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Ultrastructural Differentiation of the Midgut Epithelium in Female *Aedes aegypti* (L.) (Insecta, Diptera) Imagines

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Introduction

Female mosquitoes of the *Culicidae* family are responsible for the transmission of various important parasitic diseases, such as filariasis, malaria and yellow fever. Transmission is performed in conjunction with blood meals and blood digestion by the mosquitoes. Blood digestion, therefore, has to be thoroughly understood in order to elucidate part of the existing host-parasite interrelations. For this reason, various authors (BERTRAM & BIRD 1961, ROTH & PORTER 1964, FREYVOGEL & STAEUBLI 1965; FREYVOGEL & JAQUET 1965; STAEUBLI, FREYVOGEL & SUTER 1966) studied the histological structures of the midgut epithelium and its changes in connection with the digestion of blood and the formation of the "peritrophic membrane", at microscopical as well as submicroscopical level. Special attention was thereby given the rough surfaced endoplasmic reticulum. It is found in the shape of conspicuous "whorls" or "fingerprints" in unfed *Aedes aegypti* females, but it breaks down upon the intake of blood by the mosquito, and, is subsequently built up anew towards the end of the digestion processes. Also, it has been postulated that some membrane material needed for the restauration of the ergastoplasmic whorls originated from the Golgi complex (STAEUBLI et al. 1966).

For obvious reasons, previous work has been carried out with mosquitoes of a certain age, having had at least one blood meal. It is known, however, that newly emerged female mosquitoes are not prepared to take up blood before at least 48 hours since emergence from pupa. In consequence we set ourselves the task to find out, whether this was due to incomplete differentiation of the midgut epithelium on emergence from the pupa and, if it were so, to study the final differentiation of the ultramicroscopical structures from emergence till readiness for blood meal.

Material and Methods

The mosquito stock used in the present study is *Aedes aegypti* (L.) which has been bred for many years in this laboratory. The egg material was originally derived from Congo (Kinshasa) and was handled according to usual methods. All rearing was done at $25 \pm 1°C$ and at a relative humidity of 80–90%. The larvae were fed with powdered dog-biscuits and the adult mosquitoes were maintained on cotton plugs saturated with 10% sucrose-solution. For our purposes pupae were isolated and the emergence of ♀♀ and ♂♂ was observed to set up an accurate timetable. Samples were collected at the following intervals after emergence: 10–60 min., 6, 12, 18, 24, 36 hours, 2, 3, 4, 10, 24 days with deviations exceeding not more than 1–2 hours. It should be noted, however, that even such a precise timetable did not exclude biological variation, that is to say, that specimens

collected at the same time after emergence showed different stages of midgut epithelium differentiation (cf. p. 86).

Females destined for fixation were completely starved with the exception of those aged 10 and 24 days. These got a first bloodmeal on a guinea-pig 3 days after emergence and were allowed to oviposit. The ♀♀ of 24 days received 2 further bloodmeals every 7 days following the previous one; finally, these had completed 3 gonotrophic cycles and never had sugarwater.

At the time of fixation females were shortly anaesthesized with ether and the hind part of midgut (the stomach) was removed under a dissecting microscope. Stages, from 24 hours onward, were dissected directly in a droplet of the prefixation solution, whereas younger ones, because of their extreme softness, were dissected in saline first and then immediately transferred into the prefixation solution. In the young delicate intestinal tube often the greenish meconium could be recognized, latest till 24 hours after emergence (cf. p. 81 below).

For each age group the midguts of 2 to 4 mosquitoes were prepared. Of each midgut cross-sections were performed in 3 to 5 plains.

