the window. All these specimens, which became orientated similarly, included most of the proboscis. One was a separate proboscis with the dorsal nerve cord cut, one was a piece of the anterior end of the proboscis and one lacked the proboscis tip.

(*d*) *Responses to other stimuli*

The cells which appear to be sensory, lying at the periphery of the proboscis base and in the groove of the ciliary organ, may well be gustatory, for this is likely to be the prime sensory requirement of *Saccoglossus*. The only other areas observed to contain many cells which appeared on histological grounds to be sensory were the dorsal nerve cord of the trunk, the anterior lip of the collar, and the first part of the oesophagus. No special sensitivity could be discerned in the epithelium of the dorsal nerve cord, but the first part of the oesophagus was markedly sensitive. Touching its internal surface induced peristalsis which appeared to be concerned with compacting its contents. This was a local reflex, occurring in isolated, short portions.

As Barrington (1941) described in *Glossobalanus minutus*, the mobile anterior lip of the collar prevented large particles from entering the mouth with the respiratory current. In *Saccoglossus* this behaviour was not associated with feeding, as Barrington suggested, but occurred only when feeding was not taking place. *S. cambrensis* rarely showed it, attempting to feed at every opportunity, but *S. horsti* fed less readily and lay for long periods with a current of water flowing in at the mouth and out of the gill pores. Minute particles which had not been in contact with the proboscis continually entered the mouth with this current, but when a larger particle approached, transported by the proboscis cilia, the section of the collar's anterior lip which lay in its path was bent inwards, with a delicate and precise movement, to touch the periphery of the proboscis base. The particle was thus prevented from entering the mouth and was transported posteriorly by the collar cilia. It is doubtful whether local reflexes play an important part in this mechanism, for the inward movement of the lip often began before it was touched, whilst the approaching particle was at the periphery of the proboscis base; and when a specimen was divided transversely through the middle of the collar, the anterior lip lay immobilized and relaxed, its main nervous connexions with the remainder of the body having been removed.

Touching it elicited slight movements but not the well-defined ejector movements of intact specimens. Mutilation of the epidermis at the posterior edge of the collar on one side was also followed by immobilization of the entire anterior lip for some hours, suggesting a shock effect. During this period carmine particles were ingested automatically, with the respiratory current, except when so much mucus accumulated that it bulged out over the collar.

The sluggishness of Enteropneusta generally makes them appear very insensitive, but carmine particles settling on the proboscis of *S. horsti* invoked writhing movements and apparently increases in mucus secretion and ciliary activity, supporting Bullock's (1945) view that sensory cells are abundant in the epidermis. During burrowing of *S. cambrensis* the tip of the proboscis was often pressed downwards against the glass bottom of the dish, even if the proboscis was lying on its side, suggesting an orientation due to tactile stimuli. Relaxed individuals contracted the proboscis if the dish in which they lay was tapped, recalling the sensitivity of various species to vibrations caused by footfalls (Ritter & Davis 1904; Morgan 1894; Assheton 1908; Stiasny 1910). They often reacted to a light touch, especially on the proboscis. The typical response was a phase of activity, which would have tended, under natural conditions, to cause withdrawal from the point of stimulation. The reaction time to strong stimuli was short, crushing the posterior end of the trunk sometimes being followed within a second by an extension of the proboscis tip which initiated burrowing peristalsis. Once avoiding movements had begun they were not modified except in response to severe stimuli.

Local reactions, referred to by Crozier (1915) and Bullock (1940) as typical of the forms which they studied, were rarely observed after artificial stimuli, though local damage to the proboscis often caused elongation, as though the balance between the longitudinal muscles and the extensor system had been upset. Nothing was observed like the local spreading response recorded by Bullock (1940) from the proboscis of *Balanoglossus occidentalis*, which suggested to him the action of a nerve net. On the contrary, the peristaltic contraction which followed effective stimulation of one end made its first appearance at the other, even in small pieces of the proboscis or trunk.

(*e*) *Longitudinal reflex pathway*

Stimulation of the posterior end of the trunk often caused the proboscis to burrow (figure 19*A*), while stimulation of the proboscis usually induced retreat peristalsis (figure 19*B*). These responses were as well co-ordinated in separate pieces as in more nearly intact worms. If the ventral nerve cord was cut and the proboscis was stimulated, the retreat wave that usually followed always started at the lesion (figure 19*C*), and the trunk posterior to the cut showed no response. Apparently the impulses which initiated the retreat wave travelled backwards down the ventral nerve cord and, if interrupted by a lesion, produced a contraction there. When the posterior end was stimulated the part of the body anterior to the lesion did not respond, but waves of forward peristalsis sometimes appeared at the lesion and travelled posteriorly (figure 19*D*). It seemed that the mechanism which produced co-ordinated responses was a reflex path, involving the main longitudinal nerve tracts and operating in either direction.

A cut in the dorsal nerve cord of the trunk did not modify the normal response, unless the cut was a short distance behind the collar, when retreat waves originated there in some specimens on stimulation of the proboscis (figure 19*E*). When the neurocord was cut, the proboscis and trunk reacted independently to stimuli. When the dorsal nerve cord of the proboscis was cut and the trunk was stimulated, the burrowing waves which often followed always started at the cut (figure 19*F*), the proboscis anterior to it showing no immediate

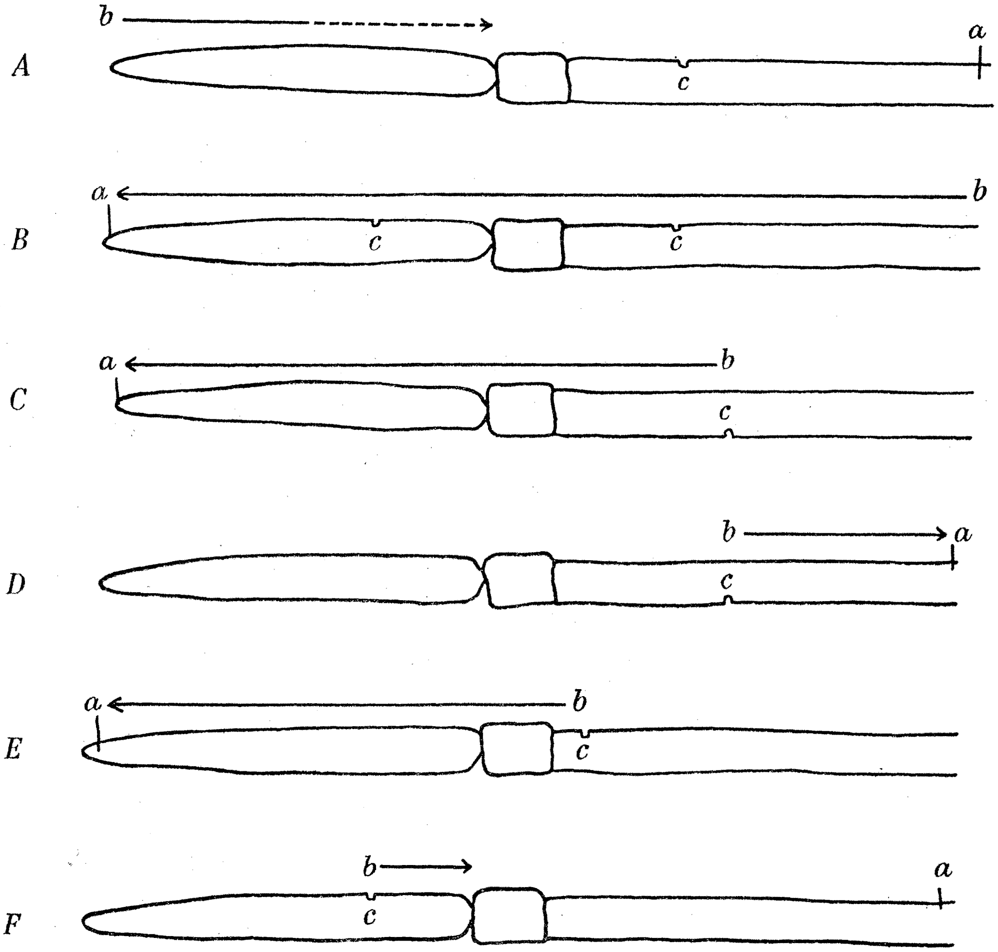

FIGURE 19. Diagrams showing the proboscis, collar and part of the trunk, to illustrate the functioning of the longitudinal reflex path. *a*, point of stimulation; *b*, starting point of the peristaltic wave which follows and which moves along the course indicated by the arrow; *c*, lesion of longitudinal nerve tract. *A*, effective stimulation of the posterior end is followed by burrowing movements, which are sometimes confined to the anterior part of the proboscis: a lesion of the dorsal nerve cord of the trunk does not interrupt the normal reaction. *B*, stimulation of the anterior end is followed by retreat peristalsis: lesions of the dorsal nerve cords of the proboscis and trunk do not affect the reaction. *C*, if the ventral nerve cord is cut stimulation of the anterior end is followed by retreat peristalsis starting at the cut; there is no reaction posterior to the cut. *D*, stimulation of the posterior end is often followed by forward peristalsis starting at the cut in the ventral nerve cord: there is no response anterior to such a cut. *E*, if the dorsal nerve cord is cut a short distance behind the collar, the retreat wave which follows effective stimulation of the anterior end often starts at the cut. *F*, if the dorsal nerve cord of the proboscis is cut, the burrowing waves which follow effective stimulation of the posterior end appear at the cut; no immediate reaction anterior to such a cut was observed.

response, though sometimes beginning to burrow later. The burrowing must have begun in response to nervous impulses travelling forward from the point of stimulation; it appears that the dorsal nerve cord conducts these impulses along the proboscis, in addition to the part which it plays in propagating burrowing peristalsis.

The presence of this reflex pathway throughout the length of the body explains the high degree of autonomy shown by separate pieces. The main longitudinal tracts of the proboscis and trunk provide the rapidly conducting association paths which call for peristalsis to begin, as well as the paths by which the peristaltic waves are slowly propagated. But the response to an effective stimulus half-way along the body, which was on different occasions an increase of activity, cessation of activity or change in type of activity, always involved the worm, or piece of worm, behaving as an integrated whole, providing its nervous system was intact. Burrowing and retreat peristalsis never occurred simultaneously.

(*f*) *Neural drive to action*

The neurocord, like that of other Enteropneusta (Bullock 1940), seems to be a mere nervous pathway, but autonomous nervous activity is to a considerable extent centred in the proboscis. Bullock (1940) observed that intact specimens of *S. pusillus* showed almost continuous writhing movements of the trunk, whilst lengths of trunk cut off from the collar and proboscis lay motionless for many seconds or longer. The proboscis behaved alike whether attached or unattached. *S. cambrensis* and *S. horsti* often embarked upon phases of peristaltic activity apparently spontaneously, but specimens which lacked the proboscis were markedly inactive compared with the collar and trunk regions of intact specimens. In dim light they were not observed to move unless stimulated, and then they responded by single movements rather than by phases of activity. The proboscis, which was the most active region, affected the activity of the rest of the body.

Loss of the anterior half of the proboscis did not modify the behaviour appreciably. The behaviour of a separate proboscis was not obviously different from that of an intact specimen, but when separated proboscides and almost intact specimens were placed together on a sand surface in dim light, the intact specimens buried themselves much more readily than the proboscides, which moved about on the surface by ciliary action. Proboscides which lacked the basal region proceeded forwards particularly persistently by ciliary action, varying their behaviour somewhat infrequently, but the contrast with complete proboscides was not marked.

When burrowing, activity developed gradually, from a few tentative movements of the proboscis tip to vigorous activity of the whole organ, which was carried on with great persistence. The contractions of retreat peristalsis also increased in strength during successive waves. The earlier movements seemed to prepare the way for the later, stronger movements, as though some process akin to facilitation or the gradual appearance of a chemical mediator was involved. Adrenaline had a striking influence on activity, in concentrations of 1:100000 or less. In stronger solutions specimens contracted abnormally and their movements became imperfectly co-ordinated, but in 1:100000 solutions they became more active within about a minute. The surface of the proboscis became uneven and bulges formed which travelled backwards at the same speed as burrowing waves. Within a few minutes vigorous burrowing had begun, and it continued indefinitely, occasionally interrupted by retreat contractions. All forms of muscular activity increased and tonus of the muscles seemed greater, for if the proboscis was damaged, addition of adrenaline made the lesions gape. Adrenaline also promoted activity in *S. horsti*; it stimulates *Arenicola marina* to burrow (Wells 1937) and affects a wide variety of animals (Hanström 1939), often stimulating muscles which are required to be active for long periods.

(*g*) *Function of the giant nerve cells*

Since it has been shown for a variety of animals that giant axons are concerned with the rapid contraction of large groups of muscles, Bullock (1944) referred to their probable function in Enteropneusta as the 'avoiding' contraction of the anterior end of the trunk, in response to touching the proboscis of a relaxed specimen, which he had described (1940) as the 'ability to jerk up the abdomen'. A similar response was observed in *Saccoglossus cambrensis* and *S. horsti*. The rapidity of the contraction contrasted markedly with the slow movement of the peristaltic waves. It was restricted to the region to which the axons of the posterior group of giant nerve cells are limited, so these probably evoke it. They may well function as a single system, since they are frequently in intimate contact with one another.

The only other reaction of this type which was observed was rapid contraction of the proboscis. Axons which are much larger than the majority of the nerve fibres run longitudinally throughout most of the proboscis, so are probably responsible for it. Their cell bodies form the small group of giant nerve cells at the anterior end of the neurocord. Rapid contraction was observed in the separate proboscis, but axons can function for some time in the absence of their cell bodies (Young 1938) and no critical observations were made on long-separated proboscides, in which the large axons would probably have degenerated.

Under natural conditions contraction of the branchio-genital region can rarely result in a jerking up of the trunk, which is laden with food material and extended in a winding burrow. Instead it must jerk back the collar and proboscis. *S. horsti* in aquaria usually lay with the anterior end near the entrance to the burrow, and some were found in a similar position in their natural habitat. An ability to jerk back the anterior end from such a position would help escape from fishes or wading birds; Devanescen & Chacko (1942) found that certain fishes fed on Enteropneusta.

EVIDENCE OF DEGENERATION AND PAEDOMORPHOSIS IN HEMICHORDATA

To give a comparative interpretation of the nervous system of Enteropneusta, particularly of the curious neurocord, it is necessary to consider the probable phylogenetic origin of the group. The idea of a relationship with other chordates and with echinoderms has been widely accepted since the time of Bateson (1885). It has been supported by evidence from embryology (Davis 1908; Colwin & Colwin 1949), biochemistry (Needham & Needham 1932), serology (Wilhelmi 1942) and the cilia of the gill-slits (Knight-Jones & Millar 1949). The points of resemblance between the ciliated larvae of Echinodermata, Enteropneusta and *Amphioxus* suggest that the common ancestor of chordates and echinoderms had a trimetameric, bilaterally symmetrical, ciliated larva. Several authors (Agassiz 1873; de Beer 1930; Van der Horst 1927–39) have remarked that this general form and symmetry persists in adult Enteropneusta, and it seems highly probable that paedomorphosis has played a part in the evolution of the group.

The essential peculiarity of the Enteropneusta is that neurulation is never completed. In other chordates which have little yolk (Urochordata, Cephalochordata and some Amphibia) the neural folds fuse posteriorly over the blastopore, enclosing the neurenteric canal, the anus appears *de novo* and the tail is formed. In the Enteropneusta the neural folds, where these appear (Morgan 1891; 1894) fuse only in the collar region, leaving open anterior and posterior neuropores. They do not enclose the blastopore, which becomes the anus, and dorsal tail formation never begins.

The neurocord differs from the neural tube of other chordates in two important respects, its shortness in being confined to one metamere and its shape in transverse section, but as Van der Horst (1927–39) pointed out, its shortness may be explained as the result of arrested development, whilst the neural tube does not develop its normal shape without a notochord beneath it and myotomes on each side. If the notochord of amphibian embryos is removed and muscular tissue substituted, the neural tube which develops is semicircular in transverse section, with the nerve fibres confined to the flat ventral side (Huxley & de Beer 1934, figure 180). This is very like the neurocord of Enteropneusta, which has nothing corresponding to the notochord beneath it (the doubtfully homologous stomochord being anterior to the collar), but which has instead a band of muscles, those of the perihaemal cavities. Apparently both neural tube and neurocord assume a similar form when developing under similar conditions.

The neurocord resembles the neural tube in having a lumen or residual lacunae, bounded by epithelial cells resembling periventricular ependymal cells, amongst which are gland cells like those in the neural tube of lampreys, sharks and teleosts (Kappers 1929). In view of the other evidence placing the Enteropneusta in the phylum Chordata, the neurocord is very probably homologous with the neural tube, and its presence as a mere conducting tract suggests that the Enteropneusta are degenerate. The fact that large unipolar ganglion cells are almost entirely confined to it, and to the dorsal nerve cord immediately behind the collar, indicates that this region was once a nerve centre, but now the nervous system is diffuse, with no centres.

It seems a reasonable hypothesis that the Enteropneusta arose from some group of more generalized chordates, that possessed a ciliated larva with three pairs of coelomic pouches and the rudiments of a notochord and neural tube. After closure of the latter in the middle somite its further development was arrested. Adaptive modifications connected with the adoption of a burrowing habit supervened. In many forms the anterior somite elongated as an organ of locomotion, in all the posterior somite elongated to house the long gut necessary for dealing with bottom deposits, whilst the ventral nerve cord appeared as a neomorph, to control the locomotory muscles and cilia. The middle somite underwent no comparable increase in length, so the neurocord contained in it now appears absurdly short.

