FURTHER STUDIES ON THE RECTAL COMPLEX OF THE MEALWORM *TENEBRIO MOLITOR*, L. (COLEOPTERA, TENEBRIONIDAE)

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CONTENTS

An electron microscopical study has been made of the rectal complex. The perinephric membrane is a complicated structure which, in the posterior region, comprises an inner and an outer sheath separated by a space containing tracheolar end cells. The outer sheath is formed of a single layer of cells covered by an external basement membrane. The inner sheath is a multi-laminate structure made up of many thin, cellular layers which in places are reduced to closely apposed plasma membranes. Anteriorly the cellular layers are reduced in number, but each layer is of greater thickness; they finally terminate where the perinephric membrane is applied to the intestine. Posteriorly the inner sheath makes contact with the rectal epithelium.

An earlier description identified three spaces within the rectal complex: the perirectal, subepithelial and peritubular spaces. The first two are true intercellular spaces, bounded by basement membranes, but the so-called peritubular space is occupied by necrotic cells.

The inner sheath of the perinephric membrane is interrupted by the leptophragmata. Each leptophragma is bounded by a prominent electron-dense ring into which the laminae of the inner sheath are inserted. The outer sheath forms a blister over the leptophragma and is completely non-cellular in this region. At the base of the blister a basement membrane covers the leptophragma itself, and the body of the leptophragma cell projects into the lumen of the tubule, with a thin layer of cytoplasm lying beneath the basement membrane. Both this layer and the cell body itself bear

microvilli. The cell has a normal complement of mitochondria, but these do not invade the microvilli.

In this last respect the ordinary tubule cells differ from the leptophragma cells in that most of their microvilli contain mitochondria, with connexions between the outer mitochondrial membrane and the plasma membrane. The tubule cells have a poorly developed endoplasmic reticulum but are filled with numerous small granules; basal infoldings are restricted to those parts of the cell which face the perirectal space.

The permeability of the perinephric membrane has been re-investigated and it is shown that the membrane is more permeable to water and solutes at the anterior end, as might be expected if the inner sheath were the main barrier.

Using preparations isolated in small volumes of haemolymph or other external media it has been shown that the rectal complex takes up potassium against a gradient of concentration. The lumen of the perirectal tubule is some 50 mV positive with respect to the external medium, so the uptake of potassium must be active. The leptophragma is freely permeable to chloride and this ion appears to enter the tubule passively.

A model of the mechanism of the rectal complex is proposed, whose main feature is that the high osmolarity of the fluids within the rectal complex is brought about by the inward secretion of potassium chloride, unaccompanied by water, at the leptophragmata. This should result in a fall in the osmolarity of the external medium. A substantial fall has been observed on occasion, but in most experiments a fall is barely detectable. It is believed that the impermeability of the leptophragmata to water is rapidly lost in a deteriorating preparation.

Introduction

The idea that the rectal complex of *Tenebrio* was concerned in the thorough removal of water from the faeces originated with Wigglesworth (1934), but was at that time unsupported by any physiological evidence. Some preliminary experimental data consistent with this hypothesis were presented by Saini (1962, 1964), and some further evidence pointing to the same conclusion was provided by one of us in a previous paper (Ramsay 1964). This earlier work, which will be summarized below (p. 358), left a number of important points unresolved, and also made apparent the need for more detailed knowledge of the structure of the rectal complex before its functioning could be understood. In this paper we present, first, the results of an electronmicroscopical investigation of the rectal complex, and, secondly, some new physiological data. The results, while not by any means fully explaining the functioning of this system, go some way towards elucidating its mode of operation, and permit some tentative correlations of structure with function.

2. Fine structure

(*a*) *Material and methods*

Mealworms—*Tenebrio molitor* L. (Coleoptera, Tenebrionidae)—were obtained from the stock cultures of the laboratory, maintained on dry bran. The ordinary culture conditions are here considered as a dry régime, but on occasions the experimental animals were subjected to even drier conditions by being placed in a desiccator over calcium chloride. A moist régime was provided by adding slices of fresh carrot to the bran. Hereinafter mealworms will be described as 'dry' mealworms or 'moist' mealworms in accordance with the régime on which they were maintained.

Good fixation of the rectal complex proved difficult to achieve and we were unable to devise a method which gave completely consistent or satisfactory results. The recurring

faults were swelling and disruption of membranes and mitochondria, shrinkage of the leptophragma cells, and separation of cell layers which we believe most probably to be in contact in life. From the physiological data, which indicate the existence of high local concentrations of solutes in certain parts of the rectal complex, it seems likely that it is osmotic swelling or shrinkage which is chiefly responsible for imperfect fixation. If this is so it is not to be expected that any one fixative, of a given tonicity, will preserve all kinds of tissues equally well, since the osmotic conditions vary widely from one part of the rectal complex to another. This certainly accords with our experience. A number of different fixatives were used, and various methods were tried for adjusting the tonicity of the fixative solution, but no marked success was achieved. The most satisfactory solutions proved to be:

(i) A mixture of 4% paraformaldehyde and 1% glutaraldehyde in 0·2M phosphate buffer at pH 7·0 for 4 h. This was followed by washing with 0·1M phosphate buffer (pH 7·0) containing 0·25M sucrose, and then post-fixation in 1% osmium tetroxide in veronal-acetate buffer (pH 7·4).

(ii) 2·5% glutaraldehyde in 0·05M cacodylate buffer at pH 7·4, containing 0·17M sucrose for 4 h. Material was washed in 0·05M cacodylate buffer containing 0·3M sucrose and post-fixed in osmium tetroxide.

Osmium tetroxide (1% in veronal-acetate buffer, pH 7·4) was also used as a primary fixative for some material.

The procedure followed in fixation was to pin out the mealworm on a wax slab, open the body rapidly by a longitudinal cut in the body wall and immediately cover the rectal complex with fixative. No anaesthetic was used. The rectal complex was then dissected out under fixative, and cut into small pieces. All fixatives were used at 4 °C. After washing, material was dehydrated in an ethanol series and embedded in Araldite via propylene oxide. For electron microscopy thin sections were double-stained with uranyl acetate and lead citrate. For light microscopy 1 μm sections were stained in 1% methylene blue in 1% borax.

The rectal complex of 'moist' mealworms appeared best fixed by the mixture of paraformaldehyde and glutaraldehyde, which gave less swelling of mitochondria and microvilli and less shrinkage of leptophragma cells. This fixative was less successful with 'dry' mealworms, which always showed some swelling of microvilli and which appeared to be as well fixed by glutaraldehyde alone as by the mixture with paraformaldehyde. No method of fixation gave entirely consistent results, however, and there was a good deal of variation both from one mealworm to another and between different parts of the same specimen.

Cytochemical observations were made either on the same material as that used for electron microscopy, after first bleaching the sections in hydrogen peroxide, or on paraffin sections of Carnoy-fixed material. The staining methods used were the standard ones described in Pearse (1960).

(*b*) *Results*

(i) *The Malpighian tubules*

The general structure of the tubules (of which there are six) has been described by Ramsay (1964) and their anatomical relations to the rectum are illustrated in figures 1 and 3 and in figure 5, plate 18. In each tubule there is a clear distinction between the

distal, perirectal part which lies within the rectal complex (here taken to include the common trunk) and the very much longer proximal part which lies free in the body cavity and opens into the gut. Towards the anterior end of the perirectal part there is a transition from a posterior thick-walled region with leptophragmata to an anterior thin-walled region without leptophragmata. For the purposes of this paper the thick-walled region with leptophragmata is of chief interest, and it will therefore be described first and in some detail. The other parts will be more briefly considered later.

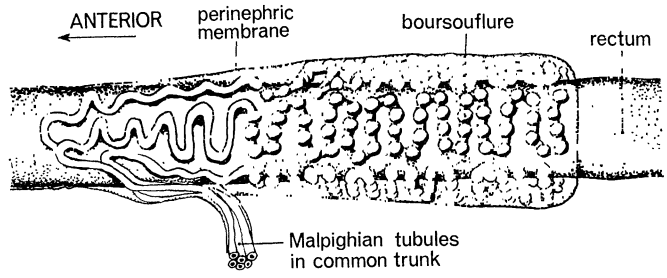

FIGURE 1. Diagram showing the rectal complex as a whole. Three of the Malpighian tubules are shown, enclosed in the perinephric membrane. For the sake of clarity only a few of the convolutions of the tubules have been drawn. The actual number of boursouflures on each tubule is about 400.

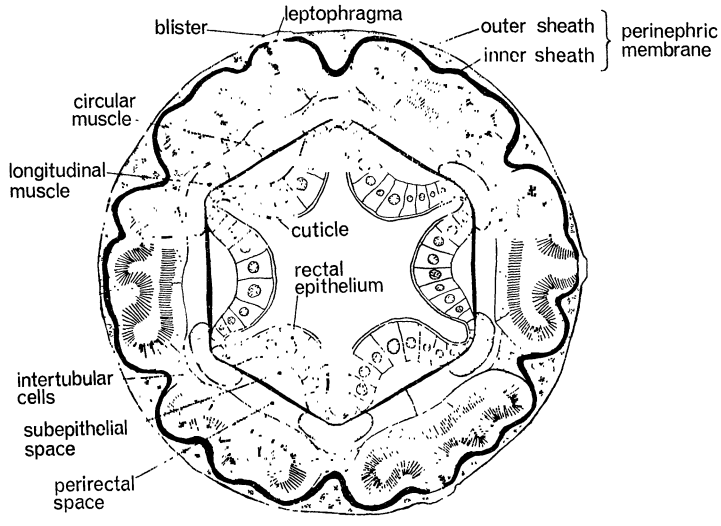

FIGURE 2. Diagram of the rectal complex as seen in a transverse section through the posterior region.

In the posterior region the tubules are highly convoluted, folded back and forth on themselves, so that a single section shows them cut in various planes. The outer walls of the tubules are pushed out at closely spaced intervals to form large diverticula, which are the button-like 'boursouflures' of Lison (1937), previously described (Ramsay 1964). The lumen of each boursouflure connects with the main lumen of the tubule and in the majority of cases is tripartite, being formed of a central compartment and two lateral ones (figure 3). All three compartments are narrow, slit-shaped spaces. The lateral ones join the central one near its base, where it arises from the main tubule lumen. Each boursouflure bears a leptophragma on its outer, apical surface, located above the central compartment. The latter is in this respect differentiated from the two lateral compartments. A few

boursouflures have only two compartments. Except in the region of the leptophragmata, the lumen of the tubule and of the boursouflures is lined by a well-developed brush border. The nuclei of the tubule cells lie in the cytoplasm of the main part of the tubule; they are not found in the boursouflures. A cross-section of a tubule and boursouflure usually includes parts of two to four cells.

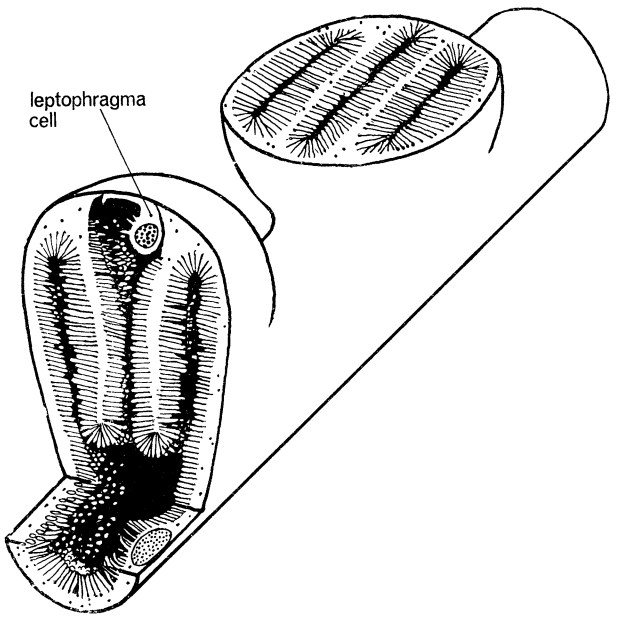

FIGURE 3. Diagram of a short section of a Malpighian tubule, with two boursouflures. These are cut open to show the diverticula of the tubule lumen. In the boursouflure at the left the leptophragma at the apex of the central diverticulum can be seen. The microvilli of the brush borders are not drawn to scale.

The brush border of the tubules is up to 10 μm thick and formed of closely packed microvilli. For the most part these are cylindrical and 0·15 to 0·3 μm in diameter (figure 6, plate 18), and each such microvillus contains a single long mitochondrion which fills most of its interior. A few microvilli, however, are smaller (about 0·05 μm in diameter) and lack mitochondria (figure 8, plate 20). Such small microvilli sometimes occur interspersed between the larger ones, or they may occur exclusively in some areas (figure 11, plate 20). Their distribution has not been traced in detail and it is not known whether they are a constant feature of all tubules or all individuals. When a mitochondrion is present (as it usually is) the plasma membrane of the microvillus is commonly closely applied to it, the two being separated by a uniform gap of only 100 to 250 Å. Fine strands run across this gap, connecting the two membranes (figure 9, plate 20). These connecting strands are often regularly spaced at intervals of 100 to 200 Å. They may be present throughout the length of the microvillus or only in small patches. They are not present (or cannot be seen) in regions where the membrane of the microvillus is widely separated from the mitochondrial membrane, and in some instances they appear to be absent altogether. The mitochondria are typical in structure and usually contain rather few, irregularly arranged cristae. A good deal of amorphous material may be present in the matrix, and there are sometimes a few small electron-dense granules.

While in many tubules the microvilli are uniform in diameter, a good many cases have been found in which their tips are swollen into spherical or ovoid knobs, up to 1 μm in diameter (figure 10, plate 20). Such swelling may include the mitochondrion which thereby becomes vacuolated at one end. The swelling at first sight looks like an artifact of fixation, and this is indeed probably the most likely explanation of it. However, it may nevertheless be of interest, since it has been found far more commonly in tubules from 'dry' mealworms than in those from 'moist' mealworms. The swelling may therefore indicate an altered physiological state of the tubules or their surrounding tissues.

In the boursouflures the microvilli on opposite sides of the tubule often almost touch, the tubule lumen being largely occluded (figure 28, plate 28). In the main part of the tubule there is commonly a more extensive lumen. The size of the lumen as it appears in fixed material may possibly be related to the physiological state of the mealworm, though we have no definite information on this point. The lumen appears empty in electron micrographs.

The cytoplasm of the tubule cells, apart from the brush border just described, appears to be fairly uniform in composition, both in the main part of the tubules and in the boursouflures. It presents some unusual features, of which the chief is the almost complete absence of cytoplasmic membranes. In low-magnification micrographs the cytoplasm appears dense and relatively homogeneous (figure 7, plate 19). At higher magnification it can be seen to consist almost entirely of small granules, together with a substantial number of microtubules. Mitochondria are almost entirely absent, except in the brush border and the cytoplasm immediately below, and the endoplasmic reticulum is represented by nothing more than a few tiny vesicles, scattered through the cytoplasm, with ribosomes on their external surfaces (figure 15, plate 22). Golgi bodies are represented by a few small clusters of flat sacs and vesicles, which have been seen in one or two sections; they are not common or present in well developed form. The only other components (apart from the small granules, and microtubules, to be described below) are some apparently empty vesicles, often present in large numbers immediately below the brush border (figure 7, plate 19; figure 10, plate 20) and large inclusion bodies, up to 2 μm in diameter, which can probably be identified as cytolysomes, and which may be present both in the cytoplasm below the brush border and elsewhere. If present, these are usually membrane-bound bodies containing the mixture of membrane fragments, vesicles, dense material and granules typical of cytolysomes (figure 16, plate 22).

The small granules which make up the bulk of the tubule cell cytoplasm include a few which can be identified with reasonable certainty as ribosomes, since they are similar in size and density to the ribosomes attached to the small vesicles of endoplasmic reticulum. The great majority of the granules, however, are smaller than these (average diameter 120 Å as against 150 Å), and are less electron-dense. They occur free in the cytoplasm, unattached either to membranes or to each other (figure 15, plate 22). Since granules of this type are so abundant attempts have been made to characterize them by applying cytochemical tests at the light-microscope level, but without success. The tubule cytoplasm stains weakly with pyronin and with protein stains such as brom-phenol blue, and it is *PAS*-negative, suggesting that the granules do not contain large amounts of ribonucleic acid, protein or polysaccharide. There is sometimes a marked difference in the over-all

electron density of the cytoplasm of adjacent cells of a single tubule (figure 7, plate 19). This probably reflects differences in the abundance of small granules. The granules are preserved following osmium fixation, though sometimes in markedly fewer numbers than after glutaraldehyde fixation (figure 17, plate 22).

The other main constituent of the tubule cytoplasm, the microtubules, are about 220 Å in diameter and of typical appearance in section. They are not localized in any particular region of the cytoplasm and usually appear to be randomly oriented (figure 15, plate 22) Over small distances, however, groups of them may run more or less parallel to each other (figure 17, plate 22). They are never arranged in tight bundles. They appear to be as well fixed by osmium as by glutaraldehyde (compare figures 15 and 17, plate 22).

The plasma membrane of the tubule cells is a typical unit membrane. Over approximately the inner half of the tubules—that is, the surface facing the rectum and the lateral borders of the tubules extending about half-way up the boursouflures—the plasma membrane is deeply infolded (figure 7, plate 19). There is a great deal of variation in the extent and form of these basal infoldings. In some cells they delimit large ramifying extracellular channels extending several microns into the cytoplasm (figure 14, plate 21). In others the infolded membranes may extend equally deeply but run closely parallel to each other, separated by a gap of as little as 100 Å (figure 12, plate 21). These are some indications that the form of the infoldings may be related to the physiological condition of the insect, since the wide, ramifying spaces have been found more commonly in material from 'moist' mealworms. This difference is not a clear-cut one, since some exceptions have been found, and there is also a certain amount of variation between the cells of a single tubule. After osmic fixation the basal infoldings are dilated and large numbers of small vesicles are present in the extracellular channels (figure 13, plate 21). The significance of this finding is unknown.

The external surface of the tubule is bounded by a non-cellular basement membrane layer, about 0·1 to 0·2 μm thick, amorphous in texture and of moderate electron density (figure 7, plate 19; figures 12 to 14, plate 21). This material does not enter the basal infoldings.

Towards the tubule lumen the membranes of adjacent cells run closely parallel to each other and are linked by septate desmosomes. More peripherally they are less closely applied to each other and desmosomes are absent.

The leptophragmata will be considered below, after the perinephric membrane has been described.

In the anterior region of the rectal complex, where leptophragmata are absent, the fine structure of the tubules differs in several respects from what has just been described. There are no boursouflures, and the tubules are cylindrical and less convoluted. The lumen is much wider, and the cells are much less thick than in the posterior region. The apical surface of the tubule cells bears a brush border, but it is less well developed than in the posterior region, the microvilli being less closely packed and at most only about 2 μm long (figure 18, plate 23). A mitochondrion is usually present in each microvillus, though not invariably so, and in one mealworm no mitochondria were found in any of the microvilli in this region. Where mitochondria are present fine strands commonly connect them to the plasma membrane, as in the posterior region (figure 20, plate 23). Basal infoldings

are present, but again are somewhat less well developed than in the posterior region. The gaps between the infolded membranes are narrow and no widely expanded spaces have been seen. The cytoplasm of the cells in the anterior region is less unusual than that in the posterior region; mitochondria are distributed throughout the cells and Golgi bodies are often present. There is, however, no well-developed endoplasmic reticulum. Free ribosomes are common, and the small granules are abundant, though less so than posteriorly. In addition to these there are extensive areas containing glycogen granules, which are not found in the posterior region. Cytolysomes are present and may occur in any part of the cell, instead of being largely restricted to the cytoplasm below the brush border. There are abundant microtubules. Adjacent cells in this region are linked at the luminal end by septate desmosomes (figure 19, plate 23). There is an external basement membrane, as in the posterior region.

A brief examination of the Malpighian tubules in the common trunk showed that their fine structure is essentially the same as in the anterior region just described.

(ii) *The perinephric membrane*

The rectal complex is bounded externally by the perinephric membrane, which forms a continuous sheath around the rectum and Malpighian tubules (figures 1 and 2; figure 5, plate 18). It is evident from light microscopy (see Ramsay 1964, and figure 5, plate 18 of this paper) that in the posterior region it is a complex structure, divisible into a thin outer layer which runs smoothly over the surface of the rectal complex, and a thicker, inner layer which is applied more closely to the surface of the tubules and is consequently a good deal convoluted, with deep infoldings extending down between the tubules and into the gaps between adjacent boursouflures (figure 2). Both of these are largely cellular layers. We shall refer to them as the *outer* and *inner sheaths* of the perinephric membrane respectively. They are separated by a space of variable dimensions, which contains cells and, more important, the abundant tracheolar supply of the rectal complex (figure 21, plate 24). There is a good deal of variation in the thickness and appearance of the various parts of the perinephric membrane in different parts of the rectal complex. In particular there are marked differences between the structure in the posterior region, where leptophragmata are present, and in the anterior region, where they are not. These two regions will be described separately. The special modifications of the perinephric membrane over the leptophragmata themselves will be considered when those structures are described. Apart from these real structural differences there is also a good deal of more problematical variation, particularly in the degree of separation of the various layers. Much of this is almost certainly the result of imperfect fixation, but we cannot be certain that this is always so. The existence of this variation complicates the task of trying to correlate physiological with structural data.

In the posterior region of the rectal complex the *outer sheath* consists principally of a single continuous layer of flattened cells (figure 22, plate 25). Their margins often taper and overlap, giving the appearance of a double layer of cells in section, but otherwise the layer is only one cell thick. The cell surfaces are smooth in outline and there are neither microvilli nor infoldings. The cytoplasm in the basal region of these cells—that is, the inner part, facing the rectum and tubules—presents no unusual features (figure 23,

plate 25). It contains abundant mitochondria, rough endoplasmic reticulum and free ribosomes, and a few small cytolysome-like bodies. This region is rather sharply distinct from the rest of the cytoplasm, forming the outer or 'apical' region of the cell, which contains no mitochondria or endoplasmic reticulum but is almost completely filled with closely packed microtubules (figure 23, plate 25). The two regions of the cell are about equal in extent. The microtubules of the apical region for the most part run parallel to the cell surface and to each other. They appear to be somewhat sinuous. Their diameter is about 230 Å, but they are not regularly arranged. The micrographs suggest that they tend to run transversely with respect to the rectal complex as a whole.

The contacts between cells of the outer sheath present no special features; neither desmosomes nor tight junctions have been seen.

Immediately external to the cells just described, and also forming part of the outer sheath, there is a prominent non-cellular basement membrane layer, about 0·2 μm thick and apparently formed of amorphous or finely filamentous material (figures 22 and 23, plate 25). As will be described below, this layer is highly developed over the leptophragmata.

In contrast to the outer sheath, just described, the *inner sheath* is composed of many cell layers. These, however, are extremely flattened, so that the total thickness is, on average, not much greater than that of the outer sheath (figure 21, plate 24; figure 22, plate 25). Most of the cells forming the inner sheath are so greatly flattened that in sections they appear to consist of little more than two apposed plasma membranes, separated by a layer of cytoplasm about 100 Å thick (figure 24, plate 26). There are about 40 such cell layers. The membranes of adjacent cells frequently make contact, the dense components of the unit membranes fusing to give rise to a five-layered junction (figure 25, plate 26). In places there are stacks of such tight junctions, resembling the myelin sheath of nerve fibres (figure 25), and it is possible that such a compact, multi-laminate structure is characteristic of much of the inner sheath in life, the separation of the layers in most sections being an artifact of fixation. However, in some areas the membranes of adjacent cells are separated by extracellular material (figure 25), and the parallel with the myelin sheath cannot be pressed too far since some cells retain a good deal of cytoplasm, in which mitochondria, groups of microtubules and occasionally other organelles can be seen. Nuclei, however, have not been found in this layer, except in some of the outermost cells bordering the space between the inner and outer sheaths (these are further discussed below). In the apparent absence of nuclei we have not been able to decide whether each layer in the sheath represents a single cell, or whether the sheath is formed by repeated folding (or perhaps coiling) of a smaller number of cells.

As already noted, the inner sheath may be deeply infolded between the tubules and boursouflures (figure 2; figure 5, plate 18). These infoldings (which are the 'pads of connective tissue' of Saini (1962)) are U-shaped in cross-section and formed of the same multiple cell layers as are found elsewhere. It should be noted, however, that such infoldings are not always present between tubules. The inner surface of the inner sheath is closely applied to the outer and lateral surfaces of the Malpighian tubules (figure 21, plate 24).

The final component of the perinephric membrane in the posterior region of the rectal complex is the space between the outer and inner sheath and the cells it contains. The

extent of this space varies a good deal. It is usually largest in the regions between two tubules, where it may be several microns wide. Elsewhere it may be much narrower. It usually contains finely filamentous extracellular material, aggregated into clumps or strands (figure 22, plate 25). The cells in the space can for the most part be identified as tracheolar end-cells (figure 21, plate 24; figure 22, plate 25). These are mostly located in the inner part of the space, adherent to the outer surface of the inner sheath. They are often flattened, with long peripheral processes and extensions, and they may grade almost imperceptibly into the completely flattened cells of the inner sheath (figure 22). Some of these cells, which cannot be assigned with certainty to either the inner sheath or the space between the two sheaths, contain nuclei, and it is possible, though by no means certain, that the nuclei of the inner sheath cell layers are all located in this peripheral region. The gross arrangement of the tracheae and the importance of the richly tracheated perinephric membrane in supplying oxygen to the rectal complex were described previously (Ramsay 1964); the present observations have merely served to define the position of the tracheolar end cells more precisely. Tracheolar end cells are present in the infoldings of the inner sheath.

In the anterior part of the rectal complex, where there are no leptophragmata, the structure of the perinephric membrane differs considerably from that just described for the posterior region. A detailed description of the structure of the membrane in this region is not relevant here, but some of the principal differences may be summarized. In general, the membrane as a whole becomes thinner passing anteriorly, and the distinction between inner and outer sheaths gradually disappears. The number of layers of cells diminishes. The membrane eventually terminates some distance anterior to the point at which the tubules leave the rectum in the common trunk (figure 1). Figure 18, plate 23, and figures 26 and 27, plate 27, illustrate the structure of the membrane at various points along the length of the anterior region. It will be seen that multiple layers of more or less flattened cells are usually present, but the proportion of flattened to non-flattened cells varies a good deal in different regions of the anterior part of the perinephric membrane, and also differs apparently from one mealworm to another. The cells forming the membrane in this region typically contain abundant mitochondria, rough endoplasmic reticulum, free ribosomes and microtubules (figure 26). Cytolysomes may be present. The presence of large intercellular spaces is characteristic of the anterior region of the perinephric membrane. Tracheolar end cells are less common than in the posterior region and occur interspersed among the other cells. In the most anterior region the membrane is often considerably thinner and includes fewer layers of cells where it is in contact with the tubules than elsewhere. At the extreme anterior end the membrane is reduced to one or two layers of thin cells, together with a good deal of extracellular space (figure 27). Basement membrane layers are present on the inner and outer surfaces. The perinephric membrane is continuous with and similar in structure to the membrane of the common trunk which encloses the Malpighian tubules after they leave the rectum.

In contrast to the situation at the anterior end of the rectal complex, at the posterior end the perinephric membrane is firmly attached to the gut, thereby sealing off the perirectal space from the haemocoel. The morphological relationships between the various structures in this region are complex and a detailed description is outside the scope of the

present paper. For present purposes, the essential feature is that immediately behind the posterior limit of the cryptonephric tubules the perinephric membrane is inflected sharply inwards, converging upon a specialized region of the gut lying between the rectum and the anal canal. Here the cuticle lining the gut (see below) is reduced in thickness (to about 1 μm) and is composed entirely of epicuticular layers. It is underlain by an annulus of elongated cells which differ in shape from the epithelial cells of the neighbouring rectum and anal canal. These elongated cells lie with their long axes at right angles to the cuticle and appear to interdigitate with the posterior terminations of the laminae of the inner sheath of the perinephric membrane. The circular muscle which elsewhere surrounds the gut is absent in this region.

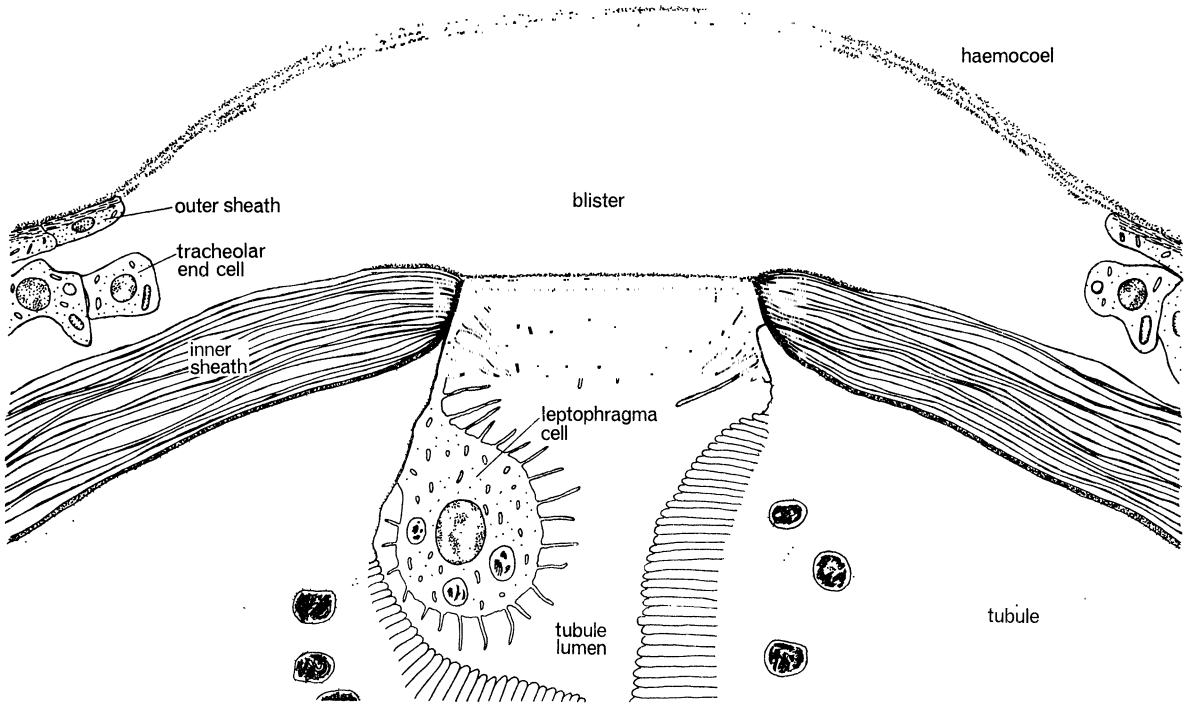

FIGURE 4. Diagram, based on electron micrographs, showing the structure of a leptophragma and its relation to the inner and outer sheaths of the perinephric membrane. Note the dense ridged ring on which the laminae of the inner sheath terminate.

(iii) *The leptophragmata and blisters*

As already noted, at the apex of each boursouflure there is a leptophragma, a small thin-walled region in the tubule wall formed by a single leptophragma cell. The perinephric membrane overlying this is modified to form a shallow, dome-shaped structure which will be termed a 'blister' (figure 4). The two structures, leptophragmata and blisters, are presumably functionally as well as structurally related, and they will be described together in this section.