Prefixation was carried out in 3% or 5% glutaraldehyde in 0,1 M cacodylate buffer (c.b., pH 7.2–7.4) at 4°C or room temperature for 2 hours. The blocks were then rinsed over night at 4°C in 0.2 M c.b., with sucrose added. Fixation followed with 2% osmium tetroxide in 0.2 M c.b. at 4°C for 2 hours. For the demonstration of acid phosphatases in lysosome-like structures (in midgut epithelial cells of 15 days old mosquitoes) a combined modification of the methods of GOMORI (1953), BARKA & ANDERSON (1962) and BLUEMINK (1967) was employed.

All blocks were stained with 1% uranyl acetate in 70% acetone, dehydrated in graded acetone and propylene oxide and embedded in Epon or Araldite. Thin sections were cut with glass knives (LKB Ultrotome I and III, Reichert OmU_2), stained with lead citrate and examined in a Philips EM 300.

Results

Midgut epithelium

In all cases examined the midgut epithelium consists of a single layer of cells. Its boundary towards the haemocoel is made up by the basement lamina, which, in turn, is surrounded by muscle and tracheal cells.

In newly emerged females the gut contains heterogenous material (fig. 1) which consists of remnants of membranous structures, myelin-like figures, multivesiculate bodies and, possibly, lysosomes. 24 hours after emergence these contents which form part of the meconium have disappeared.

Essentially, the epithelium is made up by a single type of cells, though it must be admitted that some cells are found which show more conspicuous contrast than the majority does. Around 2 days after emergence, on the basal side of the epithelium smaller cells are observed, which are characterized by their small amount of cytoplasm (fig. 31). It is presumed that these are undifferentiated regenerative cells (cf. SMITH 1968).

In early stages after emergence the epithelial cells are but loosely connected and dissimilar in appearance (fig. 2–4). Many of them show

apical extensions which bear only a few microvilli (figs. 3 and 4). Three days after emergence the cells have assumed their typical columnar shape, which they retain throughout most of the mosquito's life, with the exception of the periods of blood digestion (cf. BERTRAM & BIRD 1961, FREYVOGEL & STAEUBLI 1965, STAEUBLI et al. 1966). Older specimens – in our investigation from 24 days – exhibit a somewhat flattened epithelium, the cells assuming a cubical shape.

The connections between cells seem to grow from the apical side of the epithelium, while on the basal side extracellular cavities are to be observed for quite some time (fig. 4). Not before 24 hours after emergence the first septate desmosomes are observed in the apical zone (fig. 5). They gradually extend towards the centre. In addition, after 3 days, maculae adhaerentes (FAWCETT 1966) can be found in the zone of the basal labyrinth (fig. 6) and up to the height of the nuclei. From then the epithelium seems to be firmly fitted together. The same is true 10 days after emergence, i.e. after digestion of a blood meal (figs. 7 and 12). In mosquitoes, 24 days old, septate desmosomes, maculae adhaerentes and vesicular shaped stretches of loose contact are seen next to each other (fig. 8). The surface membranes of neighbouring cells are separated by a gap of 110–160 Å, with the exception of the vesicular shaped stretches of loose contact just mentioned (figs. 5 and 9). These tend to get smaller with the mosquitoes' further development, though they never entirely disappear (fig. 8).

Microvilli (figs. 2–5, 10, 22, 26–30)

In cross sections their diameter measures some 110 to 140 mμ. They exhibit a fine coat of intermediate electron density (fig. 5), the glycocalyx (cf. SMITH 1968). The contents seem to consist largely of very thin fibrils which extend into the apical cytoplasm of the cells (fig. 10). A connection with desmosomes could not be observed such as was seen in resorptive cells of rats' small intestine (BRUNSER & LUFT 1970).

In young females the microvilli are less numerous than in older ones and are located mostly in depressions next to the cell boundaries, in between the apical extensions of the cells (figs. 3 and 4). When these disappear the brush border attains a more regular appearance. Especially in young females, some vesicles are to be observed at the basis of microvilli.

