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ELECTRON MICROSCOPE OBSERVATIONS ON HONEYBEE SEMINAL VESICLES (*APIS MELLIFERA ADANSONII*, HYMENOPTERA, APIDAE)

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Abstract

A comparison was made between the seminal vesicles of adult males and late pupae of *Apis mellifera adansonii*. Differences were found in the epithelium, musculature, and membranous tunic.

Introduction

The seminal vesicle of the honeybee is a simple dilation of the vas deferens, where the spermatozoa are retained until copulation.

The walls are formed by three layers. The epithelium is made up of tall and narrow cells, covered by an inner sheath of circular muscle fibers, and by an outer sheath of longitudinal fibers. There is also an external membranous tunic. In the seminal vesicles, the spermatozoa have their heads embedded in the epithelium, where it was thought they attain their final form (Snodgrass, 1956).

The only work we know on the ultrastructure of seminal vesicles of insects is Bawa's (1966) on the epitelium of *Thermobia domestica*. Work on vertebrates (Deane & Porter, 1960; Deane, 1962; Dangelo & Munger, 1964; Deane & Wurzelmann, 1965) is only partly comparable to that of invertebrates.

This paper is the first of a series of reports on aspects of the ultrastructure of the organs involved in the storage and maturation of sperm.

Materials and Methods

Seminal vesicles were obtained from pupae ready to emerge and from mature adults of *Apis mellifera adansonii* Latreille. The

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animals were anesthetized by freezing, and the organs removed and placed in saline. Specimens were fixed immediately in phosphate-buffered 1% OsO_4 (Millonig, 1964), phosphate-buffered 1% OsO_4 with glucose (Caufield, 1957) and phosphate-buffered 3% glutaraldehyde followed by post fixation in 1% OsO_4 in the same buffer (Sabatini, Bensch & Barnett, 1963). After fixation the organs were quickly dehydrated in acetone and embedded in Epon. Blocks were sectioned with glass knives on a MT2 Ported Blun ultramicrotome.

Sections were double-stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and examined in a Siemens Elmiskop I Microscope, at the Section of Virology, Instituto Agronômico, Campinas, thanks to the courtesy of Drs. Alvaro Santos Costa and Elliot W. Kitajima.

Light micrographs of 7 μ paraffin sections of the seminal vesicles have been used to study the overall organization within the organ. The material was then fixed in Bouin's fluid and sections stained with Delafields's hematoxylin and eosin.

Results

The results will be described by structure rather than by age of specimens, to avoid repetition.

Whole organ structure

In the pupa each seminal vesicle consists of a slightly curved tube composed of the same layers described by Snodgrass (1956) for adults. In the adults seen the organ was dilated by the presence of spermatozoa inside it.

Figure 1 shows a longitudinal section of a seminal vesicle from an adult. One can see that the epithelium is much folded internally. In the younger animals, the folds were even more conspicuous due to the absence of spermatozoa. The cells are tall and very narrow; accordingly, the nuclei are elongated, crowded and pushed to different levels, so that the epithelium seems to be of the pseudostratified type. In the adults the spermatozoa crowded in the lumen distend the walls and push the cells against the basal membrane, resulting in a single columnar rather than a pseudostratified arrangement.

The cytoplasm of the epithelial cells is more basophilic in the pupa than in the adult. The outer fibrous membrane of the organ is also thicker in the pupa.

Cytology of the epithelium

The epithelial cells of the seminal vesicles are very tall, columnar in shape, almost filiform (fig. 2). In the pupae, they were on the average 30 μ high and 2.5-3 μ wide; in the adults, respectively 20 μ and 3.5-4 μ wide. They typically bulge somewhat into the lumen, the apical membrane forming crowded, long microvilli; these are longer (1-1.5 μ) and more crowded in the pupa than

in the adult, where they are short and irregularly spaced. The microvilli generally have a dense filament within (fig. 6). The lateral margins of the cells show slight undulations and some interdigitations, more easily seen in cross or oblique section through the epithelium (fig. 6). In the adult, intercellular spaces are also frequent. The apical portions of the cell are tightly bound together by broad, dense, junctional complexes (figs. 2, 4 and 8) that are entirely typical of the terminal bars found in most mammalian columnar epithelia (Farquhar & Palade, 1963). Although the resolution of micrographs 2 and 8 does not permit identification of the layers of membranes, there is a *zonula occludens* and an underlying *zonula adherens,* which can be seen in figure 5 (cross section). In the rest of the lateral margins there are septate desmosomes (Wood, 1959).

The epithelial cells of the seminal vesicles display prominent membrane infoldings, very elaborate on the basal side (figs. 2 and 7). A thicker basal lamina (700 mμ) of lower density to the eletrons is always present.

In pupal cells the electron density of the cytoplasm (figs. 2 and 3) is low, as compared to that of the adult (figs. 8 and 9), because of the absence of electron-dense structures.

The endoplasmic reticulum is not greatly developed even in pupal cells. Only scattered, short lengths of ergastoplasm are found in sections. These membranes associated with ribosomes occupy a very small proportion of the cell volume, chiefly around the nuclei. The greater basophilly of the pupal cells seems to be due to the clusters of ribosomes scattered in the cytoplasm. In the adult this type of ribosome almost disappears and most ribosomes appear attached to membranes. Thus, even if cell basophilly is lower in the adult, the development of the ergastoplasm in the cells is practically the same as in the pupae.

The Golgi complex (fig. 7), located around the nucleus, appears only in pupal cells, where it is small and undistended. We have never seen unequivocal enlargement of the Golgi vesicles or granule formation in the Golgi zone.

The epithelial cells show fairly abundant mitochondria. These organelles are slender and quite long (exceeding 2 μ in length) in the apical part of the cell (fig. 2), and are more rounded basally, where they are encircled by the plasma membrane infoldings (fig. 7). However, a typical association between the plasma membrane infoldings and the mitochondria has not been seen. The apical border is richer in mitochondria than the rest of the cell, and the cells of adults are richer in these organelles than those of the pupae. Opaque granules within the mitochondria are very uncommon in the younger specimens, but very frequent in adults (figs. 10 and 11). Some of the mitochondria that contain granules become greatly enlarged (fig. 11); those occur preferably in the basal portion of the epithelial cell, although they may also be found on the apical side.

The nuclei are large and ovoid-spherical in shape, depending on age. Generally they tend to be spherical in the adult. In all specimens the nucleus, cut on the appropriate plane, shows one or more nucleoli.

The epithelial cells in both pupa and adult show a few segments of microtubules (figs. 4 and 6). Apparently there is not a preferential location or orientation of these structures. Some multivesicular bodies are also seen in the pupal cells. They are absent in the adult.

No granules were found which could be interpreted as secretory material; however, in adults, the apical border frequently forms bubbles that later become free in the lumen. These bubbles were also seen, but less frequently, in the pupae. On the other hand, in the cytoplasm, above the nucleus, enormous vesicles appear, filled with a low density material which could be a secretory product (fig. 10). Small vesicles also appear but there is no evidence that they would, by fusion, originate the large ones. Structures very frequent in the adult cells are bodies made up concentric arrays of membranes (fig. 9). These organelles, if they may be so called, were described by Gabe & Arvy (1961) as parasomes.