The anterior and prebranchial nerve rings of *Saccoglossus*, encircling the body at either end of the neurocord, recall the anterior and posterior loops of the neural folds of vertebrates and of the Tornaria's circum-oral ciliated band (figure 20). The presence of these nerve tracts is therefore relevant to Garstang's (1894) hypothesis, that the neural folds of vertebrates may have been derived from the ciliated band of a larva like the Auricularia, an idea which received some support when Assheton (1896) showed that the neural folds are particularly strongly ciliated in embryos of *Rana*. In vertebrate embryos there are closely associated with the neural folds only those ganglion cells which belong especially to the sensory system; the bulk of the presumptive nervous tissue is associated not with the folds themselves, but with the neural plate between, which appears to represent the original dorsal or aboral surface. In Enteropneusta most of the surface of the body is aboral to the ciliated band, and to the anterior and prebranchial nerve rings, so the greater part of the richly nervous epidermis may well be homologous with the neural plate of vertebrates.

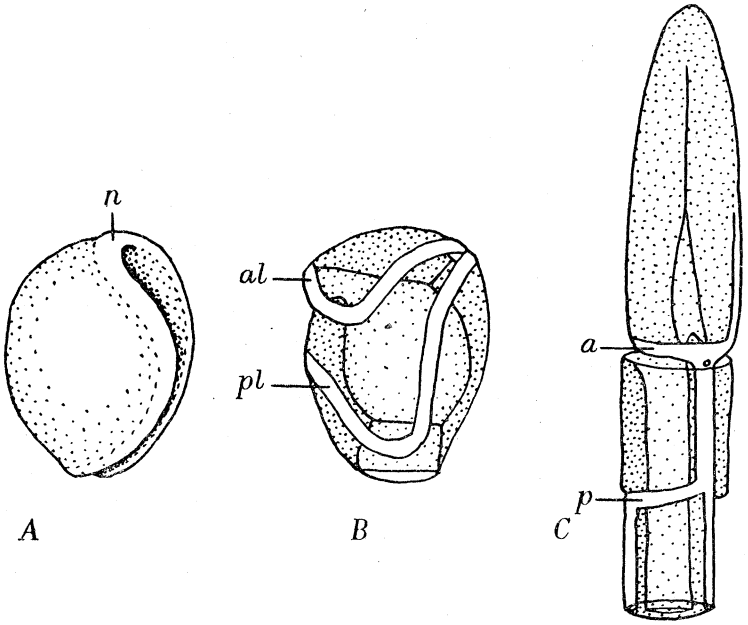

FIGURE 20. *A*, urodele embryo from Huxley & de Beer (1934) after Goerttler; *B*, early Tornaria from Morgan (1891) and *C*, anterior end of *Saccoglossus*, to illustrate the general resemblance in shape between the neural fold of a young vertebrate embryo, the circum-oral ciliated band of an early Tornaria larva and the nerve rings and neurocord in *Saccoglossus*. *a*, anterior nerve ring; *al*, anterior loop of the ciliated band; *n*, anterior loop of neural fold; *p*, prebranchial nerve ring; *pl*, posterior loop of ciliated band.

As for the Pterobranchia, they lack certain generalized chordate characters, which are present in their close relatives, the Enteropneusta. Probably they were derived from more generalized Hemichordata by further paedomorphosis and specialization. The relationship which seems to exist between echinoderms and chordates is likely to be comparatively remote.

This work was begun in 1938 in the Department of Zoology at the University College of North Wales, Bangor, at the suggestion of Professor F. W. Rogers Brambell, F.R.S., and under his supervision. I am indebted to him for this and for access to his collections, which include specimens of *Balanoglossus australiensis*, *Ptychodera flava* and *Glossobalanus hedleyi* given by Professor J. P. Hill, F.R.S., and *Saccoglossus inhacensis* given by Professor C. J. Van der Horst. The work was continued in the Department of Zoology and Comparative Anatomy at Oxford, where I held a Meyricke Scholarship at Jesus College, and later received a grant, through the Ministry of Agriculture and Fisheries, under the post-war Further Education and Training Scheme. I am indebted to Professor A. C. Hardy, F.R.S., and to my supervisors, Professor J. Z. Young, F.R.S., and, later, Dr W. Holmes, for their advice and interest; also to Mr D. A. Kempson for the photographs.

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PLATES 32 TO 35

PLATE 32

FIGURE 21. A very oblique section through the deeper part of the proboscis epidermis, more superficial at the upper left, deeper at the lower right corner of the figure, the upper margin of which is anterior. Bodian. *c*, circular muscle fibres; *b*, one of the several longitudinal bundles of nerve fibres lying in the superficial part of the nerve-fibre layer; *f*, nerve-fibre layer—apart from the longitudinal bundles the majority of the superficial fibres are transverse, but the deeper fibres are more irregular and are closely associated with the circular muscle fibres, from which they are separated only by the very thin unstained basal membrane; *l*, outer layer of longitudinal muscle fibres; *n*, nuclei occurring immediately outside the nerve-fibre layer, the majority probably belonging to primitive ganglion cells.

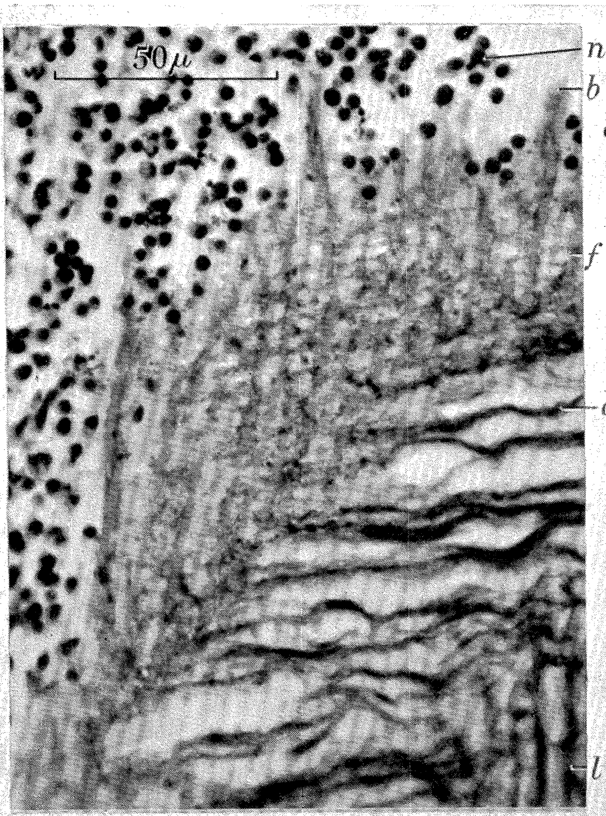
FIGURE 22. Part of a transverse section through the proboscis near its posterior end, showing the epidermis and underlying muscles. Bodian. *b*, large bundle of longitudinal nerve fibres, projecting above general nerve-fibre layer; *c*, circular muscle fibres; *ci*, rootlet bundles of cilia; *f*, nerve-fibre layer, showing about three large and seventeen small darkly stained bundles of longitudinal nerve fibres, cut transversely and traversed by transverse nerve fibres; *l*, outermost layer of longitudinal muscle fibres.

FIGURES 23, 24 and 25 all same scale, and all with right margins anterior.

FIGURE 23. Part of a horizontal section through the dorsal edge of the proboscis base, showing the epidermis and underlying tissues, cut obliquely, in dorsal view, slightly to the right of the mid-line. Bodian. *a*, bundle of fibres in anterior nerve ring; *b*, bundle of longitudinal nerve fibres, one of several which project above the general nerve-fibre layer and curve away from the mid-line to join the anterior nerve ring; *c*, circular muscle fibres; *ci*, ciliated border of epidermis; *e*, nuclei of epidermis; *f*, general nerve-fibre layer of epidermis, cut very obliquely; *l*, outermost layer of longitudinal muscle fibres; *n*, nuclei of muscles.

FIGURE 24. Part of a parasagittal section through the edge of the proboscis base on the right side, showing the epidermis and underlying tissues cut obliquely. Bodian. Letters as in figure 23, but here the longitudinal bundles of nerve fibres curve dorsally to join the anterior nerve ring.

FIGURE 25. Part of a horizontal section through the ventral edge of the proboscis base, showing the base of the epidermis cut tangentially in the mid-line. Bodian. Letters as in figure 23. Owing to slight folding the surface of the general nerve-fibre layer is cut in several places.



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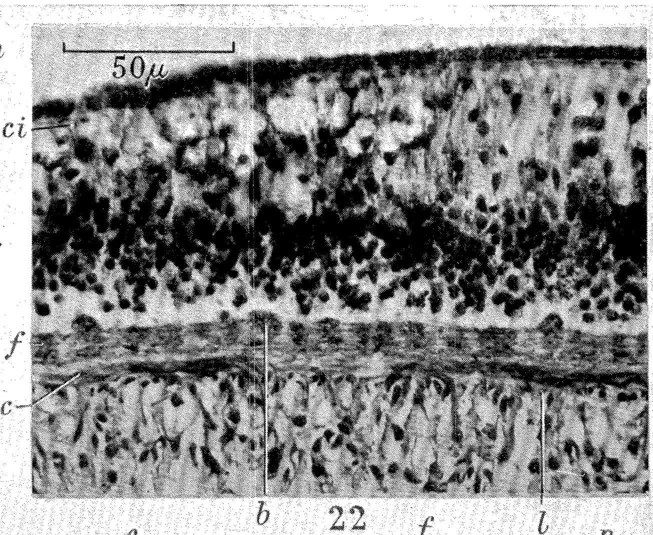

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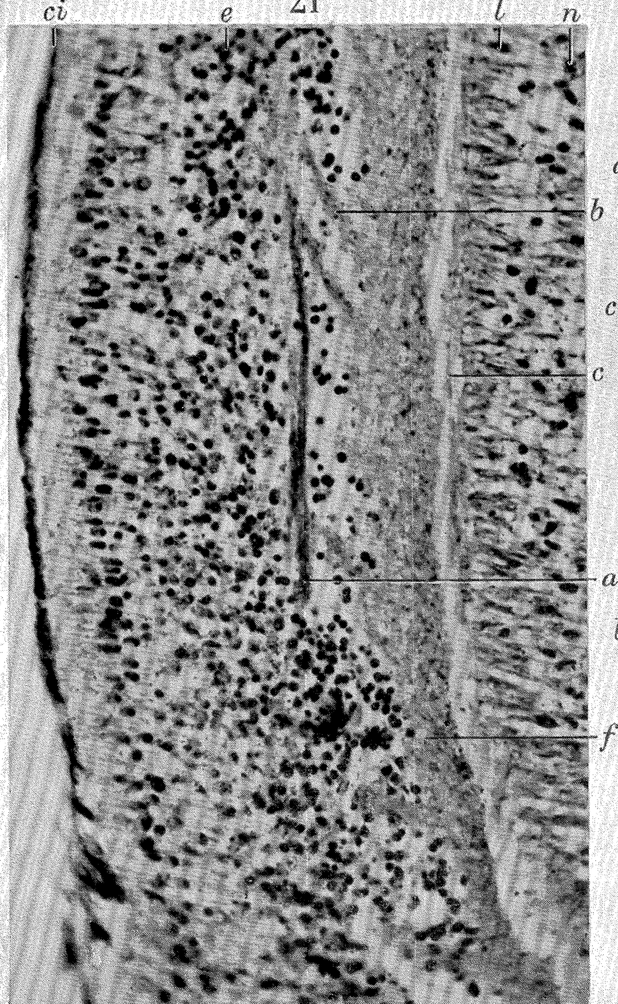

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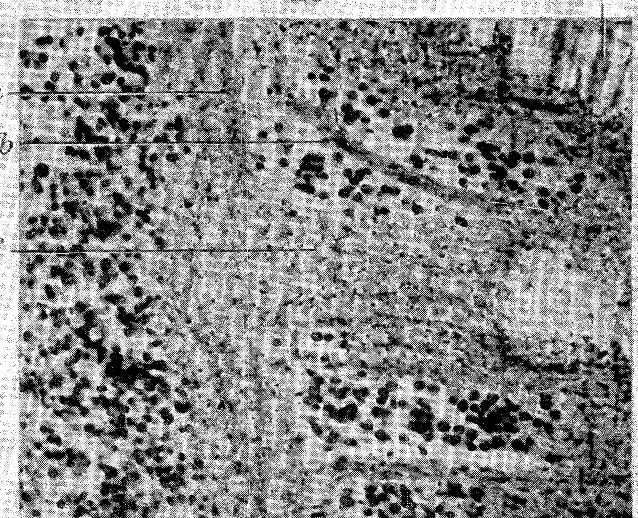

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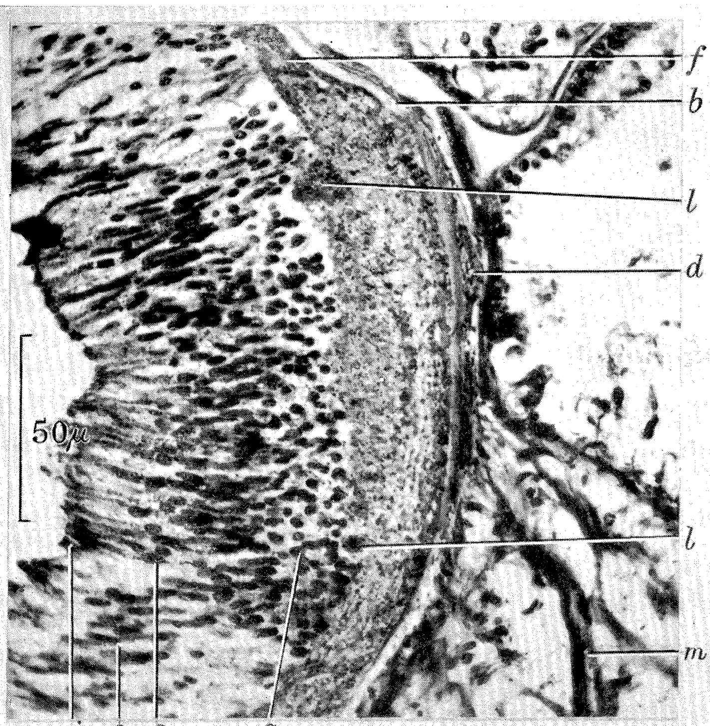

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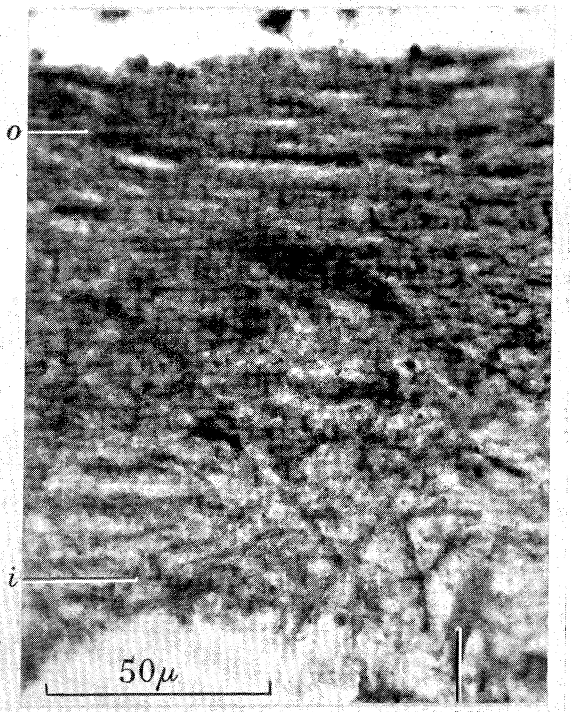

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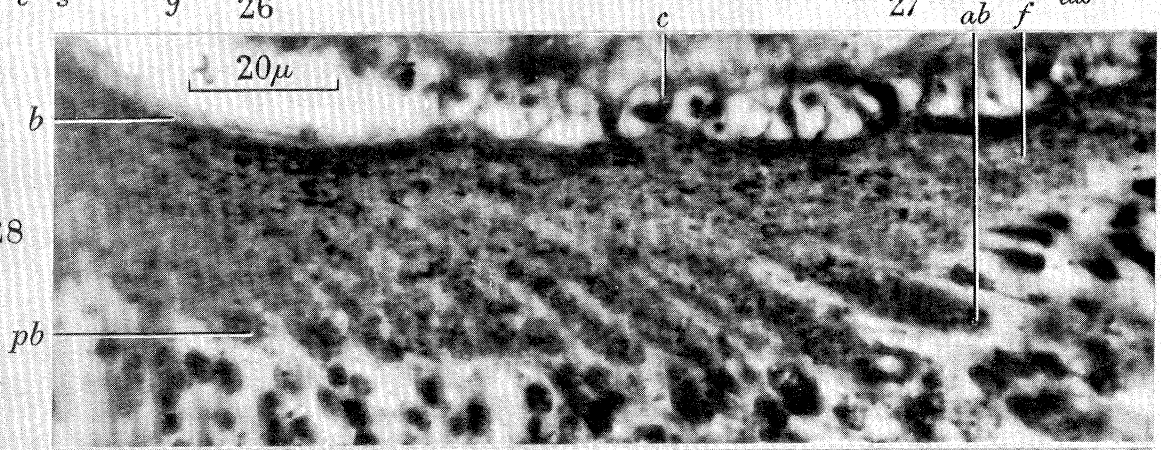

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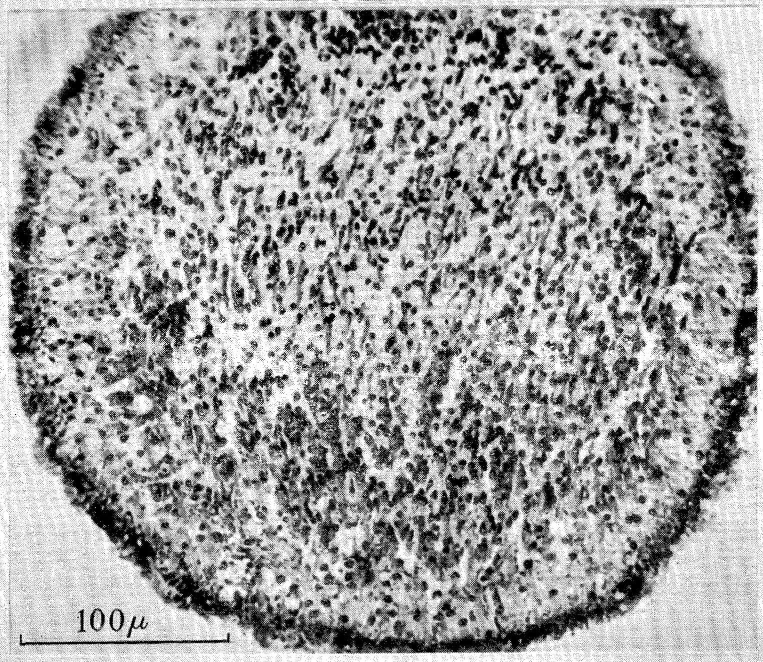

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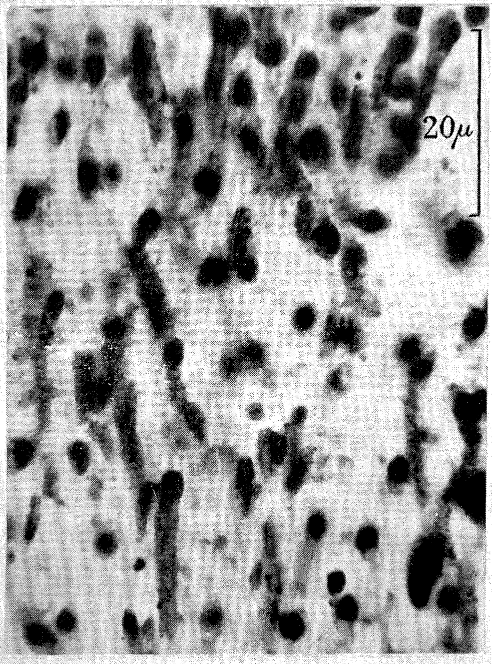

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PLATE 33

FIGURE 26. Part of a sagittal section, showing the groove of the ciliary organ cut transversely (*l* in figure 5). Bodian. *b*, basal membrane of epidermis, which is thick in this region; *c*, nuclei of ciliated cells, large and often elongated; *ci*, rootlet bundles of cilia—the cilia themselves have been torn away; *d*, fibres of doubtful function, lying between the basal membranes of the epi- and mesothelia and continuous with similar fibres in the cartilaginous tissue; *f*, general nerve fibre layer of proboscis base; *g*, concentration of nuclei associated with nerve loop of ciliary organ, probably belonging to primitive ganglion cells; *l*, borders of nerve loop of ciliary organ, cut transversely; *m*, muscle fibres; *s*, cells probably sensory in function, having slender bodies containing many granules, tapering distally and extending to surface of epidermis between adjacent rootlet bundles. Their nuclei are smaller, denser and less elongated than are those of the ciliated cells, and they lie for the most part nearer the surface of the epidermis. Although they somewhat resemble sensory cells from other parts of the body, cells conforming strictly to this type were observed only in the groove of the ciliary organ.