Basal labyrinth

This consists of numerous infolds of the cell membrane on the basal side of the epithelium. In young females the extracellular clefts between cell processes are rather wide and irregular in shape (figs. 2, 4 and 11), but become more narrow in older ones until they form nearly parallel in-

vaginations extending quite far into the cells (figs. 7, 12 and 31). Intracellularly are found mainly mitochondria, free ribosomes and microtubuli (figs. 7, 11–13 and 31). The basal labyrinth of older females (24 days), again, is less regular (fig. 13).

Basal lamina ("Basement membrane")

It is present in all stages of adult mosquito life (figs. 2, 4, 11–15 and 32), mostly consists of several layers and is often folded. It is always partly "striated", whereby the transverse structures consist of electron-dense short rods (figs. 14 and 15) as described by previous authors for *Aedes aegypti* (BERTRAM & BIRD 1961) and for *Anopheles quadrimaculatus* (VANDERBERG, RHODIN & YOELI 1967). Mainly on the haemocoel side, there are additional amorphous layers of intermediate electron density. These layers often extend into the thin basal lamina of neighbouring muscle, nerve, or tracheal cells (fig. 14). In older mosquitoes the basal lamina seems to increase in width and its "striation" gets more conspicuous.

Nucleus (figs. 2, 7, 16, 18–20, 22–25, 30 and 31)

In young females the nuclei appear quite large in comparison to the volume of cytoplasm (fig. 2). They are, then, centrally located and their contours are irregular. When the cells grow in size the nuclei assume a somewhat more basal location and become slightly ovoid. The nucleus/cytoplasm ratio is shifted in favour of the cytoplasm. The nucleus' diameter measures some 4.5–7 μ in young mosquitoes, while its longitudinal axis measures up to 10 μ in fully developped epithelial cells.

The nuclei possess two membranes forming the perinuclear cisterna traversed by nuclear pores. Ribosomes are found on the outer membrane. The nucleoplasm is flocular to granular and is of intermediate electron density. The granula's size corresponds to the size of ribosomes (figs. 16 and 17). Heterochromatin may be found neither in young (figs. 2, 4 and 16) nor in 24 days old (fig. 20) mosquitoes. Heterochromatin is, however, conspicuous in nuclei of mosquitoes 2–4 days (figs. 18 and 23) and 10 days (figs. 7, 19 and 31) old.

The nucleolus, in young females, consists of loosely connected masses of material, finer in the centre and coarser at the periphery (figs. 16 and 17). In older mosquitoes the masses have joined and form a rather compact nucleolus. It then consists of filamentous to fine granular material in its central part, which is interspersed and surrounded by more coarse grana of ribosomal size (figs. 18–20).

Ribosomes and endoplasmic reticulum

Free ribosomes are found at all stages of adult mosquito life in the epithelial cells' cytoplasm. They seem to get less numerous with age, which explains the lighter complexion of epithelial cells in older animals (figs. 20 and 29). Also, the most apical zone of the cells contain less ribosomes but, instead, filaments from the microvilli which cause the fine fibrillary appearance of this zone (fig. 10).

It is the rough surfaced endoplasmic reticulum (rer) which undergoes the most striking development in the final differentiation of the midgut epithelial cells. In young females the cells show only short bits of membranes covered with but a few ribosomes (fig. 21). These membrane pieces are located in the apical half of the cells, mainly in the vicinity of Golgi zones and of the nucleus. It is there that the formation sets in (fig. 22) of what is to become the "ergastoplasmic nebenkerns" (HAGUENAU 1958), also called "whorls" or "fingerprints" as studied in the context with blood digestion by BERTRAM & BIRD (1961) and STAEUBLI et al. (1966). Soon their tubes get longer and studded with numerous ribosomes. They, then, are found in the basal half of the cells as well. After 36 hours the tubes begin to form parallel groups (figs. 23 and 24). The formation of whorls is completed 3–4 days after emergence (fig. 25). They are seen also 10 days after emergence (fig. 26), but in 24 days old mosquitoes they are no longer found. Besides the actual whorls shorter bits of membranes with ribosomes are observed all over the cytoplasm (figs. 20, 25, 26 and 29). Such pieces are even to be seen in older females (figs. 20 and 29).