Figure 25 is a diagramatic representation of the seminal vesicle epithelium, showing the principal differences between adult and pupa.

Musculature

The musculature of the seminal vesicle is striated and arranged in two layers, one inner, circular and one outer, longitudinal. The muscle fibers are characterized by the lenght of the sarcomeres, about 6 μ from Z line to Z line. In the preparations the bands are not well set of (figs. 12 and 13), but it was possible to ascertain that band A was 5 μ long. Figure 13 shows a muscle fiber sectioned at the Z line level. Miofilaments within a given fiber may not be parallel (fig. 12) but this condition is very rare. Sarcosomes (mitochondria) are small and scarse, scattered in the sarcoplasm, among the bundles of miofibrils. The endoplasmic reticulum is also poorly developed and the ribosomes appear free in the sarcoplasm. Adjacent fibers may come into contact at certain point by projection of the sarcolemma (fig. 3); it was impossible to make sure whether there is a real connection or a simple contact. The intercellular spaces are crossed by wavy filaments (fig. 13, 14 and 15), perhaps belonging to the intercellular substances. This musculature is innervated, and frequently sections of axons are found among the muscle fibers (fig. 15); however, actual connection between muscle and axon was not seen. The muscle shows the same features in pupa and adult, with the excetion that the former has more ribosomes.

Fibrous membrane

The fibrous membrane is built of flat cells belonging to the fat body or to the haemolymph. In the pupa these cells are more rounded, rich in glycogen (fig. 16) and have also a few lipidic droplets. In the adult the cells are remarkably different. The lipidic drops are still present, and in some cases in larger numbers; besides this, there appears a membrane made of peripherical granules

with a dense peripheral zone (fig. 17). These granules vary in size (0.5-3 μ), as does the thickness of the dense peripheral zone. They are very similar to the granules usually described for serous glandular cells. Another interesting feature is the abundance of microtubules in these cells (figs. 18 and 19). The microtubules are also present in the pupa but in the adult they seem to be more numerous. They form bundles (fig. 19) and, although they are not parallel, in the same cells cross and longitudinal sections of them never appear simultaneously. They are frequently found parallel to the modified and greatly elongated mitochondria (fig. 18).

Discussion

Several points of this report seem to deserve comment.

In broad outline, the maturation of the seminal vesicle epithelium follows the expected pattern, but there are many gaps in the morphological sequence. The immature cell contains an abundance of polyribosomes but only a little endoplasmic reticulum and a small Golgi zone. The adult cells exhibit a decrease in polyribosome content, practically the only ribosomes remaining being those attached to the reticulum membrane. This fact is consistent with the evidence for the action of the polyribosomes as a messenger RNA; indeed such ribosomal aggregates must occur in great numbers before secretory activity begins.

Although it is to be expected that the cells of the seminal vesicles produce some proteic secretion, typical development of the ergastoplasm and enlargement of the Golgi saccules have never been seen. In the pupa the Golgi apparatus appears in a presecretory stage; in the adult it has already disappeared. Only in a few cases it was possible to see small vesicles in the Golgi zone; thus secretory granules were not seen. The only product that could be considered as secretion was that within the vesicles in figure 10. It must be said, here, that the fixation of this epithelium was very difficult, and that the best preparations were obtained with Caufield's fixative, which contains sucrose. According to Deane (1962) fixation of rat seminal vesicle epithelium is also difficult, and improved by sucrose. This may be due to the nature of the secretory products. In many species of vertebrates the epithelium secretes fructose, which serves as a nutrient for the spermatozoa. The presence of fructose within the cells would cause an increased intake of water from a hypotonic solution of fixative (Deane, 1962). In spite of the seminal vesicles of bees being morphologically quite different from those of the rat, their secretion has the same function, the nutrition of the spermatozoa during a phase of their life. The absence of secretory granules in the epithelium of the seminal vesicle of bees could be explained by admitting that it secretes fructose. Otherwise, in some grandular cells of bees (Cruz-Landim, 1968 and Cruz-Landim & Camargo, in press), the secretory products are so fluid that they do not form granules or vesicles, but deposits of low density in the cytoplasm; this may be the case here. Sucrose and protein eventually produced would go into the apical cytoplasm before being eliminated by bubbling through the apical membrane. This would also explain the little development of the Golgi apparatus. In this case the

resorption of the solute would occur by the microvilli (Cruz-Landim, 1967). Another fact in accordance with these hypotheses is the presence of mitochondria with dense granules in the matrix in the apical part of adult epithelial cells. It is known that these organules are specially prominent in tissues transporting large amounts of ions of water (Fawcett, 1966).

The parasomes are seen in the cells when debris must be resorbed; therefore, their presence in adult cells is taken as evidence of resorption.

The epithelial cells, as well as the peripheral ones, present a cytoskeleton of microtubules. It is known from the works of Du Praw (1965), Freed (1965), and Sandborn, Zeberenyi & Messier (1966), that they act in saltatory movements of the cytoplasm and in intracellular transport mechanisms. It seems that in the present case they perform similar functions.

Finally, the cells that form the fibrous membrane are structurally more related to the fat body than to the haemolymph. Probably they are not functionally linked to the seminal vesicle, but form only a sort of adventitia around the organ.

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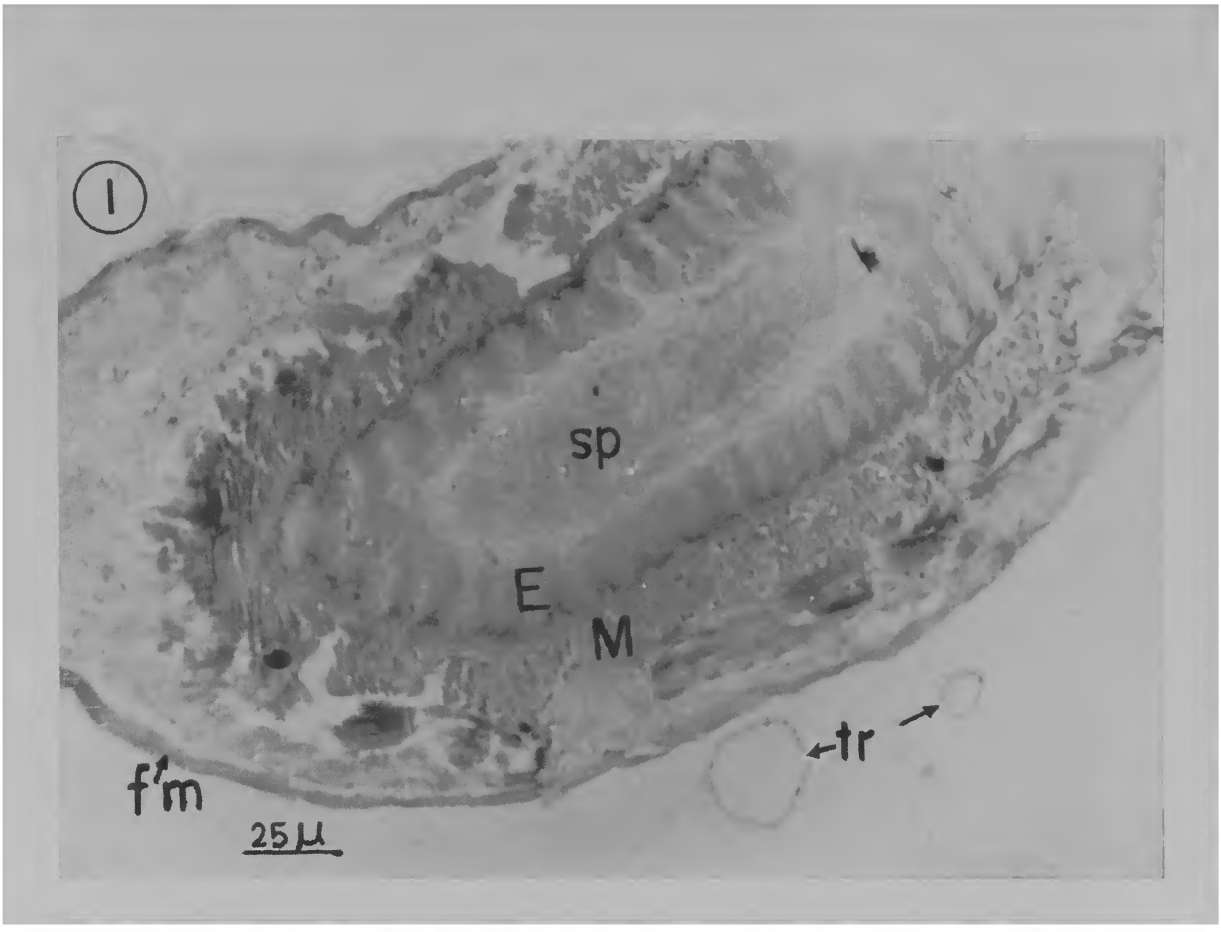