The leptophragma cell consists of two parts: a thin, flat plate with a deep rim, which is inserted into the tubule wall and forms the leptophragma itself, and a cell body containing the nucleus which hangs down into the tubule lumen from one edge of the rim (figure 4; figure 28, plate 28). The former part of the cell is an extremely delicate sheet of cytoplasm, as little as 0·1 μm thick. Its outer surface, facing the blister, is smooth, while

the opposite face bears microvilli, extending down into the tubule lumen. These are distinct from the microvilli of the tubule cells, being narrower and less closely packed. They are up to 6 μm long. The cytoplasm of this part of the cell presents no special features; it contains a few mitochondria and microtubules, some small vesicles, and free ribosomes. The cell body bears microvilli on the surface in contact with the tubule lumen. Apart from the nucleus, the cell body contains abundant mitochondria, microtubules and free ribosomes. There are also many small granules, staining less densely than ribosomes, similar to those in the tubule cells. Occasional Golgi bodies are found. The most striking feature of the cytoplasm, however, are large vacuoles, up to 5 μm in diameter, which may occupy a large part of the cell body. These contain electron-dense material in the form of irregular aggregates of granules, stacks of membranous elements, etc., but this occupies only a small part of the vacuole, which otherwise appears empty (figure 30, plate 29). These bodies can perhaps be regarded as rather sparsely filled cytolysomes, though nothing is known about their cytochemical properties. The leptophragmal cell bodies show some variation in the overall electron density of the cytoplasm. Particularly in material in which the microvilli of the Malpighian tubules are swollen, the leptophragmal cells are often dense and apparently shrunken. The large vacuoles in such cells appear to be collapsed. Swollen, empty-looking leptophragmal cells have also been seen.

The blisters in the perinephric membrane which overlie the leptophragmata are considerably larger in diameter than the leptophragmata themselves (figure 4). They are entirely non-cellular. The cellular layer of the outer sheath stops at the margin of the blister (figure 21, plate 24), while the inner sheath extends in under the edges of the blister but stops at the margin of the leptophragma (figure 28, plate 28). At the base of the blister the leptophragma is covered by a finely filamentous layer which usually continues across the inner sheath for some distance (figure 4). The basement membrane on the outer surface of the perinephric membrane is expanded to form the top of the blister. It is frequently thicker over the blisters than elsewhere, and may have a multi-laminate structure (figure 21, plate 24). The space between these two layers is continuous with the space elsewhere found between the inner and outer sheaths, and is usually filled with amorphous material (figure 21). There are no tracheolar end cells in the blister. Blisters sometimes appear collapsed and shrunken in fixed material (figure 28, plate 28).

It remains to consider the cell contacts and junctions in the region of the leptophragmata and blisters. Where a leptophragma cell adjoins a tubule cell it is linked to it by an extensive septate desmosome, which covers the entire contact area (figure 30, plate 29). The multiple cell layers of the inner sheath are inserted on a prominent ring of dense, extracellular material continuous with the basement membrane of the tubule but more electron-dense and more compact in appearance (figure 29, plate 28). This ring runs round the edge of the leptophragma and tubule cells in the immediate vicinity. Its inner surface, in contact with the leptophragma or tubule cells, is smooth, but externally it is transversely ridged, and it is on these ridges that the edges of the cells of the inner sheath are inserted. The micrographs suggest that the leptophragma cells, tubule cells and inner sheath are all firmly bound together, and this in turn might suggest that the cell contacts in these regions may be subjected to stresses from osmotic or other forces.

(iv) *The subdivisions of the perinephric space*

In the posterior region of the rectal complex most of the space between the rectum and the perinephric membrane is occupied by the Malpighian tubules. These, however, do not entirely fill it and, on the basis of light-microscopic observations and injection experiments, Ramsay (1964) suggested that the remaining space is divided into three compartments. Two of these—the perirectal and subepithelial spaces (figure 2; figure 5 plate 18)—are anatomically simple and our observations merely substantiate what was known previously and provide some additional information about the contents of these spaces. The so-called peritubular space, however, is a different matter, and since our observations cast some doubt on its existence as a real extracellular cavity we shall devote most of this section to it. Before doing this, however, it is worth recording one general observation which assists in the interpretation of these spaces in general. This is the fact that, as far as we can determine, all the truly extracellular spaces in the rectal complex are lined by a basement membrane (see, for example, figure 7, plate 19)—that is, a dense, amorphous layer of material of the kind which has already been described on the outer surface of the perinephric membrane and of the Malpighian tubules.

The so-called peritubular space. According to the previous description this is the cavity between the tubules, bordered on its outer surface by the perinephric membrane and on its inner by 'a very thin membrane which runs along the inner borders of the perirectal tubules' (Ramsay 1964). The reality of this space was taken to be demonstrated mainly by the fact that indian ink could be injected into it without spreading into the perirectal or subepithelial spaces. It is shown in figure 3 of Ramsay (1964) as a large and well-defined cavity, and an apparent space of this kind has been seen with the light microscope in Araldite-embedded material. The extent of the space varies a good deal, however, from one mealworm to another, and from one part of a section to another, and in many instances it cannot be identified at all with the light microscope. This apparent obliteration of the space can come about in two ways. Most simply, the tubules may lie close together and be separated only by the infoldings of the inner sheath, against which they are tightly pressed. This frequently accounts for the absence of the space between the lateral borders of the tubules. At a deeper level, however, around the basal region of the tubules, the region of the supposed space is often occupied by cells, plainly visible in the light microscope in sections of Araldite-embedded material. The light microscope does not permit the nature of these cells to be ascertained; they often present a fragmentary or necrotic appearance. At least in some parts of the rectal complex, therefore, the light microscope fails to reveal any obvious peritubular space, and this, of course, raises the possibility that its apparent presence elsewhere may be an artifact. We shall consider below how such an artifact might arise, and also suggest an interpretation of the injection experiments. First, however, it is necessary to describe the structure of this region as seen in the electron microscope.

Figure 31, plate 30, is a section through the lateral border of a tubule, close to its basal surface. At the bottom of the picture part of the perirectal space is visible, with its characteristic dense contents and limiting basement membrane. The region between this membrane and the tubule should, according to the previous description (Ramsay 1964), form

the peritubular space, but it is in fact filled with the cells seen in the light microscope. We shall call these *intertubular cells*. Most of them present a necrotic appearance; the cytoplasm typically contains extensive empty regions, and organelles such as mitochondria are usually swollen and disrupted. The plasma membrane of these cells usually remains intact, however. In some micrographs many of the intertubular cells are reduced to little more than seemingly empty vacuoles, surrounded by a membrane (figure 32, plate 30). In most cases, however, sufficient cytoplasmic organelles remain to make the identification of these structures as cells unequivocal. The intertubular cells are most abundant at the apex of the infoldings of the inner sheath between the tubules, where they may form a fairly solid tissue. They extend from this as a thin layer of cells against the lateral border of the tubule, stopping where the inner sheath makes contact with the tubule. They also extend for some distance along the basal edge of the tubule (figure 7, plate 19), but do not form a continuous layer there. Where they are not present in this basal region the basement membrane of the tubule is in contact with that limiting the perirectal space (see below).

It seems likely, therefore, that there is no true peritubular space, and this conclusion is supported by the fact that there is no basement membrane on the inner surface of the inner sheath, except where it is in contact with the Malpighian tubules.

The apparent peritubular space described previously has probably originated in a variety of ways. It must be remembered that the rectal complex as a whole is extremely difficult to fix adequately, and that many of the earlier observations were certainly made on imperfectly preserved material. Even in glutaraldehyde-fixed material the inner sheath separates readily from the tubules, leaving a substantial gap, and this, coupled with the fact that the intertubular cells are often necrotic and empty, probably accounts for most of the so-called peritubular space, previously described. If the perinephric membrane can readily separate from the tubules it would not be surprising if indian ink could be injected between them, and the intertubular cells would hardly offer much resistance to its passage. Injection experiments also suffer from the fact that the precise location of the tip of the pipette cannot be determined, and it is possible that in some of the experiments previously reported ink could have been injected into the space between the cell layers of the inner sheath, since these sometimes separate in fixed material.

To summarize, we see no reason for regarding the peritubular space as anything but an artifact, though it is one which can arise very easily.

The perirectal space. As can be seen from figure 5, plate 18 and figure 7, plate 19, the Malpighian tubules do not generally make direct contact with the muscles surrounding the rectum. They are separated from it by a space, which is called the perirectal space (Ramsay 1964). Internally this is bounded by the basement membrane covering the muscle cells; externally it is limited either by the basement membrane covering the basal surface of the tubules or by a sheet of basement membrane which runs between them (figure 7). The intertubular cells lie on the other side of this basement membrane, and are presumably responsible for forming it.

The contents of the perirectal space are preserved following glutaraldehyde fixation and stain fairly strongly with mercuric brom-phenol blue. In electron micrographs the space appears filled with amorphous material of moderate electron density (figure 7,

plate 19; figure 31, plate 30). These observations are consistent with the presence in the perirectal fluid of a protein, the existence of which has also been inferred from physiological data to be presented below. In addition to this material there are occasional large spherical droplets, reminiscent of lipid droplets (figure 5, plate 18).

Occasional strands of basement membrane material can be seen in sections, crossing the perirectal space and linking its outer and inner walls (figure 5). These appear to be fragile and readily disrupted by fixation. They appear to be isolated strands rather than continuous sheets of material and there is no reason to suppose that the perirectal space is subdivided into compartments.

In the anterior part of the rectal complex, where no leptophragmata are present, the perirectal space is relatively extensive, since the diameter of the rectum is smaller and the Malpighian tubules are thinner walled and somewhat flattened. The amorphous contents are present in this region, though not so dense as posteriorly, but the spherical droplets have not been seen. Occasional groups of cells occur in the perirectal space in this region. They contain much granular endoplasmic reticulum and numerous inclusion bodies and are reminiscent of blood cells.

The subepithelial space. The space between the circular muscle and the rectal epithelium has been called the subepithelial space by Ramsay (1964). It is not a single cavity but rather a series of six separate compartments, since the circular muscles make contact with the rectum at six points around its circumference (figure 2). Like the perirectal space, the subepithelial space is lined with basement membranes, of the circular muscles externally and of the rectal epithelium internally. It contains no cells but, as in the perirectal space, amorphous material is present (figure 7, plate 19). This shows a good deal of variation in its apparent amount or electron density in different larvae and in different compartments of the subepithelial space in one specimen. It stains strongly with mercuric brom-phenol blue.

The subepithelial space diminishes in extent passing anteriorly and is obliterated anterior to the tubules, where the circular muscles are in direct contact with the rectal epithelium.

(v) *The rectum*

The rectum is approximately hexagonal in cross-section, with somewhat concave sides (figure 2; figure 5, plate 18). The rectal epithelium is extremely thin (as little as 1 μm thick) at the angles of the hexagon, and considerably thicker (up to 7 μm) between them. The angles of the hexagon are the sites of the longitudinal muscles (figure 5), which project into the perirectal space. The circular muscles form a continuous sheet around the rectum, but make contact with it only at the angles of the hexagon, where they pass beneath the longitudinal muscles. They become extremely thin at these points. Elsewhere the circular muscle is separated from the rectal epithelium by the subepithelial spaces, already described (figure 2; figure 5). In the anterior part of the rectal complex the circular muscle is thicker than posteriorly. The muscles themselves call for no further comment, apart from the fact that they are all bounded externally by a basement membrane (figure 7, plate 19). The rectal lumen usually has the form of a six-pointed star in cross-section (figure 2). It is proposed to give here a brief description of the rectal epithelium and the

cuticle which lines it, since it is across these that the initial transfer of water from the faeces takes place.

The rectal epithelium is one-cell thick. The membranes of the apical borders of the cells, below the cuticle lining the rectal lumen, are thrown into irregular folds and microvilli, which no doubt serve to increase the surface area (figure 33, plate 31; figure 35, plate 32). The membrane at the basal surface of the cell is also extensively convoluted. Here there are sometimes deep inpushings of irregular form filled with coarsely laminated extracellular material, which is also often found covering the general basal cell surface below a more normal dense basement membrane layer (figure 37, plate 32). In other instances the infoldings are narrow. Adjacent rectal cells are closely applied to each other and near their apical borders their membranes are extensively interdigitated (figure 33, plate 31). The membranes appear denser in this region than elsewhere, but distinct structural connexions between them, such as desmosomes, have not been identified. The main features of the cytoplasm of the rectal cells can be seen in figures 33 and 34, plate 31, and figure 35, plate 32. The outstanding fact is the presence of many microtubules, often grouped in bundles of 20 to 30. These are often particularly abundant near the lateral borders of the cells where they may run approximately parallel to the plasma membranes (figure 33). They are also sometimes common near the apical border, and they are a striking feature of the cells forming the thin regions of rectal epithelium below the longitudinal muscles. Cytolysomes are fairly common in the rectal cells, and often there are also extensive areas largely filled with glycogen granules (figure 35). Apart from this there are many mitochondria, distributed throughout the cytoplasm, and abundant ribosomes and rough-surfaced endoplasmic reticulum.

The cuticle lining the rectum presents no special features. It has a total thickness of about 3 to 7 μm and the various layers forming it are tentatively identified in figures 35 and 36, plate 32 (cf. Locke 1964, 1966). At the luminal surface there is a thin dense layer, about 60 Å thick, which may perhaps be a wax or lipid layer. The more or less homogeneous layer below this is regarded as the epicuticle and is limited externally by a dense layer, in places appearing double, which is perhaps to be identified as the cuticulin layer. Between the epicuticle and the endocuticle, with its typical layered structure, there is a region of irregular structure, containing light and dark areas but without any sign of lamination, which may correspond to the exocuticle. In this layer, as well as in the supposed epicuticle, pore canals are present. At the base of the endocuticle, adjacent to the epithelium, irregular areas of dense material may be present (figure 33, plate 31).

3. Physiology

(*a*) *Summary of earlier work*

Reference was made in § 1 to a previous paper by Ramsay (1964). The principal results and conclusions of this work may be summarized as follows.

(i) The osmolarity of the perirectal fluid (Δ = 0·7 to 8·0 °C) can be very much greater than the osmolarity of the medium external to the rectal complex (haemolymph, Δ = 0·7 to 1·4 °C). This difference in osmolarity is more marked in 'dry' mealworms.

(ii) The existence of this maintained difference, together with the observation that

extreme changes in the osmolarity of the external medium fail to bring about rapid water movements into or out of the rectal complex, suggest that the perinephric membrane is relatively impermeable to water. On the other hand, there is comparatively little resistance to the passage of water from the rectal lumen to the perirectal space and to the tubules.

(iii) The freezing-point depression of the tubular fluid, which follows that of the perirectal fluid very closely, is almost completely accounted for as potassium chloride; other solutes, presumed to be mainly non-electrolytes, make the main contribution to the freezing-point depression of the perirectal fluid.

(iv) The rate of flow and composition of the tubular fluid are responsive to changes in the composition of the external medium.

(v) What is known to enter the rectal complex is faecal matter suspended in a fluid which is isosmotic with haemolymph; what leaves is (*a*) dried faeces and possibly (*b*) tubular fluid and/or perirectal fluid both of which are hyperosmolar to haemolymph. It therefore follows that either the rectal complex must actively transport water into the haemolymph or it must actively take up a solute, presumably potassium chloride, from the haemolymph, somewhere on the membranes—perinephric membrane and/or leptophragmata—which separate the perinephric and tubular fluids from the haemolymph.

(*b*) *The permeability of the perinephric membrane*

Poll (1934) came to the conclusion that the perinephric membrane was highly permeable, certainly to dyes such as indigo carmine. Ramsay (1964) was unable to repeat Poll's observations on indigo carmine; and in view of the substantial difference in osmolarity of perirectal fluid and tubular fluid as compared with haemolymph he concluded that the perinephric membrane was relatively impermeable to water and to dissolved substances. Further work has now shown that Poll was right in as much as the extreme anterior region of the perinephric membrane is permeable to water and to a variety of solutes, including (under certain conditions) indigo carmine; but the posterior region appears to be relatively impermeable.

The first indication that the anterior region was more permeable than had at first been thought came from further recourse to the silver-staining method of Lison (1937), which showed that there was some degree of staining over this region, suggesting permeability to chloride. This observation was followed up using a less subjective method of assessment.

The method consisted in injecting Ringer solution (for composition see Ramsay 1964, p. 304) containing ^{3}H-labelled water and ^{14}C-labelled sucrose into the perirectal space and determining the rates at which the labels were leached out. Indigo carmine was added to the injected solution as a check against gross leakage. Two series of experiments were carried out, one using the anterior part of the rectal complex and the other using the posterior part. For the anterior part a ligature was tied around the sleeve of the perinephric membrane just anterior to the origin of the common trunk, which was turned forward so as to be included in, and therefore closed by, the ligature; the injection was made from a pipette thrust through the posterior part of the rectal complex into the perirectal space, and when this space was fully distended a second ligature was tied around

the rectal complex at the region of the transition between the thin-walled and the thick-walled parts of the tubules (about one third of the length of the complex, measured from the origin of the common trunk). For the posterior part a ligature was first tied around the anal canal. The injection pipette was inserted through the sleeve of the perinephric membrane and was passed backwards until its tip lay in the posterior part of the peri-rectal space. A second ligature was tied around the transitional region and was drawn down tight on the pipette shank. After the injection the pipette was withdrawn and the second ligature was finally tightened and made fast. In both cases the ligatured region was cut out and immediately transferred to 0·1 ml. of Ringer, as a drop resting upon a waxed surface, in which it was continuously agitated so as to reduce unstirred layers.

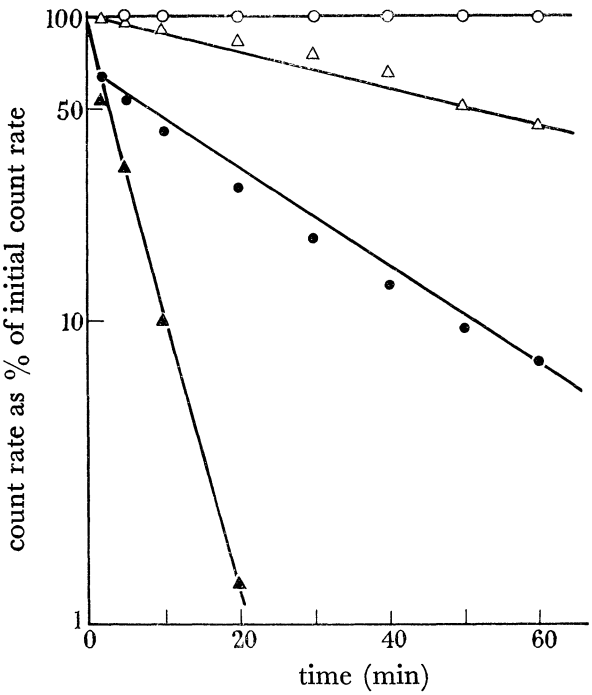

FIGURE 38. Effluxes from the isolated rectal complex. ○, ^{14}C-labelled sucrose from the posterior half; △,^{3}H-labelled water from the posterior half; ●, ^{14}C-labelled sucrose from the anterior half; ▲, ^{3}H-labelled water from the anterior half.

At intervals the preparation was moved to a fresh drop of Ringer, and at the end of the experiment it was torn apart under Ringer and 15 min were allowed for the remaining radioactive material to leach out. The drops of Ringer were sealed up in glass tubes, the water was separated from the dissolved material by distillation, and water and solids were counted by conventional methods which need not be described.

The time course of washing-out, as may be seen from figure 38, indicates that the permeability is very much greater over the anterior part than over the posterior part. Over the anterior part the half-times of exchange were 3 and 20 min, for water and sucrose respectively; over the posterior part the half-time of exchange of water was 50 min, while that of sucrose was too long to measure. These experiments were carried out on 'dry' mealworms.

The differences in permeability may be interpreted in terms of the structural differences

between the anterior and posterior parts, which have been described in § 2 (*b*). The inner sheath of the perinephric membrane suggests itself as the main barrier to diffusion not only by virtue of its multi-laminate appearance but also in that it is progressively reduced in thickness over the anterior part. From the point of view of the mechanism of the rectal complex the important point is that the relative impermeability over the posterior part is confirmed, and this relative impermeability probably applies in some measure to the leptophragmata as well as to the perinephric membrane itself. It is of less significance that the perinephric membrane is relatively permeable over the anterior part since in any case the perirectal space is in open communication with the haemocoel via the sleeve.

The reason for Ramsay's earlier failure to observe the passage of indigo carmine through the perinephric membrane, as observed by Poll, remains to be considered. The simplest way to examine the permeability of the perinephric membrane is to inject a concentrated solution of the dye into the perirectal space (a ligature having first been tied around the sleeve of the perinephric membrane) and then to see whether the dye leaches out into the external medium. With 'dry' mealworms no colour was ever seen to leach out; but with 'moist' mealworms colour has on occasion been seen to leach out, not through any detectable perforations but apparently from the general surface of the perinephric membrane. The earlier failure to confirm Poll is explained by the fact that only 'dry' mealworms were used in the earlier experiments on the permeability of the perinephric membrane.

It was therefore of interest to repeat other earlier observations upon the effect of immersing the rectal complex in concentrated media (Ramsay 1964, p. 295). In these experiments the rectal complex was ligatured around the sleeve and was then flooded with 3 M sucrose made up in Ringer. Using 'dry' mealworms some decrease in the distension of the anterior perirectal space and some loss of turgor by the tubules could be detected in about 30 min after the rectal complex had been flooded with the sucrose solution. Using 'moist' mealworms the same changes could be detected in 5 min.

These observations, which indicate that the permeability of the anterior part of the perinephric membrane varies in accordance with the mealworm's state of water balance, are further discussed in § 4.

(*c*) *Movement of ions between rectal complex and haemolymph*

In this study the methods of analysis were the same as those used in the earlier work: freezing-point depression (Ramsay & Brown 1955); sodium and potassium (Ramsay, Brown & Falloon 1953); chloride (Ramsay, Brown & Croghan 1955).

The possibility of following net movements of ions and water between rectal complex and haemolymph almost inevitably depends upon the possibility of setting up the rectal complex, as an isolated preparation in good physiological condition, in a limited volume of haemolymph or other external medium. Such isolated preparations were examined during Ramsay's earlier investigation and were found to remain in good condition, as judged by the production of tubular fluid, for periods of about 1 h. In respect of long-continued function the isolated preparation was found to be markedly inferior to the rectal complex *in situ* in the opened animal with its tracheal connexions intact (Ramsay 1964, pp. 302–304). The *in situ* preparation was therefore preferred for all the earlier

experiments, previously reported upon, which were intended to show the effect of changes in the composition of the haemolymph upon the composition and rate of flow of the tubular fluid. But because the volume of the haemolymph is not accurately known, and because its composition may be affected by exchanges with other tissues of the body, the *in situ* preparation is unsuitable for the present purpose and the disadvantage of the isolated preparation in limiting the duration of experiments has to be accepted.

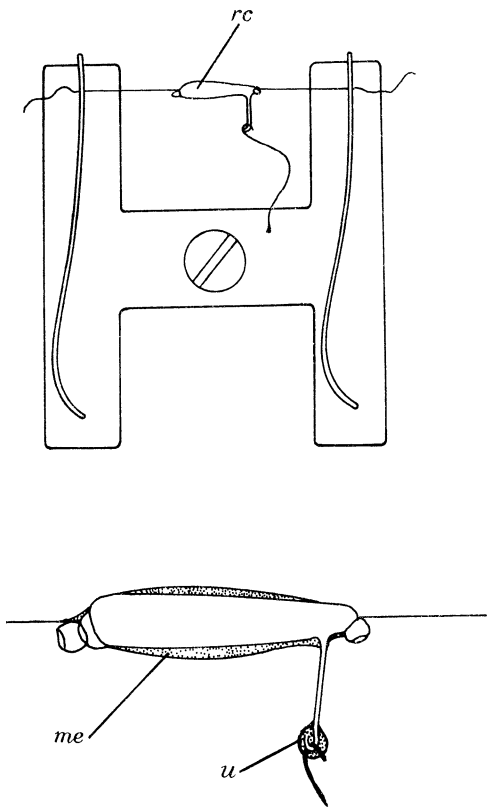

FIGURE 39. Metal frame carrying isolated rectal complex (for details see text). *me*, external medium; *rc*, rectal complex; *u*, tubular fluid.

The isolated preparation is easily made. The tracheal connexions of the rectal complex with the rest of the body are cut and ligatures are tied around it, one around the anal canal and the other around the sleeve of the perinephric membrane just anterior to the common trunk. The rectal complex is freed by cutting the anal canal and the intestine just beyond the ligatures. It is then fastened by means of the ligatures to a metal frame (figure 39) submerged in liquid paraffin which is saturated with oxygen at atmospheric pressure. The frame with the rectal complex attached is rotated at about 6 rev/min, thus maintaining some circulation of the liquid paraffin and some oxygen supply to the rectal complex.

After the rectal complex has been set up as described above, and before the frame is set into rotation, as much as possible of the adherent haemolymph is sucked away with a fine pipette. Then a measured volume (0·2 to 0·4 μl.) of medium, which may be haemolymph or some physiological saline, is applied to it. The frame is set into rotation for some chosen interval of time, after which it is stopped and a sample of measured volume

is taken from the medium. This may be repeated as desired. The cut ends of the tubules may be hooked up on a fine platinum wire, as shown in figure 39, for the collection of tubular fluid, or the common trunk may be tied down under the adjacent ligature so that tubular fluid is retained.

With this preparation it was possible to follow changes in the freezing-point depression and in the concentrations of sodium, potassium and chloride in the external medium; and since this was of small volume substantial changes could be observed over intervals as short as 5 min. The interpretation of changes in concentration is made easier if information is also available about changes in volume of the external medium. Assessment of changes in volume is best made by following changes in the concentration of some non-penetrating solute; Phillips (1964) made effective use of ^{131}I-labelled serum albumin for this purpose. When this method was tried in the present context the results showed that

TABLE 1. ISOLATED RECTAL COMPLEX OF 'MOIST' MEALWORMS—ANALYSES OF EXTERNAL MEDIUM

Na, K in m-equiv./l., Δ in °C

no.		Na	K	Δ
1	haemolymph	87	33	0·945
	5 min	73	16	0·865
	35	72	9	0·915
	45	75	9	0·995
	65	80	9	1·155
2	haemolymph	66	36	0·755
	5 min	46	44	0·765
	10	49	30	0·725
	20	48	24	0·685
	30	52	21	0·655
3	haemolymph	72	37	0·925
	5 min	74	49	1·035
	10	76	52	1·135
	25	80	54	1·245
	45	86	51	1·355
4	haemolymph	83	34	0·965
	5 min	85	15	0·935
	10	86	11	0·925
	20	79	11	0·985
	30	78	10	1·025
	40	82	8	1·195
5	haemolymph	74	28	0·875
	5 min	64	16	0·775
	10	61	8	0·765
	20	59	7	0·785
	30	58	8	0·805
	45	62	10	0·885
6	haemolymph	80	39	1·105
	5 min	66	37	1·075
	10	62	33	1·105
	20	54	25	1·145
	30	61	27	1·185
	45	57	22	1·305
7	haemolymph	78	27	0·865
	5 min	72	36	0·885
	10	73	31	0·855
	20	72	29	0·875
	30	73	25	0·855
	45	73	25	0·875

some activity was taken up into the rectal complex, where it was associated with the perinephric membrane. It seems likely that albumin can pass through the thin walls of the blisters and so reach the space between the outer and inner sheaths of the perinephric membrane. Be that as it may, the immediate consequence is that ^{131}I-labelled serum albumin cannot be relied upon to provide a measure of volume change. No satisfactory method of accurately measuring volume change could be found.

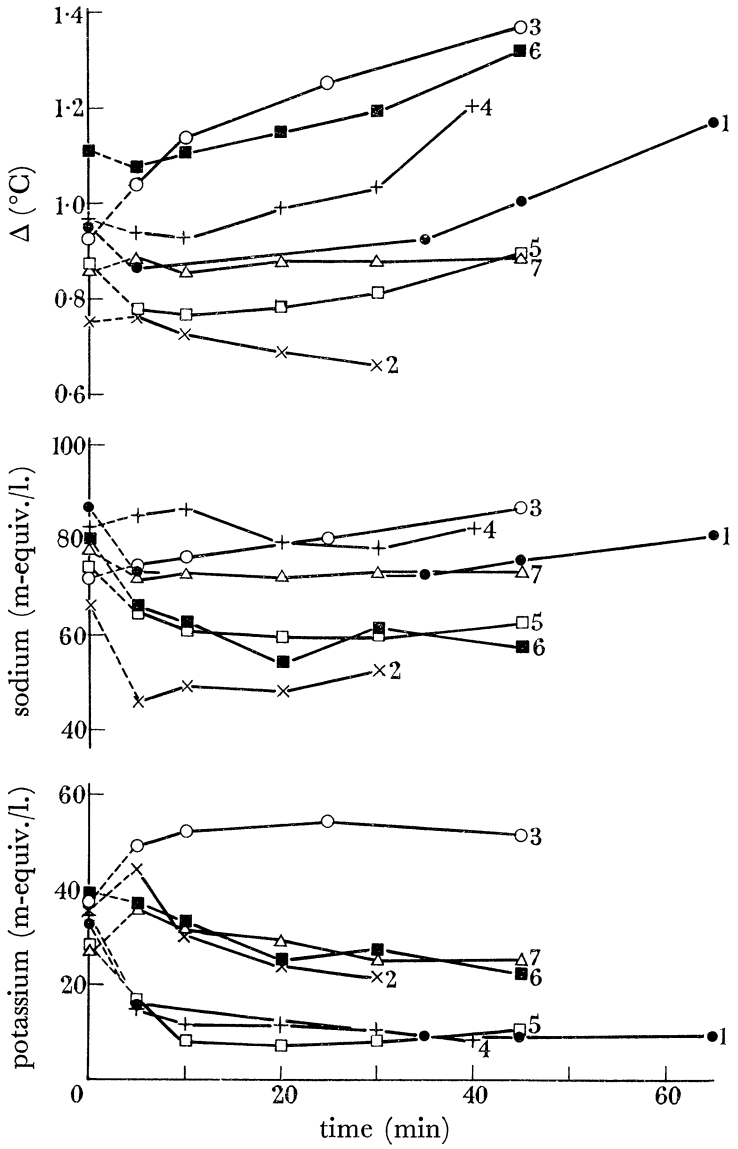

FIGURE 40. Changes in freezing-point depression and in concentrations of sodium and potassium in a small volume of haemolymph bathing the isolated rectal complex (details in table 1).

In the first series of seven experiments carried out along these lines 'moist' mealworms were used and the external medium applied to the rectal complex was haemolymph taken from the same mealworm. The results are set out in detail in table 1 and are shown plotted in figure 40. Although it might seem appropriate to use the haemolymph values to represent the external medium at zero time, the external medium in fact probably includes the fluid in the intersheath spaces of the perinephric membrane, whose composition

may be changed by physiological activities during the preparation of the rectal complex; we have therefore thought it better to restrict consideration to changes in the external medium from the first collection onwards.

Directing attention first to the concentrations of ions, we note that the concentration of sodium in the external medium remains more or less unchanged, increasing slightly in four experiments and decreasing slightly in three experiments. The changes in the concentration of potassium are greater, and in all experiments except one the concentration of potassium decreases, suggesting that potassium is taken up into the rectal complex.

This suggestion is supported by the results of other experiments in which 'dry' mealworms were used and in which various physiological salines were used in place of haemolymph as external medium. Two of these will be described in full.