The smooth endoplasmic reticulum consists of a few vacuoles and remains inconspicuous.

Golgi zones (figs. 9, 20–26 and 29)

Several such zones are already present immediately after emergence from the pupa in the apical half of the cell (figs. 9, 21 and 22). Later a few are also found in the region of the basal labyrinth. In 24 days old mosquitoes they appear vesicular and light (figs. 20 and 29).

Multivesiculate bodies and lysosomes (figs. 9, 20, 22–29)

Multivesiculate bodies (fig. 27) are found mainly in the apical portion of the cells, and, throughout the adult mosquito's life.

In young females only a few dense bodies with heterogenous contents such as lamellar and membranous structures are to be seen (figs. 9 and 22). In older mosquitoes lysosome-like bodies are more numerous, more varied in size and shape and may also contain myelinlike figures.

In 24 days old females the cells show numerous and partly very extensive catabolic organelles, and, in addition, often light patches, which seem to be connected to those organelles. These light patches have no membrane and appear to be located there, where fingerprint-like whorls are found in younger mosquitoes (figs. 20 and 29).

The test for acid phosphatases was carried out with 15 days old mosquitoes. The presence of acid phosphatases could be demonstrated (fig. 28). Thus it can be assumed that our various lysosome-like bodies are, in fact, lysosomes (DE DUVE 1963, NOVIKOFF 1967 a and b).

Mitochondria (figs. 2–3, 7, 10–13, 20–32)

Mitochondria are numerous in the midgut epithelial cells. In young females their surface is irregular, the cristae are wide and not parallel. The matrix appears of varying density and contains small granules (fig. 30). It seems that the cristae get arranged in parallel fashion earlier in mitochondria located in the basal portion of the cells than in those located in the apical portion. Approximately 2 days after emergence mitochondria of various size, but with parallel cristae are found in substantial number. They attain the highest number in the apical and basal portions of the cells; they are less numerous in the central part.

Microtubules (figs. 9 and 12)

Microtubules can be detected in between the other organelles, all over the cells. They are often camouflaged by free ribosomes.

Regenerative cells

They can be observed from 2 days after emergence. Their nuclei show much heterochromatin. They contain little cytoplasm, which is of higher electron density than the cytoplasm in the typical epithelial cells (fig. 31). It contains free ribosomes, rarely short bits of rough endoplasmic reticulum and few mitochondria.

Further observations

No "goblet cells" were seen (ANDERSON & HARVEY 1966, SMITH 1968). Neither were other cells of a secreting type found, though, in the basal half of some cells small vacuoles could be observed which might be connected with some secretion (fig. 32).

Many tracheae and tracheoles end on the midgut epithelium (figs. 11 and 31). They are closely connected with its cells, often sharing parts of the basal lamina.

The same applies to muscle cells, which surround the midgut in a net-like fashion (figs. 2, 4, 13–15 and 31). The growing regularity in the arrangement of the myofilaments and the general differentiation of the muscle cells run parallel to the development of the midgut epithelium.

Discussion

General progress of differentiation

It has been pointed out before (cf. p. 81) that time indications with respect to differentiation processes of midgut epithelial cells can be only of limited value, since there are considerable biological variations among individual mosquitoes. Nevertheless, we have tried to set up a time table of differentiation of midgut epithelial cells in *Aedes aegypti* females, in order to come to a synopsis of events such as take place after emergence (Table 1).

