Some unusual cell inclusions in the mid-gut of a moth bug, *Gyarina nigritarsis* Karsch (Homoptera : Flatidae), and their possible significance in nutrition

By A. J. P. GOODCHILD

School of Biological Sciences Bath, University of Technology

SYNOPSIS

An account is given of the histology of the anterior diverticulum, intestine and hind gut of *Gyarina nigritarsis*. The nature of rod-like inclusions in the cells of the diverticulum is discussed.

INTRODUCTION

IT is now well established that the typical alimentary canal of Fulgoroidea consists of a tubular mid-gut coiled within a membranous sheath, from the anterior end of which a diverticulum, often branched or irregularly shaped, emerges and extends forward into the thorax. There has been some diversity of opinion on the function of this diverticulum (reviewed by Goodchild, 1966), but whatever duties it has come to perform in different species, it is not unlikely that its evolutionary origin is connected with that of the sheath enclosing the intestine in order to provide a region capable of inflation in the process of ecdysis.

Fulgoroidea are also well provided with microbial symbionts contained within various special organs in the body cavity, or in a compact mass in the ventral wall of the rectum, or as yeast-like cells freely invading the extensive fat body, or in combinations of these situations (Ermisch, 1960).

On an earlier occasion, examination of a few specimens of the flatid bug *Gyarina nigritarsis* Karsch (Goodchild, 1963) revealed that the cells lining the mid-gut diverticulum were distended with an apparently homogeneous eosinophil material, similar to that observed by Kershaw (1913) in another Flatid, *Siphanta acuta* Walker. It was also observed that the mid-gut of adults contained much basophil matter, which was interpreted as being derived from the breakdown of a symbiont-containing rectal organ (the gut of sap-sucking Hemiptera being usually empty of solid material).

It was possible to collect more specimens of this species during a visit to Uganda in 1966, and a more rigorous examination of the alimentary canal has made it necessary to revise these interpretations.

MATERIAL AND METHODS

Adults and nymphs of various ages were collected from twining plants in a small grove of *Eucalyptus* and other trees on the campus of Makerere University College, Kampala. Nymphs were fixed in alcoholic Bouin's fluid after small ruptures had been made in the cuticle to assist penetration. Adults were pickled in Pampel's fluid. It was not at first intended to use the adults for histological study, but when so used, the fixation appeared to be entirely satisfactory. Nymphs were embedded whole in ordinary paraffin wax and, because of their soft cuticle, excellent sections were obtained without other treatment. Adults were embedded in Steedman's ester wax after excision of the hardest regions of the cuticle, the rostrum, leg and wing bases, and genitalia. Sections 8 μ thick were stained by triple staining techniques, the most satisfactory being a normal haematoxylin/eosin followed by a brief dip in Edicol pea green (1 per cent. in 90 per cent. alcohol), although Masson's iron haematoxylin/ponceau fuchsin/light green was also used.

Proc. R. ent. Soc. Lond. (A). **44** (1–3). Pp. 11–16, 1 fig., 2 plates. 1969.

Anterior Diverticulum of Mid-Gut

Sagittal sections of half-grown nymphs showed that the anterior diverticulum originates from the mid-gut just behind the oesophageal valve, in the second abdominal segment (fig. 1). From this point it travels forward, dorsal to the oesophagus, as a tube similar in diameter and cell type to that of the mid-gut proper, as far as the first abdominal segment, where it rapidly expands into a wide sac, filling the median region of the thorax and head and extending back above the connecting tube to the level of its origin. The cells lining this sac are mostly extremely large (up to 80 μ by 180 μ) compared with normal mid-gut cells (average 15 μ by 40 μ), their tips are swollen to near spherical proportions, and their dark-staining, coarsely granular nuclei also enlarged (33 μ by 22 μ, compared with the mid-gut nuclei of 12 μ by 8 μ). The