TABLE 2. ISOLATED RECTAL COMPLEX OF 'DRY' MEALWORM SET UP IN ROTATING FRAME UNDER OXYGENATED LIQUID PARAFFIN

External medium double-strength cloride-free Ringer (nitrate replacing chloride). Time in minutes, Na, K in m-equiv./l., Δ in °C.

time		Na	K	Δ
0	added 0·4 μl. of medium	140	96	0·93
7	removed 35 nl. for analysis	144	36	1·11
10	removed remaining medium	144	18	1·02
13	added 0·4 μl. of medium containing 10 mM/l. $AgNO_3$	152	108	1·00
18	removed 35 nl. for analysis	170	32	1·00
23	removed remaining medium	159	19	0·98
	removed tubular fluid	—	—	3·3

The results of the first of these experiments are set out in table 2 which should be self-explanatory. The analyses show a rapid and extensive (× 5) fall in the potassium concentration of the external medium. The possibility that this might be due to release of water from the rectal complex is ruled out when the sodium concentration and freezing-point depression are taken into account. These remain more or less unchanged, contradicting any suggestion of a general dilution of the external medium. In any case a fivefold increase in the volume of the external medium could not have passed unnoticed. It therefore appears that potassium is taken up by the rectal complex from the external medium and that this uptake is likely to be active since in this experiment the fluid issuing from the tubules had a freezing-point depression of 3·3 °C, indicating a potassium concentration of the order of 1 equiv./l.

One reason why this particular experiment has been selected for detailed consideration is that it shows continued uptake of potassium in the presence of silver nitrate. This is unexpected, for Koch (1938), working on the anal papillae of *Aedes*, found that these organs, which actively take up sodium, were irreparably damaged by exposure to silver ions.

In the second experiment the rectal complex was first washed for a few seconds in the external medium just before the experiment was begun, and a sample taken immediately after the definitive application of the external medium was regarded as representing the composition of the external medium at zero time. In this second experiment chloride-free Ringer was used (nitrate replacing chloride), and the changes in chloride concentration

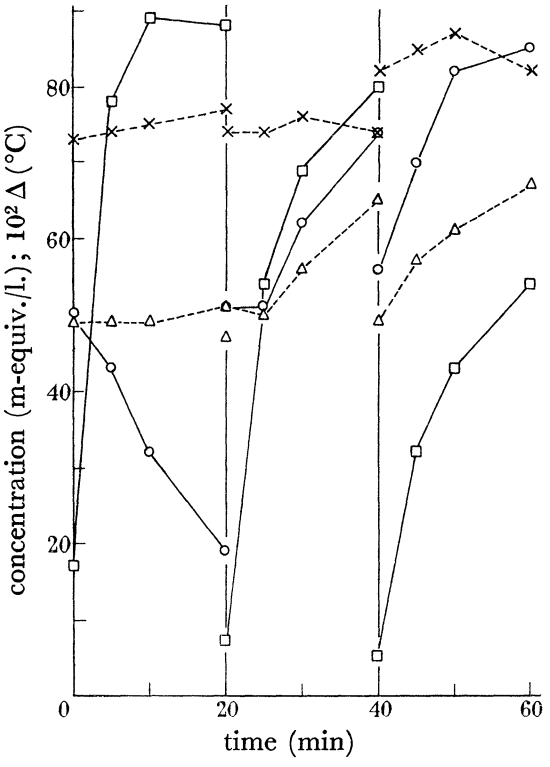

FIGURE 41. Changes in freezing-point depression (△) and in concentrations of sodium (×), potassium (○) and chloride (□) in a small volume of medium bathing the isolated rectal complex (details in table 3).

TABLE 3. ISOLATED RECTAL COMPLEX OF 'DRY' MEALWORM SET UP IN ROTATING FRAME UNDER LIQUID PARAFFIN

External medium chloride-free Ringer (nitrate replacing chloride).
Time in minutes, Na, K, Cl in m-equiv./l., Δ in °C.

time		Na	K	Cl	Δ
0	added 0·4 μl. of medium	—	—	—	—
	removed 35 nl. for analysis	73	50	47	0·49
5	removed 35 nl. for analysis	74	43	78	0·49
10	removed 35 nl. for analysis	75	32	89	0·49
20	removed remaining medium	77	19	88	0·51
	added 0·4 μl. of medium containing 10 mM/l.NaCN	—	—	—	—
	removed 35 nl. for analysis	74	51	7	0·47
25	removed 35 nl. for analysis	74	51	54	0·50
30	removed 35 nl. for analysis	76	62	69	0·56
40	removed remaining medium	74	74	80	0·65
	added 0·4 μl. of medium containing 10 mM/l. NaCN saturated with ether	—	—	—	—
	removed 35 nl. for analysis	82	56	5	0·49
45	removed 35 nl. for analysis	85	70	32	0·57
50	removed 35 nl. for analysis	87	82	43	0·61
60	removed remaining medium	82	85	54	0·67

are of some interest. The course of the experiment is described in table 3 and the results are also set out in figure 41. In chloride-free Ringer, without cyanide or ether, there is again substantial uptake of potassium but no significant change in sodium concentration or in freezing-point depression. There is also a very rapid leaching out of chloride from the rectal complex, within which it is presumably replaced by nitrate. After the addition of cyanide the movement of potassium is reversed. In the presence of ether, which presumably abolishes the selective properties of all membranes, there is immediate and rapid loss of potassium. The movements of sodium and of chloride do not seem to be so greatly affected by these poisons; the progressive change in the form of the chloride curve may be attributed to progressive reduction in the amount of chloride remaining in the rectal complex.

Restricting attention to the main theme of these experiments, the most important conclusion to be drawn from them is that the rectal complex has the ability to take up potassium against a concentration gradient.

The movements of chloride have now to be considered. The experiments just described have shown that chloride leaches out of the rectal complex into chloride-free Ringer, and from the reactions to silver-staining it may be supposed that this leaching takes place through the leptophragmata (and also to some extent through the anterior part of the perinephric membrane). In leptophragmata stained by Lison's method, which involves a very brief exposure to silver nitrate, it is not easy to decide whether the precipitate forms on the inside or on the outside of the leptophragma, and so one can only conclude that the leptophragma is permeable to chloride ion or to silver ion or to both. But when the exposure is prolonged it is clear beyond doubt that the precipitate of silver chloride accumulates on the outside of the leptophragma, which must therefore be permeable to chloride. The rapid exchange of chloride for nitrate, while the rectal complex continues to take up potassium, suggests that the permeability of the leptophragma to chloride is its normal physiological condition and is not the result of its being exposed to silver ions, as in Lison's staining method.

If this is accepted, the next question is whether the movements of potassium and of chloride are active or passive, and it is therefore necessary to know the difference of electrical potential, if any, between tubule lumen and external medium.

The measurement of this potential difference encounters certain technical difficulties by reason of the toughness and opacity of the perinephric membrane, which resists the passage of a pipette electrode and obscures the tip of the electrode when penetration has been achieved. It seemed at first that the neurophysiologist's micro-electrode, of 0·5 μm diameter at the tip, would be the ideal means of exploring the rectal complex; but attempts to pierce the perinephric membrane were always accompanied by the obvious indentation of its surface to a distance of about 100 μm before the resistance was overcome, and when the electrode was withdrawn its tip was invariably found to have been broken off. Glass micro-electrodes were therefore discarded in favour of silica pipettes of about 5 μm diameter at the tip, such as had been used in previous studies of Malpighian tubules (Ramsay 1953). The electrical system appropriate to the micro-electrode was retained. The cathode follower and pre-amplifier were to a design by Dr K. E. Machin based upon a circuit by Pugsley; this circuit was subsequently published in a modified form (Pugsley 1963). The

output was carried to an oscilloscope (Telequipment D 33 with amplifier A). A calibrating voltage could be inserted between earth and the indifferent electrode which, like the pipette electrode, was of the conventional Ag/AgCl/KCl type.

The rectal complex, dissected out and fixed by ligatures at either end to the bottom of a Perspex trough, was submerged in Ringer. The pipette electrode was mounted on a Leitz micromanipulator with its axis at a small angle to the axis of the rectal complex so that once having pierced the perinephric membrane the tip of the electrode would traverse the region occupied by the tubules for some distance before reaching the perirectal space. When a number of preparations had been penetrated in this way a certain regularity could be discerned in the profile. After the electrode had broken through the perinephric membrane it generally registered a potential some 20 mV negative to the external medium. On further advance of the electrode the potential would suddenly go positive to the external medium by 50 mV or more. Still further advance of the electrode would bring the potential negative again, and on some occasions the whole sequence was repeated in the course of a single penetration.

In order to relate this profile to the position of the electrode tip, indian ink was added to the saturated solution of potassium chloride with which the electrode was filled and the tip diameter was increased to 20 μm. After one of the significant features of the profile had been recognized a small quantity of indian ink was injected and its distribution was observed. Of twelve injections associated with a negative reading all were seen to enter the so-called peritubular space; of twelve injections associated with a positive reading all but one were seen to enter the tubule. The eleven tubule-and-positive readings ranged from +15 mV to +75 mV, with an average of +48·5 mV.

Ideally one would wish to know, for one and the same preparation, the concentrations of ions on either side of the leptophragma across which the potential is measured. All that is available is a series of measurements of potential difference and a series of analyses of tubular fluid, each made on a different preparation and showing great variation. The only concentrations which are accurately known are those in the external medium. The stock of Ringer used in these experiments was checked by analysis (flame photometry and Volhard titration) and was found to contain: sodium, 88 m-equiv./l.; potassium, 58 m-equiv./l.; chloride, 156 m-equiv./l.

Restricting consideration to 'dry' mealworms, the average of six observations on tubular fluid (Ramsay 1964, table 3, serials 1, 2, 5, 6, 7 and 8) gives a chloride concentration of 516 m-equiv./l.; the maximum chloride concentration ever recorded for tubular fluid was 2·19 equiv./l. (*ibid.* figure 12). The equilibrium potentials for chloride have been calculated from the Nernst equation, and the figures are set out in table 4, based on the average values and on the extreme values. In both cases it is seen that the observed potential exceeds the equilibrium potential, and it is therefore unnecessary to suppose that chloride is actively transported into the tubule. Transport of potassium into the tubule is clearly active.

(*d*) *Movement of water between rectal complex and haemolymph*

The position now reached is that potassium is actively transported from the haemocoel into the rectal complex, chloride following passively. In effect, potassium chloride is secreted into the tubules, and this is at least a partial answer to the question raised in

§3*a*(v), i.e. whether the mechanism of the rectal complex is based upon the inward secretion of solute or the outward secretion of water. But if this inward secretion of potassium chloride is indeed the mechanism by which high osmolarity is established within the rectal complex, then it should be possible to show that the potassium chloride is not accompanied by water—at least, not by so much water as would offset its contribution to increased osmolarity. In practical terms, it should be possible to demonstrate that the secretion of potassium chloride into the rectal complex is associated with a decrease in the freezing-point depression of the medium surrounding the rectal complex.

TABLE 4. ISOLATED RECTAL COMPLEX OF 'DRY' MEALWORM: DIFFERENCES IN CHLORIDE CONCENTRATION AND ELECTRICAL POTENTIAL BETWEEN TUBULAR FLUID AND EXTERNAL MEDIUM

Cl in m-equiv./l., *E* in mV, tubular fluid with reference to external medium.

	Cl		*E*	
	external medium	tubular fluid	equilibrium potential	observed potential
average	156	516	+31	+48·5
extreme	156	2190	+70	+75

It is first necessary to state that the maximum change in freezing-point depression which can be expected is not large. If potassium chloride alone is taken up so that its concentration in the external medium falls, say, from 40 mM to 10 mM/l. the expected change in freezing-point depression is of the order of 0·1 °C. Returning now to table 1 and figure 40, and considering the freezing-point depression from 5 min onwards, we find that it shows an over-all increase in five experiments (nos. 1, 3, 4, 5 and 6) and an over-all decrease in two (nos. 2 and 7). There is, however, a more general tendency for the freezing-point depression to decrease at first and to increase later; in four experiments (nos. 2, 4, 5 and 7) out of the six experiments in which collections were made both at 5 min and at 10 min the freezing-point depression decreased over this interval. In one case only (no. 2) the freezing-point depression decreased progressively throughout the experiment, by something of the order of 0·1 °C.

In an effort to provide more convincing evidence these experiments were repeated with the following variations: rectal complex and haemolymph from 'dry' mealworm; rectal complex from 'moist' mealworm and haemolymph from 'dry' mealworm; rectal complex from 'moist' mealworm and haemolymph from 'moist' mealworm with potassium chloride added. None of these variations except the last resulted in any substantial fall in freezing-point depression, and not even in this last case was a fall consistently observed. Figure 42 presents the results of two experiments with added potassium chloride, one showing a substantial fall followed by a rise and the other showing an insignificant fall followed by a rise; these two experiments were carried out on the same day with mealworms from the same culture.

It would be difficult to establish a case on the evidence of the slight fall in freezing-point depression which we have observed in a majority of experiments, such as nos. 4, 5 and 7 of figure 40. The case must stand or fall on the evidence of a few experiments such as

no. 2 of figure 40 and no. 1 of figure 42. Even a substantial fall in freezing-point depression if based upon a single collection, might fail to carry conviction; but in each of these experiments now under consideration a substantial fall in freezing-point depression is

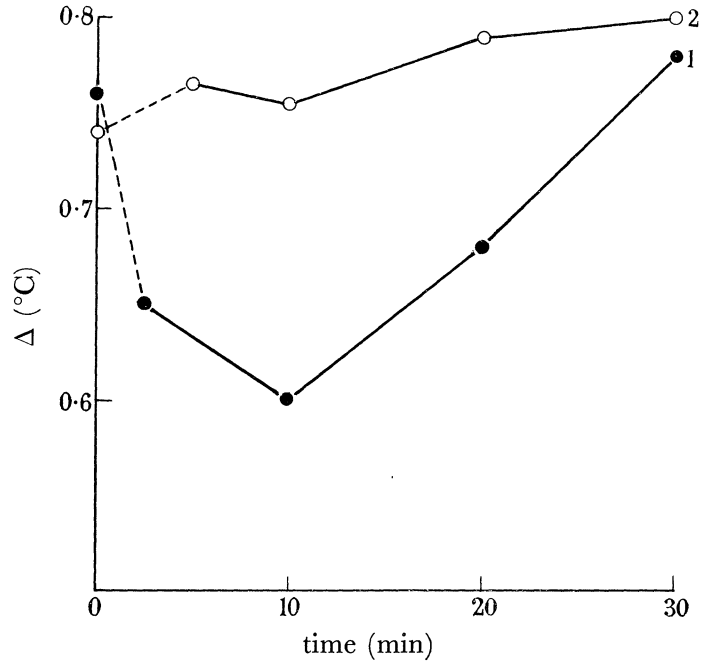

FIGURE 42. Changes in freezing-point depression in a small volume of medium bathing the isolated rectal complex. Two experiments, ○ and ●. In each case the medium was the mealworm's own haemolymph to which was added an equal volume of approximately 200 mM/l. potassium chloride (△ = 0·67 °C).

indicated by more than one point and the points are mutually supporting in the sense that they are not irregularly scattered (which might indicate experimental error) but indicate a continuous process of change with time. Although these experiments are admittedly exceptional we regard them as firm evidence of the ability of the rectal complex to lower the freezing-point depression of its external medium below the levels recorded for its internal media. The reasons for our failure to obtain equally firm evidence in a majority of experiments are further discussed in § 4.

(*e*) *Posteriorly directed flow of perirectal fluid*

After injection of a solution of indigo carmine in Ringer into the haemocoel no colour was ever observed in the perirectal fluid; this could be explained as the result of the rapid uptake of the dye into the free tubules and its rapid bleaching by the tissues. If a mealworm is injected with a slurry of solid indigo carmine suspended in Ringer the dye persists in the haemocoel for a much longer time. When mealworms thus injected were opened after 12 h, solid dye was sometimes found in the posterior end of the rectal complex, in the perirectal and subepithelial spaces. This dye presumably entered the rectal complex either through the permeable anterior end of the perinephric membrane, or by being drawn in under the sleeve as a result of the contractions of the rectal muscles; it is most unlikely that it entered the perirectal space from the rectum after having reached the

rectal lumen by way of the free tubules, for dye injected into the rectal lumen via the anus was never found in the perirectal space. The fact that the dye accumulated at the posterior end of the perirectal space suggests that there is a net movement of perirectal fluid in the antero-posterior direction.

(f) Concentration of sodium and potassium in the posterior perirectal fluid

Using 'dry' mealworms, and making special efforts to withdraw all the available fluid, it was possible to collect samples of posterior perirectal fluid ranging in volume from 0·5 to 3·0 nl., upon which it was possible to make single, and sometimes triplicate, determinations of sodium and potassium. The results listed in table 5 show that the concentrations of sodium and potassium in the posterior perirectal fluid are very variable. The average values, 134 m-equiv./l. for sodium and 163 m-equiv./l. for potassium, may be compared with the average values of 95 m-equiv./l. and 123 m-equiv./l. respectively for the anterior perirectal fluid (Ramsay 1964, table 3, serials 1, 2, 5, 6, 7 and 8). The same samples of anterior perirectal fluid have an average freezing-point depression of $\Delta = 1{\cdot}97$ °C; the average freezing-point depression of posterior perirectal fluid from 'dry' mealworms (table 6, present paper) is $\Delta = 4{\cdot}8$ °C. It is therefore obvious that the increase in the concentrations of sodium and potassium is less than would be indicated by a simple withdrawal of water from the anterior perirectal fluid, and it follows that both sodium and potassium must be taken up into the tubules as the perirectal fluid flows backwards in the rectal complex.

TABLE 5. ANALYSES OF POSTERIOR PERIRECTAL FLUID OF 'DRY' MEALWORMS

Na, K in m-equiv./l. to the nearest 5 m-equiv./l.

serial	Na	K
1	70	180
2	185	100
3	140	180
4	105	160
5	140	50
6	105	70
7	60	140
8	170	140
9	160	260
10	160	380
11	140	150
12	100	80
13	115	210
14	180	40
15	190	315
average	134	163

(g) Antero-posterior osmolarity gradient in the perirectal tubules

In the light of the earlier evidence (Ramsay 1964) showing first that the posterior perirectal fluid has a greater osmolarity than the anterior perirectal fluid, and secondly that water passes readily into the tubules from the perirectal space, it seems likely that in parallel with the antero-posterior osmolarity gradient in the perirectal space there is a similar gradient in the tubules.

Collection of tubular fluid from the posterior part of the rectal complex of 'dry'

mealworms was attempted but with limited success. The principal difficulties are first that (as aforesaid, § 3*c*) it is impossible to see the exact position of the tip of the pipette when it is thrust below the perinephric membrane, and secondly that the tubule lumen, being very much narrower here than it is anteriorly, is more difficult to penetrate and contains less fluid. In order to make the tubule visible the collecting pipette was filled with liquid paraffin stained with Sudan blue; it was thrust obliquely through the perinephric membrane into the region of the tubules and a small amount of the coloured liquid paraffin was forced out. If the tip of the pipette lay in the lumen of a tubule the colour filled the lumen for a short distance, making it possible to see the course of the injected part of the tubule and to guess at its course beyond. The pipette was then withdrawn and re-inserted so that its tip was judged to lie in the lumen of the tubule just ahead of the coloured injection. On application of slight negative pressure a very small amount of fluid could sometimes be collected. After the successful collection of a sample the tip of the pipette was withdrawn into the liquid paraffin covering the opened mealworm and a small amount of liquid paraffin was drawn in to seal the orifice. The end of the pipette containing the sample was then broken off and mounted in the freezing-point apparatus. Immediately after a successful collection samples of posterior perirectal fluid and of haemolymph were taken. The volumes of fluid collected from the tubules, being of the order of 10^{-5} to 10^{-6} μl. were much too small to be analyzed for inorganic ions, and only determination of freezing-point depression was undertaken.

TABLE 6. FREEZING-POINT DEPRESSION, Δ IN °C, OF POSTERIOR TUBULAR FLUID, POSTERIOR PERIRECTAL FLUID AND HAEMOLYMPH OF 'DRY' MEALWORMS, WITH SUBJECTIVE ESTIMATES OF ACCURACY

serial	posterior tubular fluid	posterior perirectal fluid	haemolymph
1	6·1 ±0·1	3·27 ± 0·01	0·92 ±0·01
2	9·0 ±0·5	6·5 ±0·5	0·95 ±0·01
3	7·4 ±0·25	5·0 ±0·25	0·97 ±0·01
4	>10	9·0 ±0·5	1·125 ± 0·01
5	7·5 ±0·1	5·7 ±0·5	1·045 ± 0·01
6	>10	7·0 ±0·5	1·045 ± 0·01
7	1·86 ± 0·01	1·91 ± 0·01	1·18 ±0·01
8	8·25 ± 0·2	2·72 ± 0·02	1·20 ±0·01
9	10·0 ±0·2	6·25 ± 0·2	1·20 ±0·01
10	8·25 ± 0·2	6·0 ±0·2	1·18 ±0·01
average (excluding serials 4 and 6)	7·3	4·8	

The results are assembled in table 6, each figure being qualified by a subjective estimate of its accuracy. In all cases except one (serial 7) the freezing-point depression of the tubular fluid is greater than that of the posterior perirectal fluid, and the average figures (excluding serials 4 and 6) are $\Delta = 7{\cdot}3$ °C for tubular fluid and $\Delta = 4{\cdot}8$ °C for posterior perirectal fluid. The figure $\Delta = 7{\cdot}3$ °C may be compared with the average figure of $\Delta = 1{\cdot}97$ °C for tubular fluid collected from the anterior perirectal tubules of 'dry' mealworms (Ramsay 1964, table 3, serials 1, 2, 5, 6, 7 and 8).

It is therefore clear that, as in the case of the perirectal fluid, there is a gradient of osmolarity in the tubular fluid increasing from anterior end to posterior end, and that

there is also a difference in osmolarity between the posterior tubular fluid and the posterior perirectal fluid such as would tend to draw water into the tubules from the perirectal space.

(*h*) *Anomalous behaviour of perirectal fluid on freezing*

The perirectal fluid behaves in an unusual way on freezing, as described by Ramsay (1964, p. 298, footnote). Briefly, the crystals of ice disappear normally as the temperature is raised but fail to grow in size as the temperature is lowered; the water–ice system is not reversible with temperature. A possible explanation is that the perirectal fluid contains some material which, because of surface activity, invests the ice crystals and prevents water molecules from settling upon them but does not offer the same impediment to the escape of water molecules from crystals which are melting. The presence of such material in a water–ice system would be equivalent to the presence of a non-return valve.

It is perhaps not at first sight apparent how this property could be of physiological significance in the context of water transport in ice-free systems. However, it is to be remembered that within the fine channels of porous materials water molecules are unlikely to be randomly arranged as they are in the bulk liquid phase (Derjaguin 1965), and anything which differentially affects movement from oriented layers to bulk phase and movement in the reverse direction could conceivably act as a valve in a cellular water pump. Attempts were therefore made to isolate the material responsible for this effect, and we have to record our indebtedness to Dr B. S. Hartley for assistance and advice.

Preliminary experiments showed that the 'active' material could be extracted from mealworms homogenized in 50% ethanol, that it was a protein ('activity' abolished by trypsin) and that it was negatively charged (paper electrophoresis). A crude preparation was then made according to the following procedure. 100 g mealworms homogenized in 200 ml. ethanol for 2 min; 200 ml. distilled water added and homogenization continued for 10 min. Clear fluid separated by filtration and centrifugation, concentrated by evaporation to 5 ml. Allowed to stand for 12 h., centrifuged, supernatant run on Sephadex. 'Active' fractions pooled, run on DEAE (0·02 M tris, pH 8·0 adding M NaCl). 'Active' fractions pooled, run on Sephadex. 'Active' fractions pooled, evaporated and freeze-dried. A total of 330 g mealworms yielded 0·55 g crude preparation. The method of Andrews (1964) indicated a molecular weight of 10000 to 12000.

If this material or something like it is of physiological significance in relation to a water pump it might be found in other xerophilous insects living in stored products. The body fluids of *Ephestia kuhniella*, *Tribolium confusum*, *Sitophilus granarius* and *Dermestes lardarius* (available as laboratory stocks) were tested for abnormal behaviour on freezing, but no comparable effect was found. It was then decided that the idea was not sufficiently promising to justify further investigation. The properties of this material remain for the present no more than a biophysical curiosity.

Discussion

We interpret the mechanism of the rectal complex in terms of a model which is illustrated in figure 43 and will now be described. The description, which applies to 'dry' mealworms, embodies the following propositions.

(*a*) The main input into the rectal complex is via the intestine. The intestinal fluid is isosmolar with haemolymph; it contains relatively less sodium and more potassium, and it contains faecal matter in suspension. On reaching the rectal lumen at the level of the anterior end of the rectal complex most of the fluid is rapidly absorbed and the faecal matter is formed into pellets as it passes along the rectal lumen. At the posterior end of the

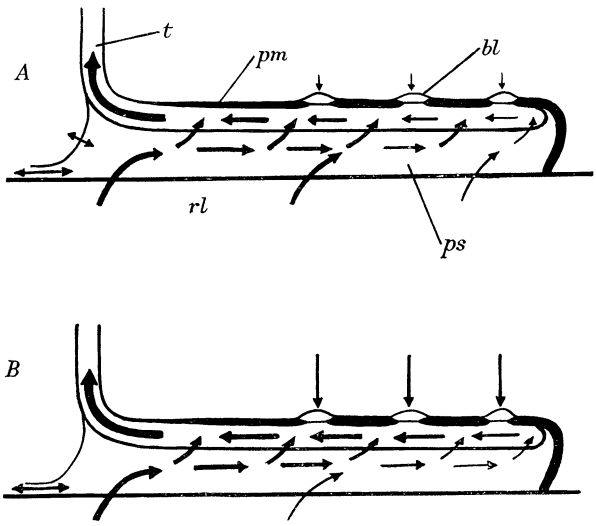

FIGURE 43. Model proposed for the mechanism of the rectal complex, showing: *A*, movements of water; *B*, movements of potassium. *bl*, blister over leptophragma; *pm*, perinephric membrane; *ps*, perirectal space; *rl*, rectal lumen; *t*, tubule. Further details in the text.

rectum the spaces between the pellets are filled with air, and removal of water from the faecal pellets is brought about by absorption of water vapour by the rectal epithelium, thus creating an atmosphere of lowered humidity into which the faecal pellets lose water by evaporation before being voided through the anal canal. This absorption of water vapour is probably an active process, facilitated by the high osmolarity of the perirectal fluid at the posterior end of the rectal complex.

(*b*) Some at least of the fluid absorbed from the rectum then flows backwards in the perirectal space. Sodium and potassium are taken up by the tubules and water follows passively. Other solutes (presumed to be mainly non-electrolytes) accumulate in the posterior perirectal fluid.

(*c*) Potassium ion is actively transported from the haemolymph into the tubular fluid via the leptophragmata, chloride ion following passively.

(*d*) While secreting potassium chloride into the tubular fluid the leptophragmata resist the tendency of water to follow. This is the cause of the high osmolarity of the fluids in the posterior part of the rectal complex.

The evidence for these propositions will now be reviewed.

(*a*) This proposition rests upon the work of Ramsay (1964), and the experimental evidence for it will not be further discussed here except in relation to the details of fine structure which have been described in § 2.

It is a requirement of the model that the rectal complex must be isolated from the haemocoel by some relatively impermeable barrier whereby the high osmolarity of the

posterior perirectal fluid is conserved. On the basis of its fine structure we feel confident that the inner sheath of the perinephric membrane constitutes this barrier. In its multilaminate nature, in which the individual laminae appear to be flattened cells, it stands in contrast to the outer sheath, formed of a single layer of cells. Indeed, above the leptophragmata the cells of the outer sheath are lacking, and only basement membranes separate the spaces between the inner and outer sheaths (and the cavities of the blisters) from the haemocoel. Further support comes from the comparison of the anterior and posterior regions of the rectal complex which shows a lower permeability in the posterior region, correlated with a greater thickness of the inner sheath. The contribution of the leptophragmata to the permeability of the posterior region is discussed under (*c*) below.

Since the publication of a paper by Wigglesworth (1932) on the function of the rectal glands in insects it has been generally accepted that these are concerned with the removal of water from the faeces, and active transport of water has been demonstrated in the rectum of the locust by Phillips (1964). With this in mind Ramsay took the view that the rectal epithelium of the mealworm probably made an active contribution to water uptake, its task being made easier by the high osmolarity of the posterior perirectal fluid. But we now find that the epithelial cells of the rectum lack many of the structural features which in other cells are thought to indicate direct participation in active transport. For example, there are no complex surface infoldings associated with mitochondria, nor are the surface membranes provided with regular arrays of small 'particles'; such features are thought to be involved in ion pumps or water pumps in the rectal papillae of other insects (Copeland 1964; Gupta & Berridge 1966). Nevertheless, it seems unlikely that the rectal epithelium is wholly passive towards the movements of water through it. Saini (1962) observed a substantial difference in freezing-point depression between rectal fluid ($\Delta = 2{\cdot}15$ °C) and perirectal fluid ($\Delta = 1{\cdot}65$ °C); these figures must relate to the anterior part of the rectal complex since (at least in 'dry' mealworms) there is no fluid in the posterior part of the rectal lumen. For this reason no comparable figures are available for 'dry' mealworms, but Ramsay's (1964) measurements of the relative humidity in equilibrium with the faeces and of the freezing-point depression of the perirectal fluid, although not made on the same animals, indicate that the activity of water is lower in the rectal lumen than in the perirectal space. In view of the ability of the mealworm to take up water from unsaturated air (88% r.h.) through the general body surface (Mellanby 1932) it would not be surprising to find that this ability extended to the rectal epithelium, which is morphologically an invagination of the surface of the body.

(*b*) The direct evidence for a posteriorly directed flow of perirectal fluid is presented in § 3 (*e*). The existence of this flow is a significant feature of the model in that it explains the conservation of the osmolarity gradient in the perirectal space. The freezing-point depression of the perirectal fluid is greater at the posterior end, and other features such as increased viscosity suggest that proteins and other organic solutes are concentrated here. Since there is free communication between the two ends of the perirectal space one would expect this difference in concentration to be dissipated by diffusion, assisted by convection caused by contractions of the rectal musculature. In the model the uptake of water and inorganic ions into the tubules is the cause of the posteriorly directed flow of fluid, and this flow in turn counteracts the dissipative tendencies. The evidence for the uptake of

sodium and potassium from the posterior perirectal fluid into the tubules is given in § 3 (*f*), and the generally higher osmolarity of the tubular fluid as compared with the posterior perirectal fluid suggests that water will move in the same direction.

In brief, we envisage the perirectal tubules as being in the same physiological relationship to the perirectal fluid as ordinary free tubules are to the haemolymph. That is to say, they take up potassium and sodium but do not maintain a large difference of osmolarity across their walls. This last statement requires qualification in the light of the figures given in table 6 where an average difference of 2·5 °C in freezing-point depression between posterior tubular fluid and posterior perirectal fluid has been recorded. It is by no means certain that in any given mealworm these pairs of collections were made at precisely the same level in the rectal complex, since they had to be made 'blind', and for this reason we are not inclined to take this difference too seriously. In any case its existence or otherwise is not of vital importance for our model.

Throughout the insects there is a good deal of variation in the appearance of the Malpighian tubules as seen with the light microscope, but as yet little progress has been made in exploring this variation with the electron microscope. Wessing (1965) has reviewed these studies. In all Malpighian tubules basal infoldings and a brush border are present. In the brush border some of the microvilli contain mitochondria. Mitochondria may be associated with the basal infoldings, but not always closely. Wessing himself, and others, have described a system of tubules of endoplasmic reticulum extending from the basal infoldings to the apical border, often opening at the tips of the microvilli. A great variety of inclusions are present in the cytoplasm, varying from one species of insect to another and from one time to another within the same species.