FIGURE 27. Oblique, nearly horizontal section through the nerve-fibre layer of the neurocord, near the posterior neuropore. Bodian. *ax*, axon of giant nerve cell; *i*, deeper fibres, irregularly arranged; *o*, more superficial fibres, mostly longitudinal.

FIGURE 28. Part of a sagittal section through base of proboscis, showing anterior nerve ring cut across ventrally (*a* in figure 5). Bodian. *ab*, most anterior of the bundles of fibres constituting the anterior nerve ring; *b*, basal membrane, thickened in the basal region of the proboscis; *c*, circular muscle fibres, attached to basal membrane; *f*, general nerve-fibre layer of the proboscis; *pb*, most posterior of the bundles in the anterior nerve ring. The spaces between the bundles are traversed by supporting fibres which are almost unstained by the Bodian method.

FIGURE 29. One of the early sections from a transverse series through the proboscis, with the dorsal side at the upper margin of the figure. Bodian. This section lay immediately above the nerve-fibre layer at the tip of the proboscis; the next was tangential to the nerve-fibre layer. Elongated cells, the majority orientated dorso-ventrally and with the nucleus near the dorsal end of the cell, are common throughout the section, except towards the periphery. These cells therefore lie parallel to and immediately outside the nerve-fibre layer at the tip of the proboscis; they appear to be bipolar ganglion cells of the type described by Hess.

FIGURE 30. Part of the section shown in figure 29. After the Bodian method the cell bodies of the elongated cells appear granular and somewhat darkly stained. In this respect they resemble the cells in the groove of the ciliary organ, which are probably sensory (figure 26).

PLATE 34

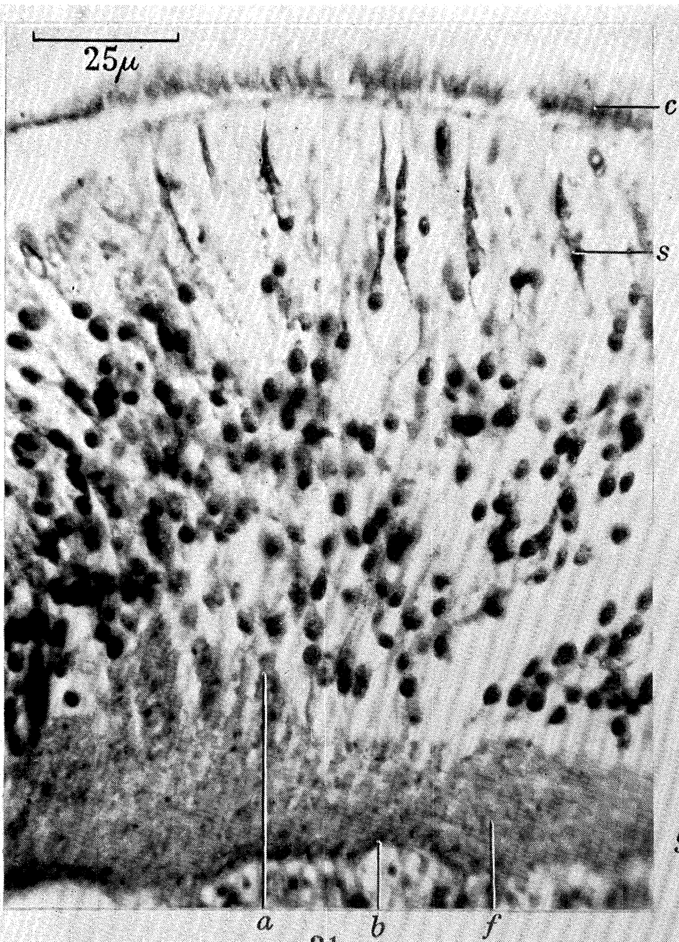
FIGURE 31. Part of a longitudinal section through the proboscis, anterior end to the right, showing the epidermis at the periphery of the proboscis base. Bodian. *a*, most anterior bundle of the anterior nerve ring; *b*, basal membrane of epidermis; *ci*, cilia; *f*, general nerve-fibre layer of proboscis; *s*, nucleus of sensory cell, one of several which appear in this section and which have darkly stained bodies tapering distally to end amongst the almost unstained ciliary rootlet bundles, usually in a small terminal swelling.

FIGURE 32. Part of a longitudinal section through the proboscis, showing the superficial half of the epidermis at the periphery of the proboscis base. Bodian. Letters as in figure 31, but showing only one of the cells which appear to be sensory.

FIGURE 33. Part of a transverse section through the collar, near its posterior end, showing the left side of the neurocord, with a giant nerve cell. Bodian. *ax*, axon of giant nerve cell passing to opposite side of neurocord, starting on the typical contra-lateral course. *b*, basal membrane bounding the neurocord, which has become separated from the nerve-fibre layer; *c*, rudimentary ciliary rootlet bundles, weakly stained, associated with a lacuna which does not appear in this section; *f*, nerve-fibre layer of neurocord; *g*, nucleus of giant nerve cell; *m*, longitudinal muscle fibres in the perihaemal cavity, a diverticulum of the trunk coelom; *n*, nuclei of supporting cells which contain a rudimentary ciliary apparatus and which may be compared with the ependyma of vertebrates.

FIGURE 34. Part of a parasagittal section through the neurocord, near its posterior end, showing parts of four or five giant nerve cells. Bodian. *a*, giant axon; *c*, rounded cell body, without dendrites; *f*, nerve-fibre layer of neurocord; *n*, vesicular nucleus with large nucleolus.

FIGURE 35. Part of a transverse section through the neurocord, showing a neuroepithelial or 'neurosensory' cell. Bodian. *c*, nucleus of a large nerve cell; *f*, fibre from the nerve cell, joining the nerve-fibre layer; *l*, lacuna, probably homologous with a fragment of the vertebrate medullary canal, with cilia projecting into its spherical cavity; *p*, process connecting nerve cell to lacuna.



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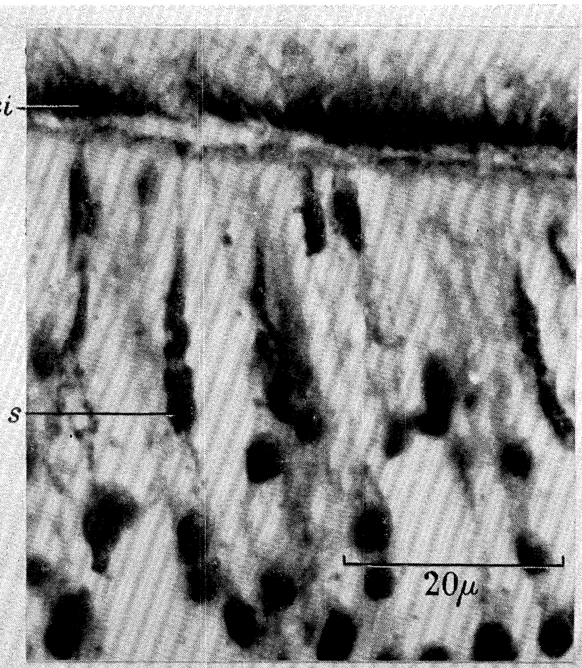

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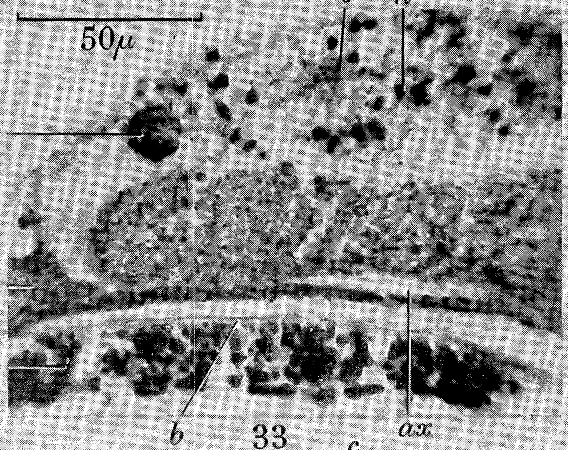

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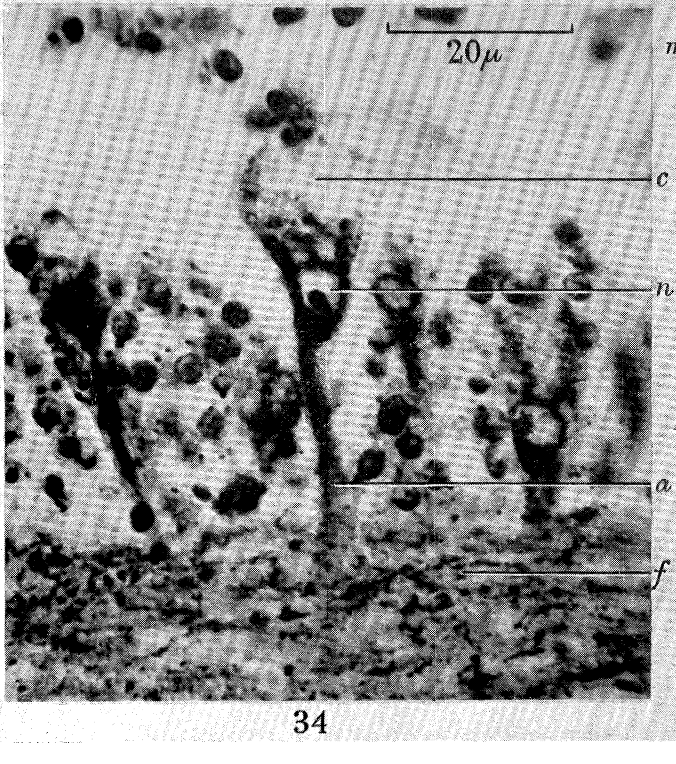

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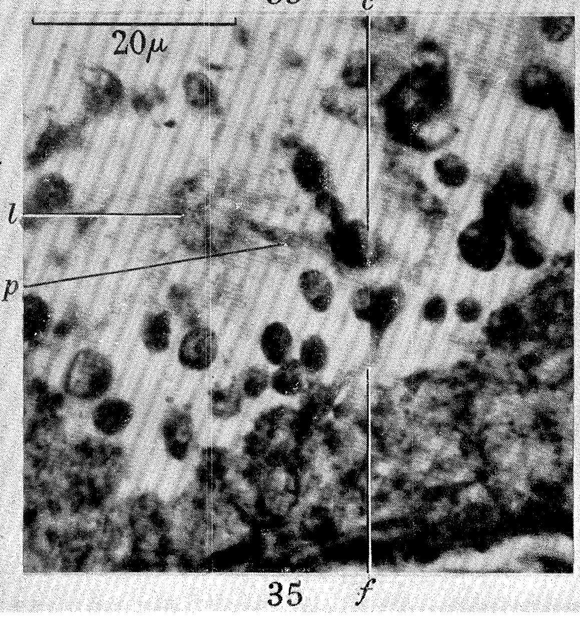

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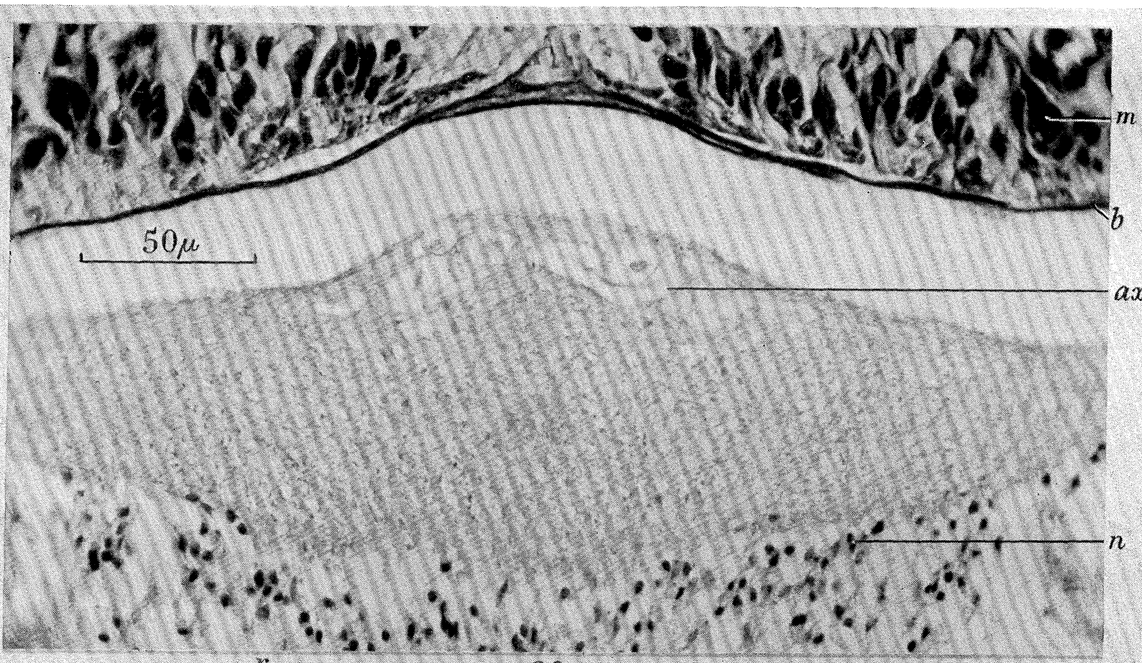

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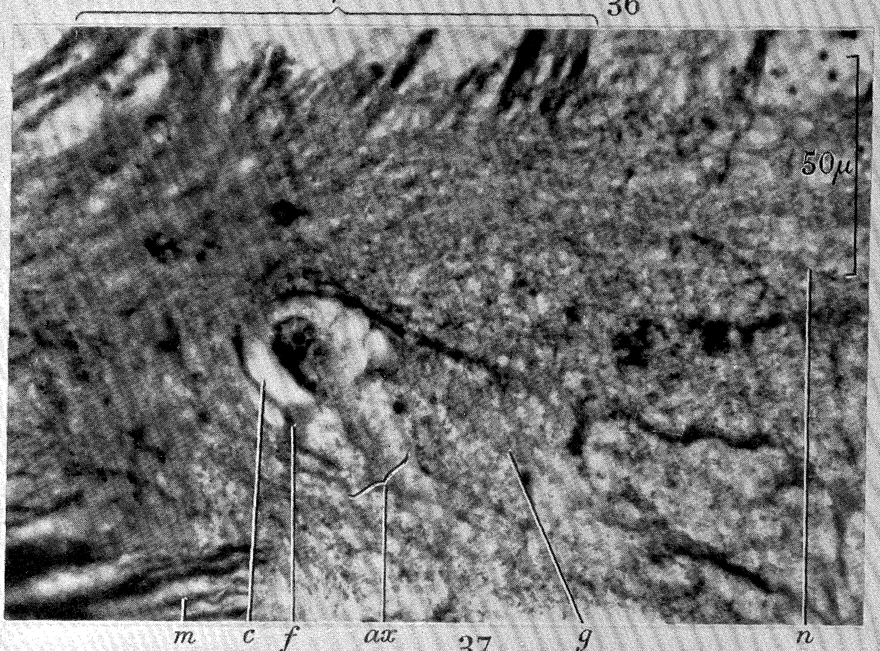