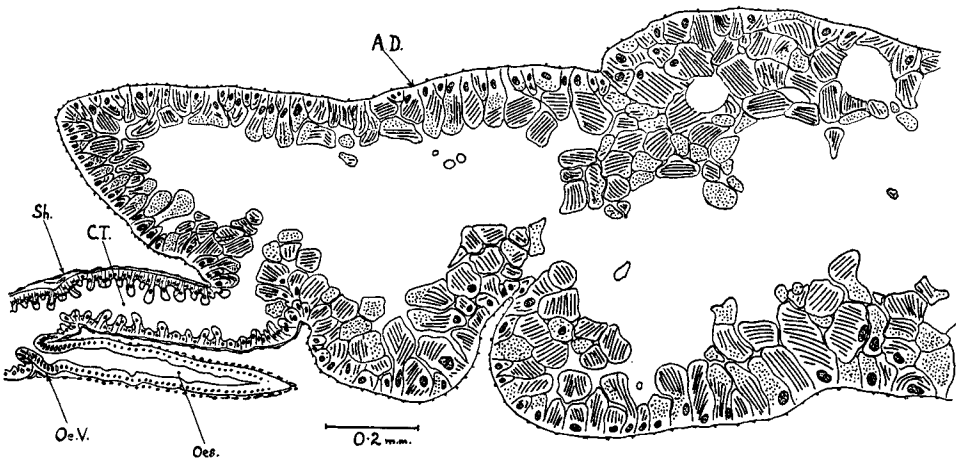


Fig. 1.—Sagittal section through posterior half of anterior mid-gut diverticulum of late nymph of *G. nigritarsis* (detail simplified for clarity). *A.D.*, anterior diverticulum, showing rod-like inclusions cut in various planes; *C.T.*, tube connecting diverticulum to mid-gut proper; *Oes*, oesophagus; *Oe.V*, oesophageal valve; *Sh*, cellular sheath of mid-gut.

cell border is a thin membrane, and the cytoplasm is completely occupied by a bundle of parallel rods, each about 5 μ in thickness and as long as the cell and sometimes longer, when they are accommodated by one or two flexures of the whole bundle, with the outside of the curve towards the lumen of the sac. The rods may be orientated parallel to the wall of the sac, or perpendicularly to it, or at slight angles to either of these directions, the orientation being generally the same in groups of several contiguous cells. In some cells there are two bundles of rods, with different orientations. Counts of transverse sections of bundles indicate that each may consist of as many as 50 rods. At the bases of some cells fragments of rods may be seen, and in the main bundles some rods may show clean transverse fractures, dividing the rod into two or more segments, which, however, retain their linear arrangement. The staining reaction of the rod substance is mildly eosinophil, that is to say, similar to that of the muscle fibres, which with eosin/pea green are pale orange, and unlike that of the storage granules of the fat body, which are bright pink with this stain. The rod structure is completely homogeneous, and neither differentiated internal structures nor distinct limiting membrane can be detected. The scanty cytoplasm around the rods is finely granular and distinctly basophil. Where the rods are crowded against the cell border they are more markedly eosinophil, or with Masson's stain a bright red (whereas deeper in the cell they are green with this stain). The rods show up extremely well under phase contrast illumination. Rods dissected from a whole preserved adult, and examined in water, seem to be of a transparent crystalline nature. Included

among the cells were abundant droplets of lipoid material, but the intact rods had no trace of affinity for fat stains.

Scattered among the bases of these large cells are other cells of all sizes down to about 20 μ by 25 μ (the nuclei of which are of normal mid-gut size). All cells contain the rod-like inclusions, running the full height of the cell distal to the nuclei or, in the smallest, on either side of the nucleus. Although fewer in number and shorter than the rods seen in the largest cells, these undeveloped rods are not much less thick (2 to 3 μ). In the region surrounding the opening of the connecting tube there is a gradual transition from normal mid-gut cells to the rod-containing cells. The anterior dorsal part of the sac wall lies close beneath the hypodermis of the head and first two thoracic nota, and its cells are obviously stretched by the expansion of the sac at ecdysis, being very wide and flattened (about 90 μ by 10–15 μ). Their cytoplasm is well stocked with rod fragments of various sizes, in some instances partly projecting through a torn cell border.