As can be seen from Table 1, upon emergence of the female mosquito, differentiation processes in the midgut epithelium are by no means terminated. Within the cells, several organelles (e.g. rough endoplasmic reticulum, mitochondria, lysosomes) are found but in an early stage of formation, while the epithelium as a whole does barely represent a well knitted unit (e.g. no septate desmosomes or maculae adhaerentes). Also, neither the microvilli nor the basal labyrinth, both likely to play an important part in the absorption and transport of food substances, have yet reached their final shape. It may well be that this state of incomplete differentiation is one of the reasons for the failure of newly emerged mosquitoes to take up blood; neither could their stomach withstand the pressure from the blood taken in, nor would digestion be possible. So, although metamorphosis has been undergone by the mosquito, its midgut epithelium is not functional until two to four days after emergence. With respect to the rough endoplasmic reticulum the final differentiation processes are the same ones which take place after each bloodmeal, as described by BERTRAM & BIRD (1961) and STAEUBLI et al. (1966).

Single organelles and various observations

Our observations confirm that the midgut contents of newly emerged females, the meconium, consist of cellular fragments, which most likely stem from the larval gut (CLEMENTS 1963).

In some of our sections undifferentiated regenerative cells were found, such as were also mentioned by STOHLER (1957). It is striking, however, that such cells were not seen before two days after emergence.

Table 1. Time table of differentiation of midgut epithelial cells in *Aedes aegypti* females

Approx. time after emergence	Status of differentiation
Upon emergence	– cells irregular in shape, with apical extensions into lumen – microvilli few, most in depressions next to cell boundaries – basal labyrinth irregular, with wide gaps – basal lamina present, faint "striation" – nucleus centrally located, irregular in shape, diameter 4,5 to 7 μ, no heterochromatin, nucleolus components loosely connected, nucleus/cytoplasm ratio high – free ribosomes all over cytoplasm – rer poorly developed, short tubules with few ribosomes – several Golgi zones present – few lysosomes (dense bodies) – mitochondria irregular, with non-aligned cristae
1 day	– septate desmosomes appearing from cell apex – microvilli more numerous, evenly distributed – rer developing
2–3 days	– cells columnar, regular in shape, without apical extensions – septate desmosomes and in addition maculae adhaerentes – nucleus shows heterochromatin, nucleolus more compact, nucleus/cytoplasma ratio lower – rer: formation into whorls – mitochondria: surface regular, cristae parallel – regenerative cells observed
4–10 days (see p. 81 for previous blood-meals)	– epithelial cells closely connected, septate desmosomes and maculae adhaerentes numerous – basal labyrinth with deep and regular, parallel invaginations – basal lamina thick, distinct "striation" – nucleus up to 10 μ (longitudinal axis) – rer: whorls fully developed – many heterogenous lysosomes
24 days	– cells cubical in appearance – basal labyrinth somewhat irregular – nucleus without heterochromatin – free ribosome density in cytoplasm diminished – rer: whorls missing (possibly remnants present) – Golgi zones vesicular – lysosomes very conspicuous (residual bodies)

It looks as if all replacement cells, as mentioned by CLEMENTS (1963), begin to differentiate towards adult epithelium around emergence. However, some few of them, seem to stop further development early in adult life and then to change into the typical regenerative cells. Similar conclusions were reached by O'BRIEN (1966) with respect to the formation of "basal cells" in early larval life.

In view of the comparatively large quantities of blood ingested by *Aedes aegypti* (FREYVOGEL & JAQUET 1965) it is obvious that the stomach must be able to resist a certain pressure from inside. It appears that the strength needed is acquired in three ways: by consolidation of the epithelial cell layer, by solidification of the basal lamina, and, by the formation of a muscle network around the midgut. Consolidation of the epithelium is thought to be brought about by the progressing alignment of its cells and by the formation of numerous septate desmosomes and maculae adhaerentes. As to the basal lamina in addition to its growing thickness, the distinct "striations" as observed by BERTRAM & BIRD (1961), VANDERBERG et al. (1967) and by ourselves is considered to be an indication of solidification. Finally, development of the muscle network takes place simultaneously with the differentiation of the midgut elements.