In the rectal complex of the mealworm it is the tubules of the anterior region which most closely conform to the generalized description of a Malpighian tubule. We have not seen precisely those developments of the endoplasmic reticulum to which Wessing has drawn attention, but the other features are characteristically developed. On the other hand, the posterior tubules are unusual in the following respects:

(1) The brush border is more highly developed than in the Malpighian tubules so far described in other insects.

(2) Basal infoldings are well developed, as in the Malpighian tubules of many (though not all) insects (e.g. Berkaloff 1961; Beams, Tahmisian & Devine 1955). But in *Tenebrio* the infoldings are not associated with mitochondria, and they are restricted in distribution to those parts of the tubule cell which face the perirectal space.

(3) The cytoplasm shows little development of endoplasmic reticulum, but is filled with numerous small granules.

The development of the brush border is probably to be understood in relation to the active transport of potassium. Active transport of potassium has been demonstrated in the Malpighian tubules of all the insects so far examined (Ramsay 1953), and the presence of mitochondria within some, at least, of the microvilli is a regular feature of the fine structure of Malpighian tubules. In this connexion attention should also be drawn to the similarities between the cells of the posterior tubules and the goblet cells of the mid-gut of *Hyalophora cecropia*, described by Anderson & Harvey (1966) and believed by them to be responsible for the secretion of potassium. The goblet cells have basal and lateral

infoldings, not associated with mitochondria; mitochondria are present in apical processes which seem to correspond to the microvilli of the tubule cells of the mealworm not only in this respect but also in the presence of connexions between the mitochondria and the plasma membrane. These connexions, as pointed out by Anderson & Harvey, may be related to the 'particles' associated with surface membranes which seem likely to be concerned in ion transport (Gupta & Berridge 1966; Noirot, Noirot-Timothée & Kovoor 1967).

What particularly distinguishes the posterior perirectal tubules is the relatively enormous concentration of potassium chloride (more than 2 mole/l.) which is maintained in the lumen. We regard the development of the brush border as the structural correlate of this physiological feature.

(*c*) The experiments described in § 3 (*c*) of this paper make it reasonably certain that potassium is transported into the rectal complex from the medium which bathes it; any alternative explanation of the data presented in tables 1, 2 and 3 would be very involved. These experiments do not tell us whereabout on the perinephric membrane the active transport of potassium takes place. Assuming that the inner sheath of the perinephric membrane is to all intents and purposes impermeable to everything, the possible routes by which potassium might enter are either via the anterior region of the perinephric membrane or via the leptophragmata. There is nothing in the fine structure of the anterior perinephric membrane which is even remotely suggestive of a site of active transport. At a time when there was no evidence of the presence of mitochondria in the leptophragma cell some thought was given to the possibility of alternative mechanisms for the accumulation of potassium, designed to place the burden of active transport upon the mitochondria of the tubules. But it has now been found that the leptophragma cell has a normal complement of mitochondria and it is known that the leptophragmata themselves are active sites of dehydrogenase activity as demonstrable with tetrazolium salts (Miss I. B. Colvin and Mrs A. M. Chase, independently, personal communication), which is suggestive of high metabolic activity. No part of the perinephric membrane itself shows this property. We therefore propose, as the simplest explanation of the observations, that potassium ion is actively transported from haemolymph to tubular fluid via the leptophragmata and that chloride ion follows passively.

The position in regard to sodium is uncertain. Assuming that there is no net movement of sodium, then if potassium chloride is secreted into the tubules without water (the freezing-point depression of the external medium falling in consequence) the sodium concentration in the external medium should remain unchanged; if the potassium chloride is accompanied by water (the freezing-point depression remaining constant) then the sodium concentration should rise. In most of our experiments both freezing-point depression and sodium concentration remain more or less constant. This can be understood if one supposes that a small amount of sodium, much less than the amount of potassium, is also secreted into the tubules, and we see no objection to this. But since so many other osmotically active solutes are not accounted for, or even identified, we do not think it profitable to discuss solute transfer any further.

While we have attributed secretory activity to the leptophragma cell we recognize that this cell does not show many of the features which in other situations are thought to be

concerned in ion transport. In fact, the only characteristic feature seems to be the presence of microvilli. Although mitochondria are present, they are not particularly abundant, nor are they located in the microvilli or specially close to any other part of the cell surface. There is no development of agranular endoplasmic reticulum, often considered to be associated with transport function (e.g. Ito 1961; Ito & Winchester 1963; Tormey 1963). The most conspicuous structural features of the leptophragma cells are the large cytoplasmic vesicles, but it has not yet been possible to ascribe a physiological role to these. It may, however, be worth mention that an ion-transport function has been suggested for complex vesicles found in the salt-secreting rectal glands of elasmobranchs (Doyle 1962).

If we are right in attributing the active transport of potassium to the leptophragma cell, there then presents itself the challenging problem that side by side in the same tissue there are two cell types of entirely different fine structure, the tubule cell and the leptophragma cell, both principally concerned in the active transport of potassium. We have no suggestions as to how these fine-structural differences may be interpreted except that any interpretation will have to take account of the different physiological properties of these cells in relation to the passage of water, now to be discussed.

(*d*) If we take the view that the inward secretion of potassium chloride via the leptophragmata is the cause of the high osmolarity of the tubular and perirectal fluids, then we must also take the view that the leptophragmata are relatively impermeable to water. Whereas the tubule cell secretes potassium chloride into the lumen, allowing water to follow passively, the leptophragma cell secretes potassium chloride into the lumen but must resist the tendency of water to follow passively, otherwise the osmolarity of the tubular fluid would not rise above that of the haemolymph. This conclusion appears to be inescapable.

Unquestionably the weakest part of the evidence by which we seek to support our model is that relating to this requirement. As already described, we have only exceptionally been able to demonstrate a substantial fall in the freezing-point depression of the medium bathing the rectal complex; and it is perhaps surprising that these exceptional experiments involved 'moist' mealworms, since it might be supposed that the effect would be more marked, and therefore more readily demonstrable, in 'dry' mealworms. A possible explanation is that the impermeability of the leptophragma to water may be an 'active' property in the sense that it depends upon the cell being in good physiological condition. Even when the rectal complex is studied as an *in situ* preparation with its tracheal connexions intact, it is observed (Ramsay 1964) that the freezing-point depression of the tubular fluid declines steadily over the course of some hours, as though the uptake of water outpaces the uptake of potassium chloride in a deteriorating preparation. In the experiments herein reported, in which the isolated rectal complex was deprived of its tracheal supply, the rate of deterioration may well have been much more rapid. This would be in line with the general tendency of the freezing-point depression of the external medium to rise towards the end of the experiment after an initial fall. It would also account for our failure to observe any fall of freezing-point depression when 'dry' mealworms were used, since in this case the higher osmolarity of the fluids in the rectal complex would draw water in still more rapidly, entirely masking the effect of uptake of potassium chloride.

In 'moist' mealworms all the fluids collected from the rectal complex tend to become isosmolar with haemolymph. This could be explained as a consequence either of a cessation of the inward secretion of potassium chloride at the leptophragmata or of an increase in the permeability of the anterior perinephric membrane and the leptophragmata to water. In view of the evidence for the continued uptake of potassium by the isolated rectal complex in 'moist' mealworms (table 1 and figure 40) we favour the second explanation. We have evidence, admittedly qualitative only, that the permeability of the anterior perinephric membrane is increased in 'moist' mealworms, and this might well explain the fact that all the fluids in the anterior part of the rectal complex become isosmolar with haemolymph. Whether alone it can explain the fall in osmolarity of the posterior perirectal fluid, without it being necessary to suppose that there is also an increase in the permeability of the leptophragmata to water, is less certain. But we have no evidence of differences in the permeability of the leptophragmata in 'dry' as compared with 'moist' mealworms. In the case of the anterior perinephric membrane it certainly seems likely that its permeability varies in accordance with the mealworm's state of water balance and it may be that its permeability changes are part of a general hormonal control of water balance, such as has been demonstrated in other insects (Berridge 1966; Maddrell 1964; Núñez 1956).

From the point of view of the model the most significant structural features which have come to light are: first, the inner sheath of the perinephric membrane, which strongly suggests an impermeable barrier; and secondly, the presence of mitochondria and microvilli in the leptophragma cell, which allow us to think that it may participate in active transport. On the physiological side this paper contributes two main pillars of evidence in support of the model: first, that potassium chloride is actively transported into the rectal complex from the haemolymph; and secondly, that the rectal complex is able to lower the osmolarity of the haemolymph. This latter we have not observed except upon occasion, but we are convinced of its reality.

We are only too well aware that we have not been able to show that these two effects are sufficient to account for the large differences in osmolarity which we have recorded; we are also well aware that when our data are examined in detail certain minor inconsistencies appear. But we believe that the principal evidence supports the model; we are not aware of any telling evidence against it; and where evidence is lacking the model does not appear to us to involve any implausible assumptions.

The question may well be asked, however, as to whether the model is applicable to the cryptonephric systems of other insects. In our model the burden of creating a condition of high osmolarity within the perinephric membrane is laid upon the inward secretion of potassium chloride by the leptophragmata; but the leptophragmata are not invariably present in cryptonephric systems (Saini 1962, 1964, and earlier workers quoted therein). In a recent review Kirschner (1967) has raised the possibility that the rectal complex operates as a hairpin countercurrent multiplier. It seems to us that since the tubular and perirectal fluids move in opposite directions, the osmolarity gradient in the rectal complex might arise by the multiplication of a unit effect whereby at all levels the osmolarity of the perirectal fluid would be raised above that of the tubular fluid by appropriate transfer of water or solutes; and that by multiplication this would produce a gradient of osmolarity

rising in the antero-posterior direction. In support of this possibility may be cited the observation by Ramsay (1954)* that in the isolated tubules of the stick insect the tubular fluid is slightly hypo-osmolar to the external medium; this, if general, could be the basis of a unit effect which might then be multiplied to produce an antero-posterior gradient of osmolarity within a cryptonephric system, whether it was provided with leptophragmata or not. On this view one might suppose that the primitive mechanism of cryptonephric systems is a countercurrent multiplier which may be reinforced by the inward secretion of potassium chloride at leptophragmata where these are present. But in the case of the rectal complex of the mealworm such evidence as we have (table 6) indicates that at all levels the tubular fluid is hyperosmolar to the perirectal fluid, and if this difference were ascribed solely to exchange between perirectal fluid and tubular fluid the effect of multiplication would be to produce an osmolarity gradient not increasing in the antero-posterior direction, but decreasing. The rectal complex of the mealworm, as compared with the cryptonephric systems of other insects, is notably well supplied with leptophragmata; and if, as we suggest, they secrete potassium chloride into the tubules this activity could mask any unit effect upon which countercurrent multiplication might be based. We do not reject the countercurrent-multiplier hypothesis, but as far as they go our observations do not lend support to it.

Our thanks are due to the following for valuable suggestions and stimulating discussion; Professor Sir Vincent Wigglesworth, F.R.S., Professor L. B. Kirschner, Dr B. L. Gupta and Dr S. H. P. Maddrell. We are also indebted to Dr D. S. Smith for providing some fixed material, to Mr W. B. Amos for help with the line drawings and to Mr D. W. Chapman for technical assistance.

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* This paper bears the unfortunate title of 'Active transport of water by the Malpighian tubules of the stick insect'. The movement of water against an osmotic gradient is an active process when regarded from the standpoint of classical thermodynamics, but need not necessarily be so when regarded from the standpoint of steady-state thermodynamics in the context of the simultaneous active transport of solute.

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Description of plates 18 to 32

All figures (except figure 5) are electron micrographs. Material was fixed as follows:

(i) glutaraldehyde and paraformaldehyde, followed by osmium tetroxide (figures 5, 8, 9, 11, 14, 18, 20–24, 28, 30, 33 and 35–37;

(ii) glutaraldehyde, followed by osmium tetroxide (figures 7, 10, 12, 15, 16, 19, 25–27, 29, 31, 32 and 34);

(iii) osmium tetroxide alone (figures 6, 13 and 17). All sections were stained with both uranyl acetate and lead citrate. Figures 18–20, 26 and 27 are of the anterior region of the rectal complex. All other figures show the posterior region.

Abbreviations

bb	brush border
bi	basal infolding of tubule cell
bl	blister over leptophragma
bm	basement membrane
bo	boursouflure
cl	cytolysome
cm	circular muscle
cu	cuticle
en	endocuticle
ep	epicuticle
ex	exocuticle
g	glycogen
ic	intertubular cell
is	inner sheath
l	lipid body
lc	leptophragma cell
lm	longitudinal muscle
m	mitochondrion
mt	microtubule
n	nucleus
os	outer sheath
pm	perinephric membrane
ps	perirectal space
r	ribosome
re	rectal epithelium
rl	rectal lumen
sd	septate desmosome
ss	subepithelial space
t	tubule
tc	tracheolar end cell
tl	tubule lumen
v	vacuole

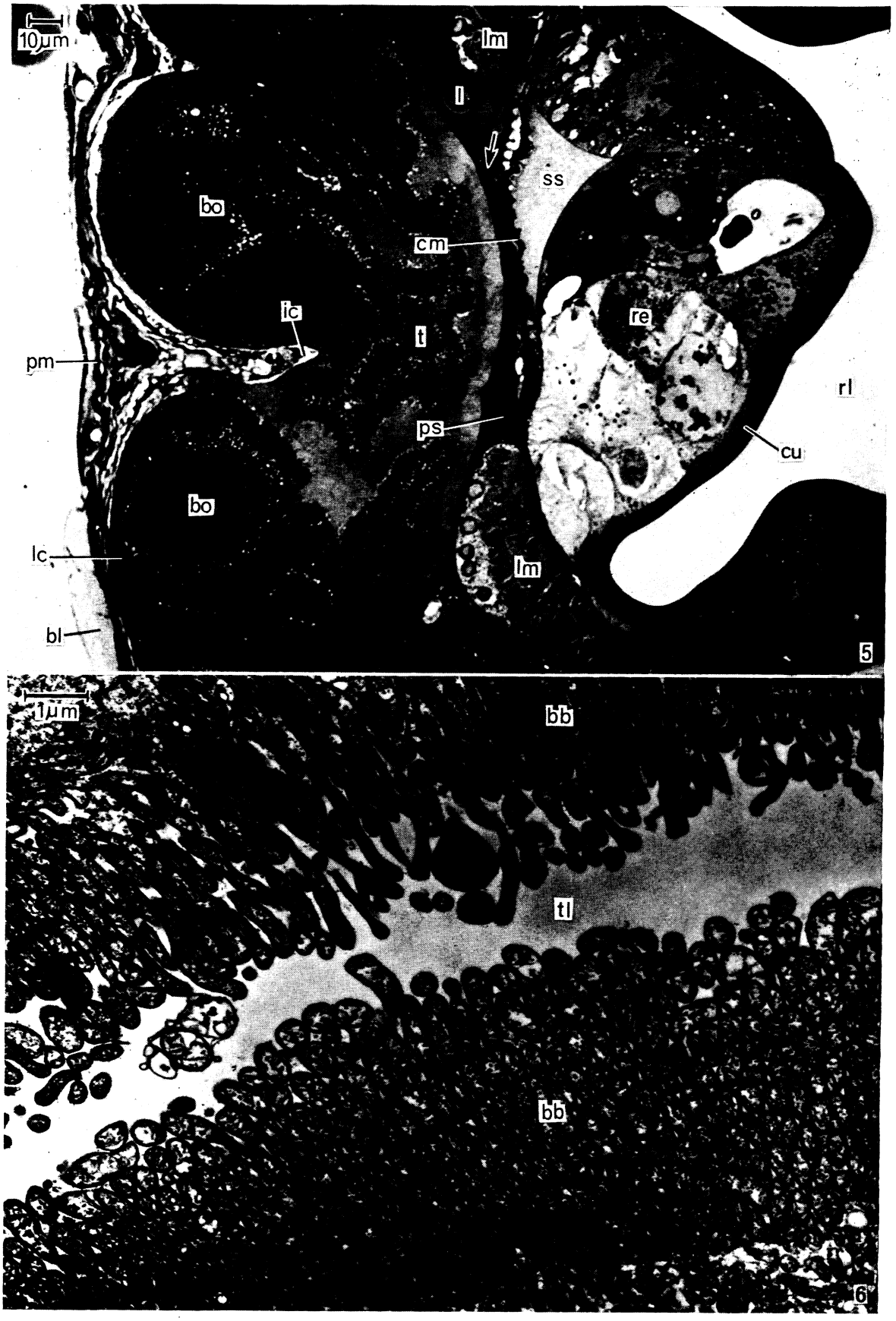


FIGURE 5. Photomicrograph of a 1 μm Araldite section stained with methylene blue, showing the main components of the rectal complex in transverse section. Parts of three boursouflures, all belonging to one tubule, are visible. The dark areas within these are the brush borders, lining the narrow lumen of the tubules. Note the homogeneous contents of the perirectal and subepithelial spaces, the former in this instance being more densely stained than the latter and containing spherical, possibly lipid, bodies. Compare these spaces with the supposed peritubular space, in which intertubular cells but no homogeneous contents can be seen. Note the connexion (arrow) between the tubule and circular muscle. × 620.

FIGURE 6. Low-magnification micrograph showing the lumen and brush borders of what is assumed to be a well fixed tubule. × 11000.

(*Facing p.* 382)

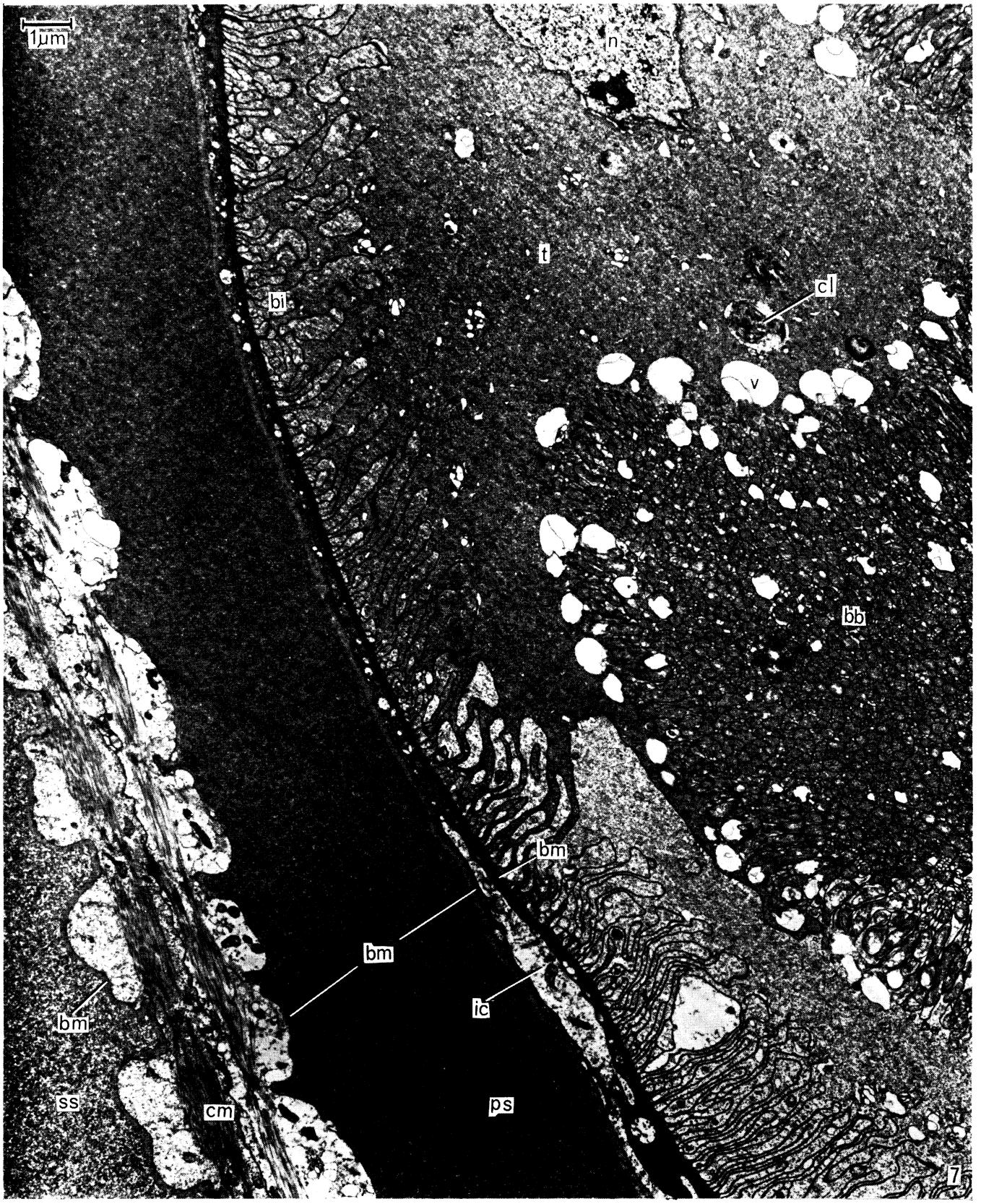


FIGURE 7. Survey micrograph showing part of a tubule and its relation to the perirectal and subepithelial spaces. Note the thin layer of intertubular cells between the basement membrane of the tubule, and that lining the perirectal space. Empty vacuoles are present below the brush border of the tubule. × 9000.

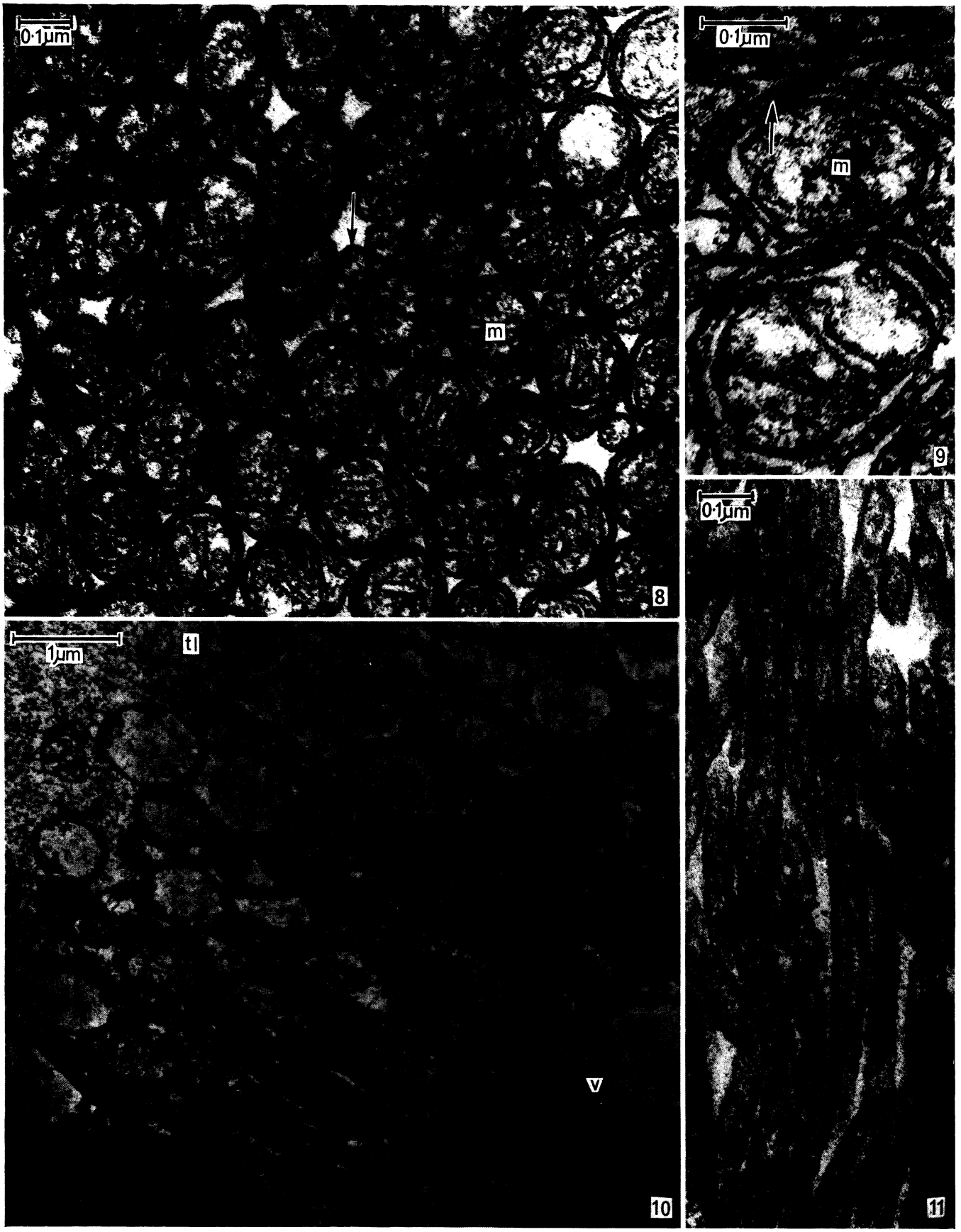


FIGURE 8. Micrograph showing the microvilli of the tubule brush border in transverse section. Note that while most of them contain a mitochondrion, a few (arrow) do not. × 100000.

FIGURE 9. Higher magnification micrograph of microvilli of tubule brush border, showing the connexions (arrow) between the outer mitochondrial membrane and the plasma membrane. × 160000.

FIGURE 10. Brush border of a tubule, showing swollen microvilli and vacuoles in the cytoplasm below. × 20000.

FIGURE 11. Micrograph showing a group of tubule cell microvilli without mitochondria. × 100000.

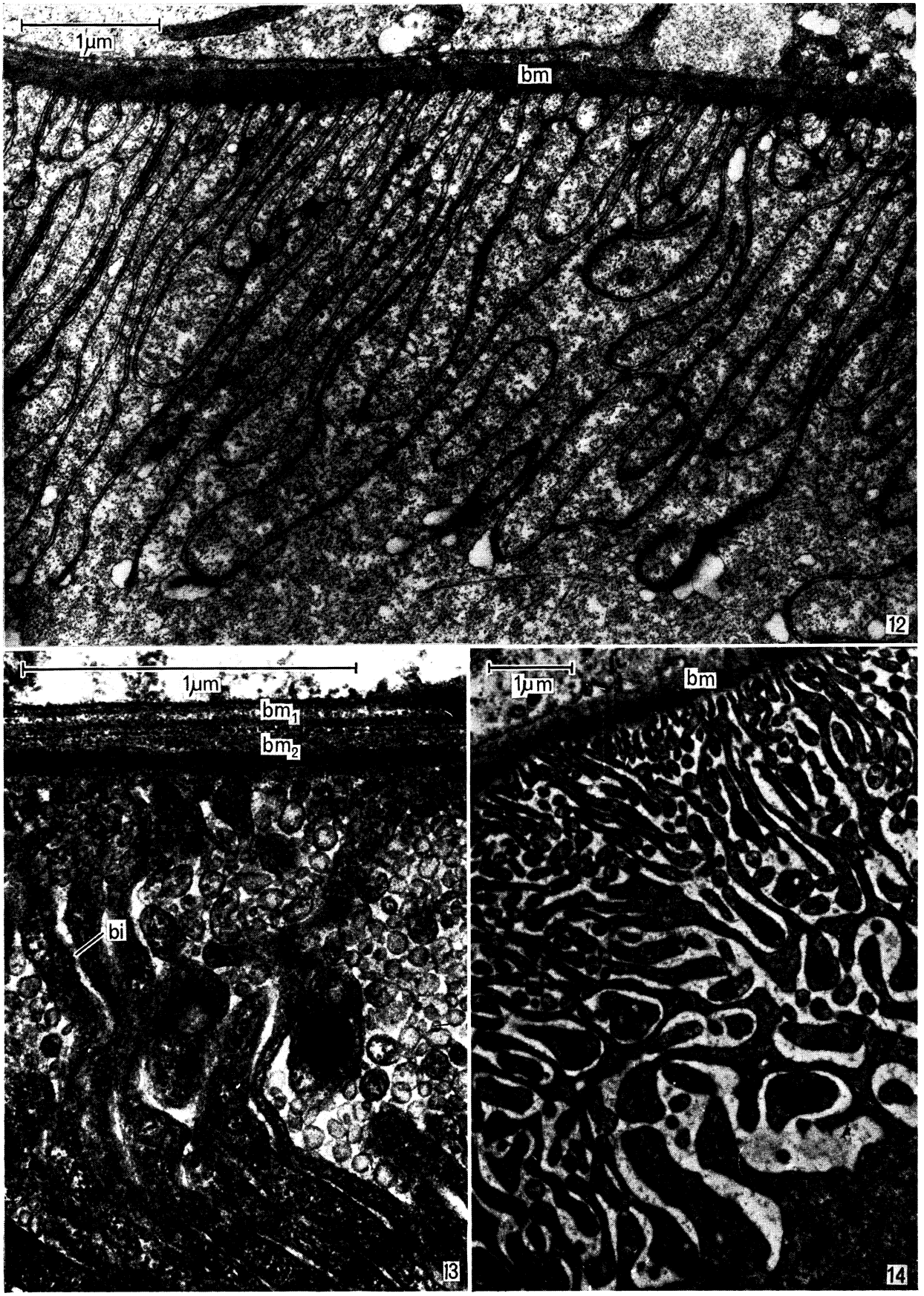


FIGURE 12. Section showing the basal region of a tubule cell, with basal infoldings in the form of closely apposed membranes. 'Moist' mealworm. × 25000.

FIGURE 13. Basal region of a tubule after fixation in osmium tetroxide. In addition to the infoldings, numerous small, apparently extracellular vesicles are present. (bm_1, basement membrane of longitudinal muscle; bm_2, basement membrane of tubule). × 60000.

FIGURE 14. Micrograph showing basal infoldings in the form of inflated channels in a tubule cell from a 'dry' mealworm. Compare this with figure 12. × 15000.

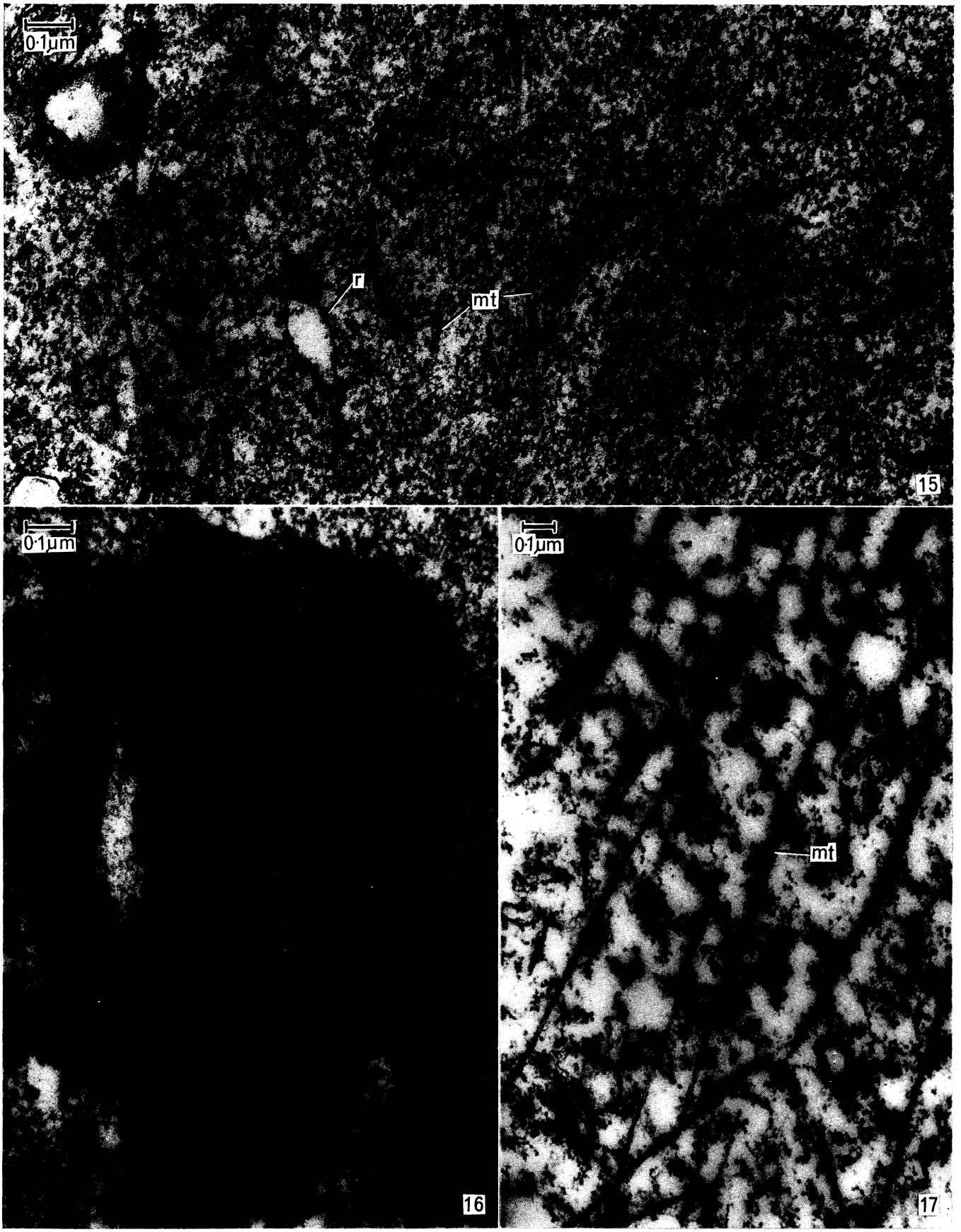


FIGURE 15. Micrograph showing the typical appearance of tubule cell cytoplasm after glutaraldehyde fixation. Note the abundant microtubules, and the marked difference in size and electron density between the ribosomes and the small granules filling much of the cytoplasm. × 90000.