Plate 1: Light microscope micrograph of the seminal vesicle of an adult bee. Spermatozoa (sp) appear in the lumen; the epithelium (E) is folded and the nuclei basally placed; two sheaths of muscle (M) can be seen the inner circular, and the outer longitudinal; envolving the whole organ is a fibrous membrane (fm); some tracheoles (tr) are also seen.

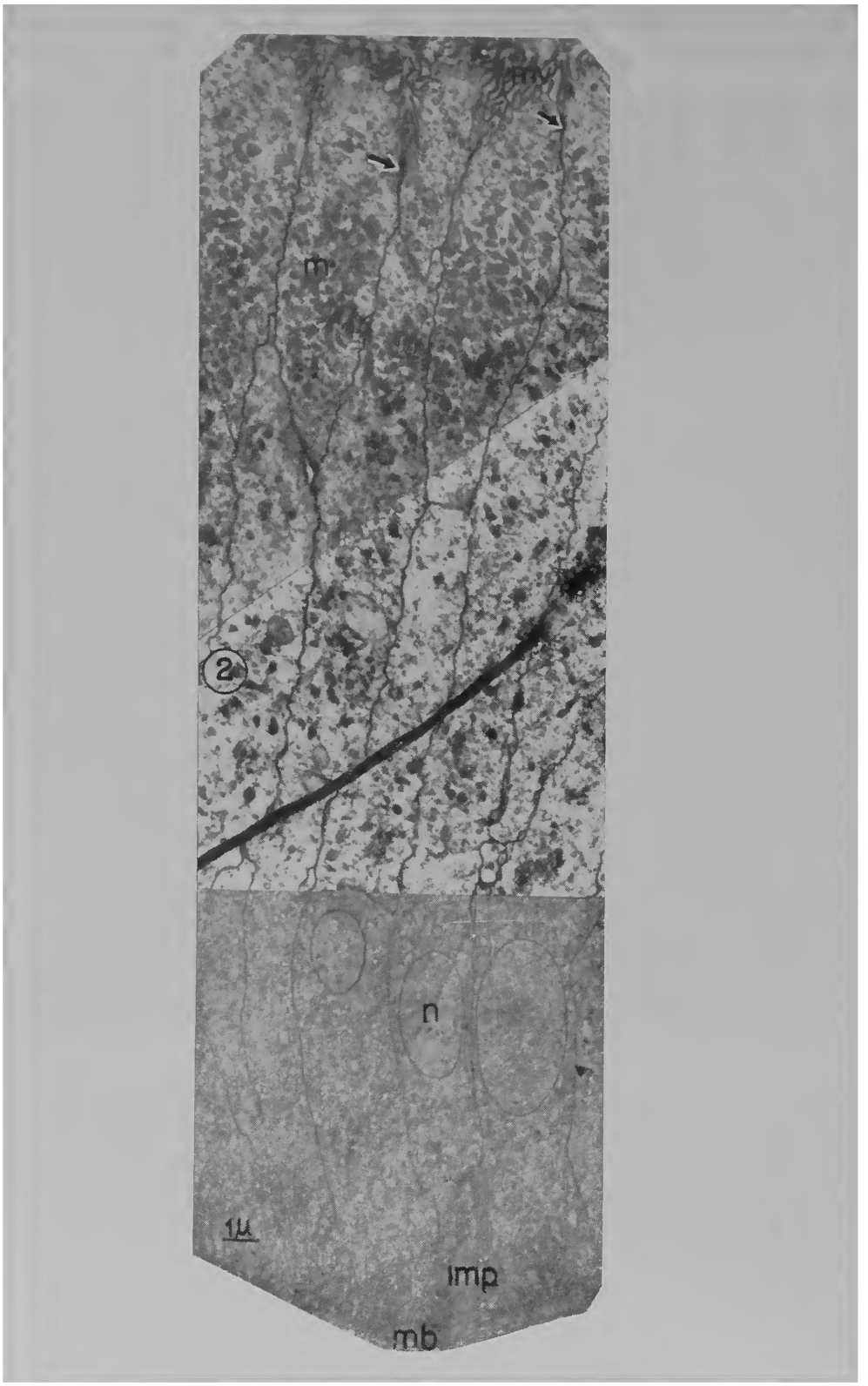

Plate 2: Ultrathin section of the seminal vesicle of a pupa seen at the electron microscope under low magnification. The epithelial cells appear very tall; the pseudostratified organization is clear; all calls are similar, with basal nuclei (n), sinuous lateral contacts, basal infoldings of the plasma membrane (imp), great quantity of mitochondria (m) in the apices, and microvilli (mv) in the apical membrane; the arrows point to the terminal bar; the epithelium basal membrane is labelled mb.