The presence of microvilli means an enormous increase of the absorbing cell surface. The microvilli are first often found in depressions next to cell boundaries. It seems conceivable, that the cell surface first forms extensions towards the gut lumen and, then, from the "surplus" of membrane material, part of the microvilli. GANDER (1968) observed cell extensions in conjunction with blood digestion in *Anopheles stephensi*. Such extensions seem to be formed only at certain times of the digestion process. This finding might indicate the possibility of reverse transformation of microvilli into extensions. Finally, it may be mentioned that FREYVOGEL & STAEUBLI (1965) described epithelial midgut cells of unfed *Aedes aegypti* with a swollen apex, thus giving the epithelium a cobblestone-like appearance. They considered this phenomenon as being an artefact. Even then their observation might be of some relevance in this context, for, artefacts, too, must find an explanation in the physico-chemical properties of the tissues concerned. Thus, artificial conditions might lead to a transformation of microvilli into extensions of cell apices.

As to the basal labyrinth, with its invaginations deep into the cells' bodies, one of its main functions is considered to consist in shortening the way of transport. Various authors have discussed its role in cells dealing with transport and osmotic regulation in different animals (e.g. PEASE 1956, RHODIN 1958, TSUBO and BRANDT 1962, WIGGLESWORTH & SALPETER 1962, ROTH & PORTER 1964, ANDERSON & HARVEY 1966, HECKER et al. 1969).

Numerous mitochondria are found in the area of the cell apex and of the invaginations of the basal labyrinth: they are concentrated where most energy is wanted for transport of substances. It is noteworthy that the formation of the microvilli, the basal labyrinth as well as the final differentiation of the mitochondria all take place between 1 and 3 days after emergence, i.e. prior to the first intake of blood.

Up to 2 days after emergence the nuclei of the midgut epithelial cells show no heterochromatin and their nucleoli appear rather loose. It is concluded that in this early stage of the mosquito's life these nuclei exert a high metabolic activity, which is likely to be associated with the cells' differentiation. From 2 or 3 days after emergence the nuclei change in appearance, in that they exhibit heterochromatin and in that the nucleoli become more compact. Their activity apparently lessens. In their investigation with the light microscope FREYVOGEL & STAEUBLI (1965) have seen changes in size and shape of nuclei and nucleoli alike in conjunction with blood digestion. These observations were confirmed with electronmicrographs by STAEUBLI et al. (unpublished). They showed that in connection with blood digestion the nuclei undergo changes similar to those reported in this paper. It is presumed that the changes observed in connection with blood digestion also indicate a higher activity of the nuclei, to be correlated with the re-organization of, for instance, the endoplasmic reticulum. This organelle seems to reach its characteristic fingerprint-like shape in the same way the first time after metamorphosis as the subsequent times, after each bloodmeal. Its formation has been discussed extensively by STAEUBLI et al. (1966). These authors have postulated the role of the Golgi zone as a possible membrane pool for the formation of the endoplasmic reticulum. We are able to confirm the close association of these two organelles. We like to point out that the nucleus, too, is in close relationship with the endoplasmic reticulum and the Golgi zones. NOVIKOFF (1967 a and b) called attention to the close connection of the Golgi zones, the endoplasmic reticulum and the lysosomes ("GERL") in neurons. In midgut epithelial cells of mosquitoes, too, the lysosomes are formed in the immediate surroundings of the nucleus, the Golgi zone and the endoplasmic reticulum. Therefore, what has been stated by NOVIKOFF for neurons seems to apply to cells of various types.

Deterioration processes

As has been shown by our results, in 24 days old mosquitoes it looks as if deterioration processes could be observed. The midgut epithelial cells assume cubical appearance. The basal labyrinth shows irregularities. The nucleus shows no heterochromatin. The Golgi zones appear vesicular. Ergastoplasmic whorls cannot be found, and, lyso-

somes, probably residual bodies, are very conspicuous. To our mind, such processes could be expected in old mosquitoes and could then be considered as symptoms of ageing. However, our mosquitoes were only 24 days old and had gone through only three gonotrophic cycles. which must be considered as being little (CHRISTOPHERS 1960). No conclusion can be reached at present, for, either the observed deterioration processes are independent of age and due to reasons unknown yet, or the mosquito strain used in this investigation becomes old physiologically rather soon, for what, again, no reason could be given at the present time.