In the posterior part of the sac a delicate meshwork of muscle strands can be detected among the cell bases, and there is also a thin peritoneal membrane, with scattered small nuclei, around the whole sac.

In the smallest nymphs studied (possibly first instar), all the cells of the anterior mid-gut diverticulum were small, but most contained typical rod promordia. In males and immature females the posterior part of the sac is immensely inflated, the cells stretched into a pavement epithelium, and the organ extending far back into the abdomen, between the gonad and the mid-gut. It penetrates amongst, and is in immediate contact with, the ovarioles (Plate I, fig. A), but is wholly ventral to the testes, from which it is separated by a layer of fat body. The middle part of the sac, in the thorax, is restricted by the development of the longitudinal muscles, but in the head it remains expanded and there the cells are also flattened. The narrow neck of the sac in the thorax is filled with swollen cells, some of which are no longer attached to the epithelium and may also be enucleate. Among them and posteriorly towards the clearly defined spherical margin of the air bubble is a mass of finely granular basophil material enclosing clusters of rods (Plate II, figs. A, B). At the edge of the air space the rods are densely crowded, tangentially orientated and with indistinct outlines. Intermediate stages in the degenerative process can be found. From the typical cell with a bundle of long rods, there appears to be a change towards a break-up of the rods into lengths of 20 to 40 μ, with rounded ends, and an increase in the amount of normal cytoplasm present so that the rods are less closely packed. The whole mass may be liberated by the dissolution of the cell membrane. Also present to a greater or less degree are cell tips (no nuclei being visible), in which the rods remain long and closely packed but their outlines become less distinct; on the dissolution of the membrane a structureless eosinophil mass, containing poorly defined thread-like fibres, is released. The distinction between the two modes is not sharp, however. In some specimens bundles of eosinophil rods may be found among the debris, still within an enclosing membrane, and in one specimen such a bundle was seen in the anterior part of the mid-gut proper (Plate I, B). The nuclei in these degenerating cells may be swollen and misshapen, and often surrounded by a zone of normal basophil cytoplasm. It may be that the increase of cytoplasm only occurs when the nucleus is present, cells that lose their nuclei undergoing the second mode of break-down. Over the surface of the cells in the posterior part of the sac, and within the margin of the air bubble where it is in contact with the rod-laden debris, is a narrow zone of strongly eosinophil granules and strands, in many instances fusing into an eosinophil membrane on its inward side.

In the mature female a similar situation obtains in the anterior and thoracic region of the sac, but with the growth of the eggs the posterior region contracts and its epithelium returns to a cuboid or columnar appearance (although the cells are separate, except near their bases). Few of these cells contain rods, but many have frayed tips

indicating the loss of their distal part. The nuclei are of various sizes, densely granular and basophil. The cytoplasm is also basophil. The muscular layer is thick and conspicuous, and many of the nuclei appear as if trapped in it and distorted to a dumb-bell shape.