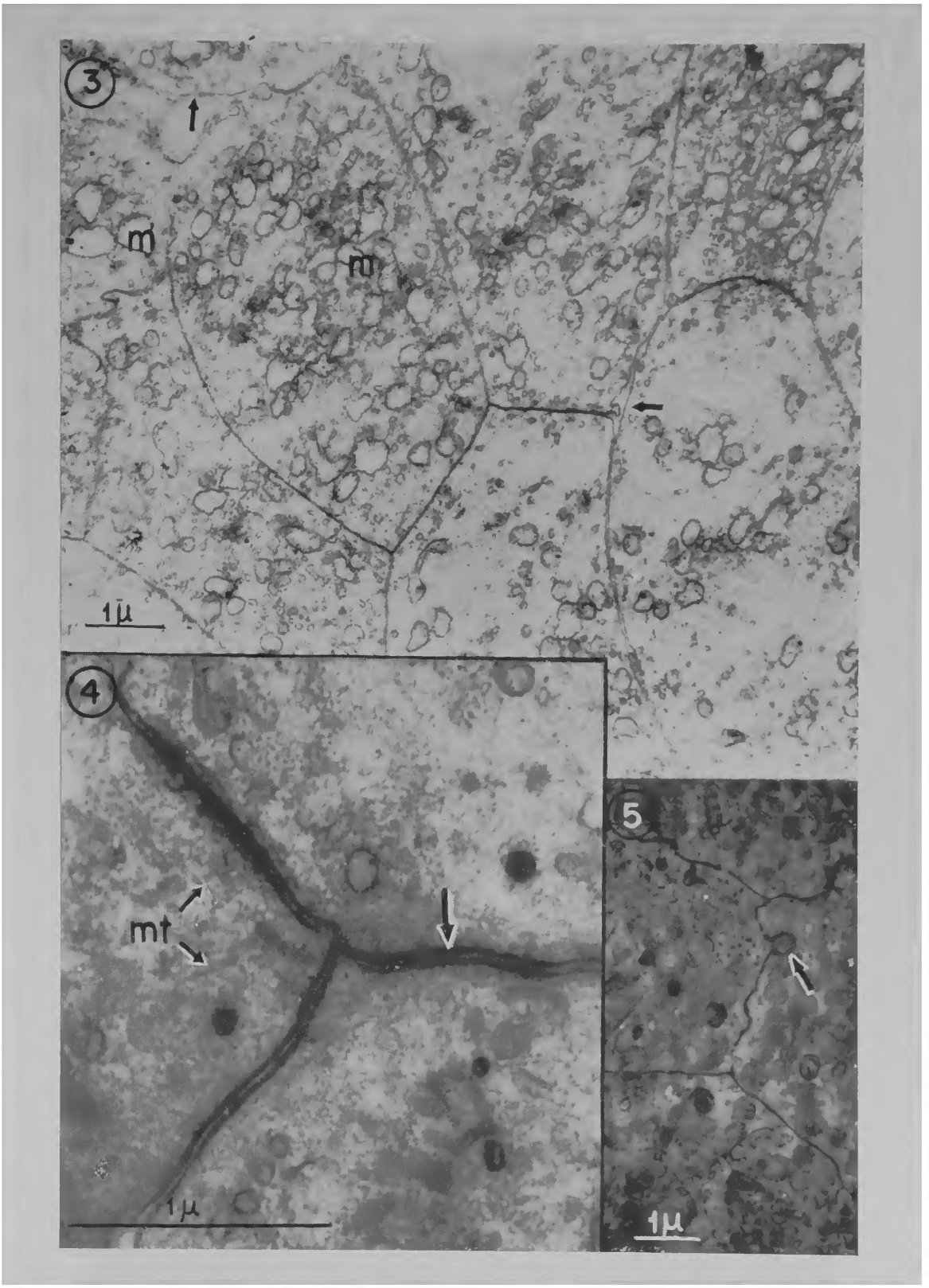

Plate 3: Cross sections of pupal seminal vesicles. 3, section through the zone that bulges into the lumen, just below the microvilli; cytoplasm very rich in mitochondria (m); the arrows point to remains of microvilli. 4, section through the junctional complex (terminal bar); microtubules (mt) present in the cytoplasm. 5, section below the preceding one showing *zonula adherens* (arrow).

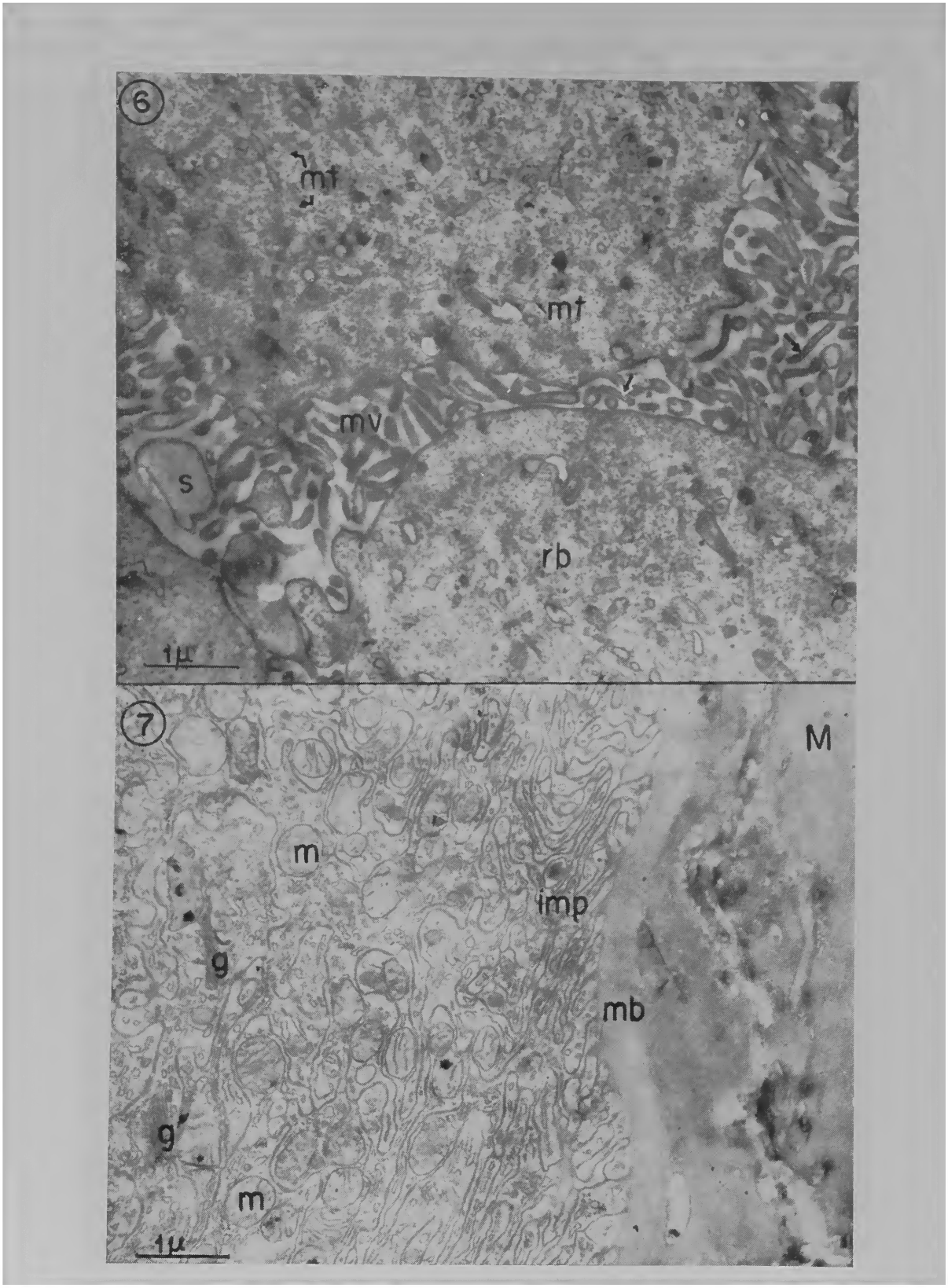

Plate 4: Pupal seminal vesicles. 6, apical portion of epithelial cells showing microtubules (mt), free ribosomes (rb), and microvilli with a dense filament within (arrow); the apical membrane forms bubbles presumed to be filled with secretion (s). 7, basal infoldings of the plasma membrane (imp) with some mitochondria surrounded by them; a few Golgi (g) zones are also shown.