FIGURE 16. Cytolysome from a tubule cell. × 90000.

FIGURE 17. Tubule cell cytoplasm after osmium fixation. Microtubules are present, as in figure 15, but in this preparation fewer of the small granules have been preserved. × 60000.

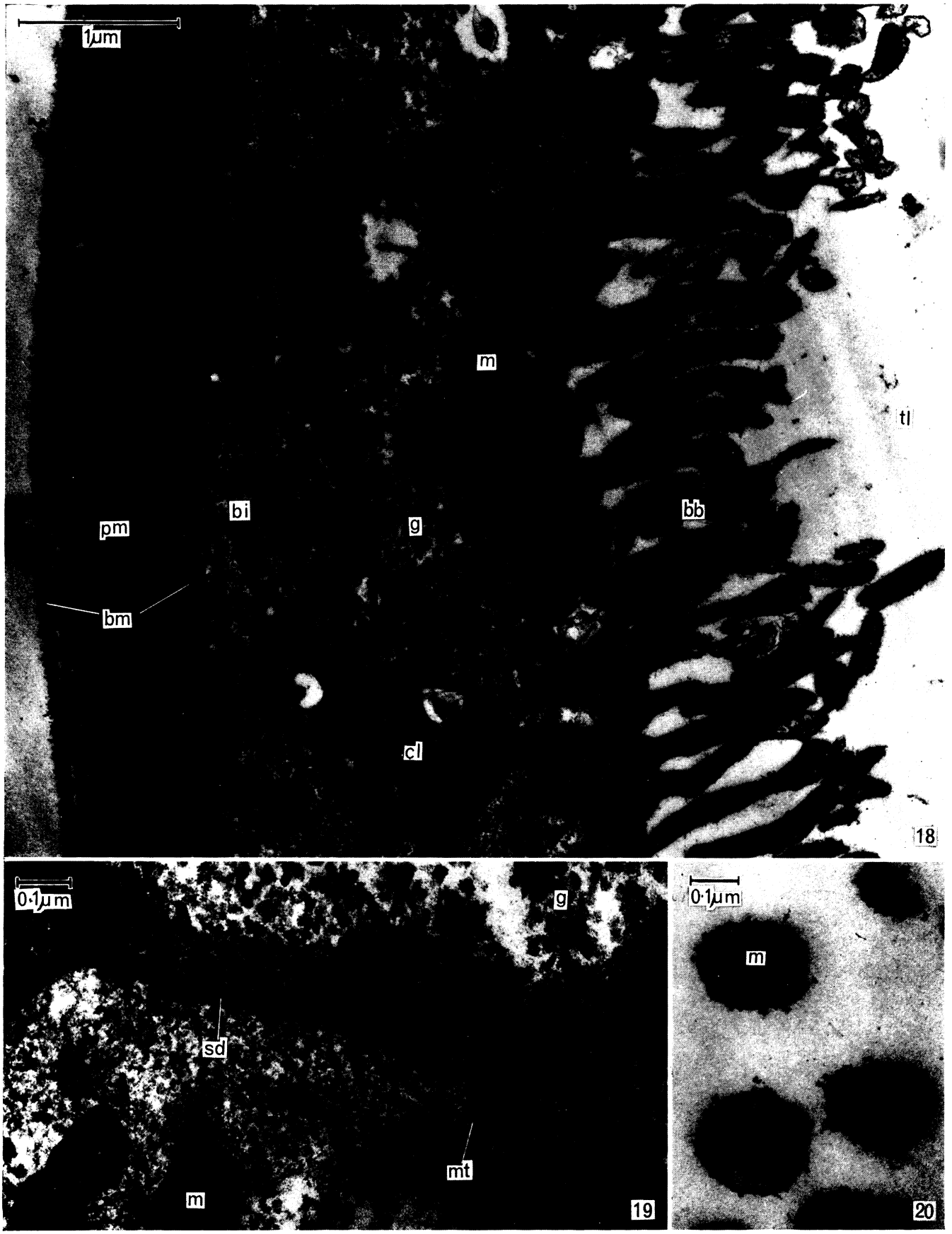


FIGURE 18. Micrograph showing part of a tubule and the surrounding perinephric membrane, from the anterior region of the rectal complex. Compared with the posterior region, the tubule wall is much thinner, with less well-developed brush border and basal infoldings, and the perinephric membrane is less complex. × 30000.

FIGURE 19. Junctional region between the apical ends of the lateral borders of two tubule cells. Towards the tubule lumen (left) there is a septate desmosome. Elsewhere the two cell membranes are more widely separated. × 100000.

FIGURE 20. Transverse section of microvilli with mitochondria from the brush border of the anterior region of a tubule. × 90000.

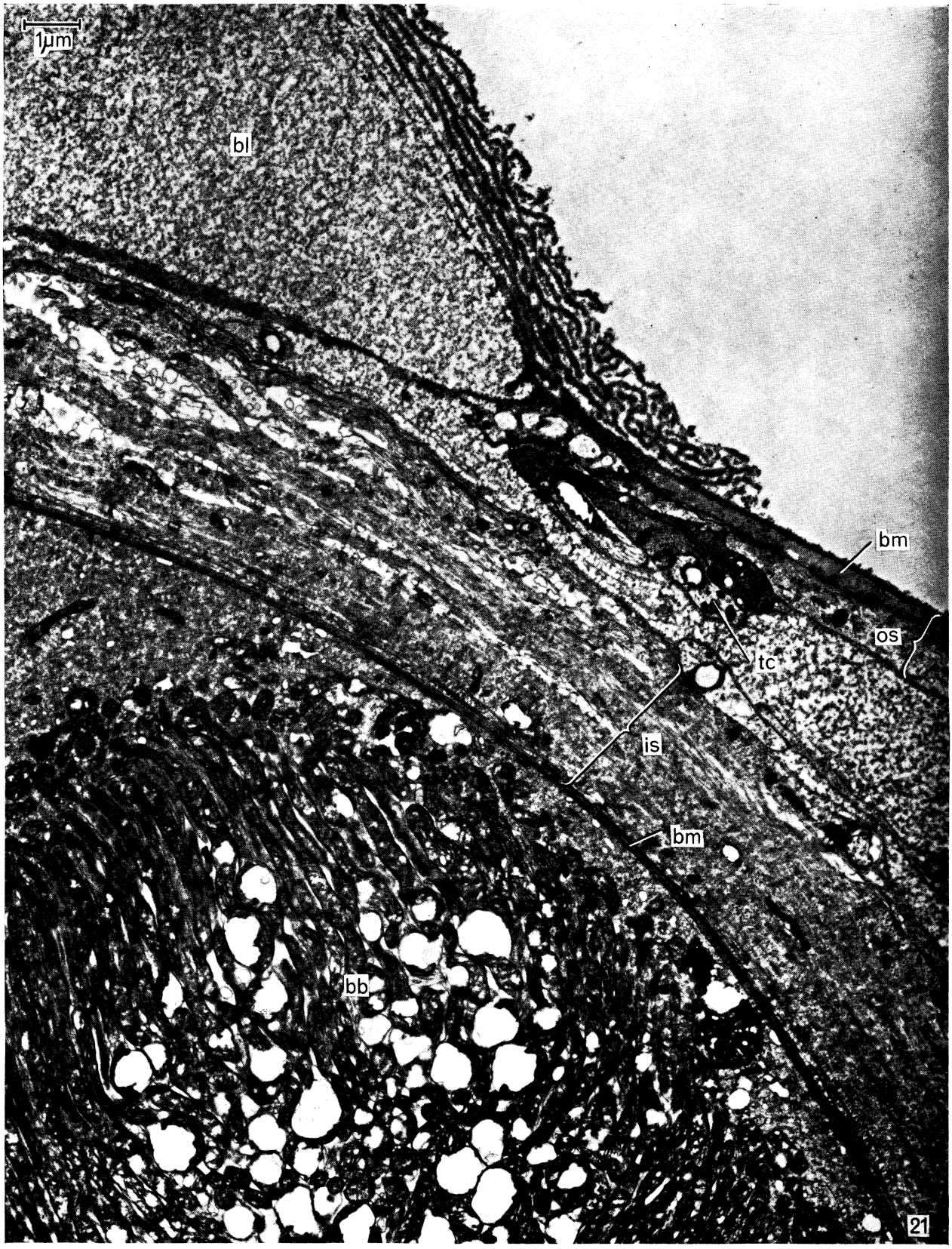


FIGURE 21. Survey micrograph showing part of a tubule, and the perinephric membrane, formed of inner and outer sheaths with tracheolar end cells in the space between. The section includes the edge of a blister overlying a nearby leptophragma. Note the multilaminate basement membrane covering the blister. × 10000.

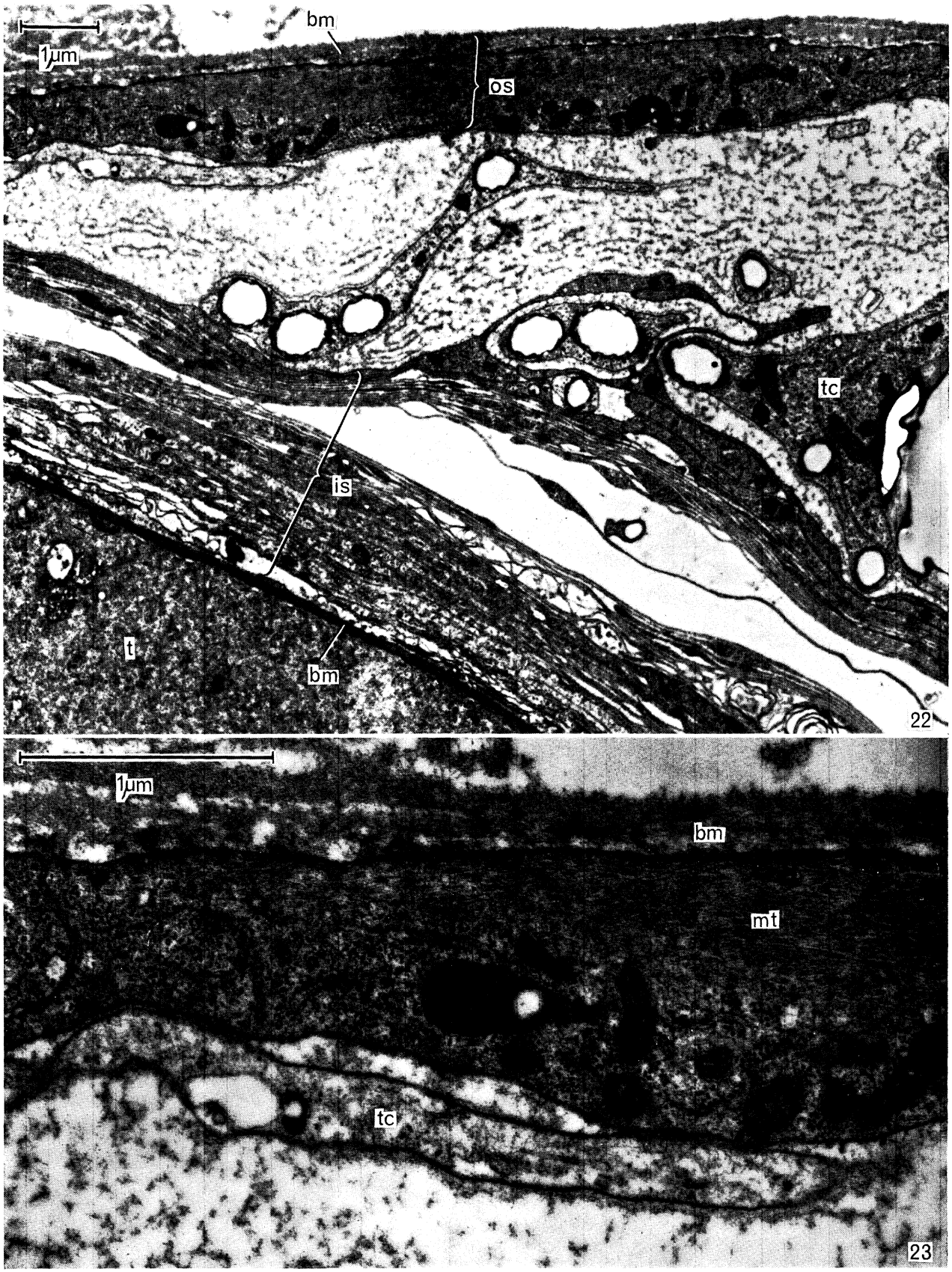


FIGURE 22. Micrograph showing the perinephric membrane. Note the difference in structure of the inner and outer sheaths. The gap in the former is probably an artifact, but shows plainly the form of one of the cell layers, which has become detached from its neighbours. × 14000.

FIGURE 23. Enlargement of figure 22 showing the single cell layer forming the outer sheath. Note the densely packed microtubules in the outer region of these cells; the inner region of the outer sheath contains mitochondria and endoplasmic reticulum. × 45000.

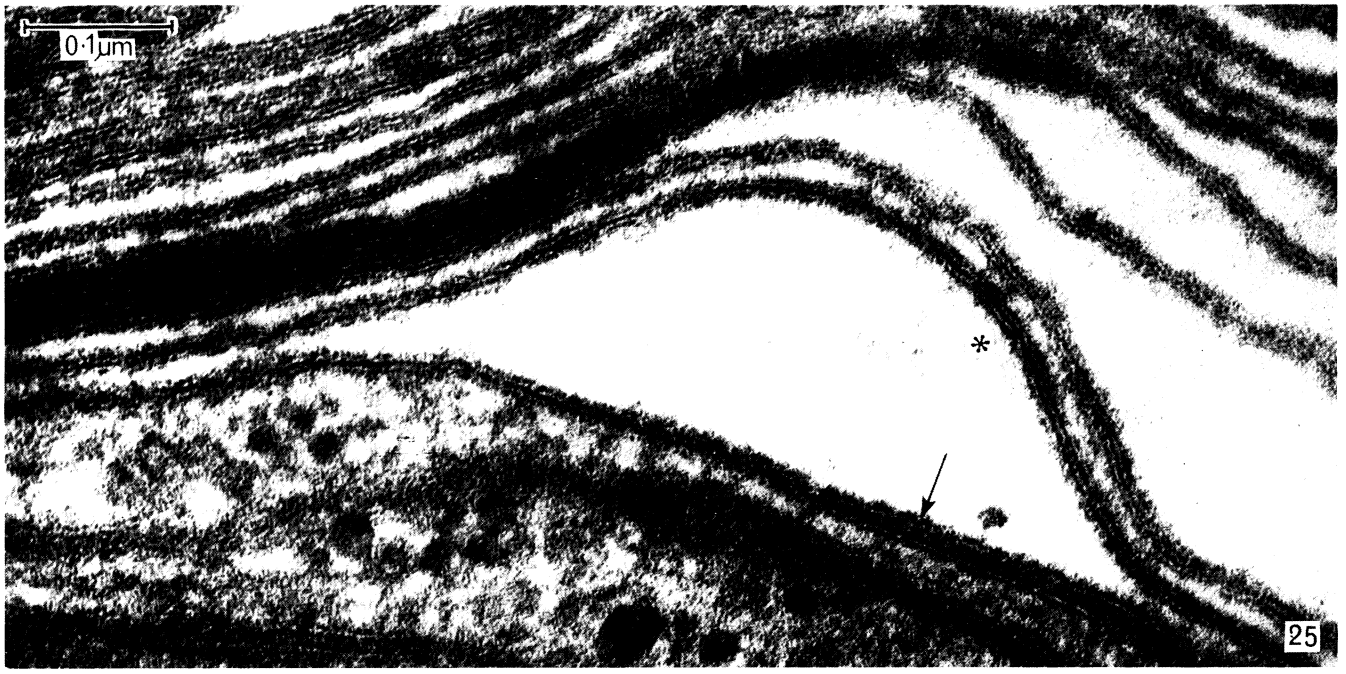


FIGURE 24. Micrograph showing part of the inner sheath. It is composed of closely packed cell layers, most of which are reduced to little more than their plasma membranes. Microtubules are visible in some layers. × 90000.

FIGURE 25. A region of the inner sheath at high magnification. While in places the plasma membranes are separated by dense extracellular material (arrow), in some regions there is apparent fusion of the plasma membranes to form five-layered structures (asterisk). Note also the region of very close and regular packing of fused membranes. × 205000.

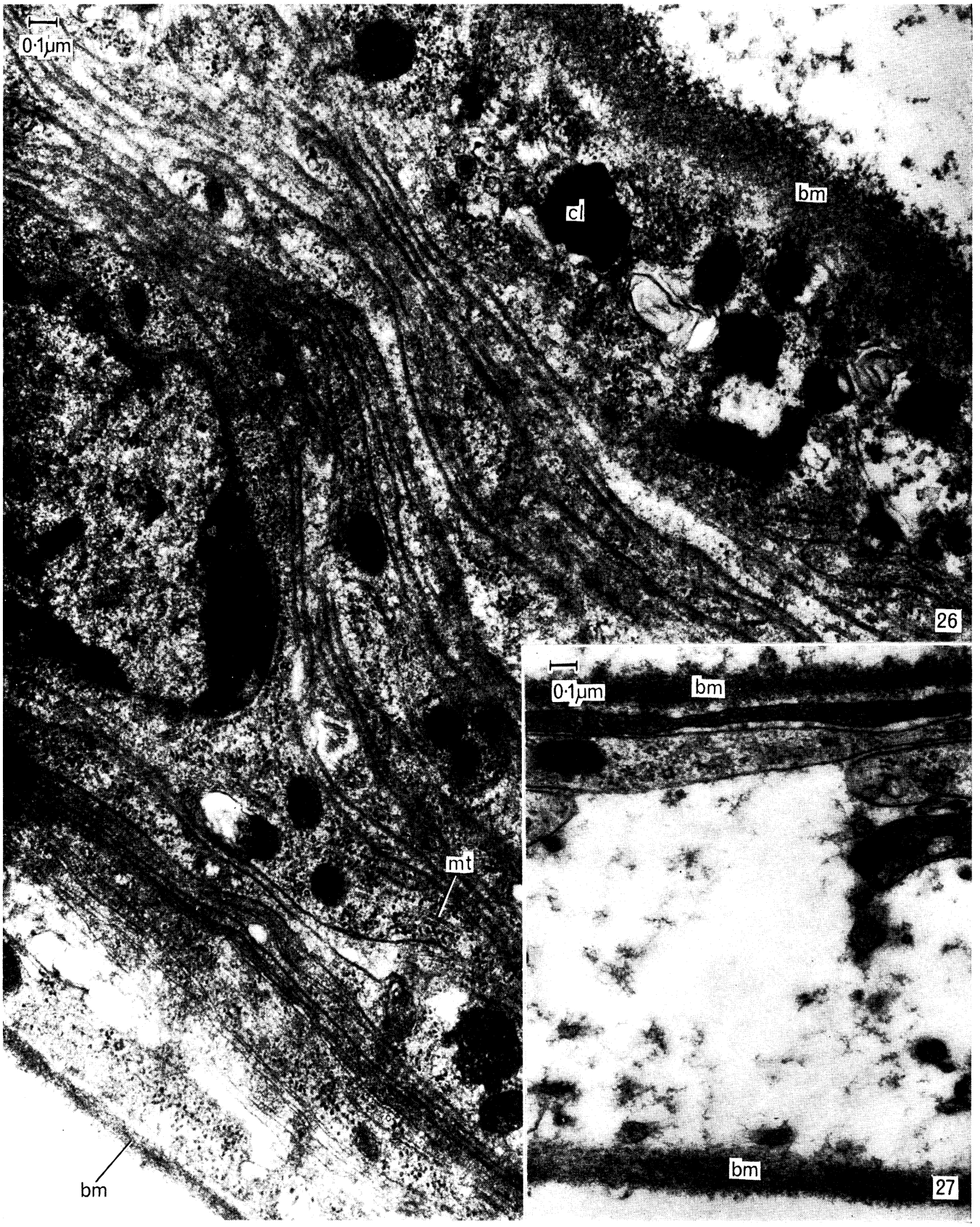


FIGURE 26. Micrograph showing the perinephric membrane in the anterior region of the rectal complex (compare with figure 18). There is no division into inner and outer sheath in this region, though in one area (bottom, left) there are some close-packed cell layers. × 45000.

FIGURE 27. Micrograph showing the perinephric membrane at the extreme anterior end of the rectal complex. Here the membrane is reduced to one or two layers of thin cells, together with the basement membranes always found on the inner and outer surfaces. The inner basement membrane is in this case separated from the cells by a large space. × 50000.

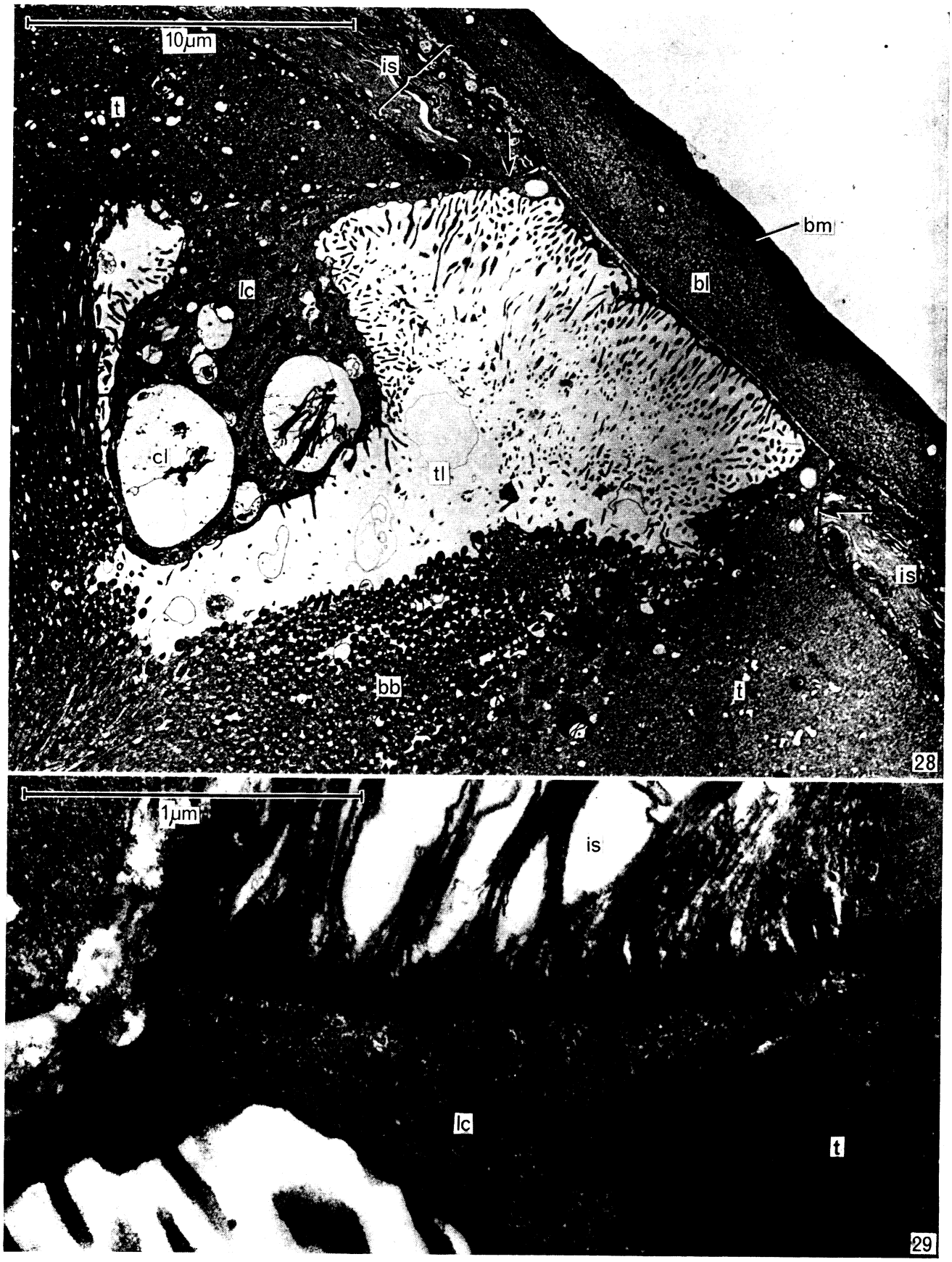


FIGURE 28. Survey micrograph showing a leptophragma. Note the extremely thin sheet of cytoplasm which forms the leptophragma itself, and the leptophragma cell body hanging down into the tubule lumen. The blister overlying the leptophragma is in this case less pronounced than usual and the outer basement membrane and underlying space are more condensed. The arrows indicate the site of the dense ridged annulus at the junction of the inner sheath with the leptophragma cell and adjacent tubule cell (see figure 29). × 6000.

FIGURE 29. Section showing the insertion of the laminae of the inner sheath into the ridged annulus at the margin of the leptophragma. × 62000

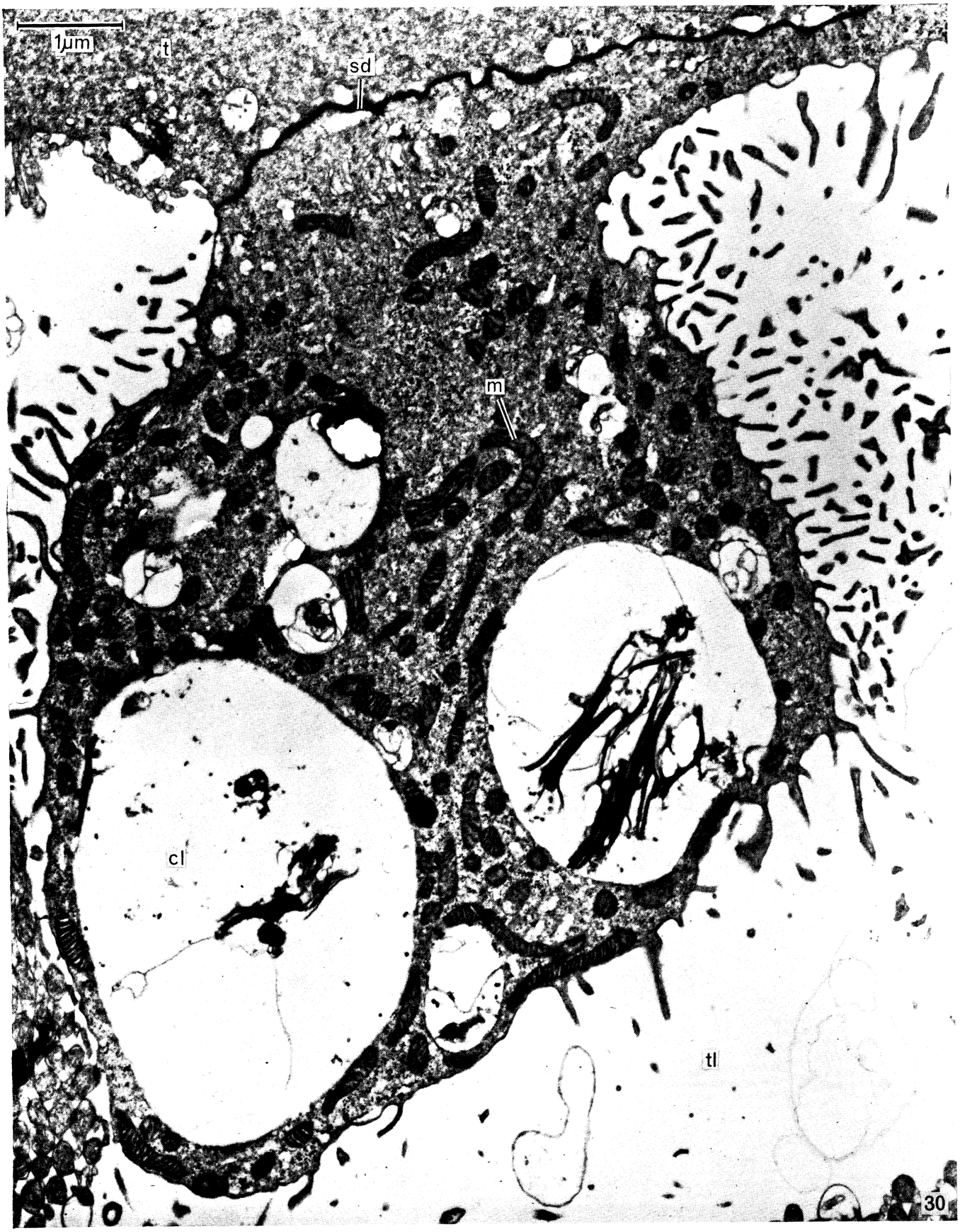


FIGURE 30. Micrograph showing the leptophragma cell of figure 28 at higher magnification. The microvilli arising from the cell surface are much thinner and more widely separated than those of the tubule brush border. The large vacuoles in the cytoplasm are interpreted as cytolysomes. In other micrographs the dense junctional area with the tubule cell has been shown to be a septate desmosome. × 20000.