The Intestine and Hind Gut

The mid-gut proper is a sinuous tube of even diameter, which leads backwards from the oesophageal valve to near the posterior end of the abdomen on the ventral side, then returns forward and loops over its anterior end before turning back again to join the hind gut in the middle of the abdomen. As in other Fulgoroidea, it is enclosed in a sheath, which in this species takes the form of a thin pavement epithelium, better developed in the nymph than in the adult. In the latter it cannot be followed continuously around the gut coils, and is reduced in thickness to an eosinophil membrane with occasional nuclei. Close around the gut wall there is also a thin basement membrane with scattered small nuclei. The cells lining the mid-gut are essentially of a uniform nature, but appear to undergo cyclical changes, possibly in waves of activity passing along the length of the gut. This conclusion is based on the fact that such differences as can be seen are not constantly associated with particular sections of the gut. The cells are columnar, with bulbous tips projecting into the lumen, sometimes singly but more usually in groups of about six cells forming a single lobe. Around the circumference of the gut there may be six to ten such lobes, separated by much shallower cells. The cell tips contain finely granular basophil cytoplasm, usually with many tiny vacuoles, and have a well defined brush-like border. Particularly in the middle region of the mid-gut, the cell tip may discharge a large, thin-walled globule of cytoplasmic material into the lumen. The tallest cells are about 50 μ by 10 μ, and the free lumen between the tips is about equal to the cell height in nymphs and young adults. The lumen is empty except for a few eosinophil granules in immature forms, but in maturing adults it becomes filled and its width increases three or four times, the cells simultaneously being stretched into a wider and lower shape. The anterior half of the mid-gut of adults contains scattered eosinophil granules, which on close examination appear to be arranged in regular circular or part-circular patterns of about 20 μ diameter (Plate I, fig. A). The impression is given that these are relics of spherical shells of eosinophil material, irregular in thickness, which surrounded a globule of a substance leached out by the processes of histological preparation. Similar material can be traced forwards along the connecting tube and into the anterior diverticulum. In the posterior part of the mid-gut the shell-like structures are much less frequent, and the few remaining are embedded in a dense mass of finely granular basophil material with which the gut is noticeably distended. In the middle region of the gut a transition zone is present, with coarse granules of varying degrees of eosinophilia among the basophil contents. In some specimens the posterior part of the gut was empty, while the anterior part contained abundant eosinophil material, as if the digestive process was in an early stage. In some very mature females the gut was mostly empty throughout, and the anterior diverticulum shrunken and with little rod debris. This suggests that the gut contents are voided as the ovarian system expands.

The hind gut is a single sac-like rectum, the walls being composed entirely of rather flattened cells with nuclei somewhat larger than those in the mid-gut cells. It is thus more in the nature of rectal gland epithelium than the usual rectal syncytium. In all adults, and some larger nymphs, the rectum was strongly contracted and the lining thrown into complex folds, with little free lumen visible, and surrounded by a thick muscle layer. Some granular, neutral staining, debris was seen in the lumen or rectal valve region in a few specimens. No symbiont-containing rectal organ could be detected.

Discussion

When the results of this investigation are compared with the early account by Kershaw (1913), many points of similarity can be found. Although Kershaw did not observe rod-like inclusions in the anterior diverticulum ("reservoir" in his terminology) of *Siphanta acuta*, his finding of the epithelium as constantly in a state of degeneration and renewal suggests that, as in the present writer's earlier studies of *Gyarina*, the fixation methods were unsuitable. Kershaw gives no details of his procedure in this respect, but in the writer's experience dissected guts of small Hemiptera easily suffer from over-fixation or post-fixation damage, and vastly better results have been obtained from the fixation and embedding of whole animals. Furthermore, it does not seem that Kershaw used a triple staining technique, without which the structures in *Gyarina* are far less evident.

Whether or not Kershaw's material could have been rod-like, the remainder of his description of cell detachment, breakdown into a viscid fluid and passage along the mid-gut, is in full agreement with what appears to happen in *Gyarina*. On the other hand the situation in *Gyarina* does not lend itself to interpretation in the way that Kershaw put forward for *Siphanta*. He regarded the secretion of the reservoir as primarily a digestive secretion, with the possibility that the organ might aid in getting rid of the waxy matter secreted by the insects. The rod-like inclusions of *Gyarina* are totally unlike any known digestive secretion of insects, the normal appearance of secreting epithelium being that of the mid-gut proper in this species. The crystalline style of molluscs has some similarity, perhaps, but there seems to be no reason why such a structure should arise in an insect. As regards the waxy nature of the reservoir contents, the presence of lipoid droplets in the diverticulum of *Gyarina*, and the probability that the gut contents are partly of this nature, corroborates the observations on *Siphanta*, although the ingestion of wax secreted by the cuticle of the insect itself must be regarded as unlikely.

Rod-like intra-cellular structures, especially in such insects as Homoptera, must always be suspected of being a form of symbiotic micro-organism, but in *Gyarina* the large size reached, the lack of nuclear structures, and the brittle texture, make it difficult to accept such an hypothesis. Furthermore, the anatomical situation would be unique among Fulgoroid symbionts, and there is no evidence of a mechanism of transmission, whether transovarial or by spore formation.