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Zusammenfassung

Zum Zeitpunkt des Schlüpfens der Imago ist das Weibchen von *Aedes aegypti* (L.) nicht imstande, Blut aufzunehmen. Es wird gezeigt, daß dies wohl auf dem Umstand beruht, daß die endgültige Differenzierung der Mitteldarmepithelzellen zwei bis vier weitere Tage nach dem Schlüpfen beansprucht.

Die Differenzierungsprozesse werden elektronenmikroskopisch untersucht und tabellarisch zusammengefaßt. Der Zellverband entsteht und erhält seine Festigkeit; lumenwärts bildet die Zellmembran zunächst Auswüchse, dann Microvilli; auf der basalen Seite entsteht das ausgedehnte basale Labyrinth. Die Kern/Cytoplasma-Relation verändert sich zugunsten des Cytoplasmas. Die auffallendste Entwicklung macht das rauhe endoplasmatische Reticulum durch. Dabei handelt es sich im wesentlichen um dieselben Vorgänge, wie sie von andern Autoren im Zusammenhang mit der Blutverdauung beschrieben worden sind. Bei alten Mücken wird auf Veränderungen im Zellbild des Mitteldarmepithels hingewiesen.

Résumé

Au moment de son éclosion la femelle d'*Aedes aegypti* (L.) n'est pas encore capable de prendre un repas sanguin. Cela est vraisemblablement dû au fait que la différenciation définitive de l'épithélium intestinal ne s'achève que deux à quatre jours après l'éclosion.

Les phases de cette différenciation sont étudiées au microscope électronique et résumées à l'aide d'un tableau. Le tissu comme tel se développe et sa cohérence s'accentue. Du côté de la lumière intestinale, la membrane cellulaire présente d'abord des protubérances, puis des microvillosités. Du côté basal évolue le labyrinthe basal. La relation nucléo-cytoplasmique se modifie au profit du cytoplasme. C'est le reticulum endoplasmique granulé qui montre les transformations les plus importantes. Il s'agit là pour la plupart de phénomènes déjà décrits par d'autres auteurs au cours de la digestion du sang. Les auteurs évoquent également les modifications de l'image cytologique enregistrées chez les moustiques âgés.

Legends to fig. 1–32

Fig. 1. Aedes aegypti female, newly emerged: part of the meconium in the gut lumen containing lysosomes (ly), multivesicular bodies (mvb), myelin-like figures (my) and rests of membranes (rm). 15,000 ×.

Fig. 2. 6 hours old female: epithelial cells, non-columnar, few microvilli (mv), basal labyrinth (lab) irregular in shape, nucleus (nu) without heterochromatin, mitochondria (mi), basal lamina (→); muscle cell (mc) with poorly developed myofibrils. 15,000 ×.

Fig. 3. Newly emerged: apical extensions (ae) of the epithelial cells, microvilli (mv) mostly in depressions near cell boundaries (→); lumen of the gut (lu), mitochondria (mi). 15,000 ×.

Fig. 4. 6 hours: cells connected apically (→) and separated by basally deep extracellular cavities (x); apical extensions (ae), basal labyrinth (lab), nuclei (nu), muscle cell (mc). 7,000 ×.

Fig. 5. 1 day: apical septate desmosome (sd), vesicular shaped stretches of loose contact (lc); microvilli (mv) with glycocalyx (→). 90,000 ×.

Fig. 6. 4 days: macula adhaerens (ma) in the zone of the basal labyrinth; free ribosomes (ri). 130,000 ×.