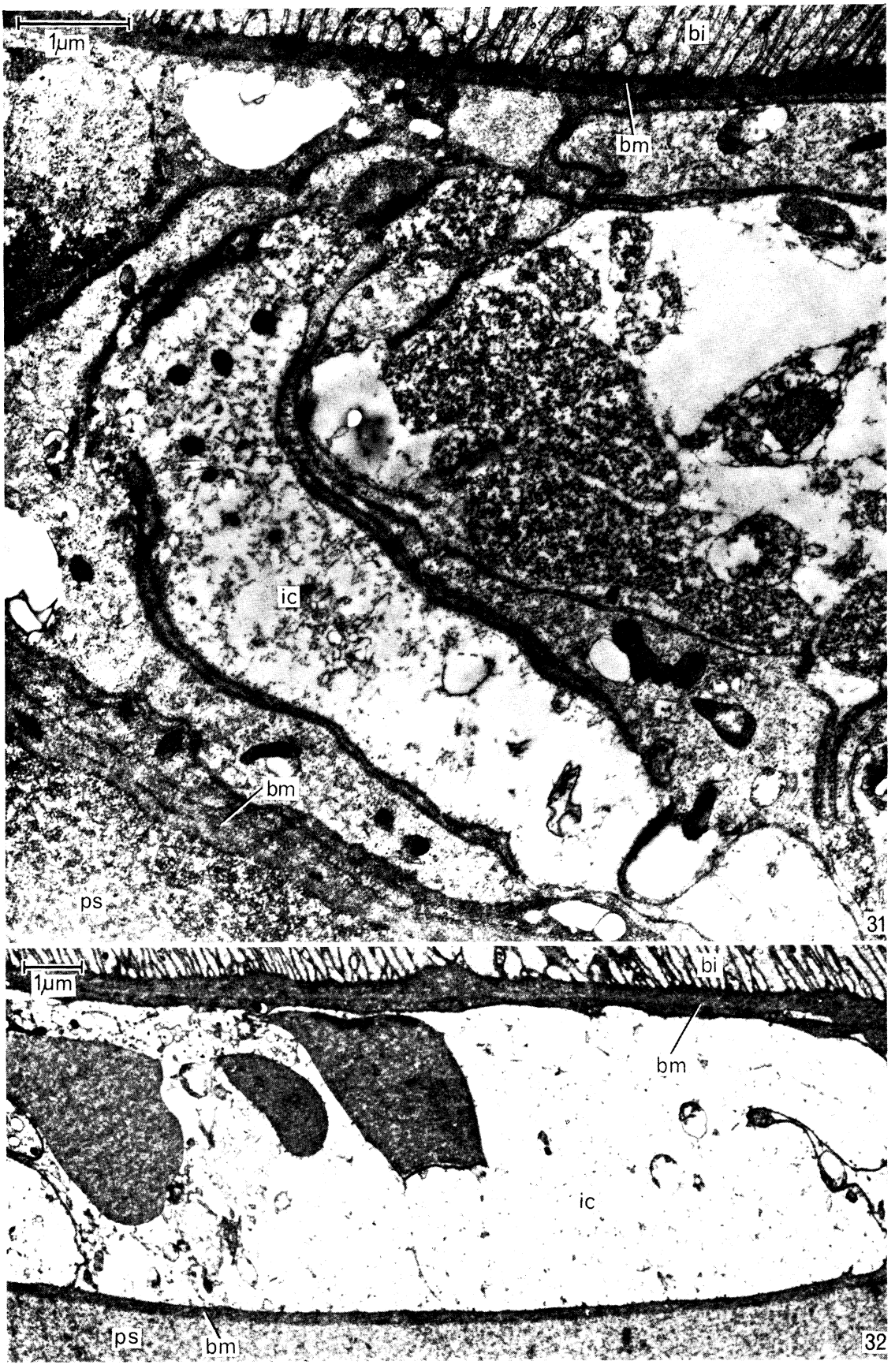


FIGURE 31. Section showing the intertubular cells found in the supposed peritubular space. Note that the cytoplasm of some of these cells contains no recognizable organelles. At bottom left of the picture, the homogeneous contents of the perirectal space can be seen. × 19000.

FIGURE 32. Micrograph similar to figure 31, showing an almost empty intertubular cell. × 10500.

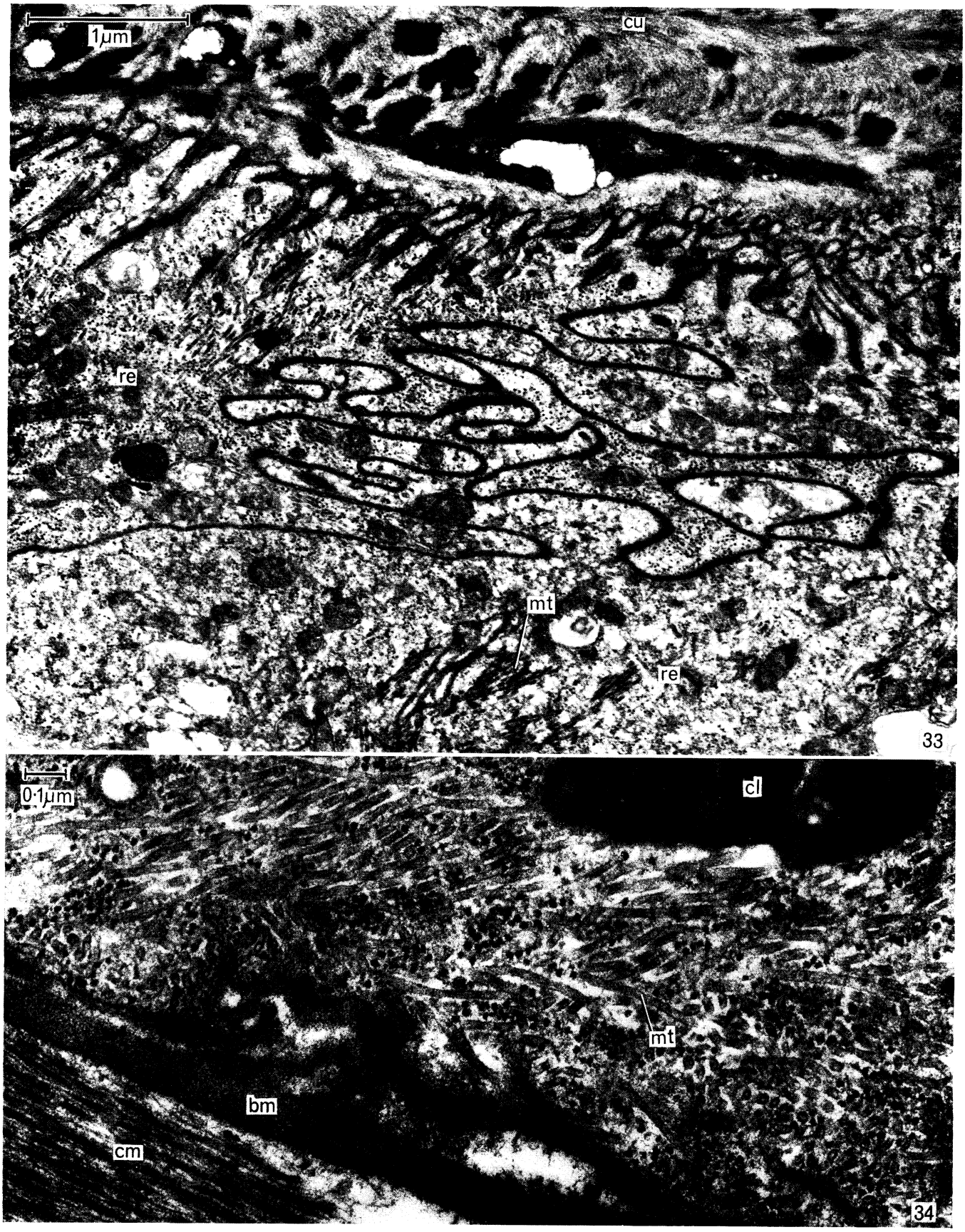


FIGURE 33. Section showing part of the apical surfaces of two rectal cells, with the basal portion of the overlying cuticle. The lateral borders of these cells interdigitate extensively in the densely staining junctional zone, lying near the luminal cell surface. Note the dense material in the basal region of the cuticle. × 30000.

FIGURE 34. Basal region of a rectal cell and adjacent circular muscle. The basement membranes of these are fused. Note the abundant microtubules, with ribosomes interspersed between them. × 77000.

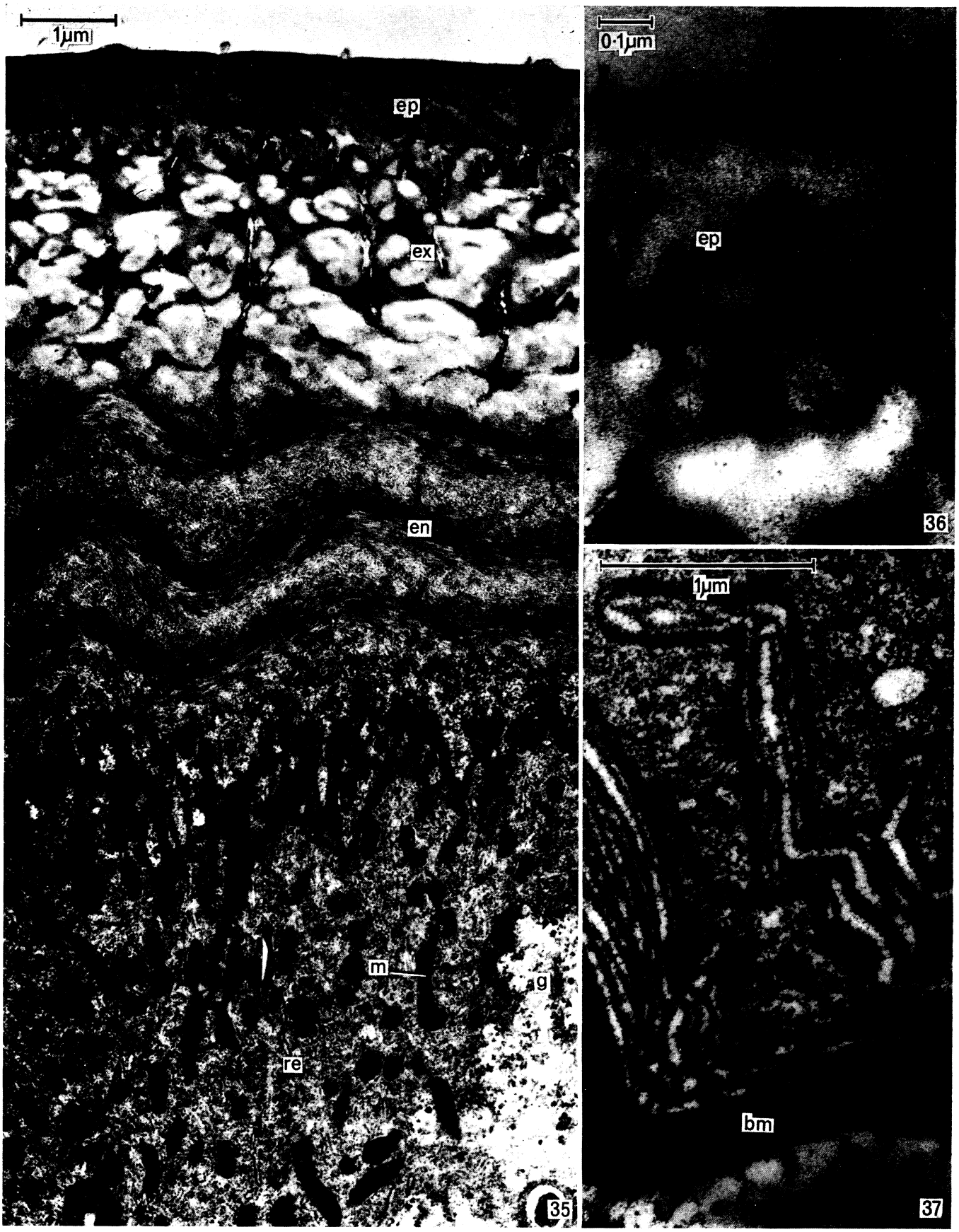
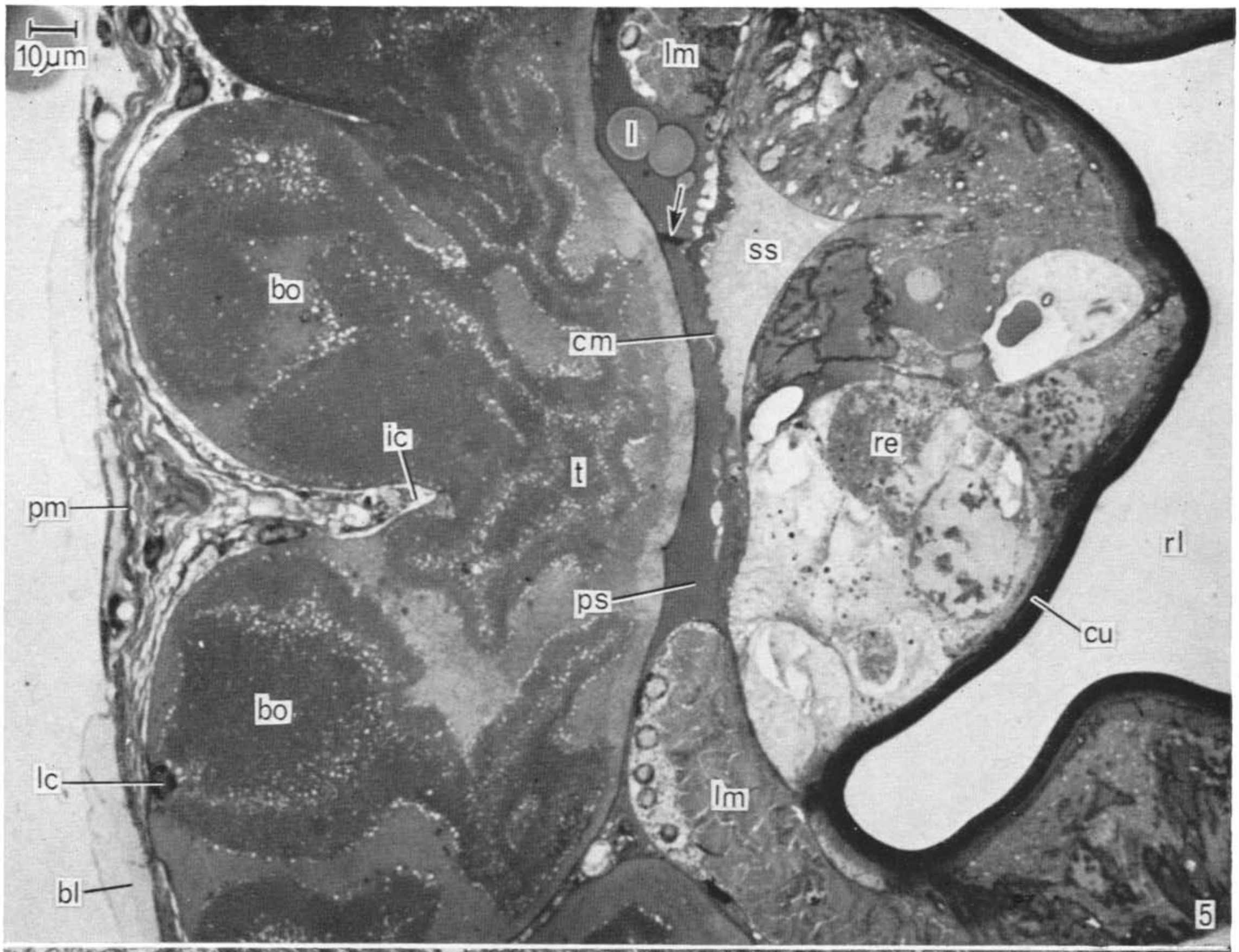


FIGURE 35. Section showing the structure of the rectal cuticle. The identification of the layers is tentative. Note below the cuticle the folds and microvilli at the apical surface of the rectal epithelial cell. × 18000.

FIGURE 36. Micrograph to show the supposed cuticulin layer (in the form of two dense layers) at the surface of the epicuticle. × 100000.

FIGURE 37. Section through the basal region of a rectal cell, showing deep, irregular infoldings filled with laminated extracellular material lying below the general basement membrane. × 44000.



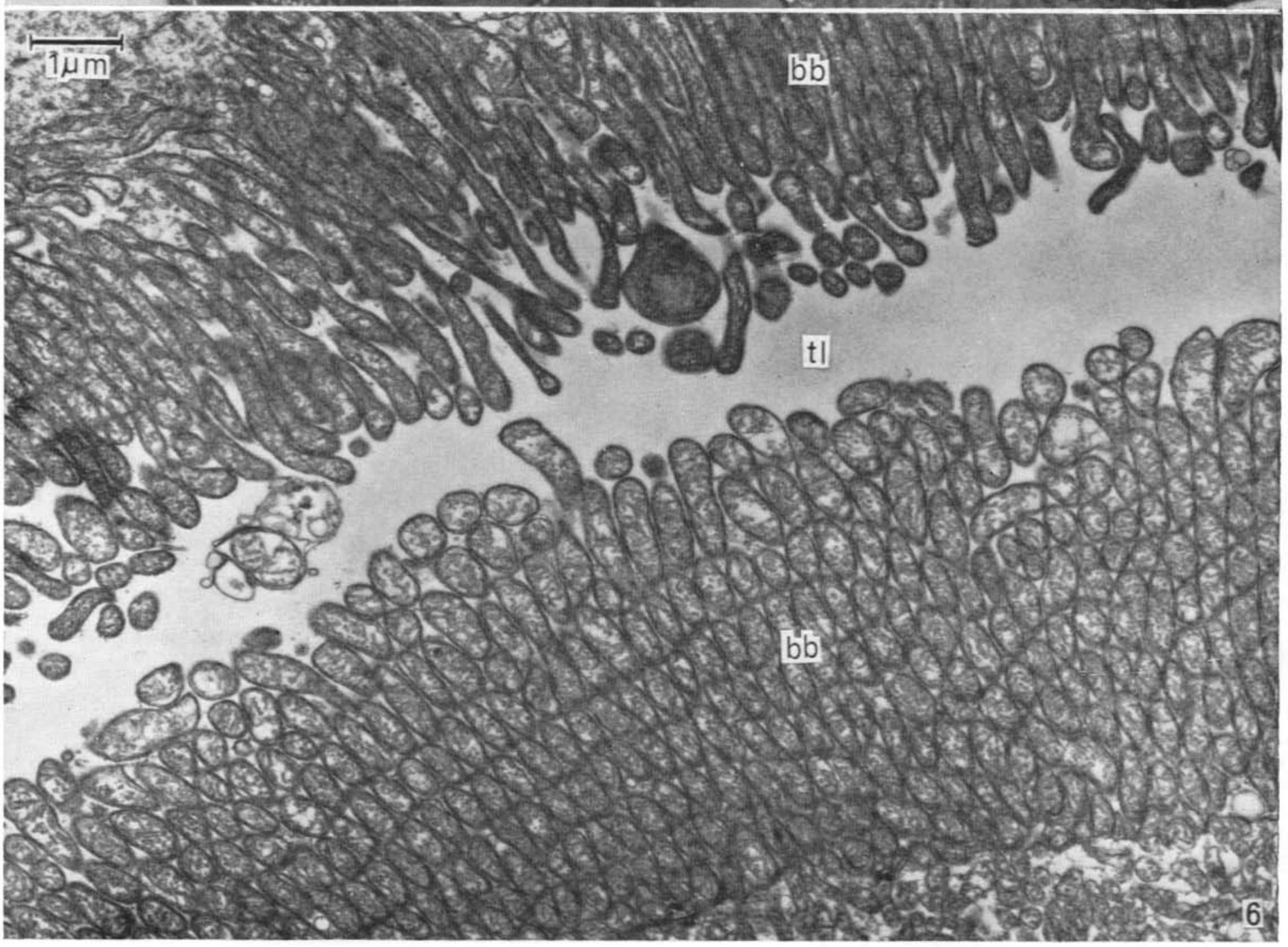


FIGURE 5. Photomicrograph of a 1 μm Araldite section stained with methylene blue, showing the main components of the rectal complex in transverse section. Parts of three boursouflures, all belonging to one tubule, are visible. The dark areas within these are the brush borders, lining the narrow lumen of the tubules. Note the homogeneous contents of the perirectal and subepithelial spaces, the former in this instance being more densely stained than the latter and containing spherical, possibly lipid, bodies. Compare these spaces with the supposed peritubular space, in which intertubular cells but no homogeneous contents can be seen. Note the connexion (arrow) between the tubule and circular muscle. × 620.

FIGURE 6. Low-magnification micrograph showing the lumen and brush borders of what is assumed to be a well fixed tubule. × 11000.

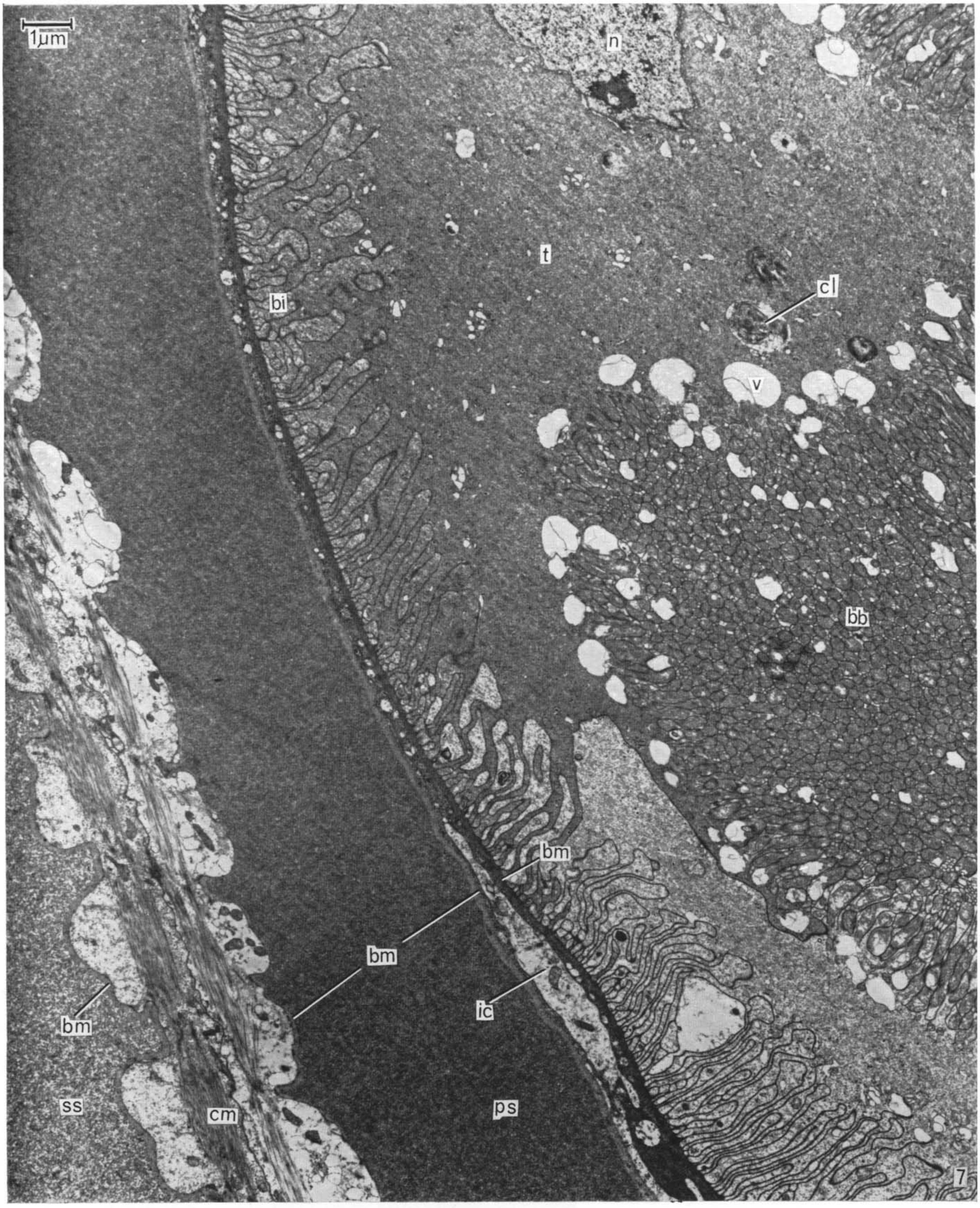
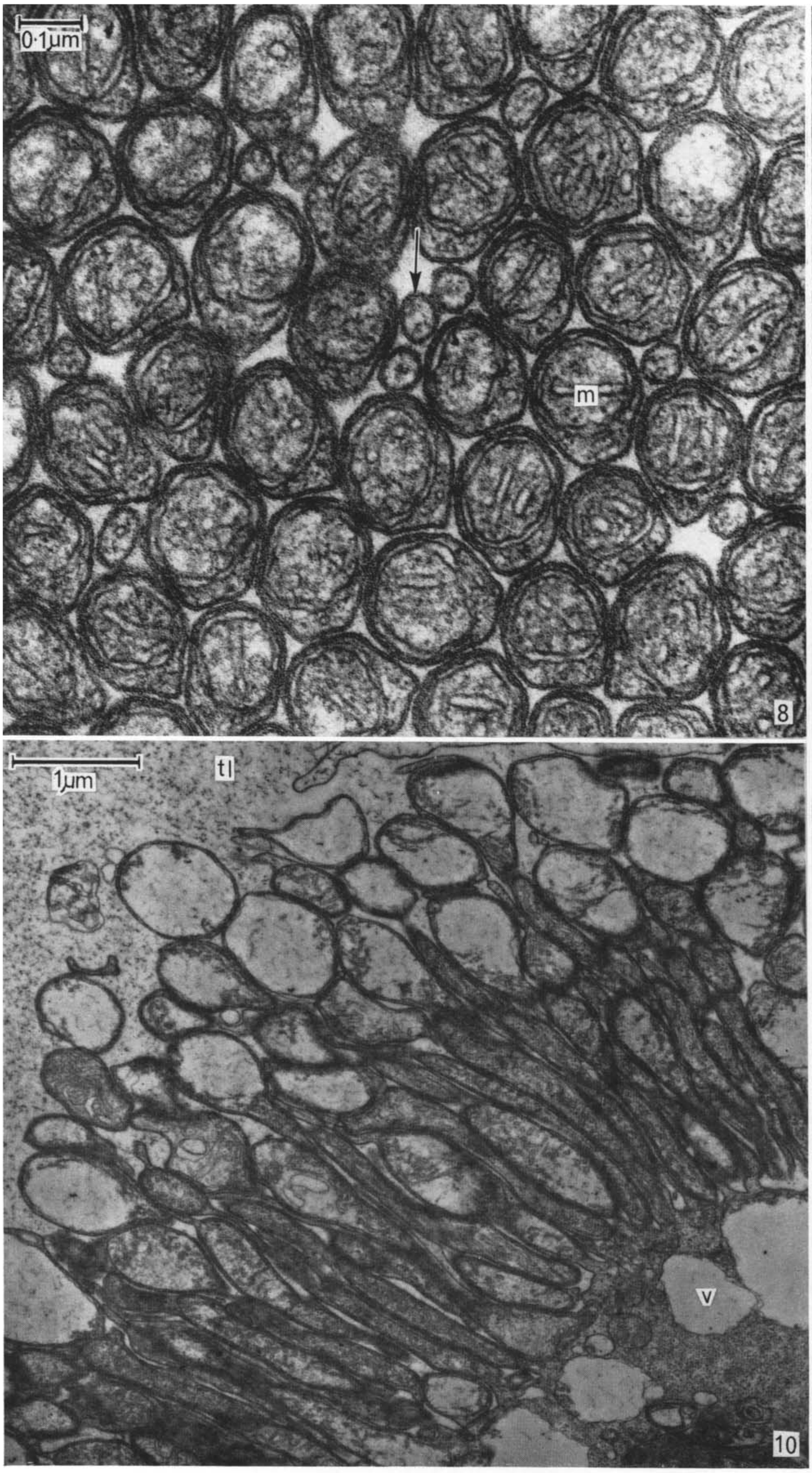


FIGURE 7. Survey micrograph showing part of a tubule and its relation to the perirectal and subepithelial spaces. Note the thin layer of intertubular cells between the basement membrane of the tubule, and that lining the perirectal space. Empty vacuoles are present below the brush border of the tubule. × 9000.



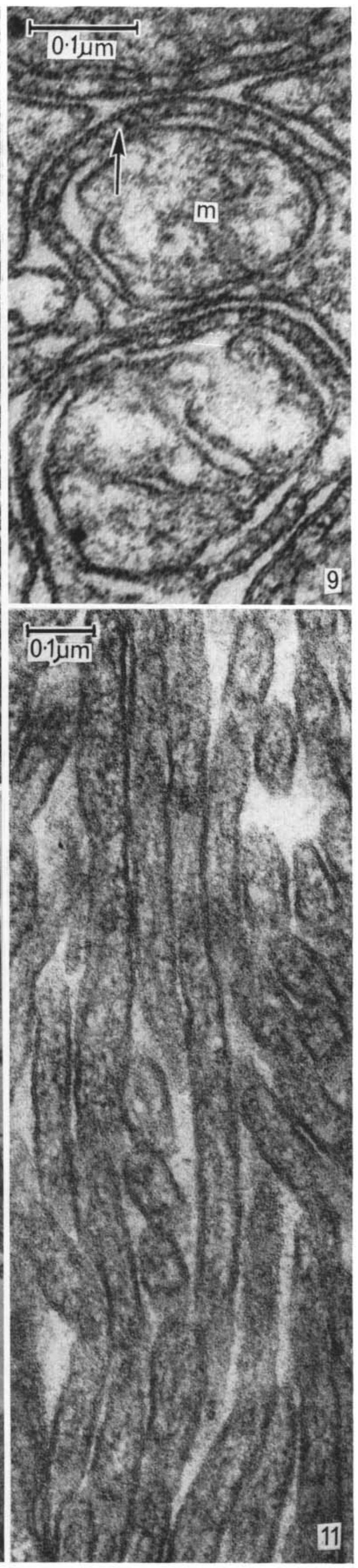


FIGURE 8. Micrograph showing the microvilli of the tubule brush border in transverse section. Note that while most of them contain a mitochondrion, a few (arrow) do not. × 100000.

FIGURE 9. Higher magnification micrograph of microvilli of tubule brush border, showing the connexions (arrow) between the outer mitochondrial membrane and the plasma membrane. × 160000.

FIGURE 10. Brush border of a tubule, showing swollen microvilli and vacuoles in the cytoplasm below. × 20000.

FIGURE 11. Micrograph showing a group of tubule cell microvilli without mitochondria. × 100000.