Supposing, therefore, that the rod-like inclusions are formed by the cells themselves, they could be a form of storage, either of an excretory nature, or for subsequent utilisation. The breakdown and passage through the gut could be compatible with an excretory product, if there was need for space in the body for the maturing reproductive system, but the histological evidence suggests that a digestive process is taking place. As Kershaw pointed out, certain plants, and particularly the eucalyptids on which *Siphanta acuta* fed, contain an abundance of lipid or resinous substances, which may be disposed of by intra-cellular deposition in the insect. In many Cercopidae (Goodchild, 1966), the hinder part of the mid-gut is clogged with hypertrophied cells containing excretory matter. On the other hand, nymphs of the Flatidae secrete such an abundance of wax on their external surface that it is difficult to imagine there being a surplus for internal deposition. If it is stored for use at the time of maturation of the reproductive system, why is it not stored in the fat body, as in most insects? In *G. nigritarsis* the fat body is very densely infested with yeast-like symbionts, which seem to be undergoing continual destruction and absorption, judged by the variation in staining reaction and refractivity under phase-contrast illumination. It may be that this specialisation of the fat-body prevents its acting as a storage depot in the usual way. Incidentally, no other form of symbiont or symbiont-containing organ was found in this species. The rods may be a lipoid-protein complex, which separates in the breakdown phase into a lipoid droplet surrounded by a protein skin and is digested in the mid-gut in this form. The dense basophil granular contents of

the posterior mid-gut would then be the final stages in this process and would not, as previously suggested, be caused by the breakdown of a rectal symbiont organ.

It has been tentatively suggested (Goodchild, 1966) that the secretory activity of the cells of the anterior diverticulum might be a reaction to resist osmotic flow into the haemocoele in this region, which is not enclosed in the intestinal sheath. In *G. nigritarsis* the activity seems too exaggerated to be directed to this end alone, and the presence, in nymphs at any rate, of a definite constriction, with conspicuous muscle layers in the connecting tube, suggests that flow of the ingested plant sap into the diverticulum may be restricted, if not entirely prevented.

It is regrettable that this account is based on histological evidence alone, and there seems to be a case for more detailed examination of the nutrition of Flatidae by those in a position to study the living insects.

Summary

(1) In the anterior diverticulum of the mid-gut of *Gyarina nigritarsis* Karsch (Fulgoroidea, Flatidae), the cells are swollen to accommodate large rod-like inclusions.

(2) In maturing adults these rods break down and pass into the posterior mid-gut, where the contents appear as shells surrounding presumed lipoid droplets, and grade into finely granular basophil material in the hindmost part of the mid-gut.

(3) It is suggested that these inclusions are formed by the cells themselves for subsequent utilisation.

References

Ermisch, A., 1960, Vergleichend-anatomische Untersuchungen über die Endosymbiose der Fulgoroiden mit besonderer Berücksichtigung der Araeopiden. *Z. Morph. Ökol. Tiere* **49** : 47–138.

Goodchild, A. J. P., 1963, Some new observations on the intestinal structures concerned with water disposal in sap-sucking Hemiptera. *Trans. R. ent. Soc. Lond.* **115** : 217–37.

—— 1966, Evolution of the alimentary canal in the Hemiptera. *Biol. Rev.* **41** : 97–140.

Kershaw, J. C., 1913, The alimentary canal of *Flata* and other Homoptera. *Psyche, Camb.* **20** : 175–88.