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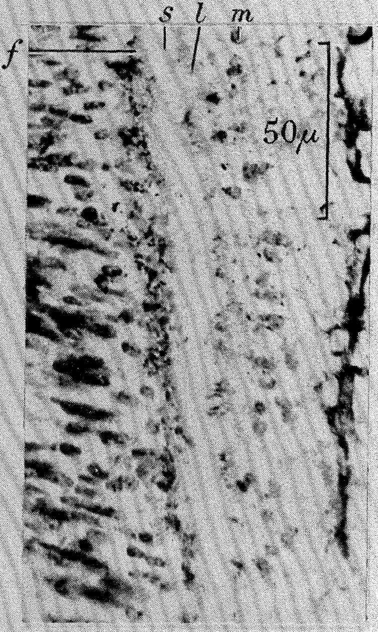

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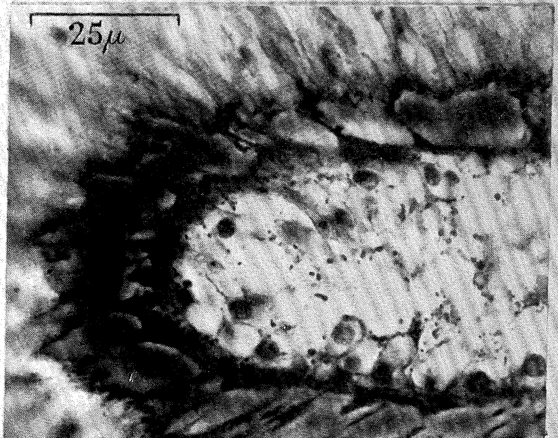

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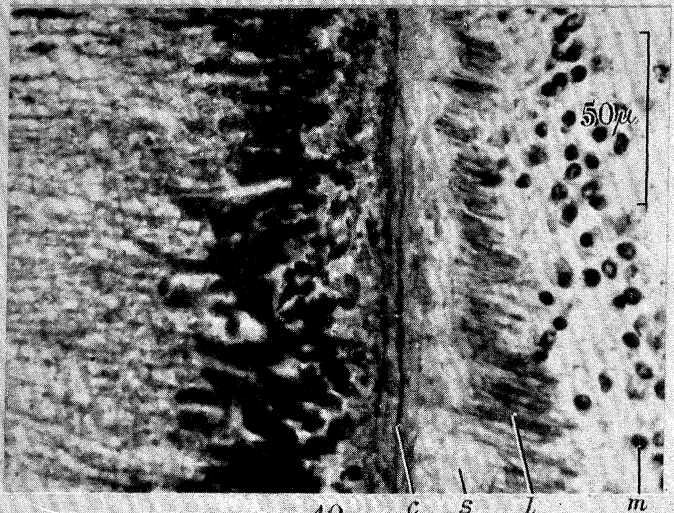

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PLATE 35

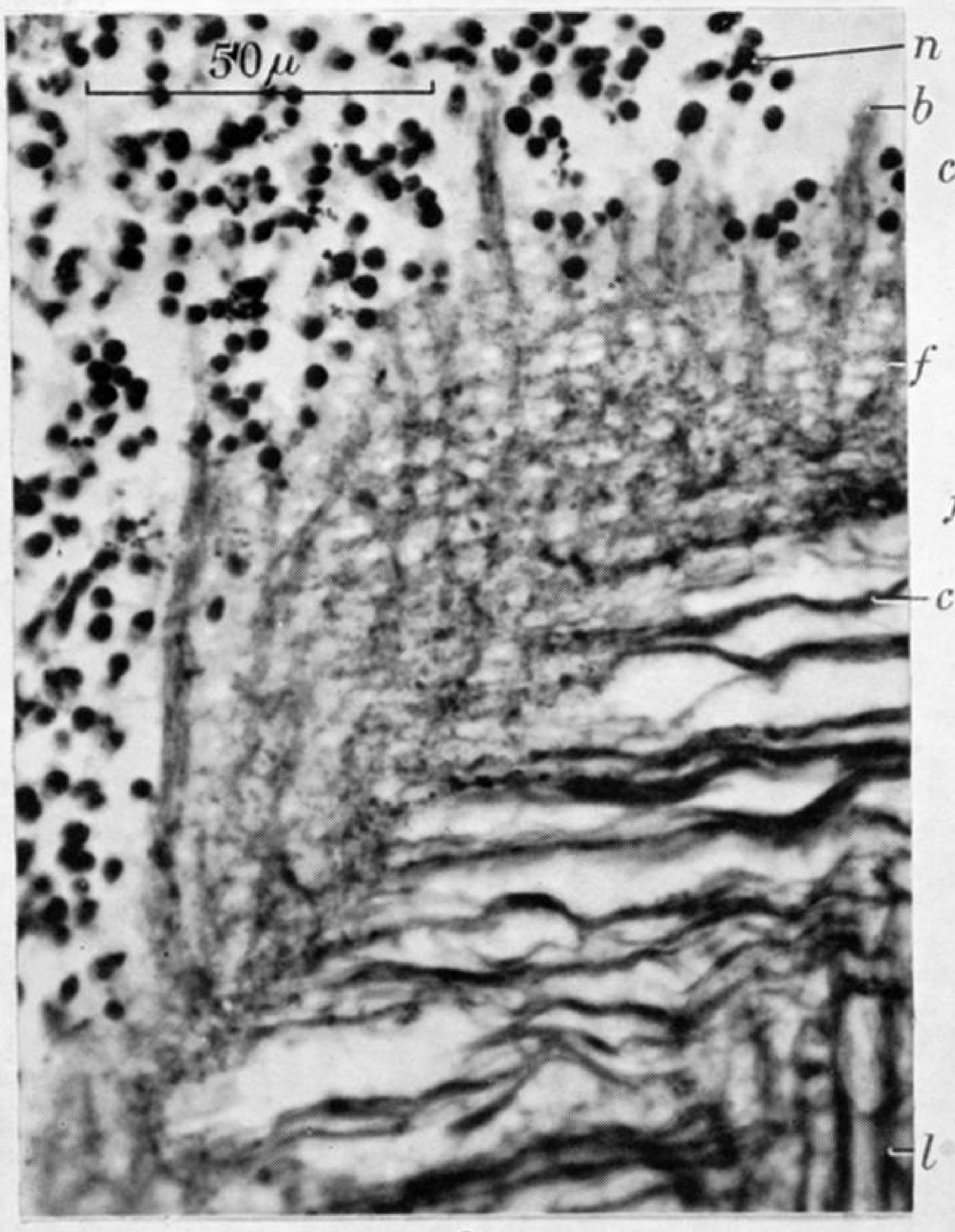
FIGURE 36. Part of a transverse section through the trunk showing the basal part of the epidermis in the mid-ventral line. Mallory's triple stain. The ventral nerve cord occupies most of the figure. The basal membrane, which is thick in this region, has become separated from the cord by shrinkage. About ten giant axons are seen near the mid-line, the majority adjoining the thin layer of irregular or transverse nerve fibres which occurs next to the basal membrane. *ax*, axon which follows an obviously convoluted course; *b*, basal membrane; *m*, longitudinal muscle fibres; *n*, nuclei of epidermis, the majority probably belonging to primitive ganglion cells.

FIGURE 37. Part of a nearly horizontal section through the dorsal nerve cord at the posterior edge of the collar, showing a giant nerve cell embedded in the nerve-fibre layer (an abnormal situation). Left margin of figure is anterior. Bodian. *ax*, axon of giant nerve cell, with reticular protoplasm and more densely stained core; *c*, giant nerve cell with vesicular nucleus and distinct nucleolus; *f*, giant fibre, stained fairly darkly, associated with giant nerve cell; *g*, another giant fibre, which can be followed to the bottom right-hand corner of the photograph, as it runs posteriorly and towards the left side, and which is also stained darkly and associated with the giant nerve cell but apparently separated from it by a thin septum; *m*, longitudinal muscle fibres; *n*, nerve fibres of the dorsal nerve cord of the trunk; *r*, bundles of nerve fibres turning laterally down the right side as part of the prebranchial nerve ring.

FIGURE 38. Base of the gut epithelium from a transverse section of the third part of the oesophagus, which passes along the ridge of one of the septal folds projecting into the gut lumen. Bodian. *f*, layer of darkly stained fibres, which are probably nerve fibres, adjoining the basal membrane of the endothelium; *l*, longitudinal muscle fibres adjoining the basal membrane of the mesothelium, which are weakly stained by the Bodian method and shown more clearly in figure 40; *m*, nuclei of cells in the splanchnic mesothelium, the bases of which contain the longitudinal muscle fibres of the gut wall; *s*, space between the basal membranes of the endo- and mesothelia, which constitutes a blood sinus.

FIGURE 39. Part of a horizontal section through the hepatic region of the gut, showing the blood sinus which runs along the ridge of each septal fold, cut transversely: the left side of the figure is nearest to the gut lumen. Bodian. Darkly stained fibres, which may well be supporting trabeculae, join the two basal membranes bounding the sinus.

FIGURE 40. As figure 38, but this section passes more obliquely through the ridge of a septum and is stained with Heidenhain's haematoxylin. The longitudinal muscle fibres of the gut mesothelium are therefore shown more clearly. *c*, circular fibres lying in base of gut epithelium, presumably endodermal muscle fibres. Other letters as in figure 38.



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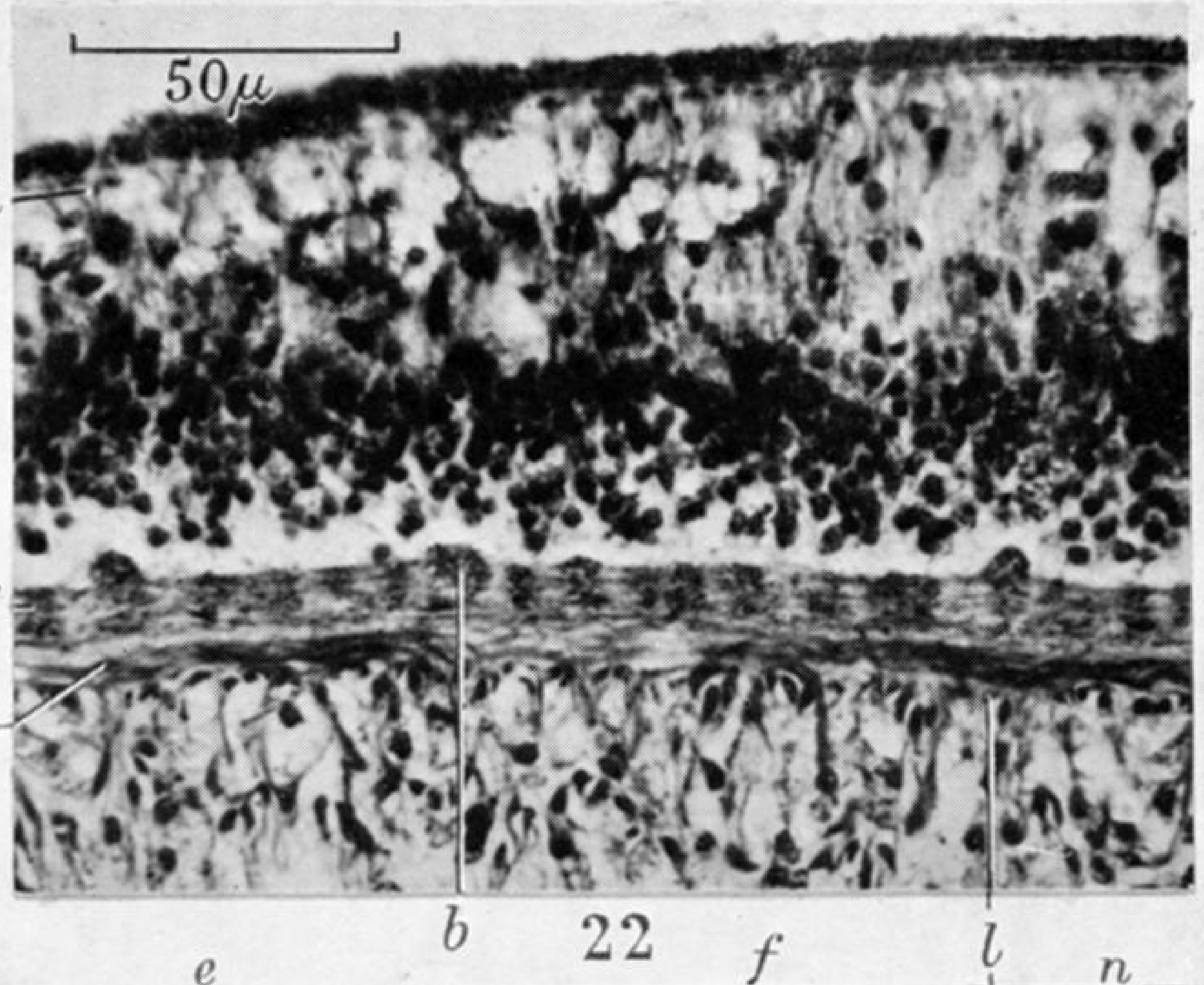

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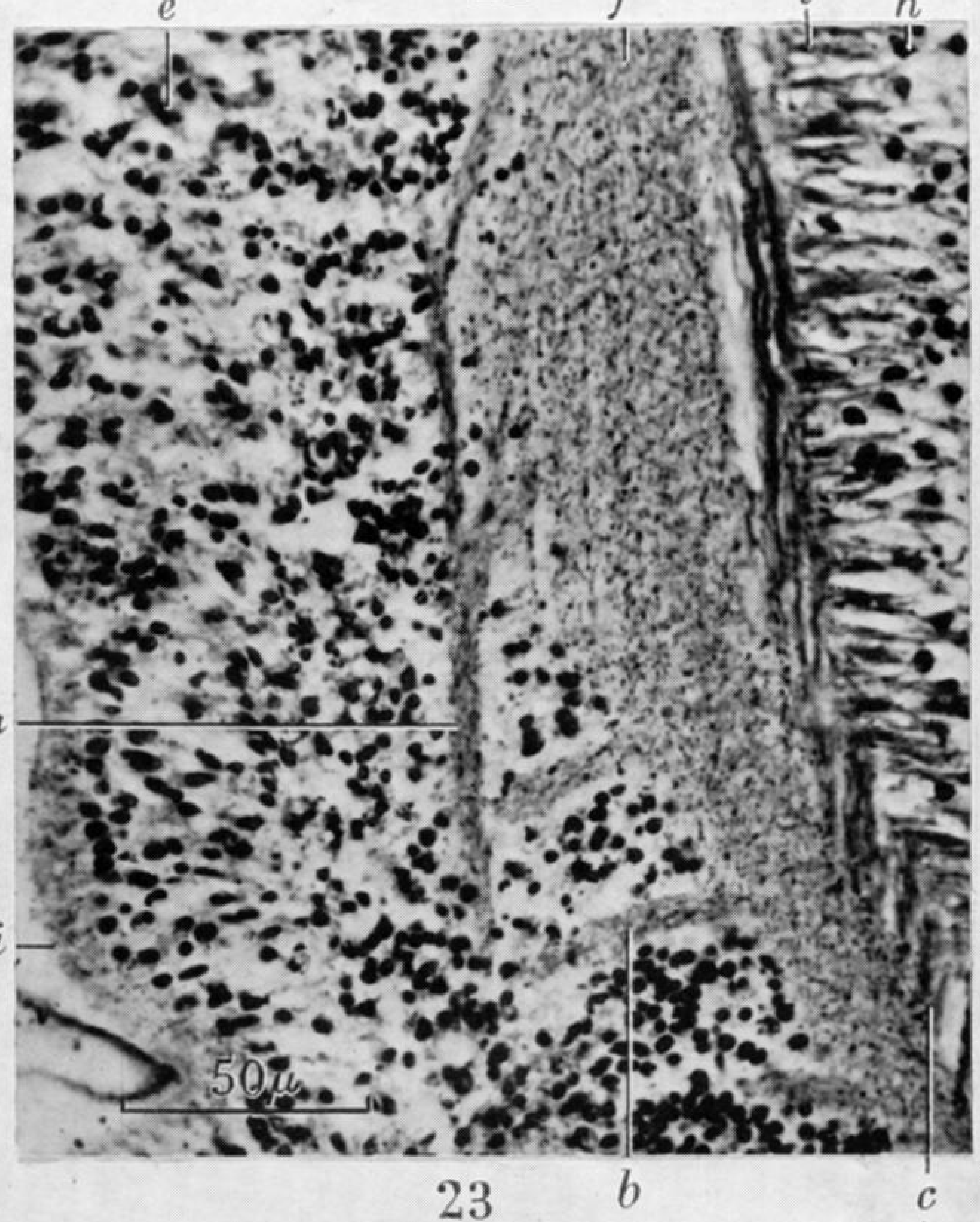