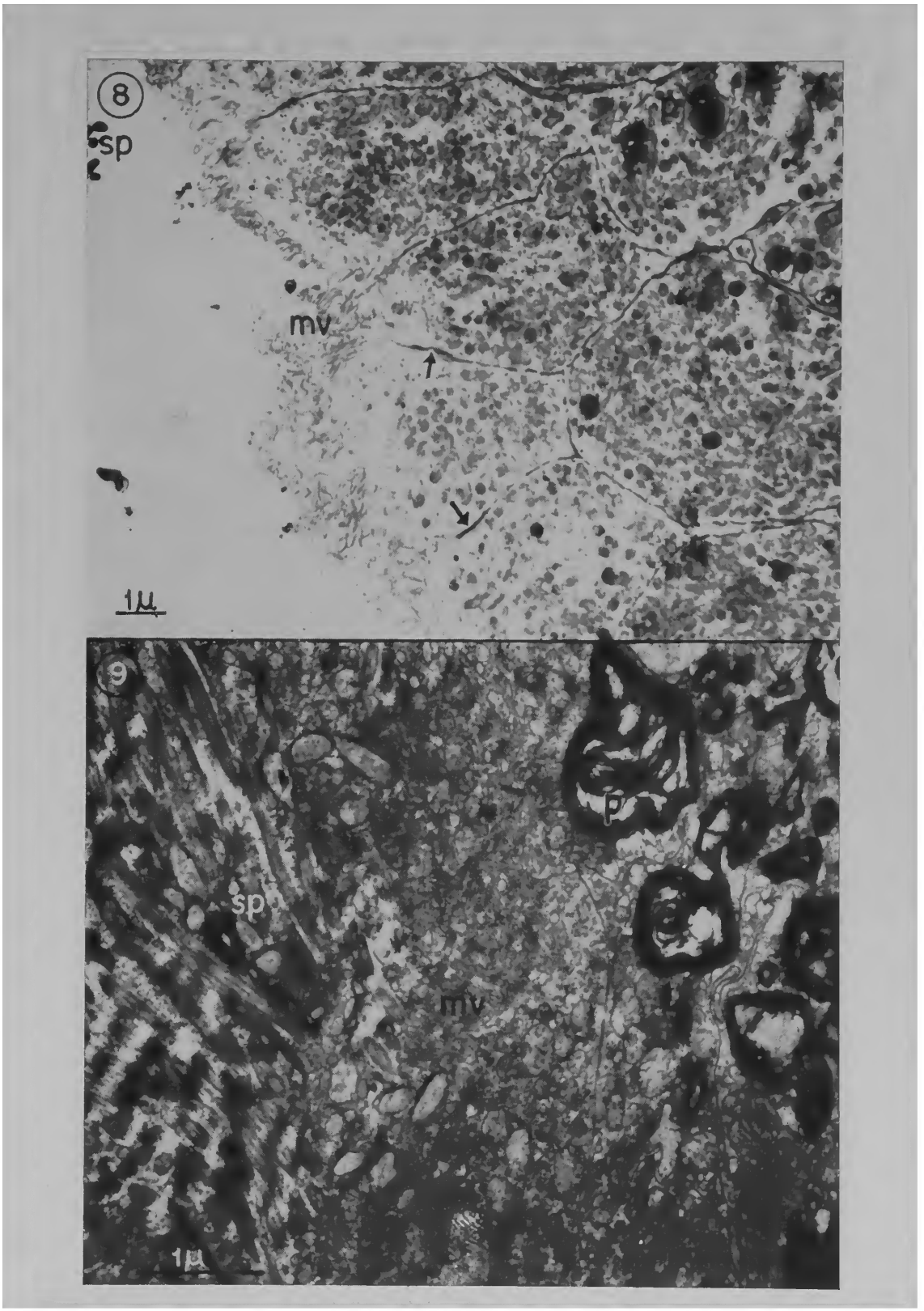

Plate 5: Apical portions of epithelial cells of adults. 8, the cells present several dense corpuscules (p) in the apical cytoplasm, better seen in 9; also concentric arrays of dense lamellae (parasomes); spermatozoa (sp) present in the lumen.