(Manuscript received 28th February, 1968)

Plate I

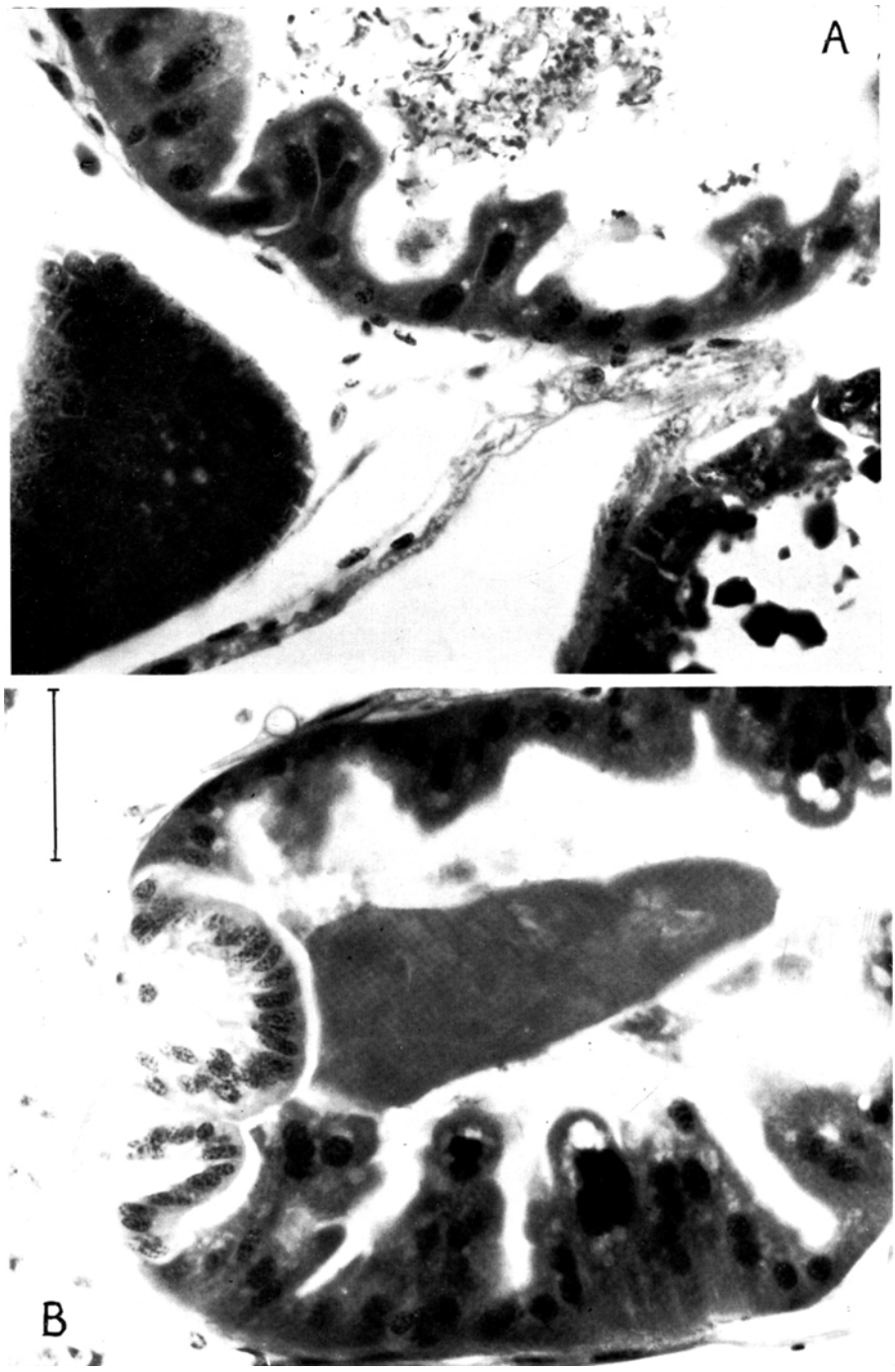
Fig. A.—Upper part: part of transverse section through mid-gut, showing lining cells and "eosinophil shell" contents. Lower part: fold of stretched anterior diverticulum between two ovarioles.

Fig. B.—Clump of rod debris from anterior diverticulum, in anterior end of mid-gut adjacent to oesophageal valve (left hand edge). Scale, 50 μ.