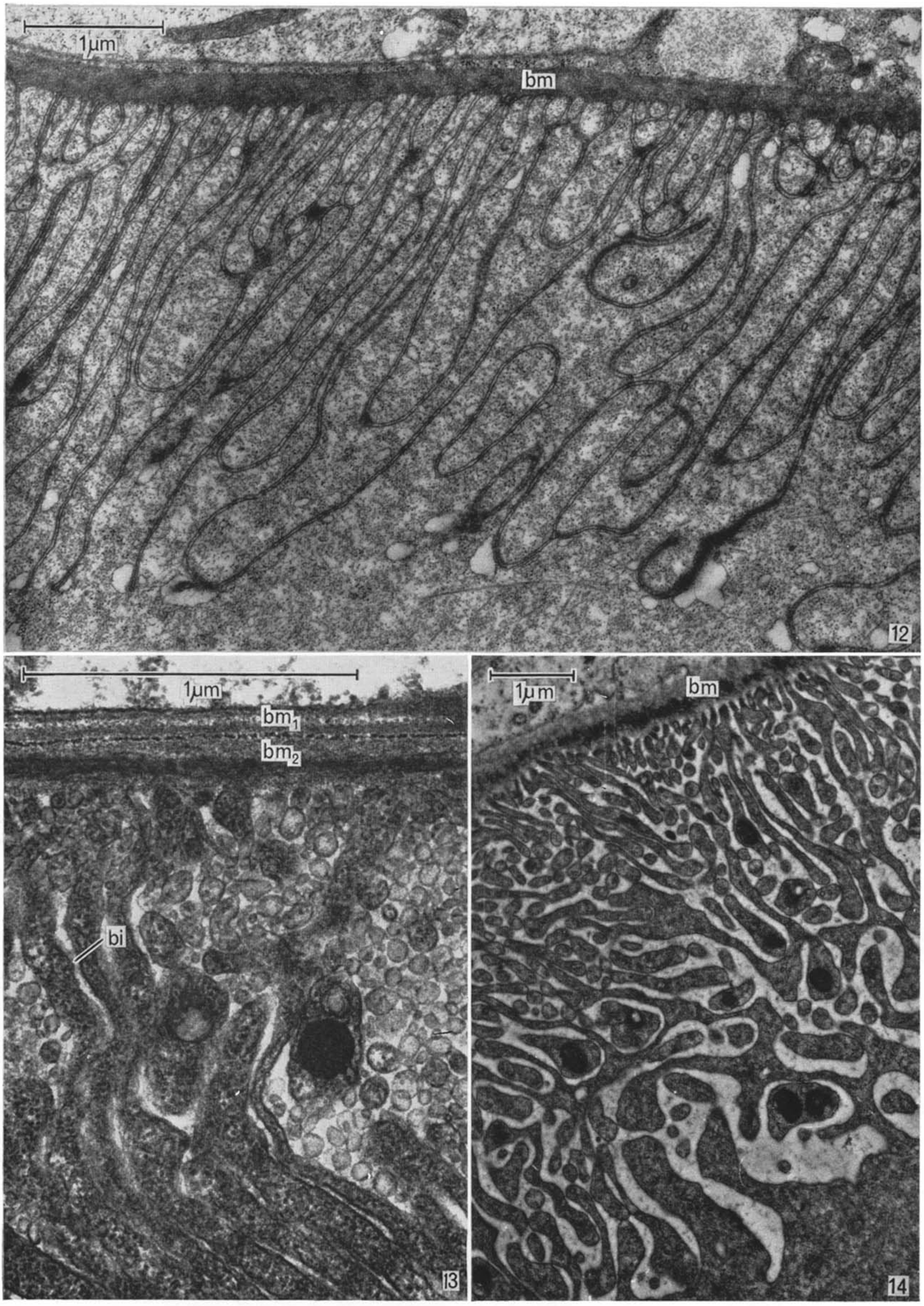


FIGURE 12. Section showing the basal region of a tubule cell, with basal infoldings in the form of closely apposed membranes. 'Moist' mealworm. × 25000.

FIGURE 13. Basal region of a tubule after fixation in osmium tetroxide. In addition to the infoldings, numerous small, apparently extracellular vesicles are present. (bm_1, basement membrane of longitudinal muscle; bm_2, basement membrane of tubule). × 60000.

FIGURE 14. Micrograph showing basal infoldings in the form of inflated channels in a tubule cell from a 'dry' mealworm. Compare this with figure 12. × 15000.

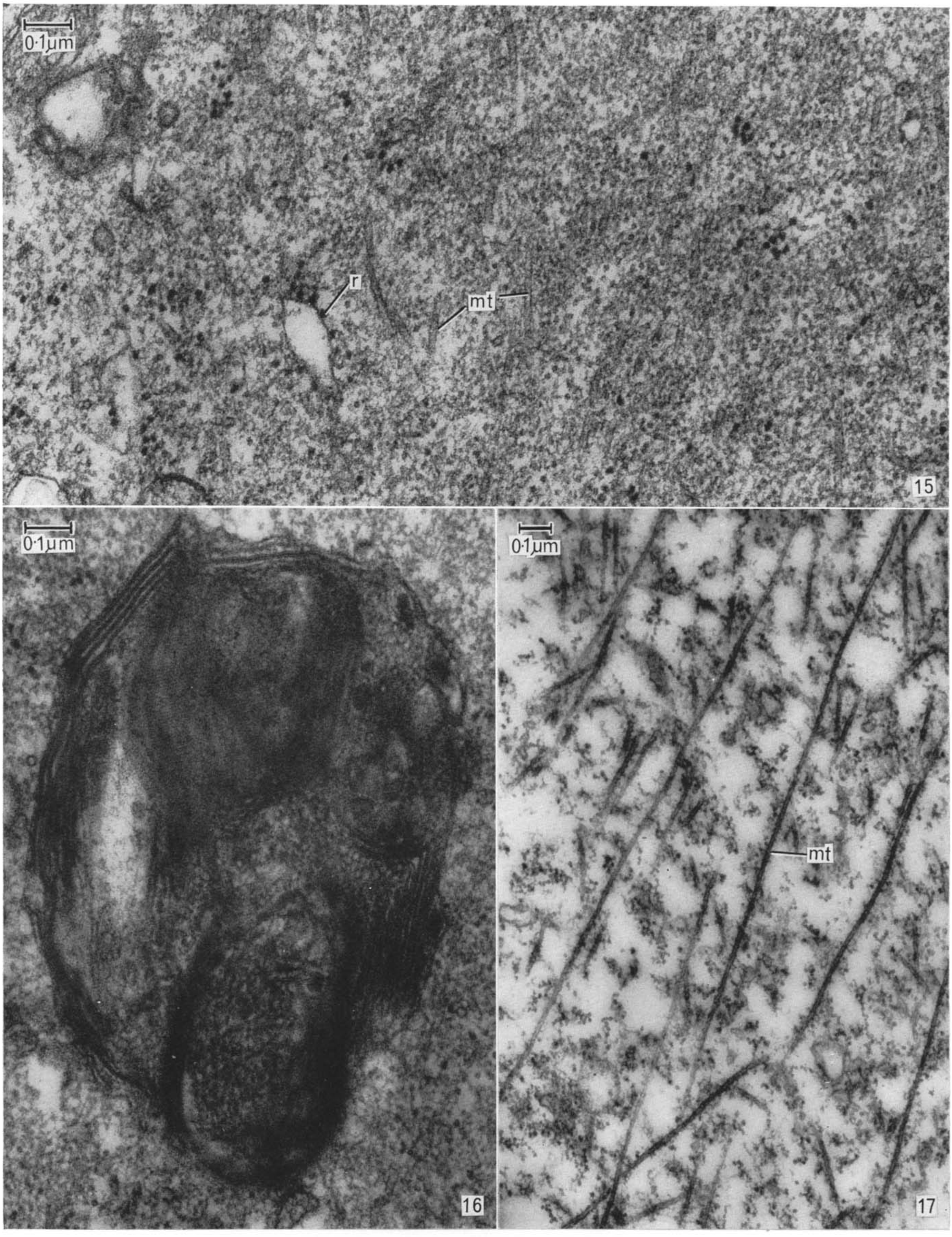
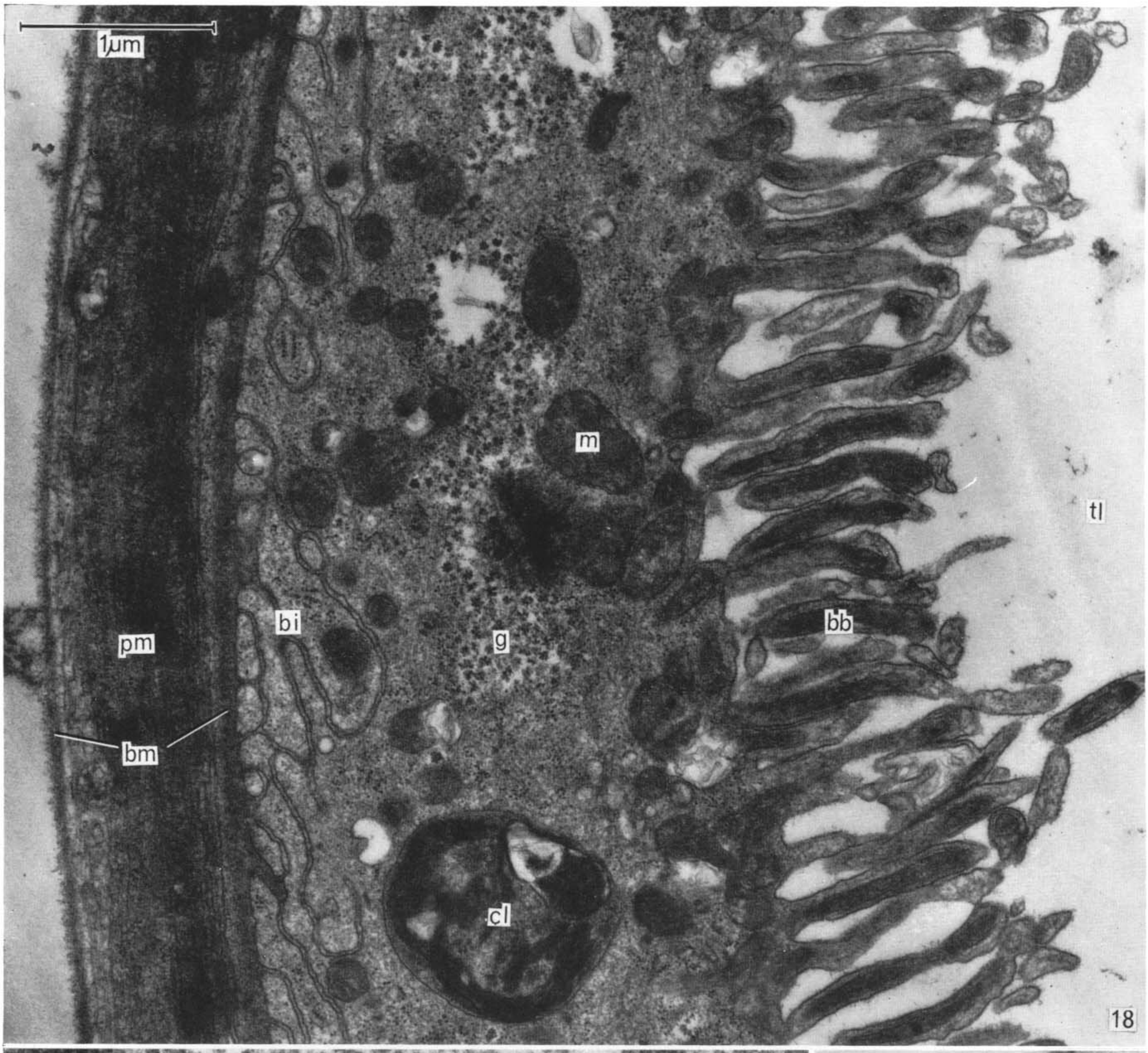
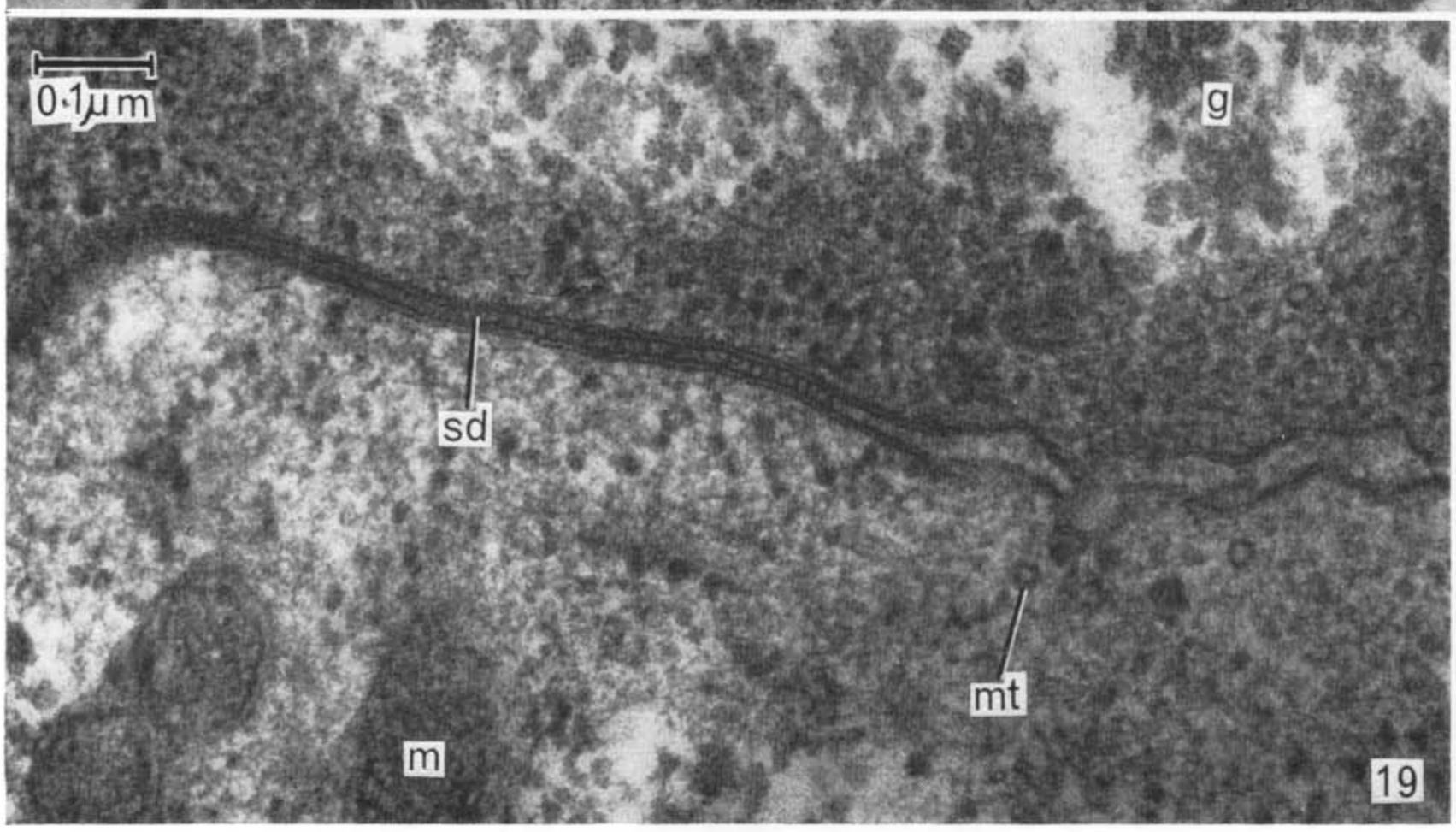


FIGURE 15. Micrograph showing the typical appearance of tubule cell cytoplasm after glutaraldehyde fixation. Note the abundant microtubules, and the marked difference in size and electron density between the ribosomes and the small granules filling much of the cytoplasm. × 90000.

FIGURE 16. Cytolysome from a tubule cell. × 90000.

FIGURE 17. Tubule cell cytoplasm after osmium fixation. Microtubules are present, as in figure 15, but in this preparation fewer of the small granules have been preserved. × 60000.





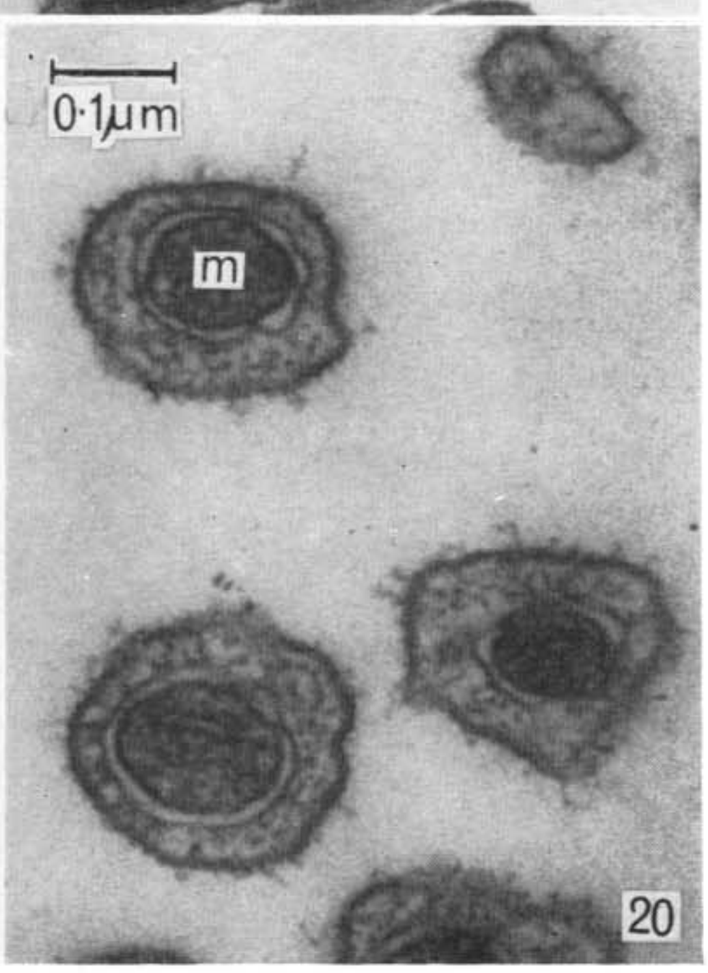


FIGURE 18. Micrograph showing part of a tubule and the surrounding perinephric membrane, from the anterior region of the rectal complex. Compared with the posterior region, the tubule wall is much thinner, with less well-developed brush border and basal infoldings, and the perinephric membrane is less complex. × 30000.

FIGURE 19. Junctional region between the apical ends of the lateral borders of two tubule cells. Towards the tubule lumen (left) there is a septate desmosome. Elsewhere the two cell membranes are more widely separated. × 100000.

FIGURE 20. Transverse section of microvilli with mitochondria from the brush border of the anterior region of a tubule. × 90000.

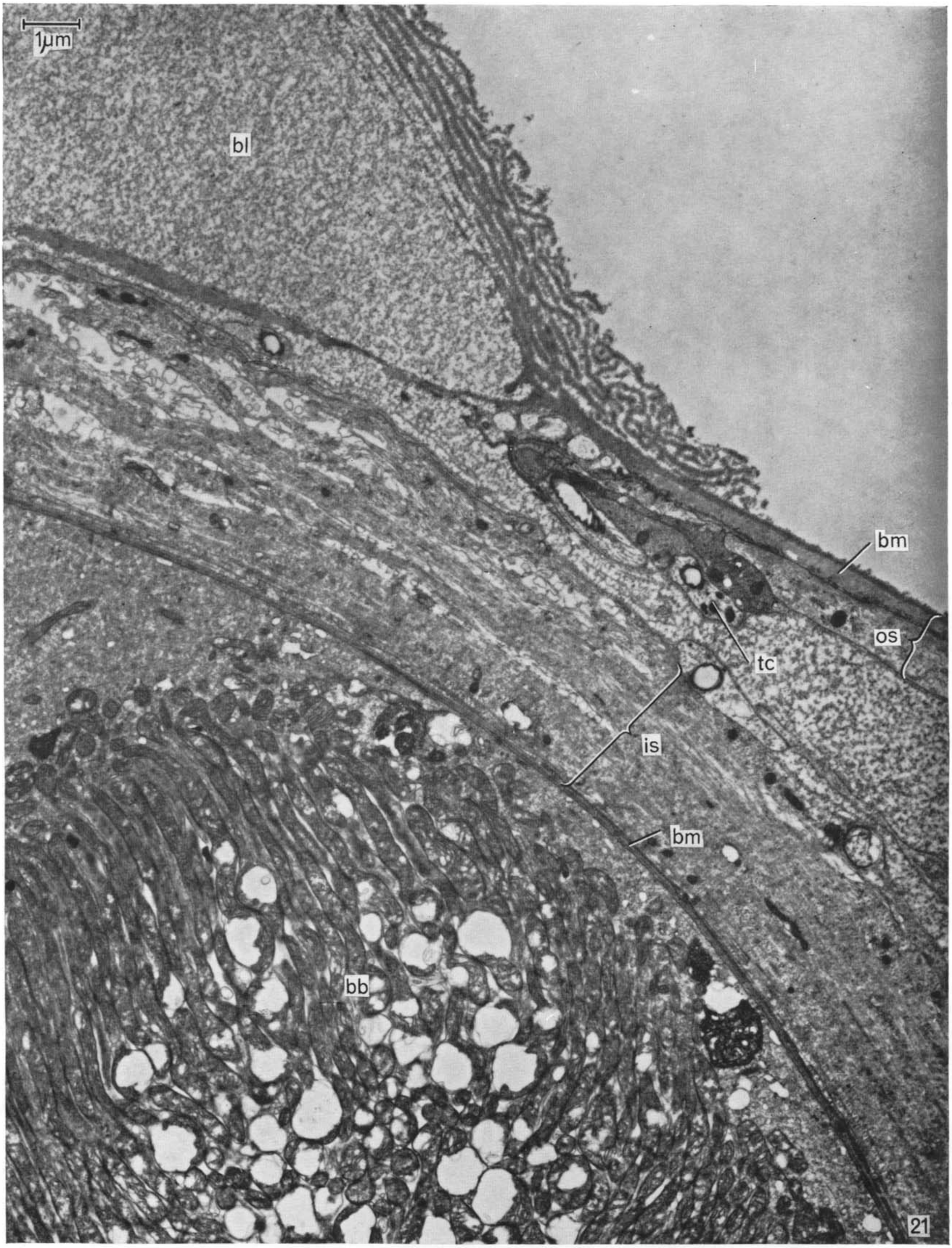
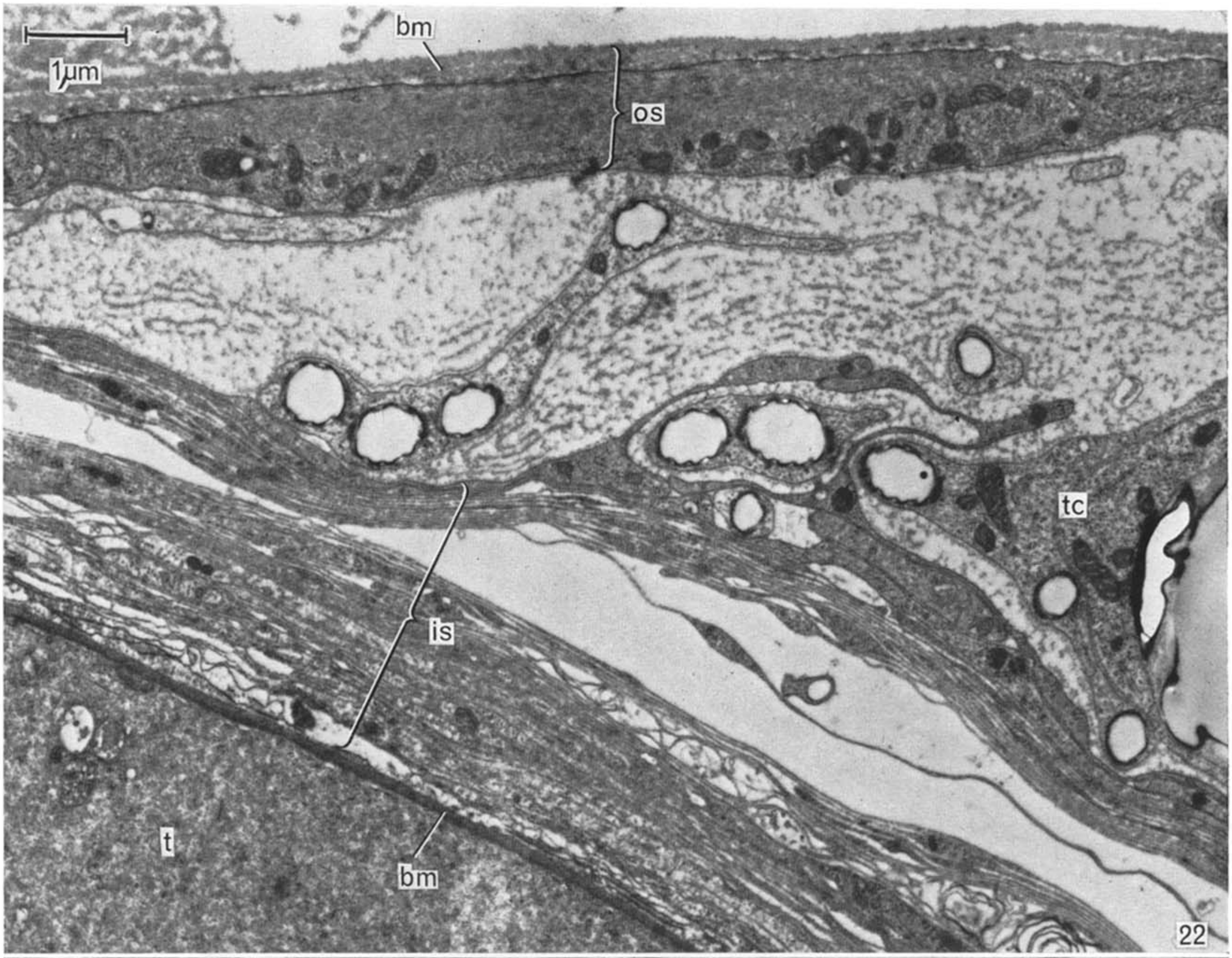


FIGURE 21. Survey micrograph showing part of a tubule, and the perinephric membrane, formed of inner and outer sheaths with tracheolar end cells in the space between. The section includes the edge of a blister overlying a nearby leptophragma. Note the multilaminate basement membrane covering the blister. × 10000.



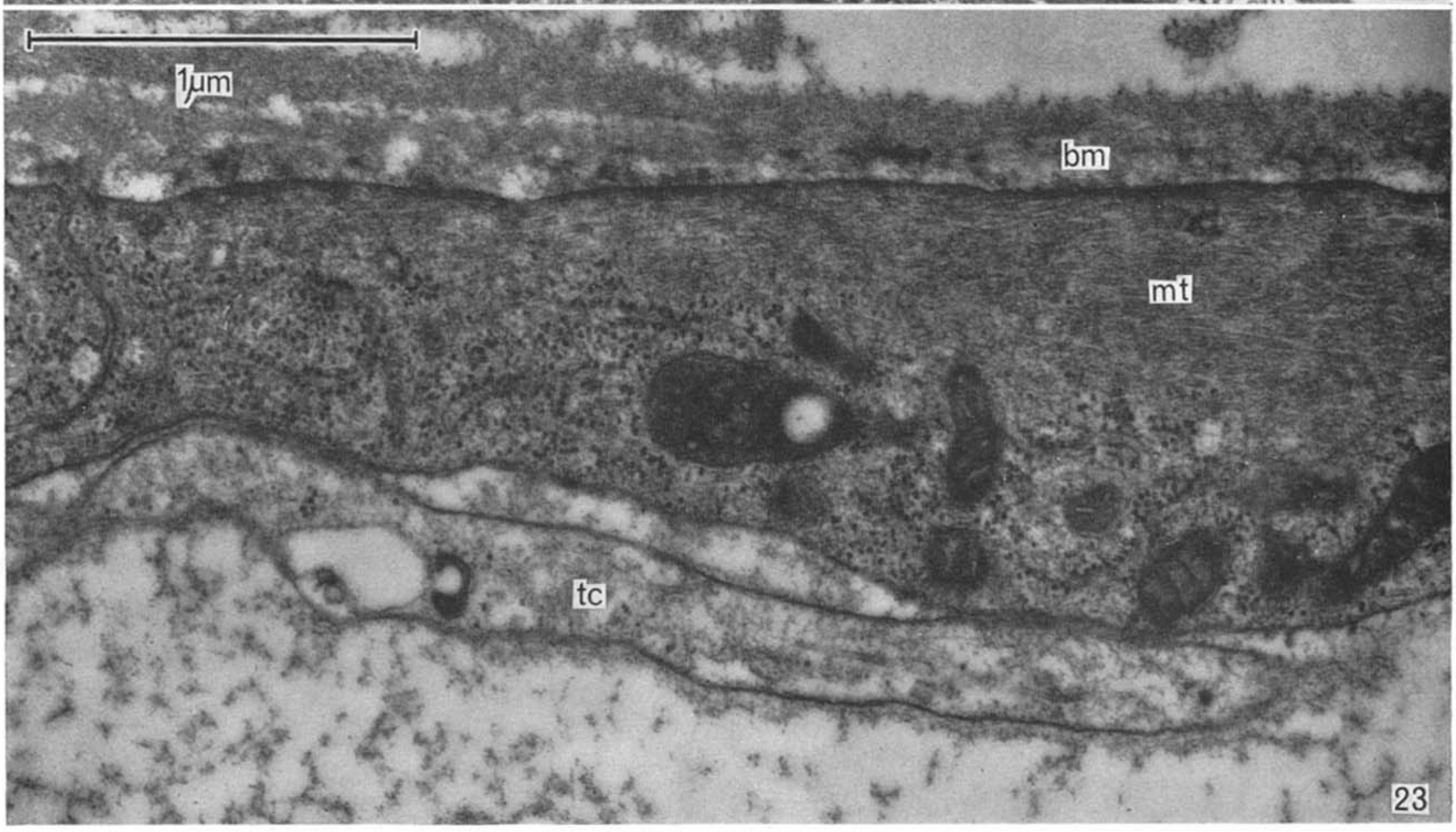


FIGURE 22. Micrograph showing the perinephric membrane. Note the difference in structure of the inner and outer sheaths. The gap in the former is probably an artifact, but shows plainly the form of one of the cell layers, which has become detached from its neighbours. × 14000.

FIGURE 23. Enlargement of figure 22 showing the single cell layer forming the outer sheath. Note the densely packed microtubules in the outer region of these cells; the inner region of the outer sheath contains mitochondria and endoplasmic reticulum. × 45000.