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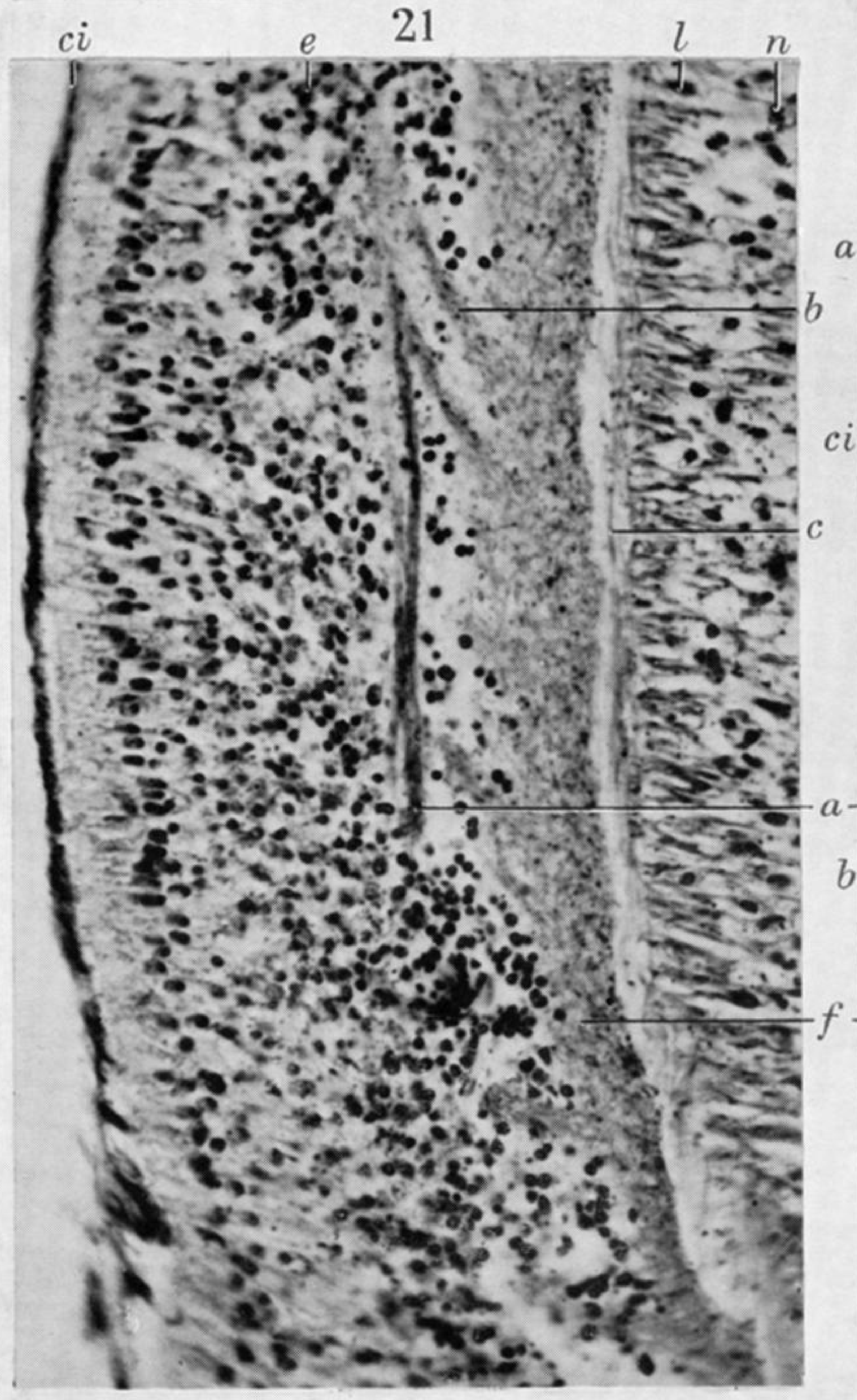

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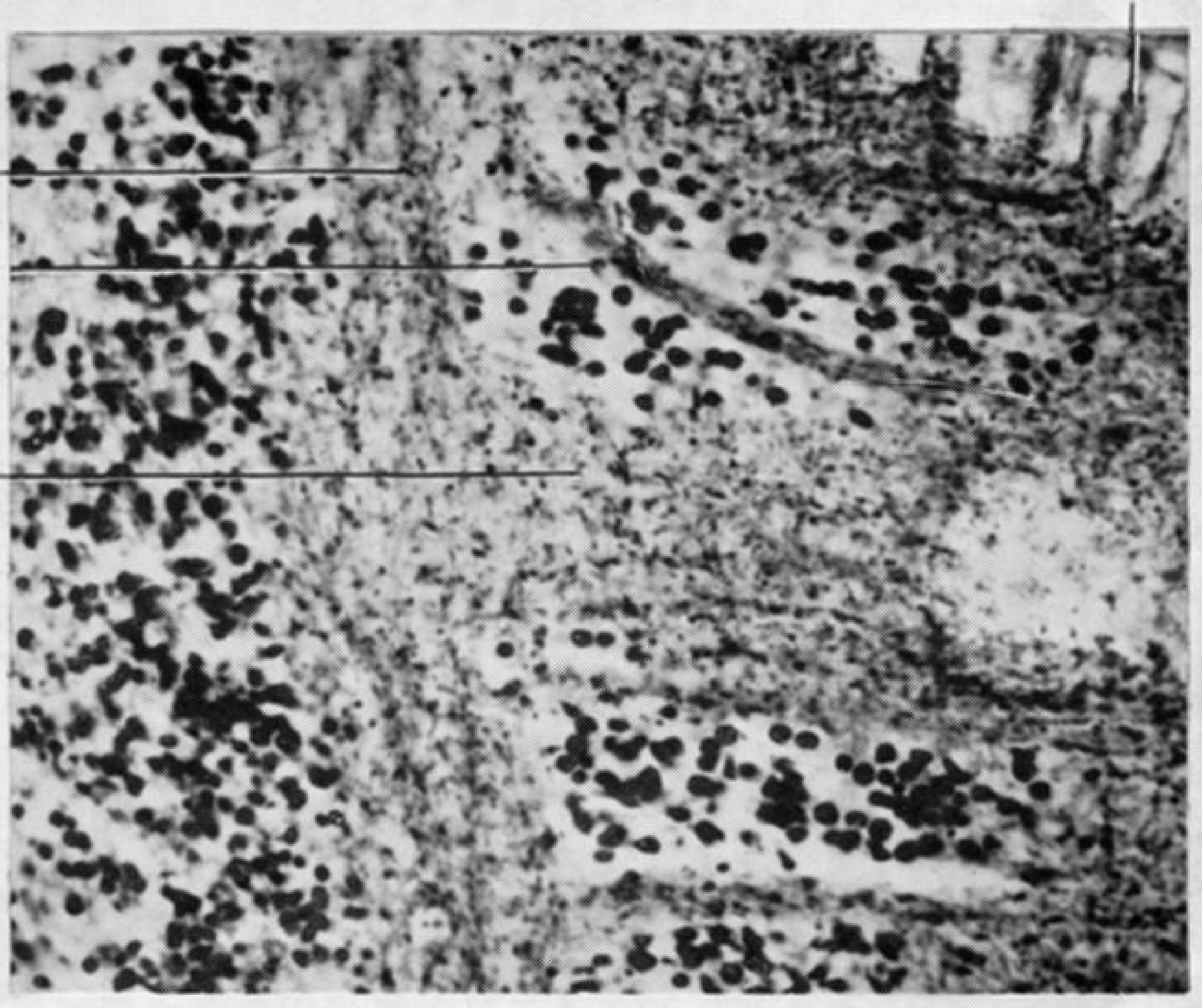

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PLATE 32

FIGURE 21. A very oblique section through the deeper part of the proboscis epidermis, more superficial at the upper left, deeper at the lower right corner of the figure, the upper margin of which is anterior. Bodian. *c*, circular muscle fibres; *b*, one of the several longitudinal bundles of nerve fibres lying in the superficial part of the nerve-fibre layer; *f*, nerve-fibre layer—apart from the longitudinal bundles the majority of the superficial fibres are transverse, but the deeper fibres are more irregular and are closely associated with the circular muscle fibres, from which they are separated only by the very thin unstained basal membrane; *l*, outer layer of longitudinal muscle fibres; *n*, nuclei occurring immediately outside the nerve-fibre layer, the majority probably belonging to primitive ganglion cells.

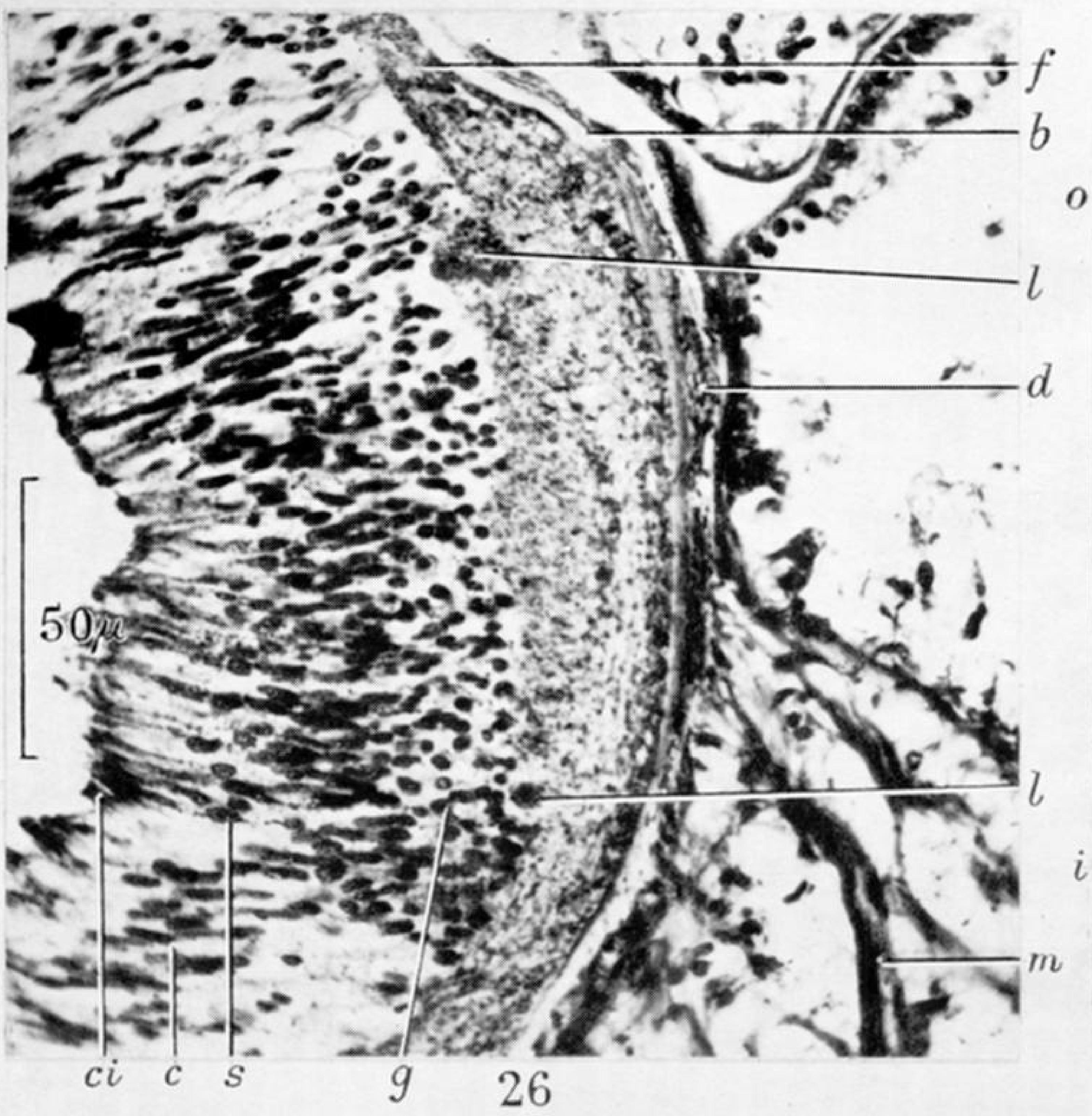
FIGURE 22. Part of a transverse section through the proboscis near its posterior end, showing the epidermis and underlying muscles. Bodian. *b*, large bundle of longitudinal nerve fibres, projecting above general nerve-fibre layer; *c*, circular muscle fibres; *ci*, rootlet bundles of cilia; *f*, nerve-fibre layer, showing about three large and seventeen small darkly stained bundles of longitudinal nerve fibres, cut transversely and traversed by transverse nerve fibres; *l*, outermost layer of longitudinal muscle fibres.

FIGURES 23, 24 and 25 all same scale, and all with right margins anterior.

FIGURE 23. Part of a horizontal section through the dorsal edge of the proboscis base, showing the epidermis and underlying tissues, cut obliquely, in dorsal view, slightly to the right of the midline. Bodian. *a*, bundle of fibres in anterior nerve ring; *b*, bundle of longitudinal nerve fibres, one of several which project above the general nerve-fibre layer and curve away from the midline to join the anterior nerve ring; *c*, circular muscle fibres; *ci*, ciliated border of epidermis; *e*, nuclei of epidermis; *f*, general nerve-fibre layer of epidermis, cut very obliquely; *l*, outermost layer of longitudinal muscle fibres; *n*, nuclei of muscles.

FIGURE 24. Part of a parasagittal section through the edge of the proboscis base on the right side, showing the epidermis and underlying tissues cut obliquely. Bodian. Letters as in figure 23, but here the longitudinal bundles of nerve fibres curve dorsally to join the anterior nerve ring.

FIGURE 25. Part of a horizontal section through the ventral edge of the proboscis base, showing the base of the epidermis cut tangentially in the mid-line. Bodian. Letters as in figure 23. Owing to slight folding the surface of the general nerve-fibre layer is cut in several places.



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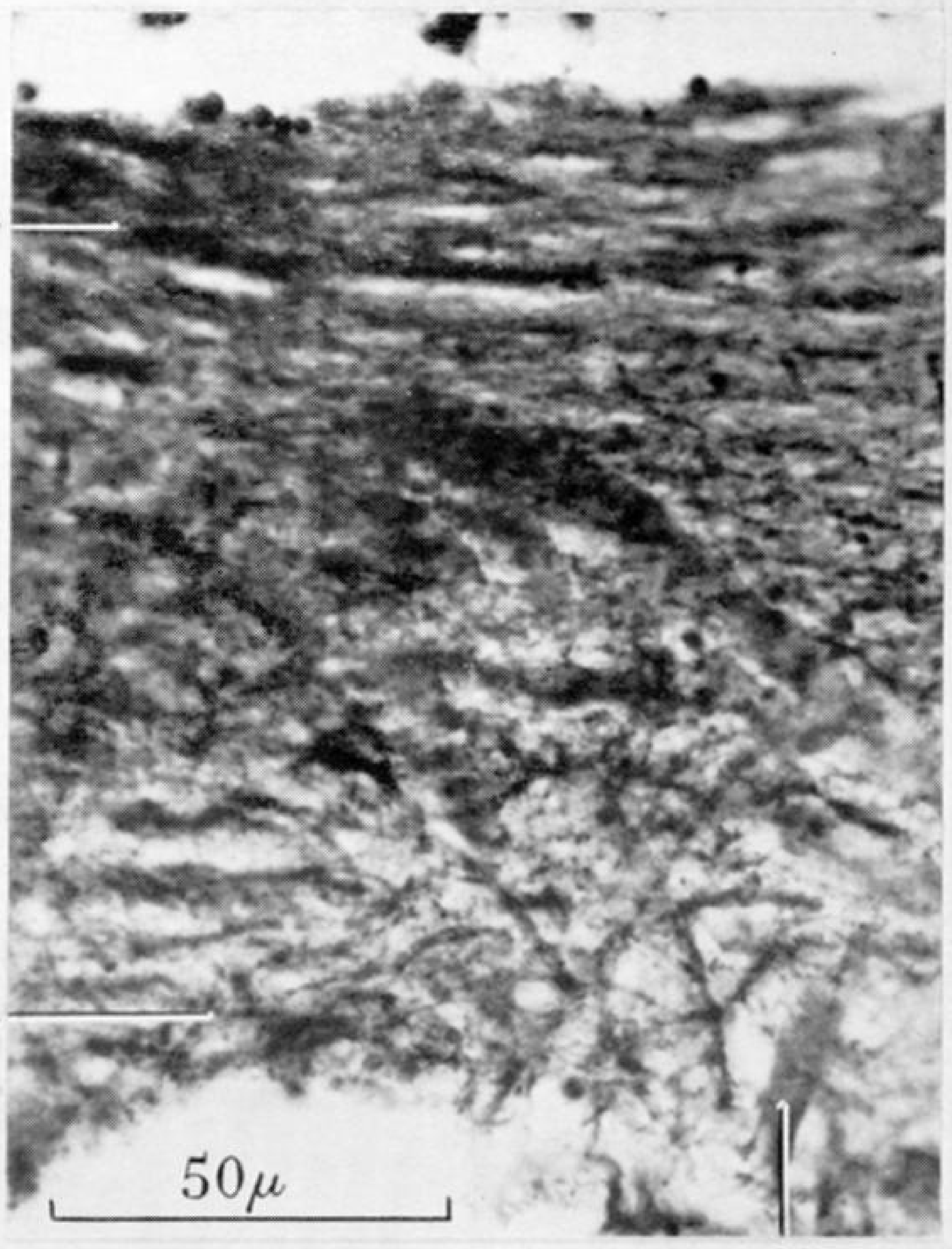

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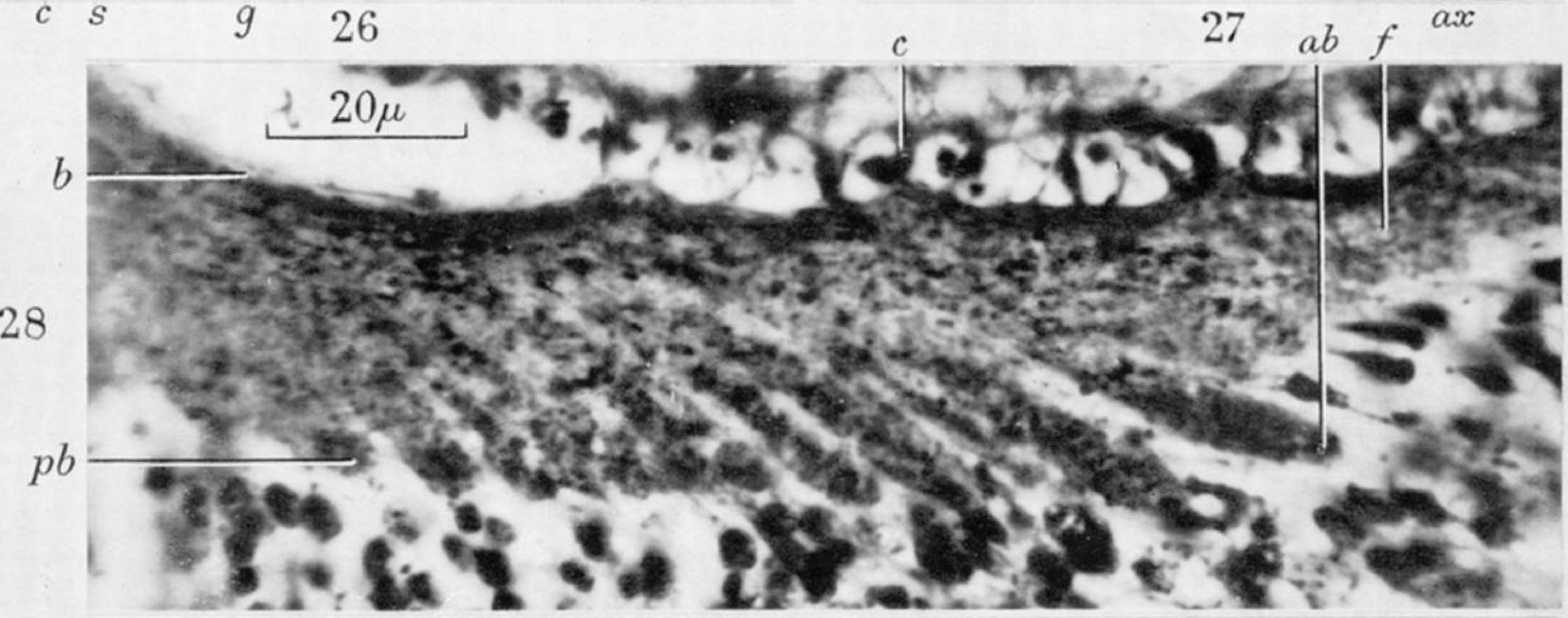