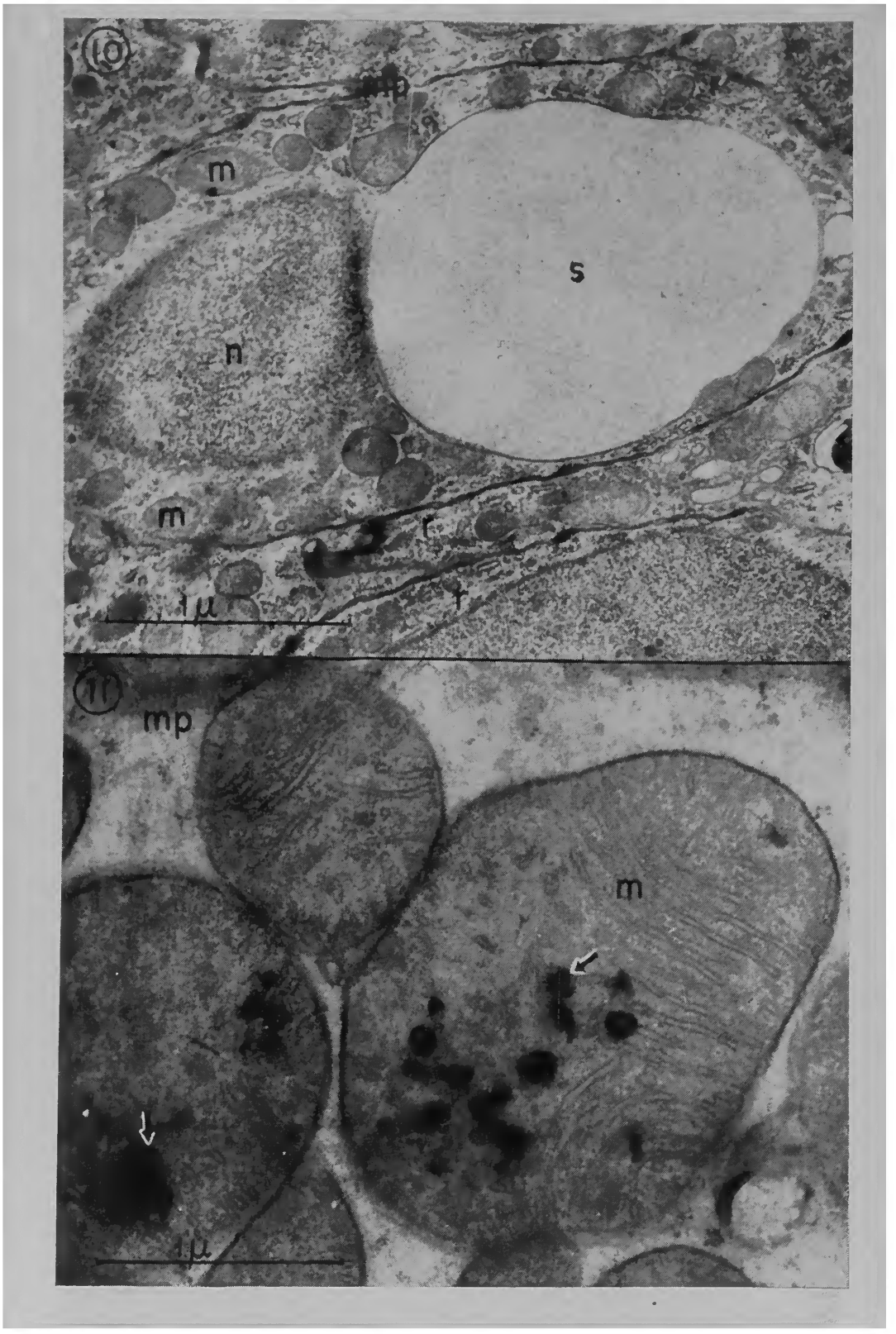

Plate 6: Two aspects of epithelial cells of an adult. 10, this cell besides the normal components had a great vesicle with secretion (s) above the nucleus (n); ribosomes are more frequently attached to the endoplasmic reticulum (r) in this phase. 11, some mitochondria from the apex of the epithelial cells with dense granules (arrows) within them; the short segment of the plasmic membrane (mp) shown in this picture shows septate desmosomes.

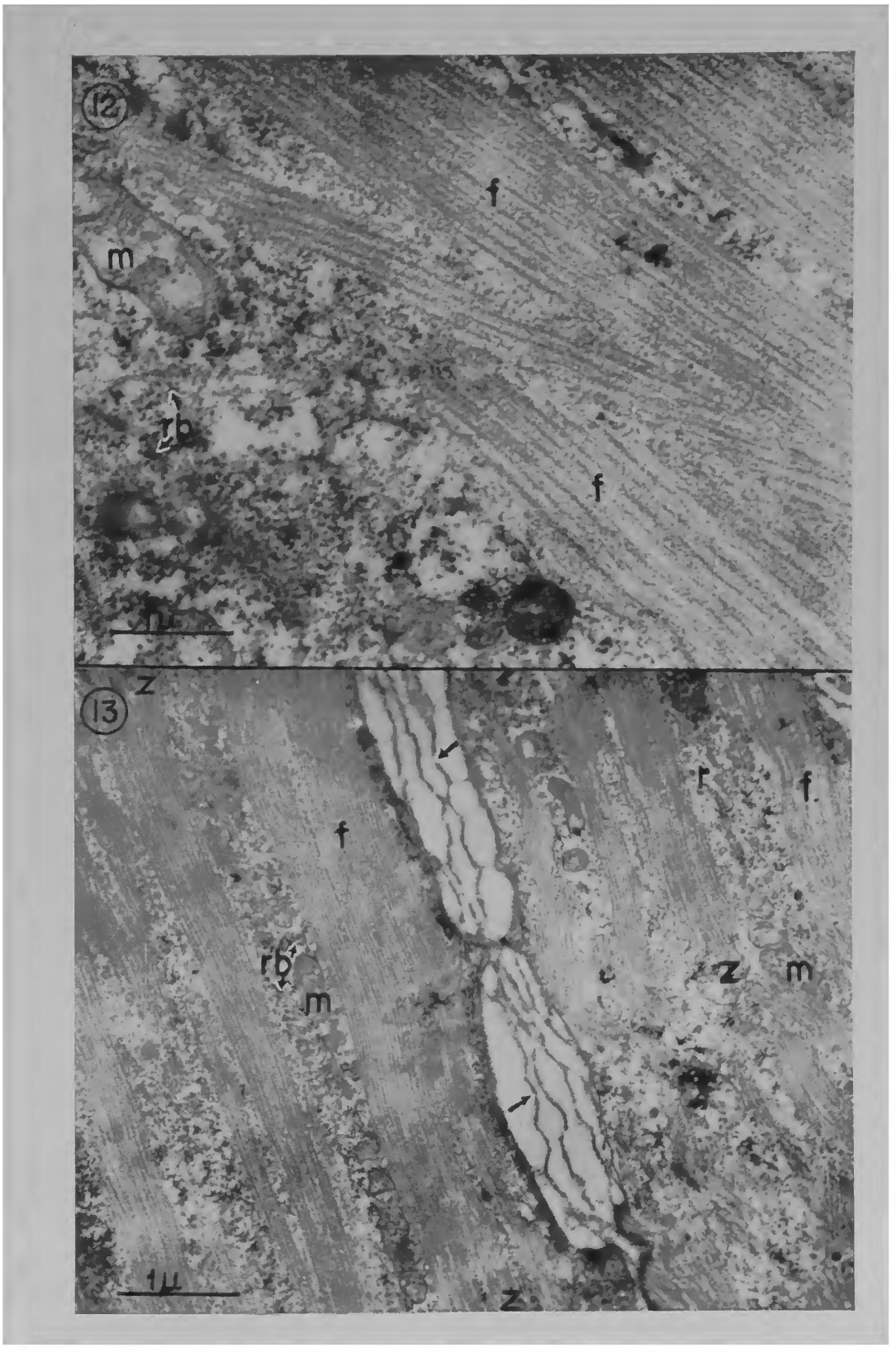

Plate 7: Musculature of a pupal seminal vesicle (longitudinal sections). 12, sarcoplasm rich in ribosomes (rb) and unexpected array of miofilaments (f). 13, a more typical picture; the muscular fibers have very long sarcomeres, few sarcosomes (m) and also little sarcoplasmic reticulum (r); filaments are frequent in the intercellular spaces (arrows).