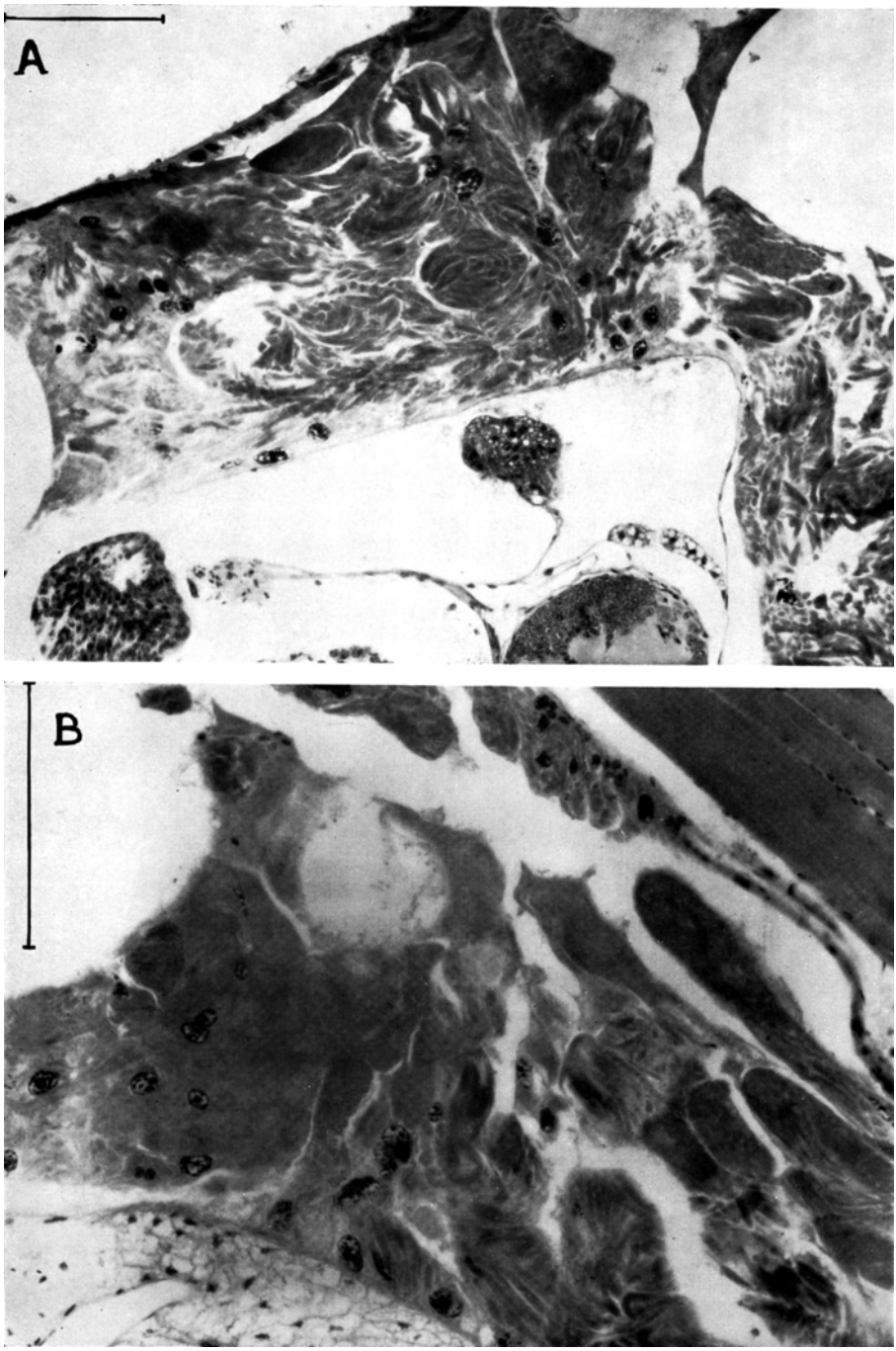
Plate II

Fig. A.—Sagittal section through head and thoracic region of the anterior diverticulum of a mature female, showing rod-containing cells in various stages of breakdown.

Fig. B.—Sagittal section of thoracic region of anterior diverticulum of a mature male, showing cell breakdown. Scale on each fig., 0·2 mm., anterior end to right.



Gyarina nigritarsis



Gyarina nigritarsis