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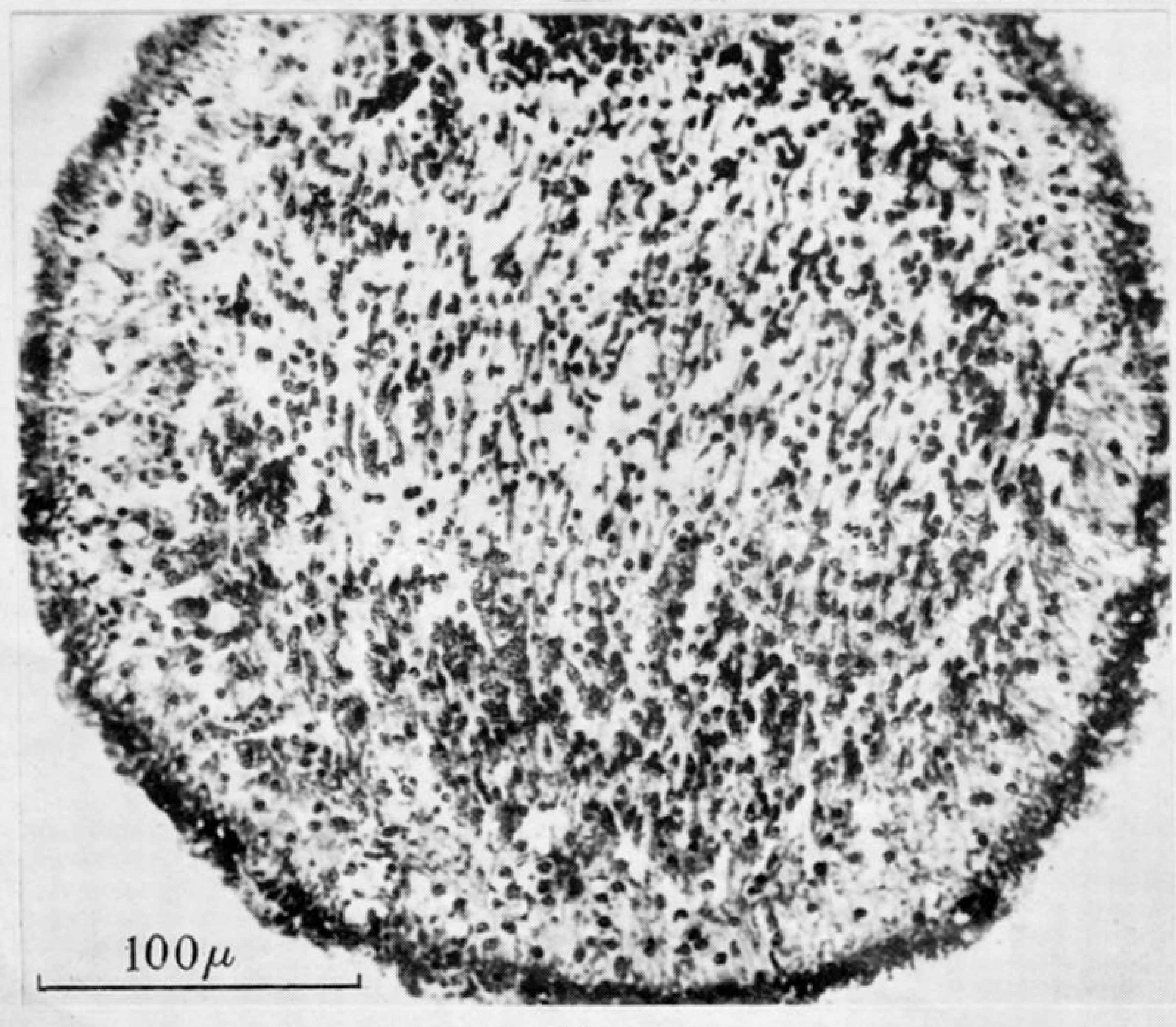

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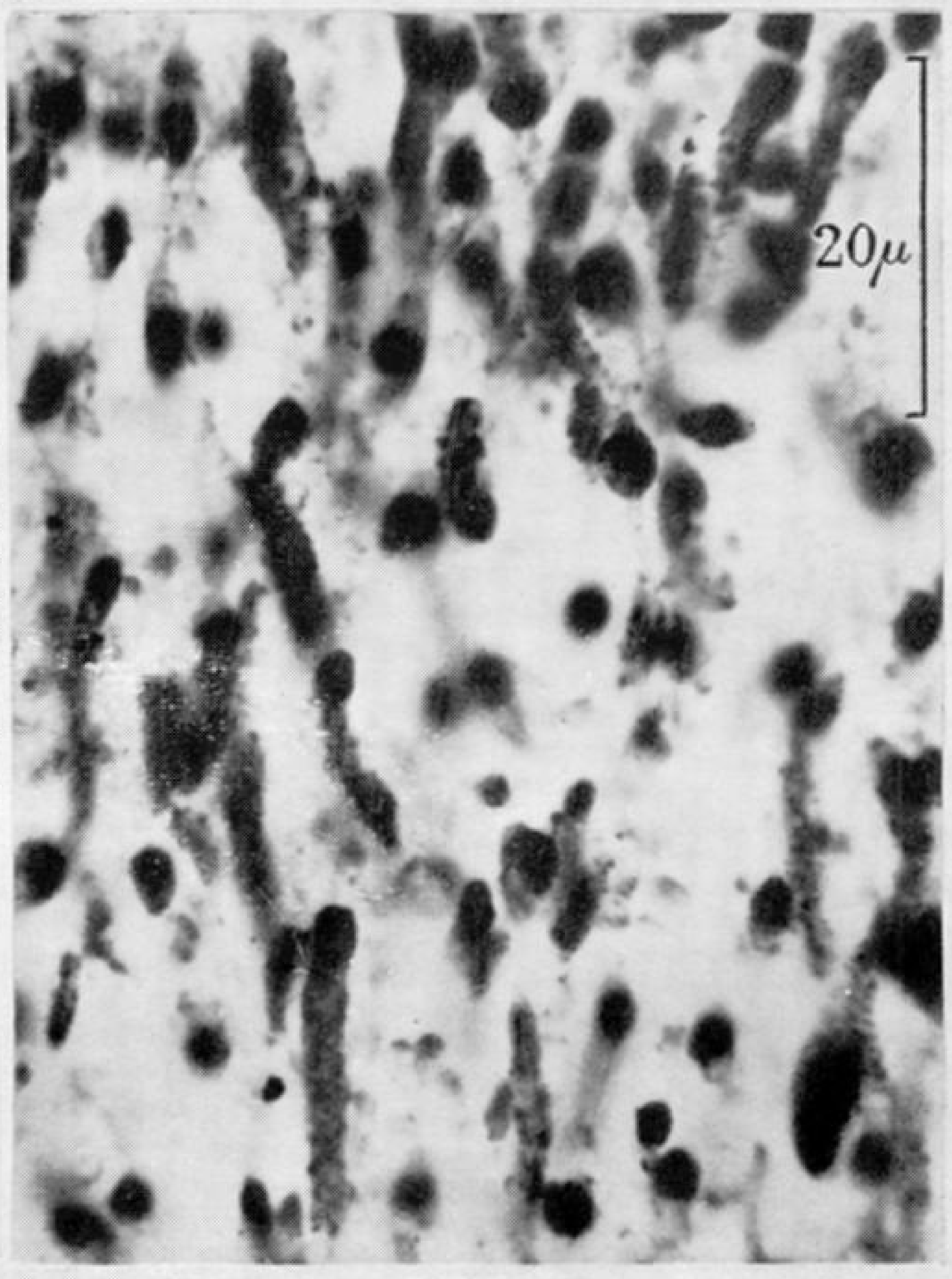

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PLATE 33

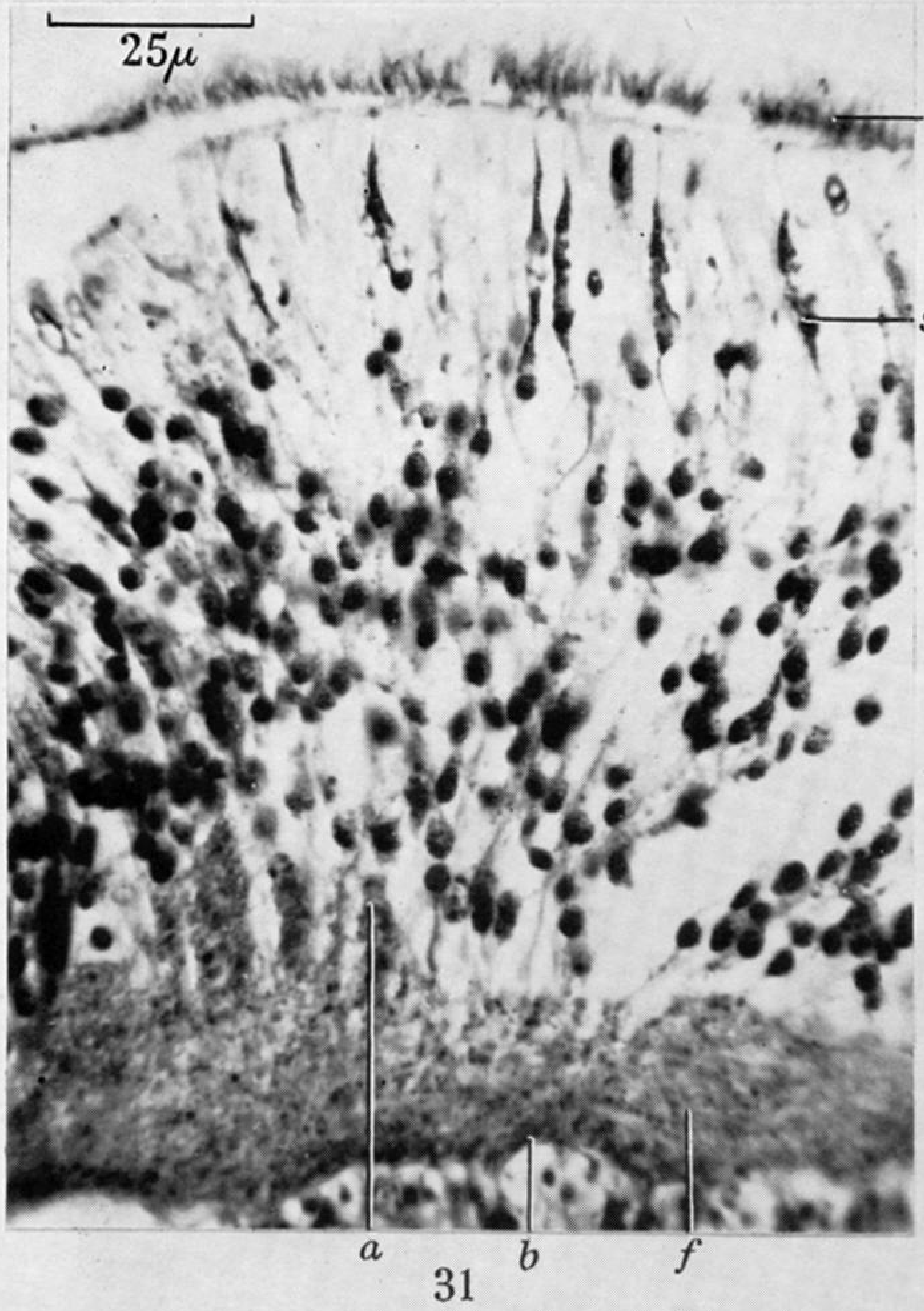
FIGURE 26. Part of a sagittal section, showing the groove of the ciliary organ cut transversely (*l* in figure 5). Bodian. *b*, basal membrane of epidermis, which is thick in this region; *c*, nuclei of ciliated cells, large and often elongated; *ci*, rootlet bundles of cilia—the cilia themselves have been torn away; *d*, fibres of doubtful function, lying between the basal membranes of the epi- and mesothelia and continuous with similar fibres in the cartilaginous tissue; *f*, general nerve fibre layer of proboscis base; *g*, concentration of nuclei associated with nerve loop of ciliary organ, probably belonging to primitive ganglion cells; *l*, borders of nerve loop of ciliary organ, cut transversely; *m*, muscle fibres; *s*, cells probably sensory in function, having slender bodies containing many granules, tapering distally and extending to surface of epidermis between adjacent rootlet bundles. Their nuclei are smaller, denser and less elongated than are those of the ciliated cells, and they lie for the most part nearer the surface of the epidermis. Although they somewhat resemble sensory cells from other parts of the body, cells conforming strictly to this type were observed only in the groove of the ciliary organ.

FIGURE 27. Oblique, nearly horizontal section through the nerve-fibre layer of the neurocord, near the posterior neuropore. Bodian. *ax*, axon of giant nerve cell; *i*, deeper fibres, irregularly arranged; *o*, more superficial fibres, mostly longitudinal.

FIGURE 28. Part of a sagittal section through base of proboscis, showing anterior nerve ring cut across ventrally (*a* in figure 5). Bodian. *ab*, most anterior of the bundles of fibres constituting the anterior nerve ring; *b*, basal membrane, thickened in the basal region of the proboscis; *c*, circular muscle fibres, attached to basal membrane; *f*, general nerve-fibre layer of the proboscis; *pb*, most posterior of the bundles in the anterior nerve ring. The spaces between the bundles are traversed by supporting fibres which are almost unstained by the Bodian method.

FIGURE 29. One of the early sections from a transverse series through the proboscis, with the dorsal side at the upper margin of the figure. Bodian. This section lay immediately above the nerve-fibre layer at the tip of the proboscis; the next was tangential to the nerve-fibre layer. Elongated cells, the majority orientated dorso-ventrally and with the nucleus near the dorsal end of the cell, are common throughout the section, except towards the periphery. These cells therefore lie parallel to and immediately outside the nerve-fibre layer at the tip of the proboscis; they appear to be bipolar ganglion cells of the type described by Hess.

FIGURE 30. Part of the section shown in figure 29. After the Bodian method the cell bodies of the elongated cells appear granular and somewhat darkly stained. In this respect they resemble the cells in the groove of the ciliary organ, which are probably sensory (figure 26).



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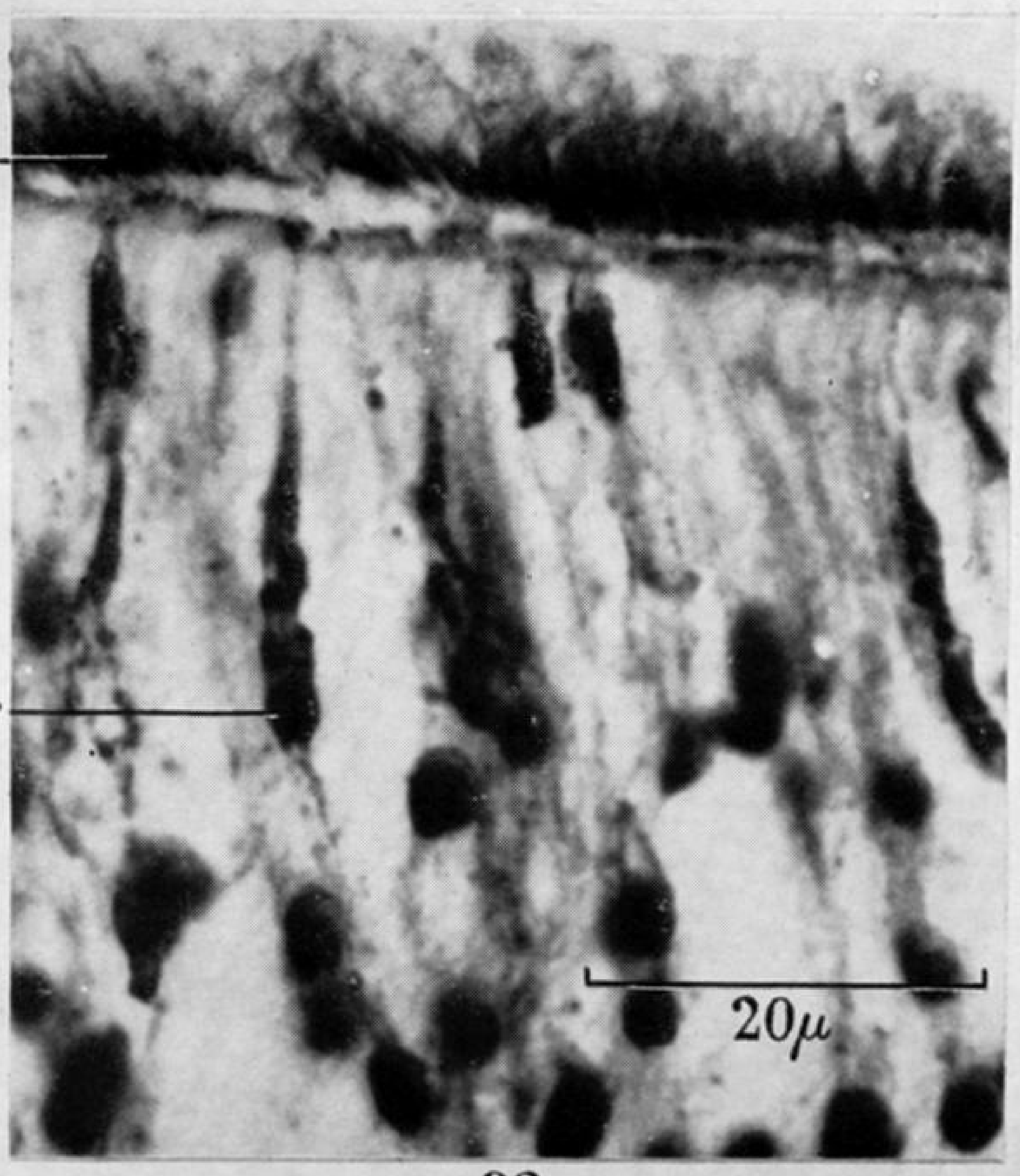