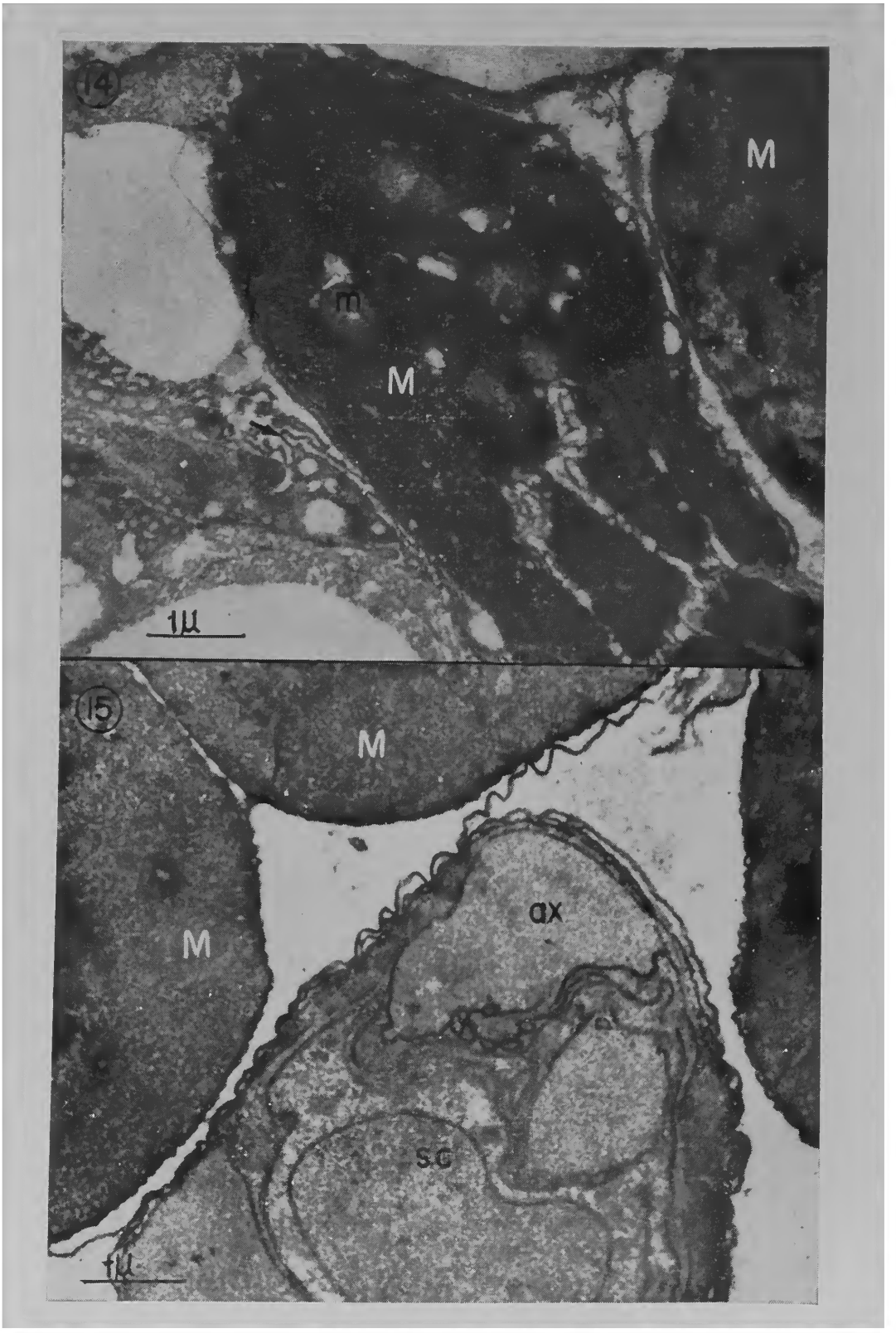

Plate 8: Cross section of adult muscles (m). 14, cross section through the Z line of a muscle fiber. 15, cross section of an axon (ax) among the muscle fibers; sc, nucleus of Schwann cell.

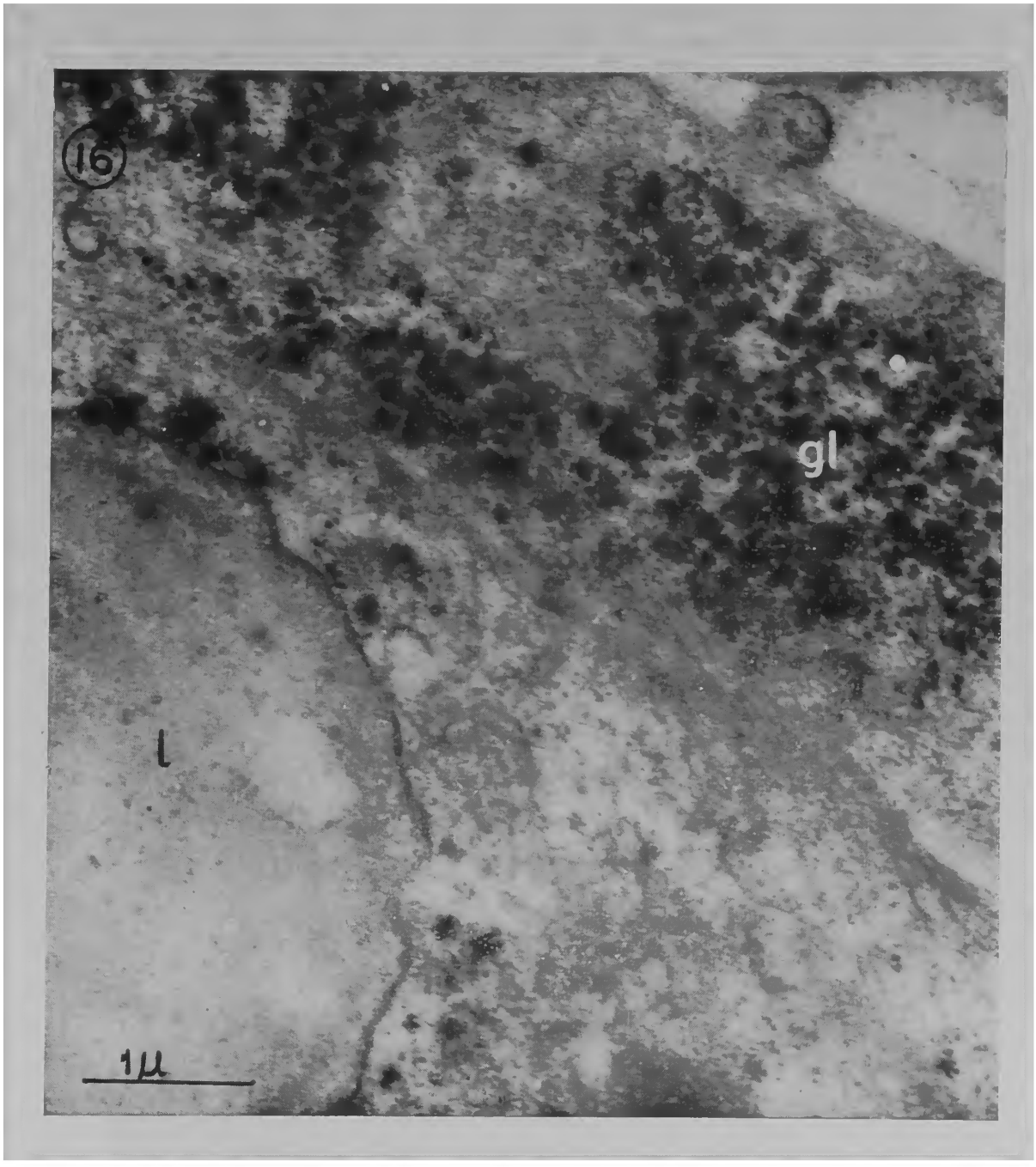

Plate 9: Cell of the fibrous membrane of a pupal seminal vesicle, showing glycogen (g) deposits and a lipid (l) droplet.