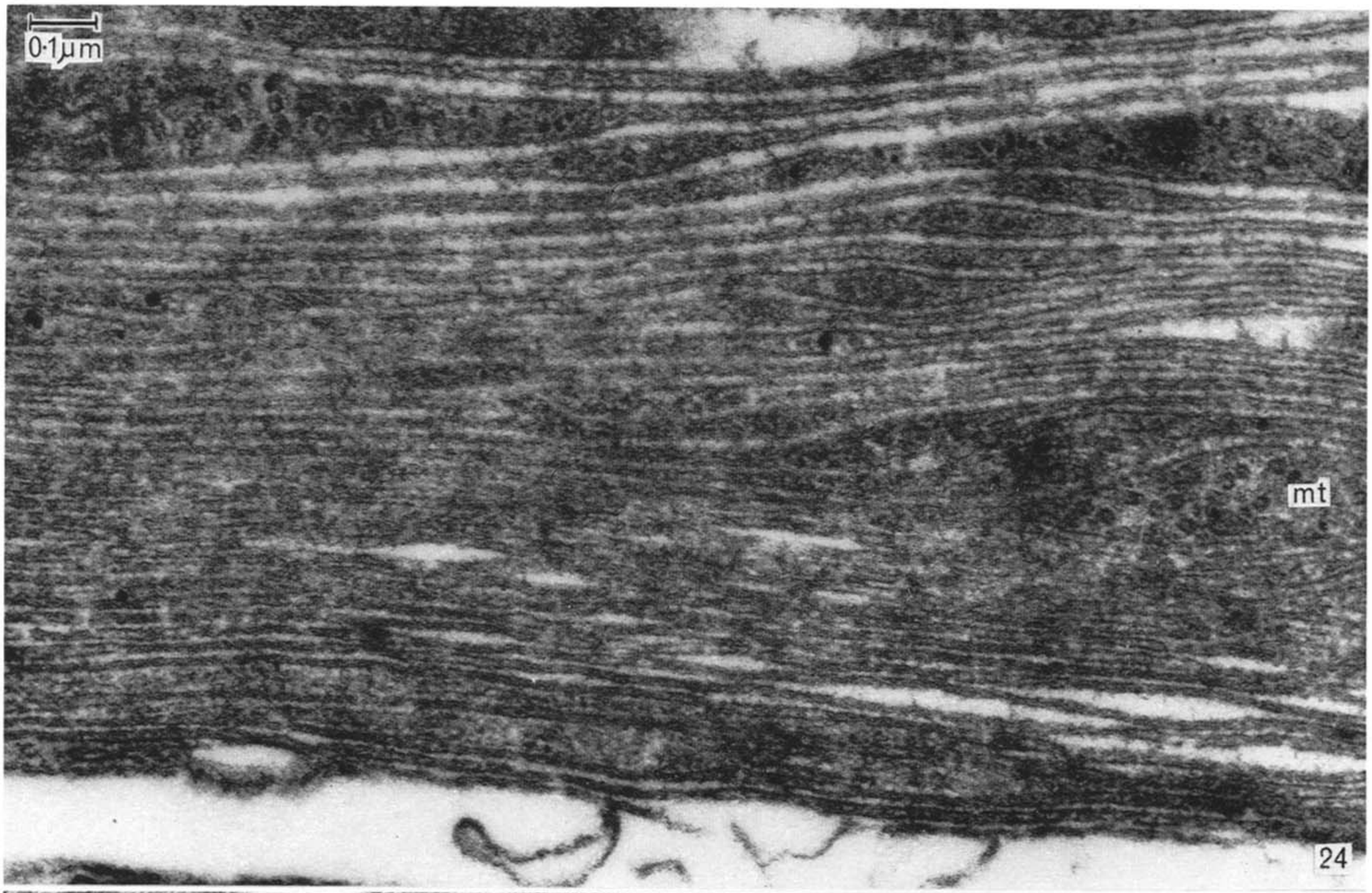
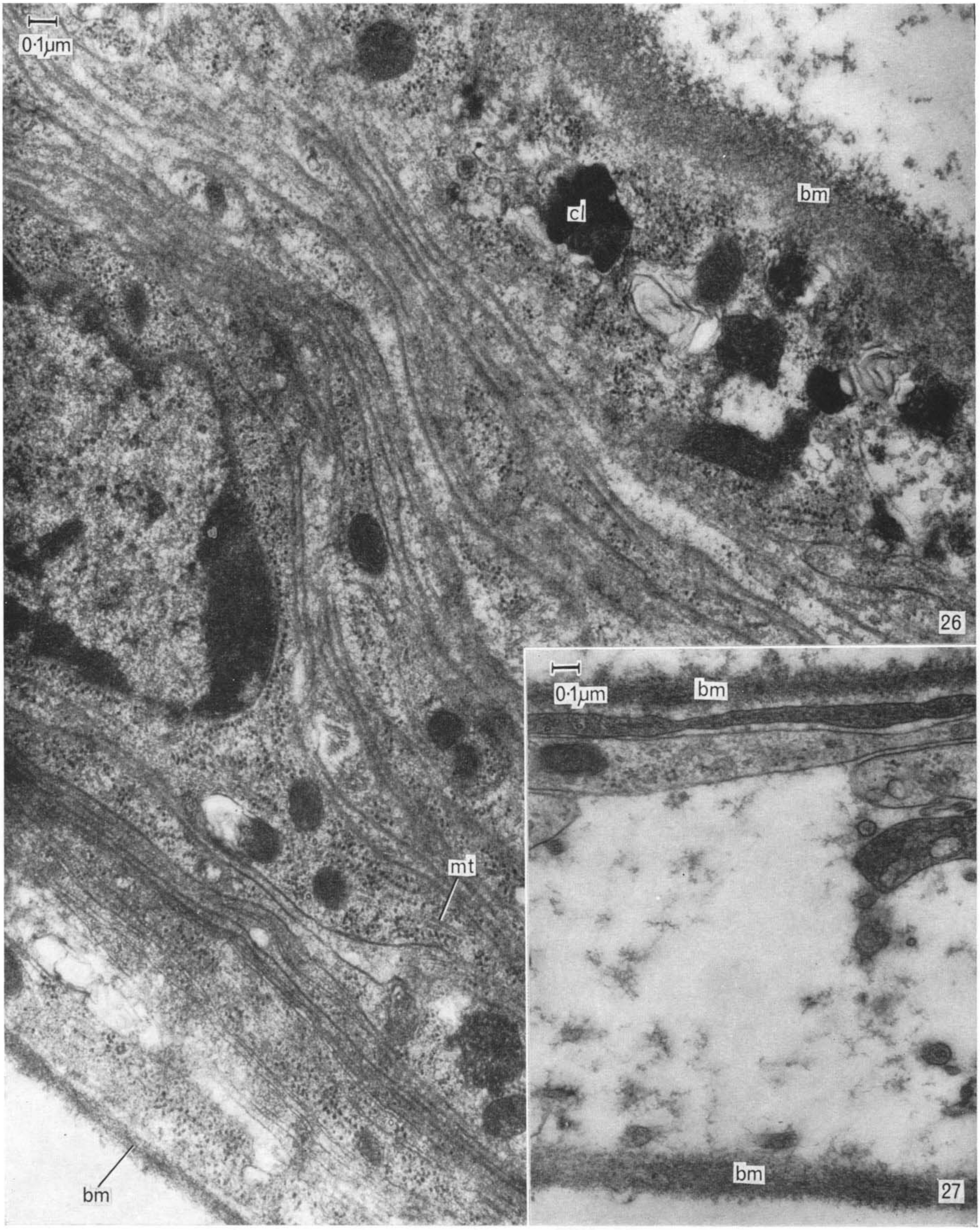


FIGURE 24. Micrograph showing part of the inner sheath. It is composed of closely packed cell layers, most of which are reduced to little more than their plasma membranes. Microtubules are visible in some layers. × 90000.

FIGURE 25. A region of the inner sheath at high magnification. While in places the plasma membranes are separated by dense extracellular material (arrow), in some regions there is apparent fusion of the plasma membranes to form five-layered structures (asterisk). Note also the region of very close and regular packing of fused membranes. × 205000.



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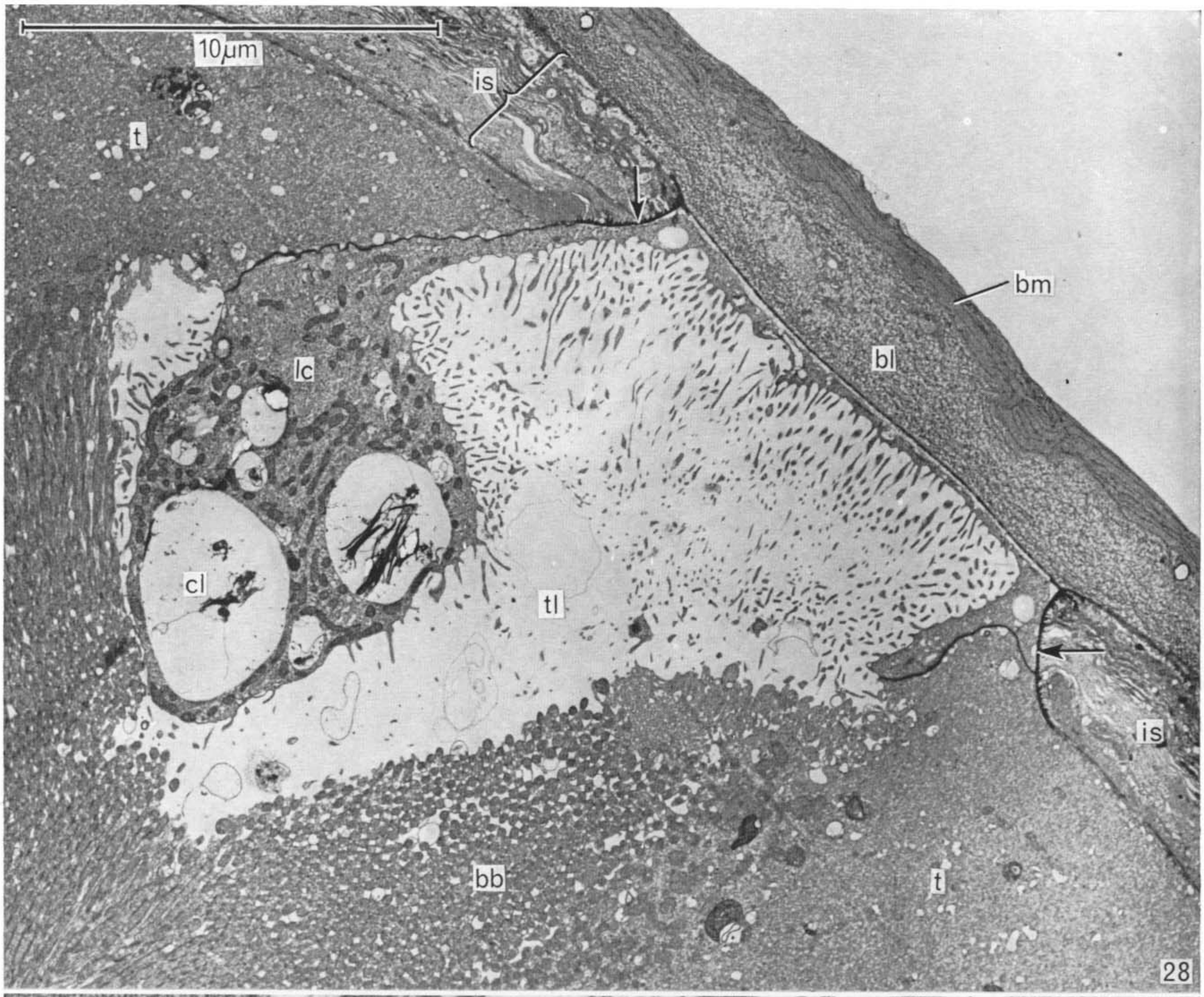

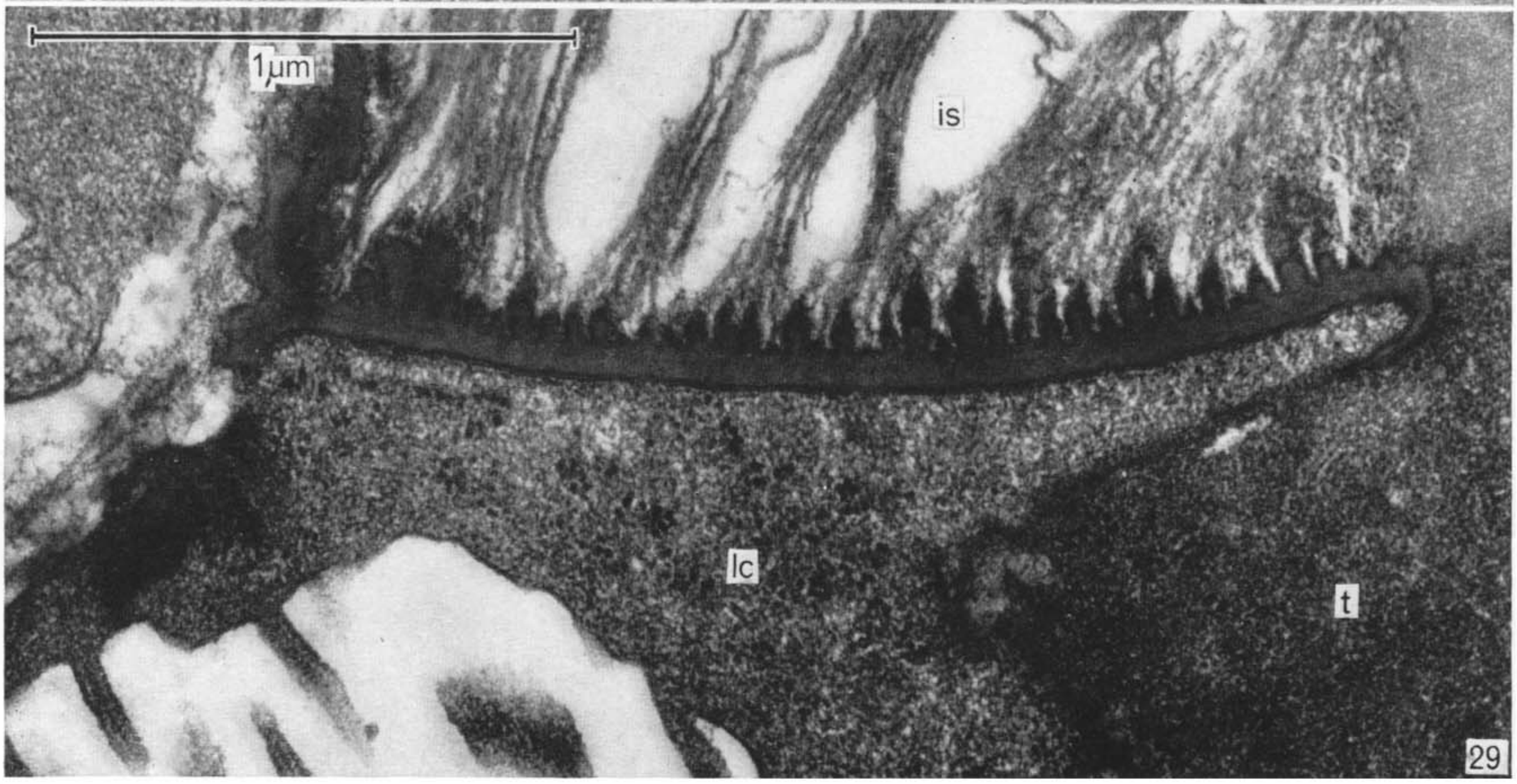

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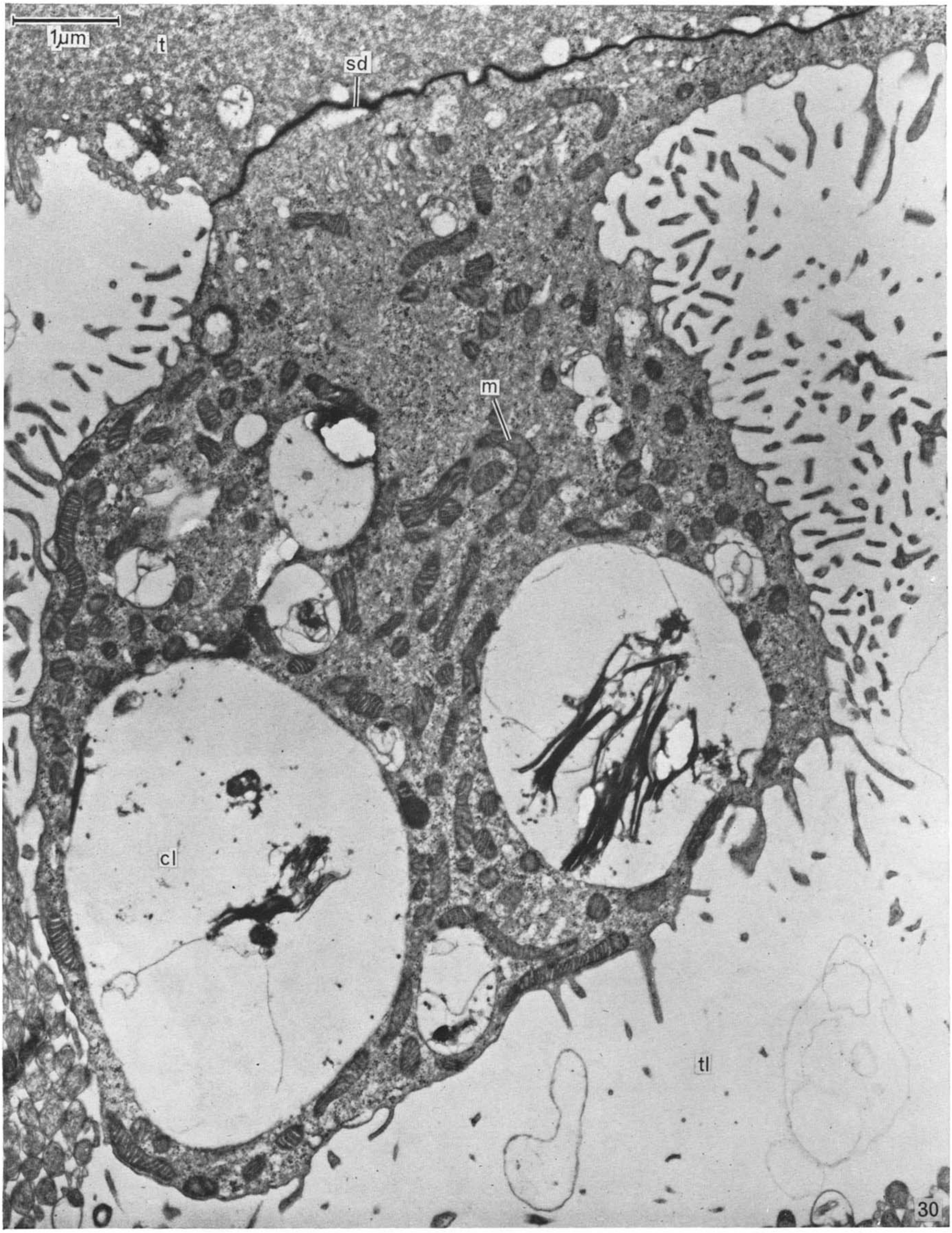

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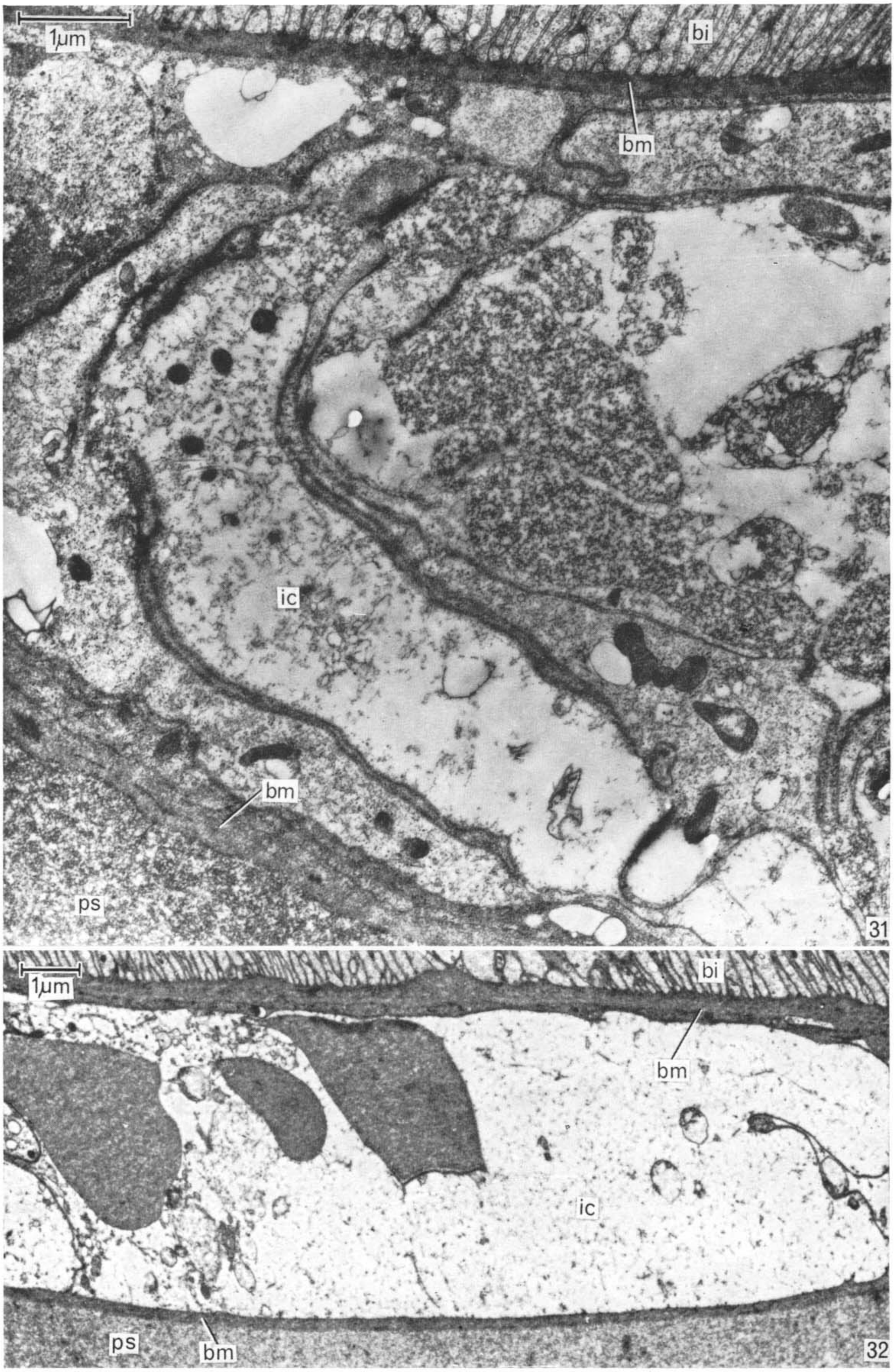

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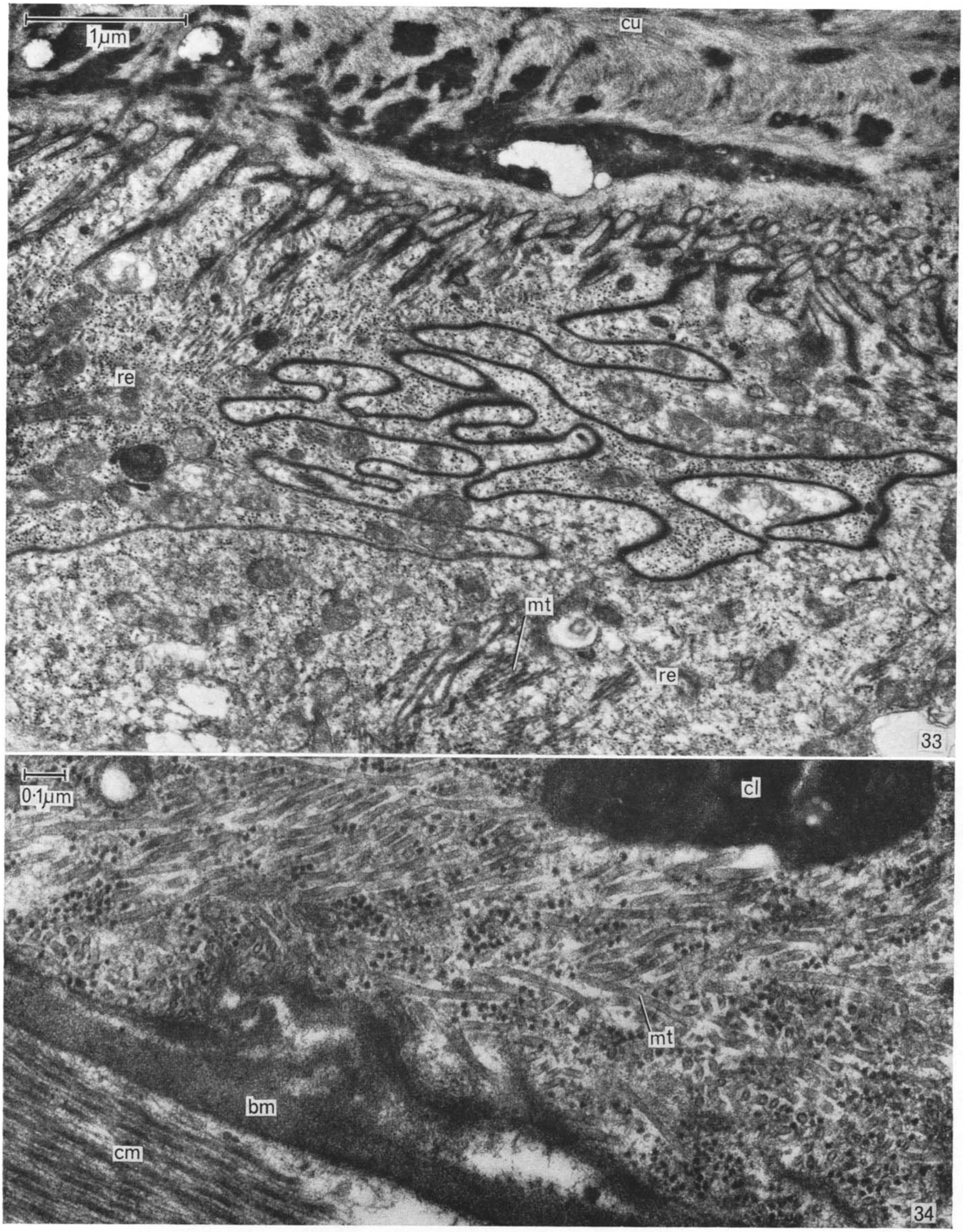

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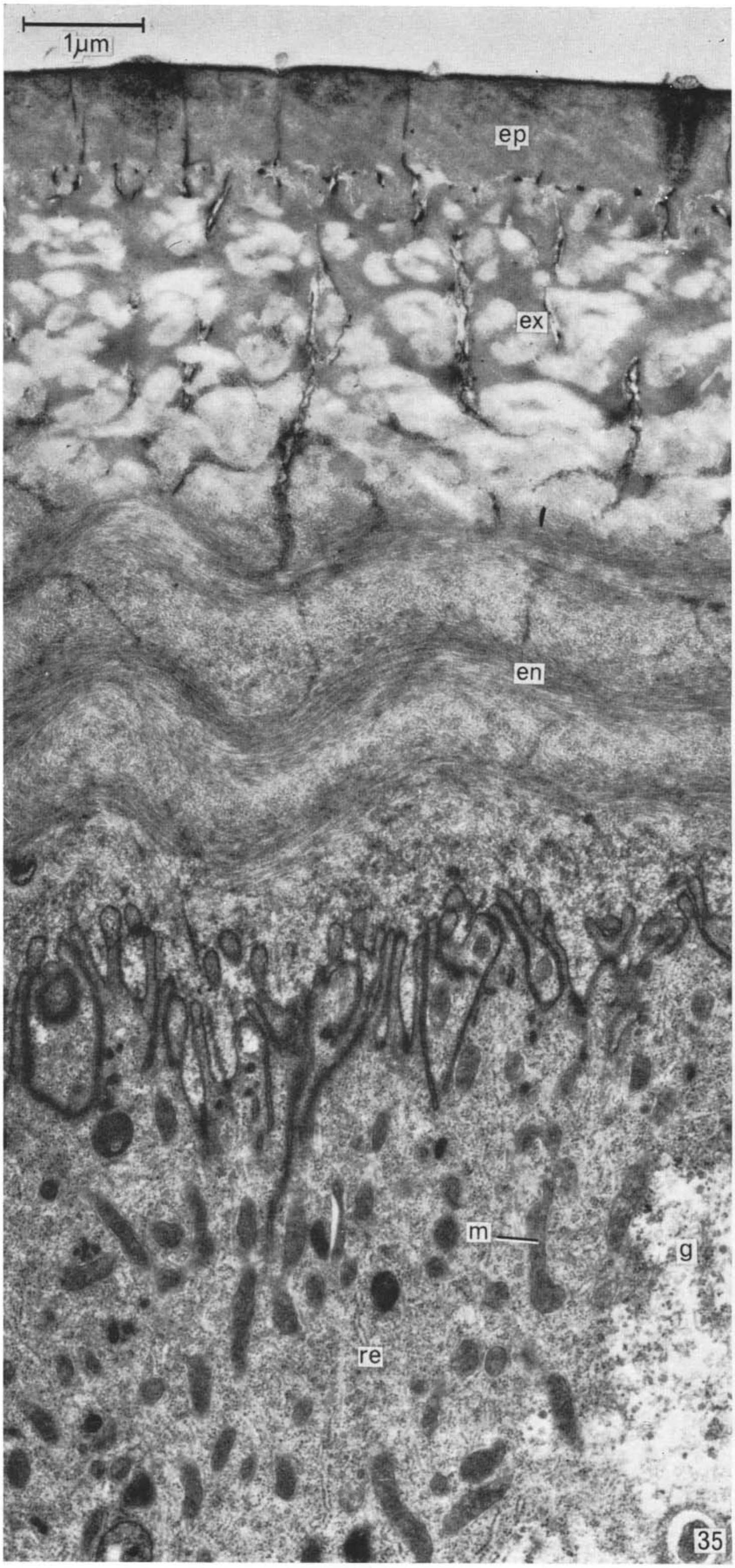

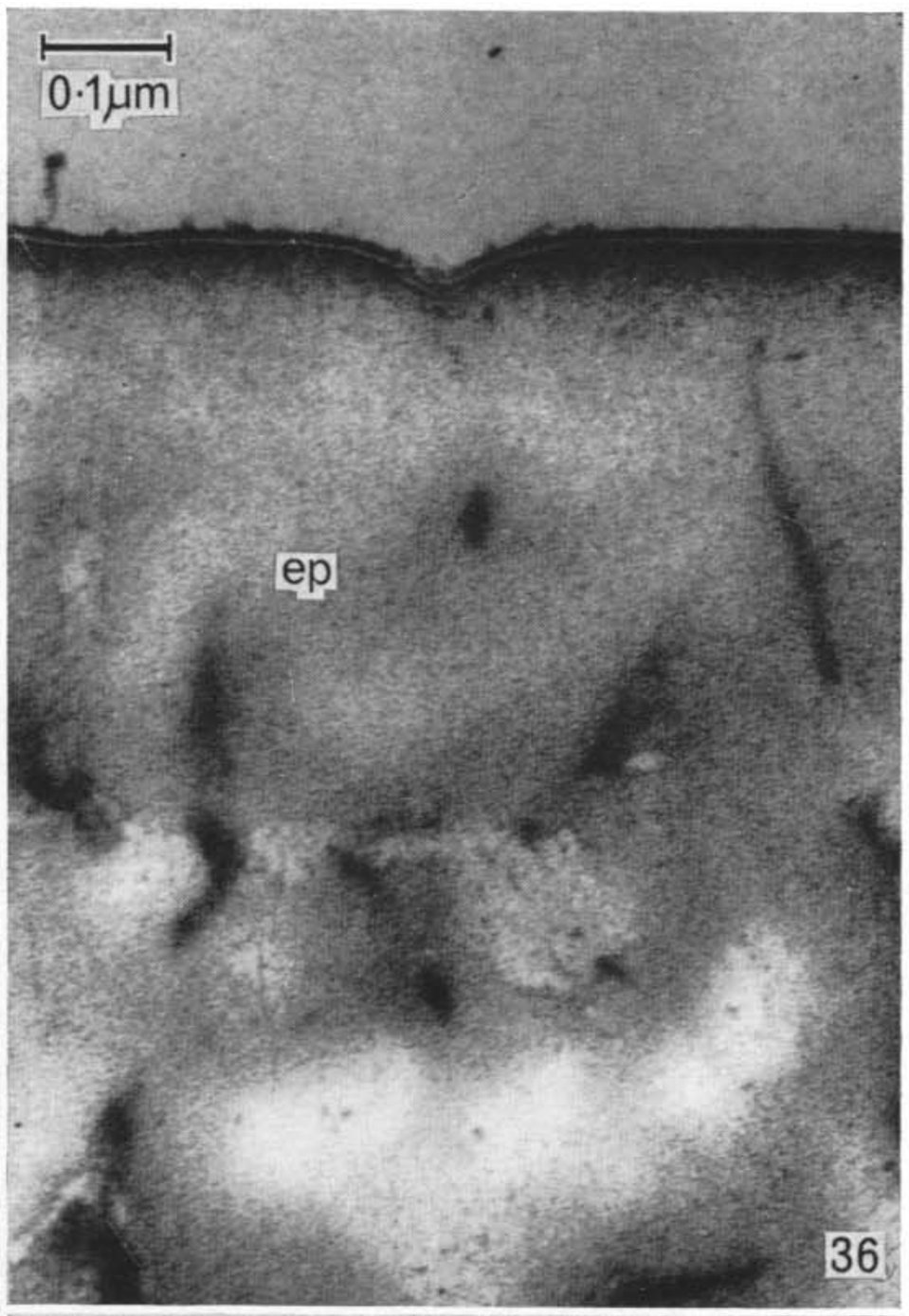

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