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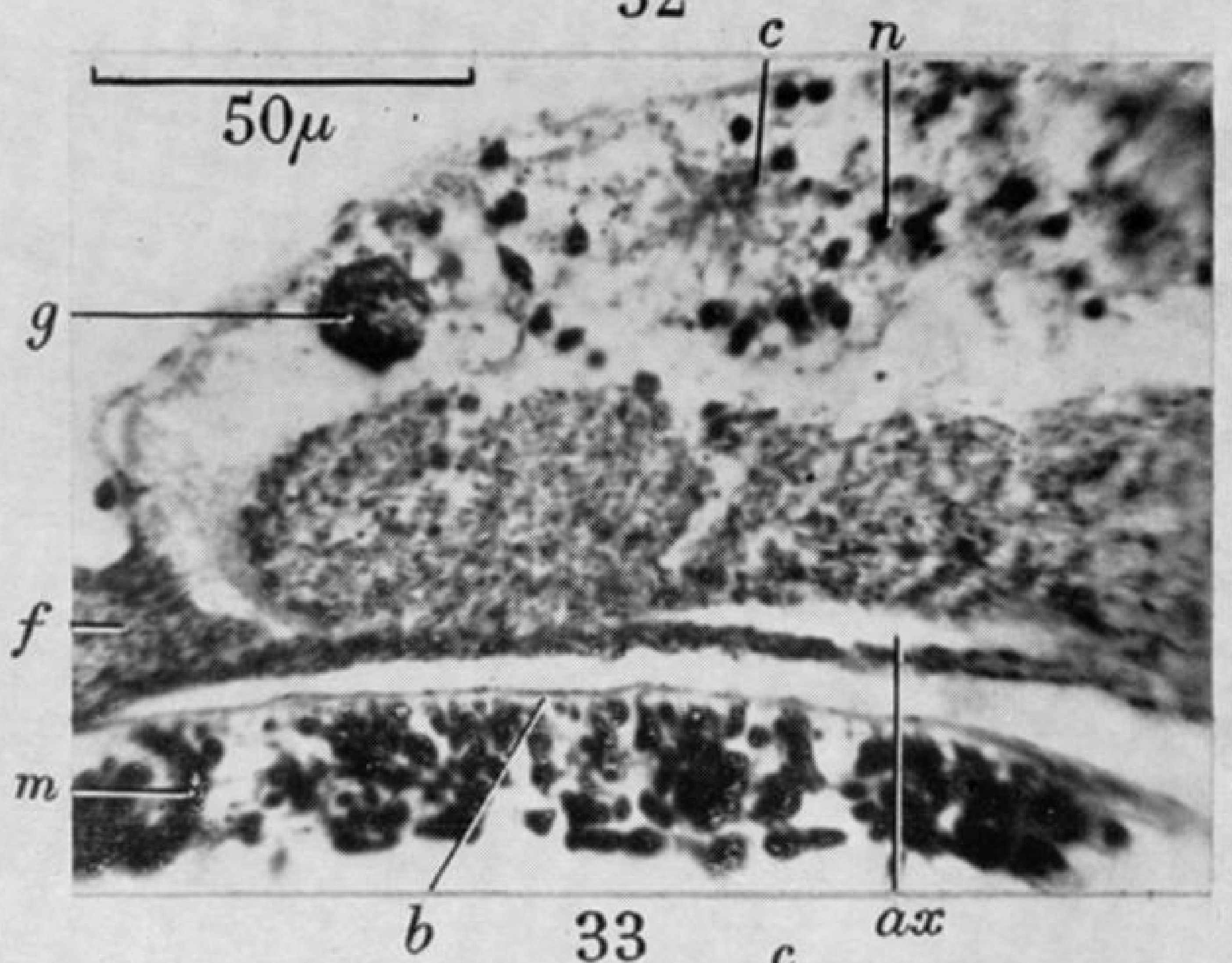

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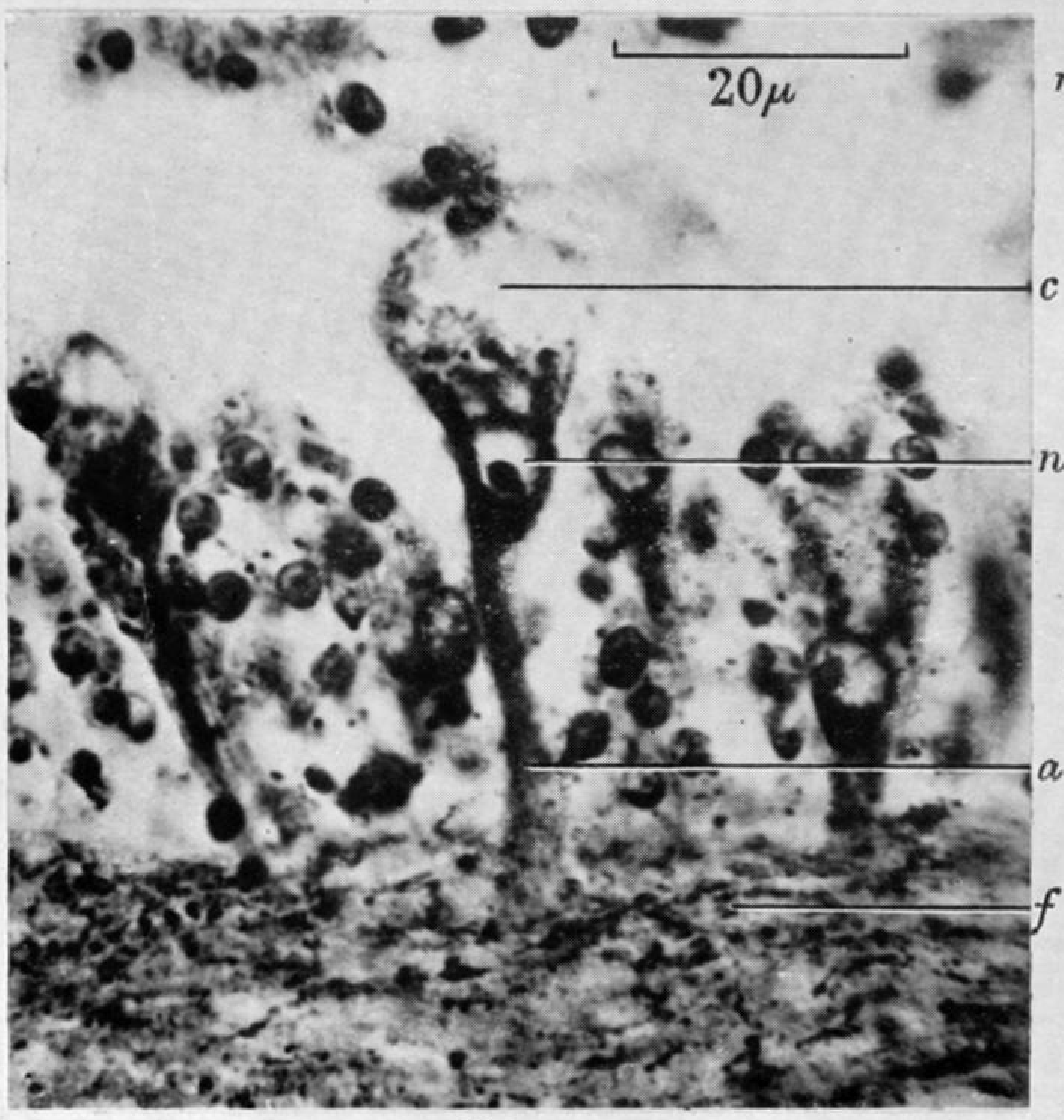

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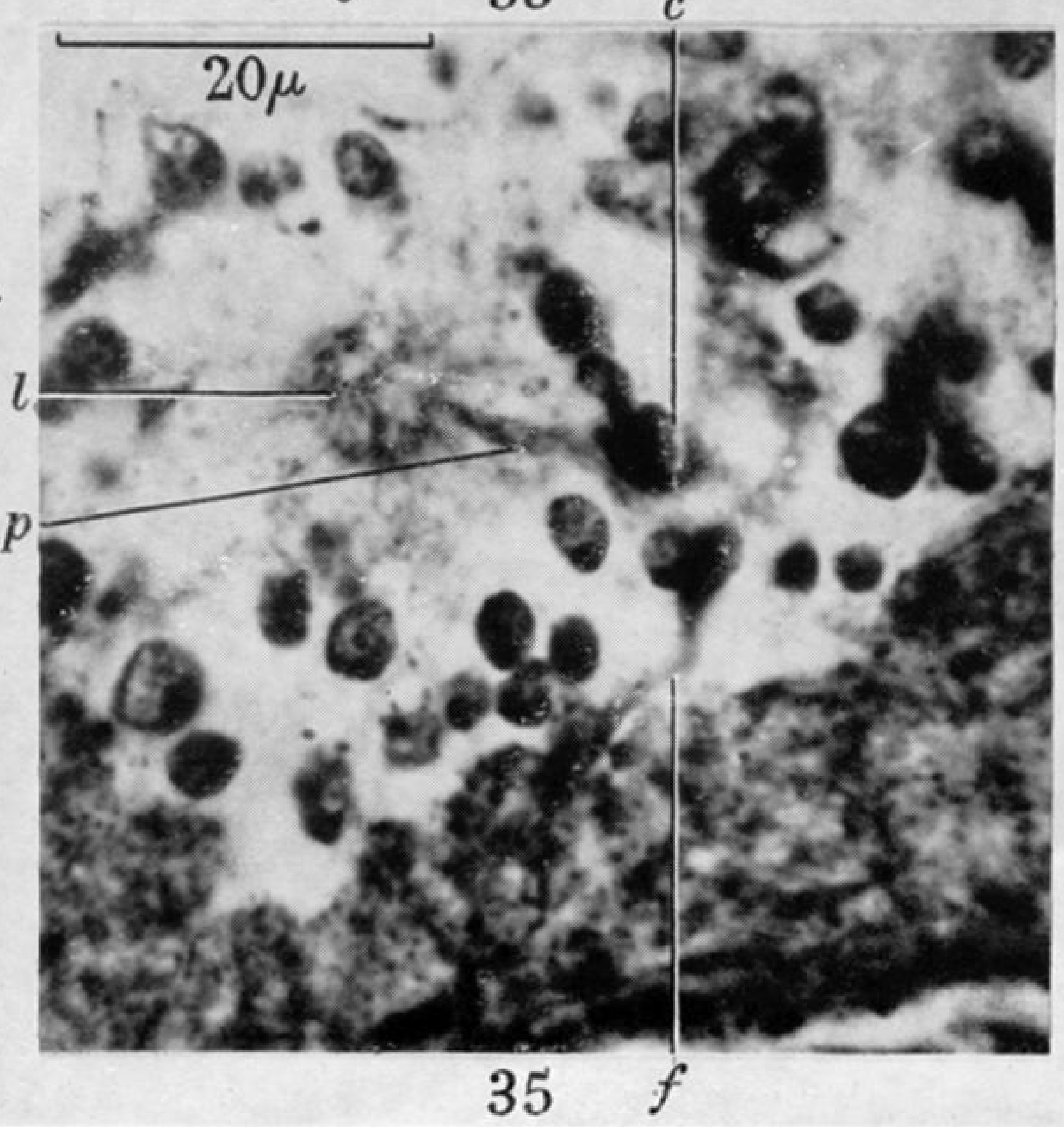

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PLATE 34

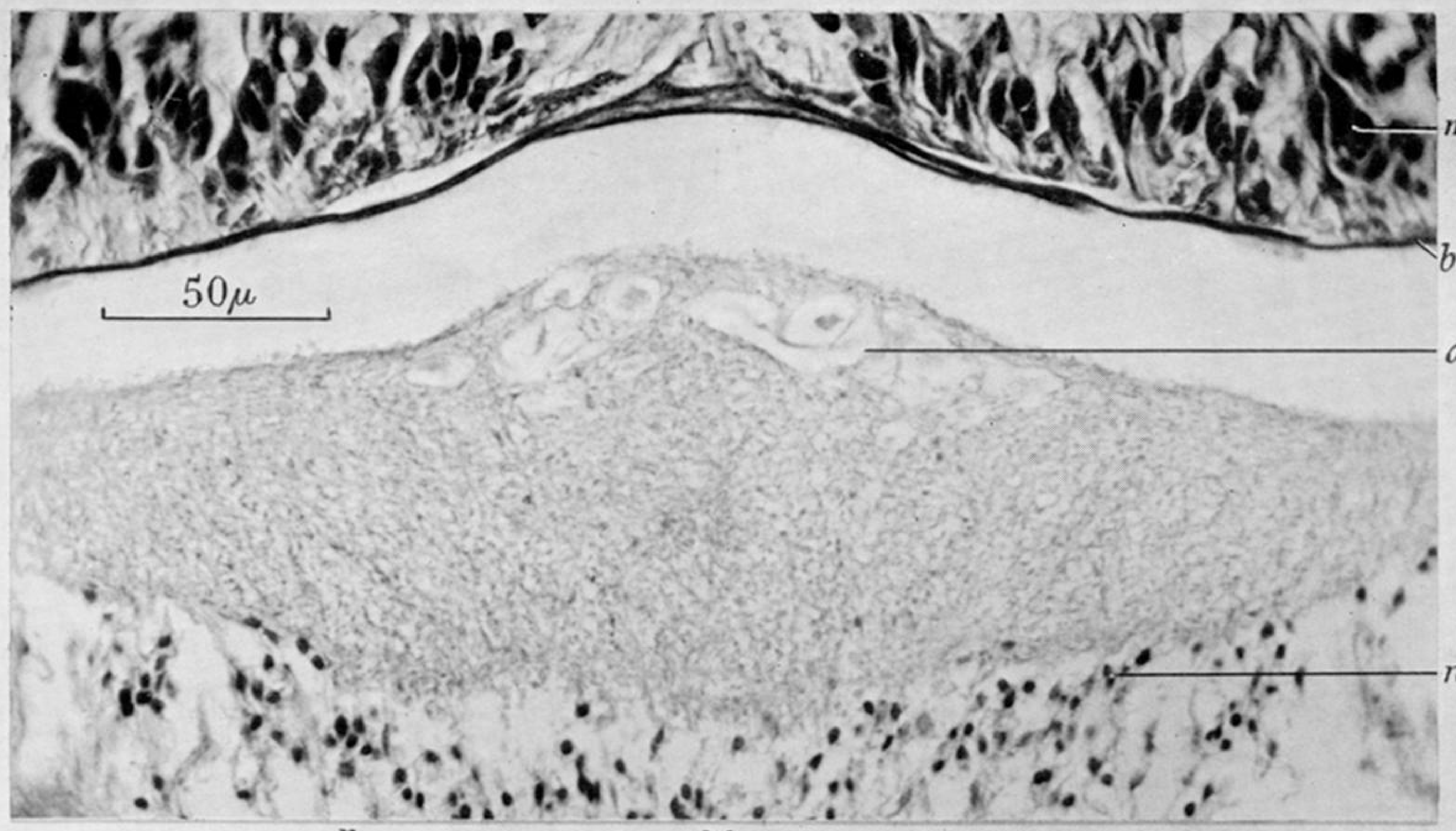
FIGURE 31. Part of a longitudinal section through the proboscis, anterior end to the right, showing the epidermis at the periphery of the proboscis base. Bodian. *a*, most anterior bundle of the anterior nerve ring; *b*, basal membrane of epidermis; *ci*, cilia; *f*, general nerve-fibre layer of proboscis; *s*, nucleus of sensory cell, one of several which appear in this section and which have darkly stained bodies tapering distally to end amongst the almost unstained ciliary rootlet bundles, usually in a small terminal swelling.

FIGURE 32. Part of a longitudinal section through the proboscis, showing the superficial half of the epidermis at the periphery of the proboscis base. Bodian. Letters as in figure 31, but showing only one of the cells which appear to be sensory.

FIGURE 33. Part of a transverse section through the collar, near its posterior end, showing the left side of the neurocord, with a giant nerve cell. Bodian. *ax*, axon of giant nerve cell passing to opposite side of neurocord, starting on the typical contra-lateral course. *b*, basal membrane bounding the neurocord, which has become separated from the nerve-fibre layer; *c*, rudimentary ciliary rootlet bundles, weakly stained, associated with a lacuna which does not appear in this section; *f*, nerve-fibre layer of neurocord; *g*, nucleus of giant nerve cell; *m*, longitudinal muscle fibres in the perihaemal cavity, a diverticulum of the trunk coelom; *n*, nuclei of supporting cells which contain a rudimentary ciliary apparatus and which may be compared with the ependyma of vertebrates.

FIGURE 34. Part of a parasagittal section through the neurocord, near its posterior end, showing parts of four or five giant nerve cells. Bodian. *a*, giant axon; *c*, rounded cell body, without dendrites; *f*, nerve-fibre layer of neurocord; *n*, vesicular nucleus with large nucleolus.