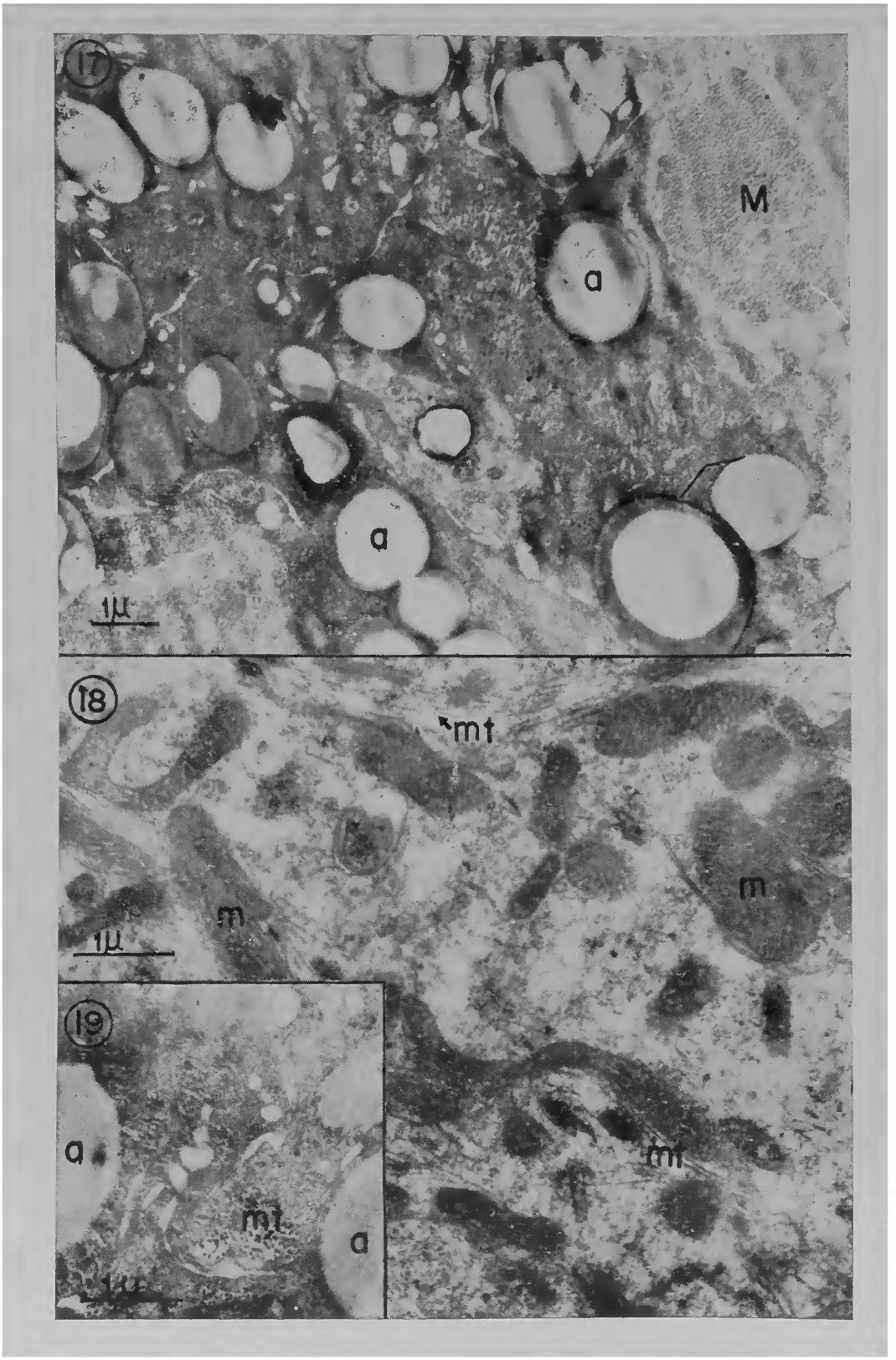

Plate 10: Fibrous membrane of adult, showing numerous unexplained inclusions (a) in 17 and bundles of microtubules in 18; 19 shows a bundle of microtubules cross sectioned.

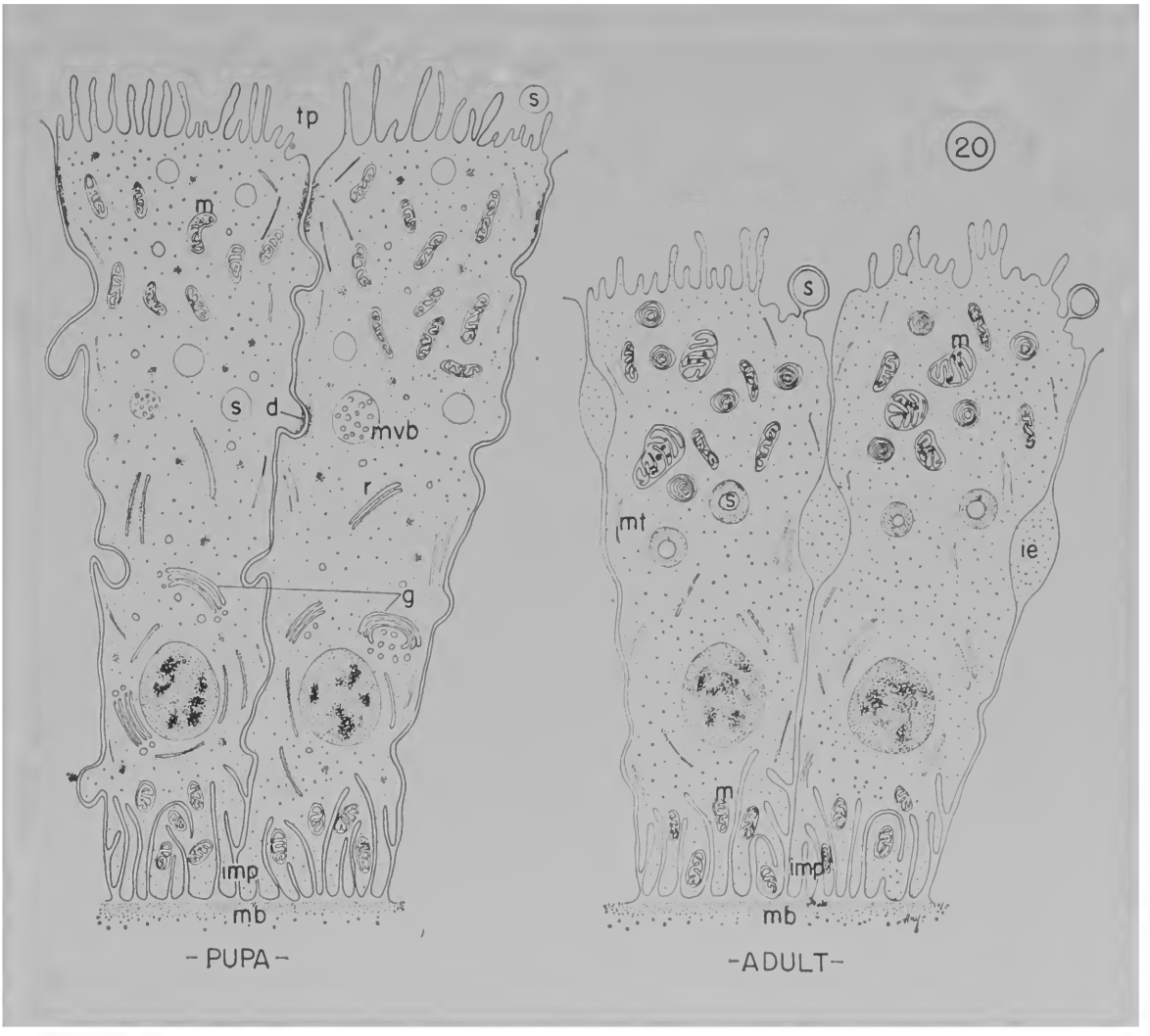

Plate 11: Diagrammatic representation of epithelial cells of pupa (left) and adult (right), showing certain features that are not seen in the fotographs, as well as the presence of intercellular spaces (ie) in the adult epithelium and multivesicular bodies (mb) in the pupal cytoplasm.