FIGURE 35. Part of a transverse section through the neurocord, showing a neuroepithelial or 'neurosensory' cell. Bodian. *c*, nucleus of a large nerve cell; *f*, fibre from the nerve cell, joining the nerve-fibre layer; *l*, lacuna, probably homologous with a fragment of the vertebrate medullary canal, with cilia projecting into its spherical cavity; *p*, process connecting nerve cell to lacuna.



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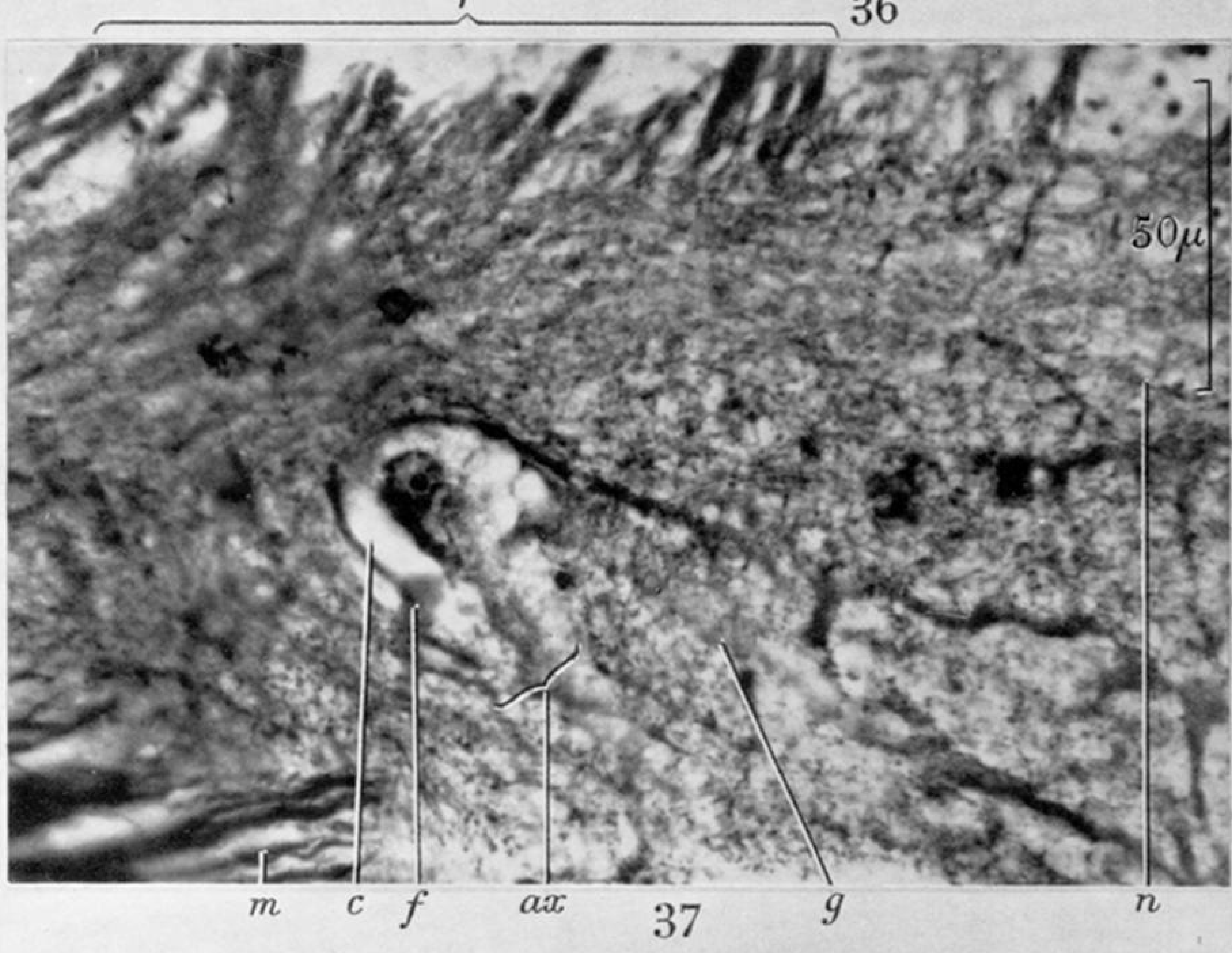

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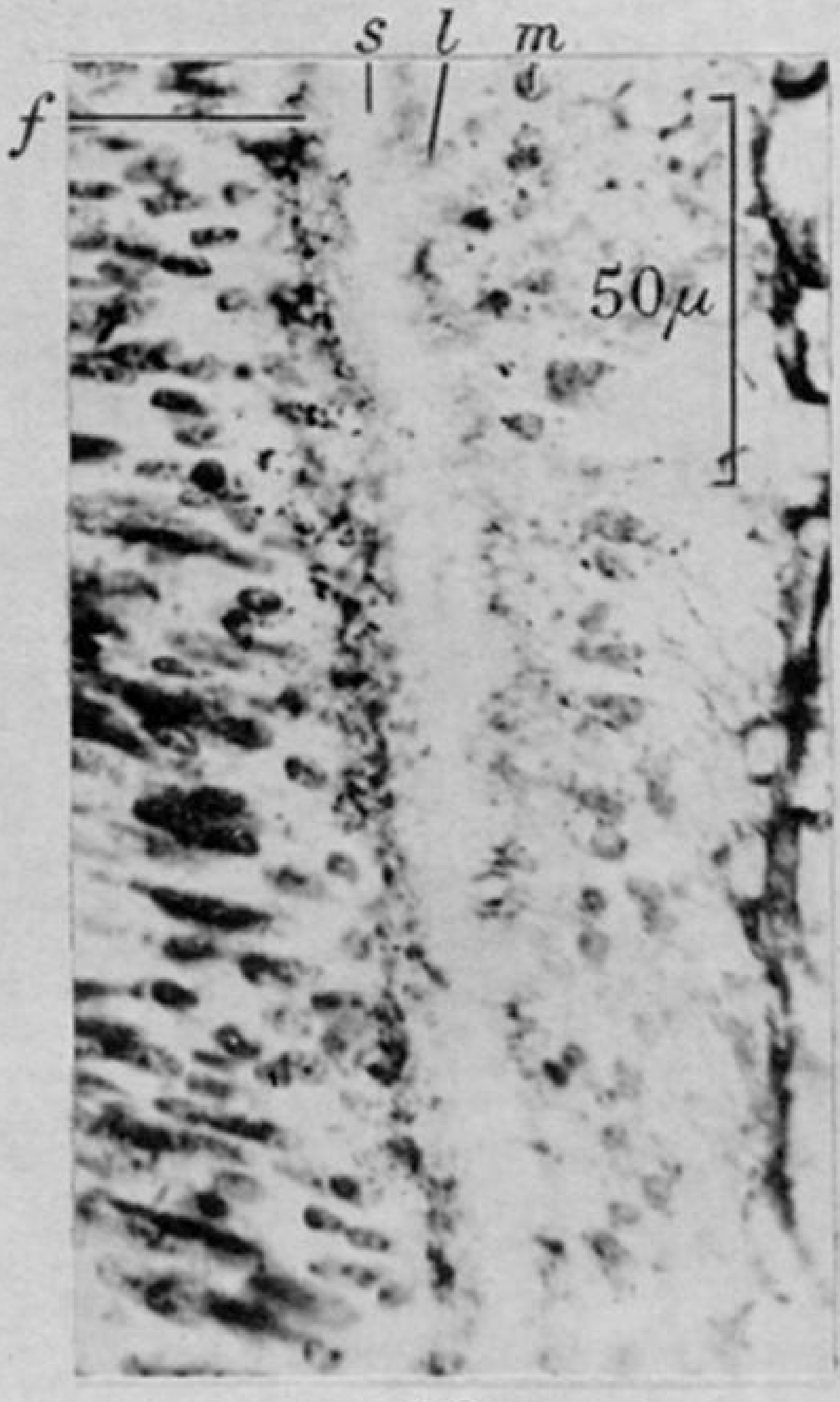

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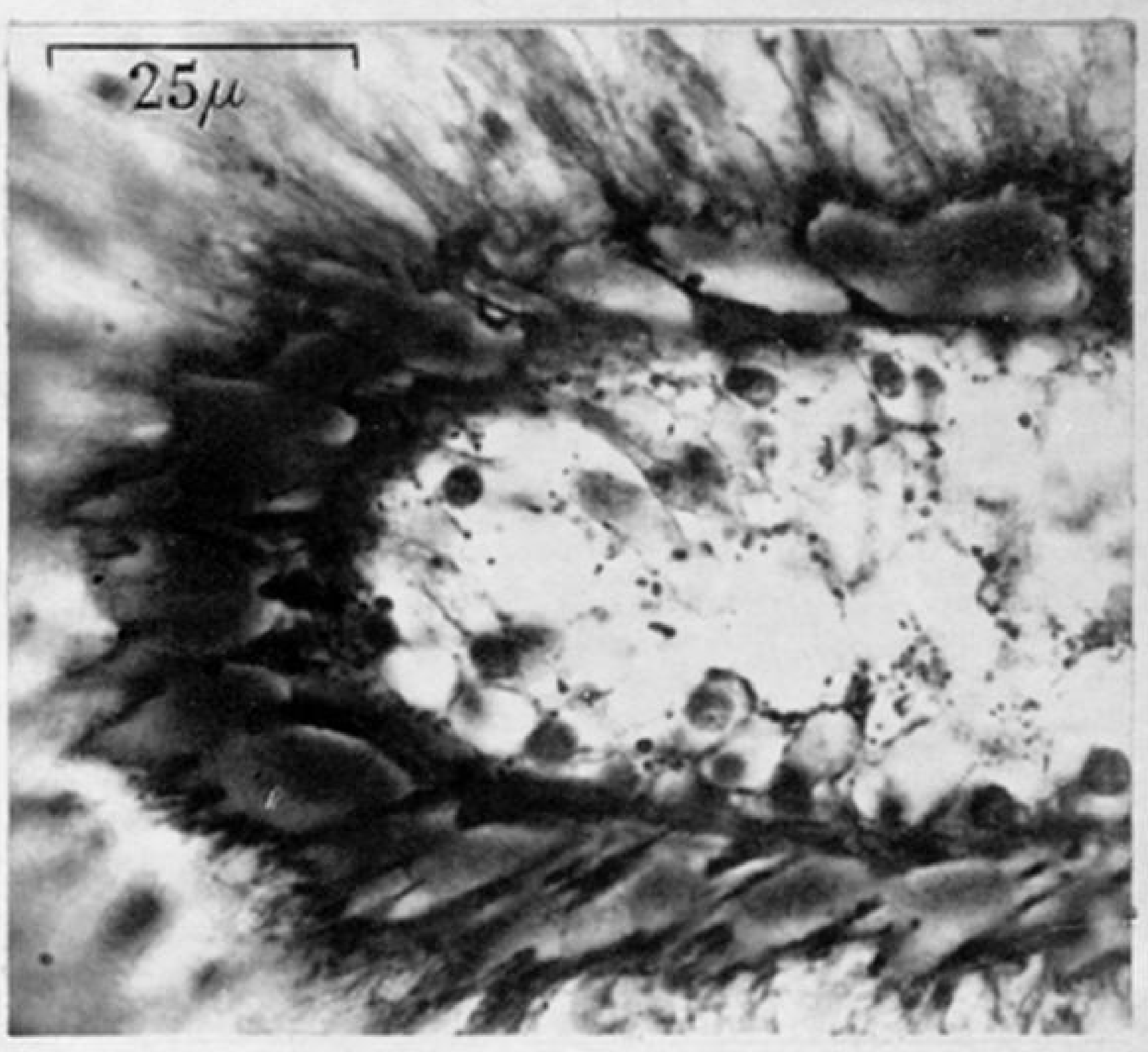

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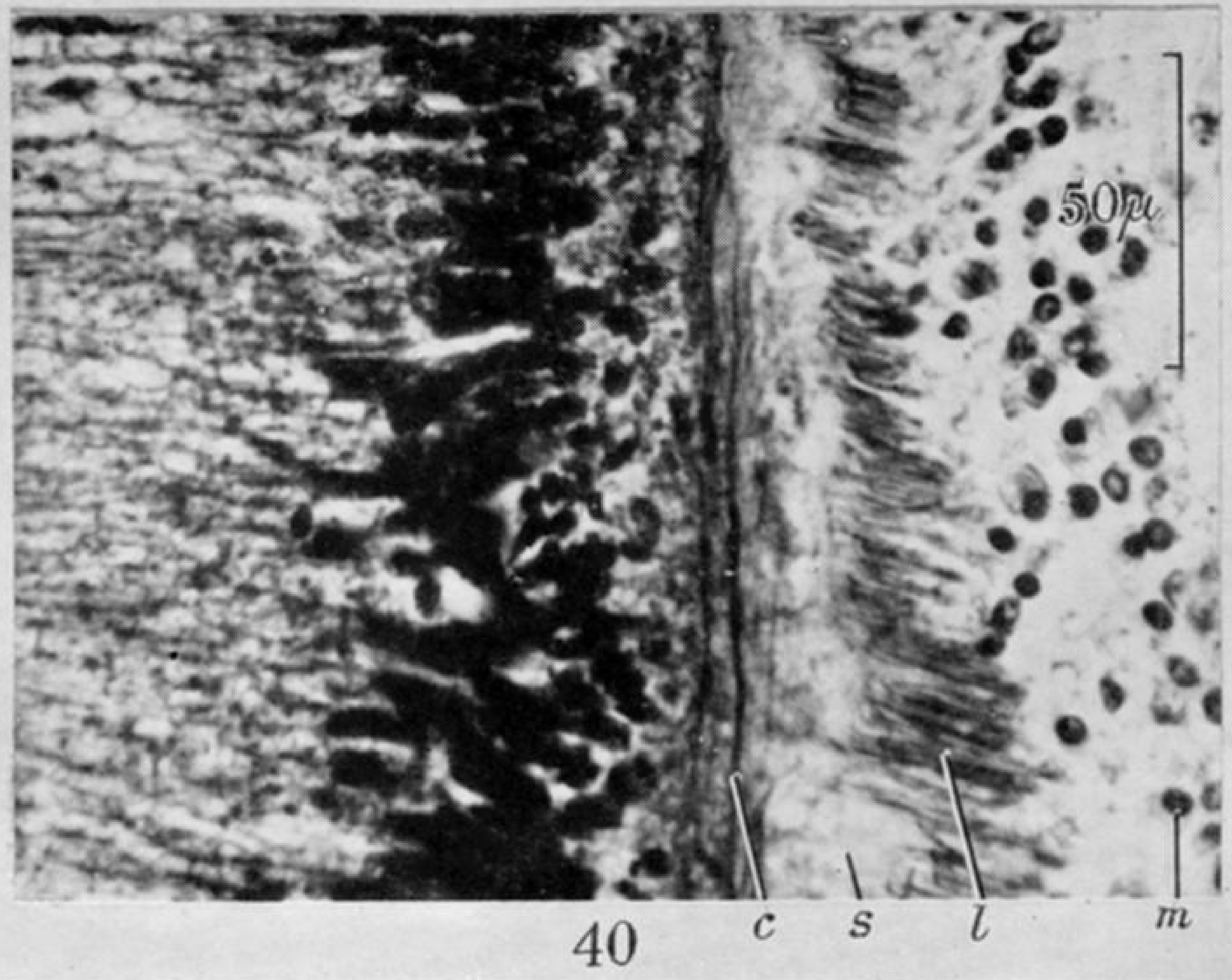

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PLATE 35

FIGURE 36. Part of a transverse section through the trunk showing the basal part of the epidermis in the mid-ventral line. Mallory's triple stain. The ventral nerve cord occupies most of the figure. The basal membrane, which is thick in this region, has become separated from the cord by shrinkage. About ten giant axons are seen near the mid-line, the majority adjoining the thin layer of irregular or transverse nerve fibres which occurs next to the basal membrane. *ax*, axon which follows an obviously convoluted course; *b*, basal membrane; *m*, longitudinal muscle fibres; *n*, nuclei of epidermis, the majority probably belonging to primitive ganglion cells.

FIGURE 37. Part of a nearly horizontal section through the dorsal nerve cord at the posterior edge of the collar, showing a giant nerve cell embedded in the nerve-fibre layer (an abnormal situation). Left margin of figure is anterior. Bodian. *ax*, axon of giant nerve cell, with reticular protoplasm and more densely stained core; *c*, giant nerve cell with vesicular nucleus and distinct nucleolus; *f*, giant fibre, stained fairly darkly, associated with giant nerve cell; *g*, another giant fibre, which can be followed to the bottom right-hand corner of the photograph, as it runs posteriorly and towards the left side, and which is also stained darkly and associated with the giant nerve cell but apparently separated from it by a thin septum; *m*, longitudinal muscle fibres; *n*, nerve fibres of the dorsal nerve cord of the trunk; *r*, bundles of nerve fibres turning laterally down the right side as part of the prebranchial nerve ring.

FIGURE 38. Base of the gut epithelium from a transverse section of the third part of the oesophagus, which passes along the ridge of one of the septal folds projecting into the gut lumen. Bodian. *f*, layer of darkly stained fibres, which are probably nerve fibres, adjoining the basal membrane of the endothelium; *l*, longitudinal muscle fibres adjoining the basal membrane of the mesothelium, which are weakly stained by the Bodian method and shown more clearly in figure 40; *m*, nuclei of cells in the splanchnic mesothelium, the bases of which contain the longitudinal muscle fibres of the gut wall; *s*, space between the basal membranes of the endo- and mesothelia, which constitutes a blood sinus.

FIGURE 39. Part of a horizontal section through the hepatic region of the gut, showing the blood sinus which runs along the ridge of each septal fold, cut transversely: the left side of the figure is nearest to the gut lumen. Bodian. Darkly stained fibres, which may well be supporting trabeculae, join the two basal membranes bounding the sinus.

FIGURE 40. As figure 38, but this section passes more obliquely through the ridge of a septum and is stained with Heidenhain's haematoxylin. The longitudinal muscle fibres of the gut mesothelium are therefore shown more clearly. *c*, circular fibres lying in base of gut epithelium, presumably endodermal muscle fibres. Other letters as in figure 38.