Fig. 7. 10 days: distribution of maculae adhaerentes (➢) in the zone of the basal labyrinth (lab); nucleus (nu), mitochondria (mi). 15,000 ×.

Fig. 8. 24 days: septate desmosomes (sd), macula adhaerens (ma) and small vesicular shaped stretches of loose contact (lc) next to each other; free ribosomes (ri). 130,000 ×.

Fig. 9. 6 hours: large vesicular shaped stretches of loose contact (lc) between cell membranes; numerous free ribosomes (ri), Golgi zone (go), dense body (db), microtubules (➢). 45,000 ×.

Fig. 10. 12 hours: microvilli (mv) containing very thin fibrils which extend into the apical cytoplasm (➢); mitochondria (mi). 45,000 ×.

Fig. 11. 18 hours: extracellular clefts of the basal labyrinth (lab) wide and irregular in shape; basal lamina (bl), free ribosomes (ri), mitochondria (mi), tracheole (tr). 15,000 ×.

Fig. 12. 10 days: basal labyrinth with nearly parallel invaginations; macula adhaerens (ma), basal lamina (bl), mitochondria (mi), cross sections of microtubules (➢). 45,000 ×.

Fig. 13. 24 days: basal labyrinth (lab) similar as in fig. 11; basal lamina (bl), mitochondria (mi), muscle cell (mc). 15,000 ×.

Figs 14 and 15. 10 days: basal lamina (bl) consisting of several layers partly "striated" (= alternating light and dark banding), amorphous layer (➤) continuous with basal lamina of muscle cell (mc); oblique section of basal lamina (bl'). 45,000 ×.

Fig. 16. 12 hours: nucleus with two membranes (nm), nucleoplasm (npl) containing granules of ribosomal size, nucleolus (nl) consisting of loosely connected masses of material (cf. fig. 17), heterochromatin indistinct. 15,000 ×.

Fig. 17. Detail from fig. 16: loosely connected masses of nucleolar material, finer in the centre *(a)*, more granular (grana of ribosomal size) at the periphery *(b)*. 45,000 ×.

Fig. 18. 4 days: nucleus with heterochromatin (hc), nucleolus more or less compact with zones *a* and *b* (cf. figs 17 and 19). 15,000 ×.

Fig. 19. 10 days: part nucleus, nucleolus compact, zone *a* filamentous to finely granular, zone *b* granular (grana of ribosomal size), heterochromatin (hc), free ribosomes (ri). 45,000 ×.

Fig. 20. 24 days: nucleus (nu without visible heterochromatin, nucleolus compact zones a and b), short pieces of rough surfaced endoplasmic reticulum (rer), no whorls present, light patches (x) located where whorls are found in younger mosquitoes, lysosomes (ly) = probably residual bodies, vesicular Golgi zone (go), mitochondria (mi). 15,000 ×.

Fig. 21. 6 hours: formation of whorls: short pieces of *rer* with few ribosomes present, elements of the Golgi zones (go) possibly transformed into elements of rer (➤), free ribosomes (ri), mitochondria (mi). 45,000 ×.

Fig. 22. 12 hours: formation of whorls: concentration of *rer* elements near Golgi zones (go) and nucleus (nu); lysosome (ly), mitochondria (mi), microvilli (mv). 15,000 ×.

Figs 23 and 24. 2 and 3 days resp.: formation of whorls: parallel grouping of *rer* elements into whorls; nucleus (nu), Golgi zones (go), lysosomes (ly), mitochondria (mi). 15,000 ×.

Figs 25 and 26. 4 and 10 days resp.: rer whorls built up, pieces of rer besides the whorls (➤), nucleus (nu), Golgi zones (go), lysosomes (ly) and mitochondria (mi) numerous, microvilli (mv). 15,000 ×.

Fig. 27. 12 hours: multivesicular bodies (mvb) in the apex of an epithelial cell. 15,000 ×.

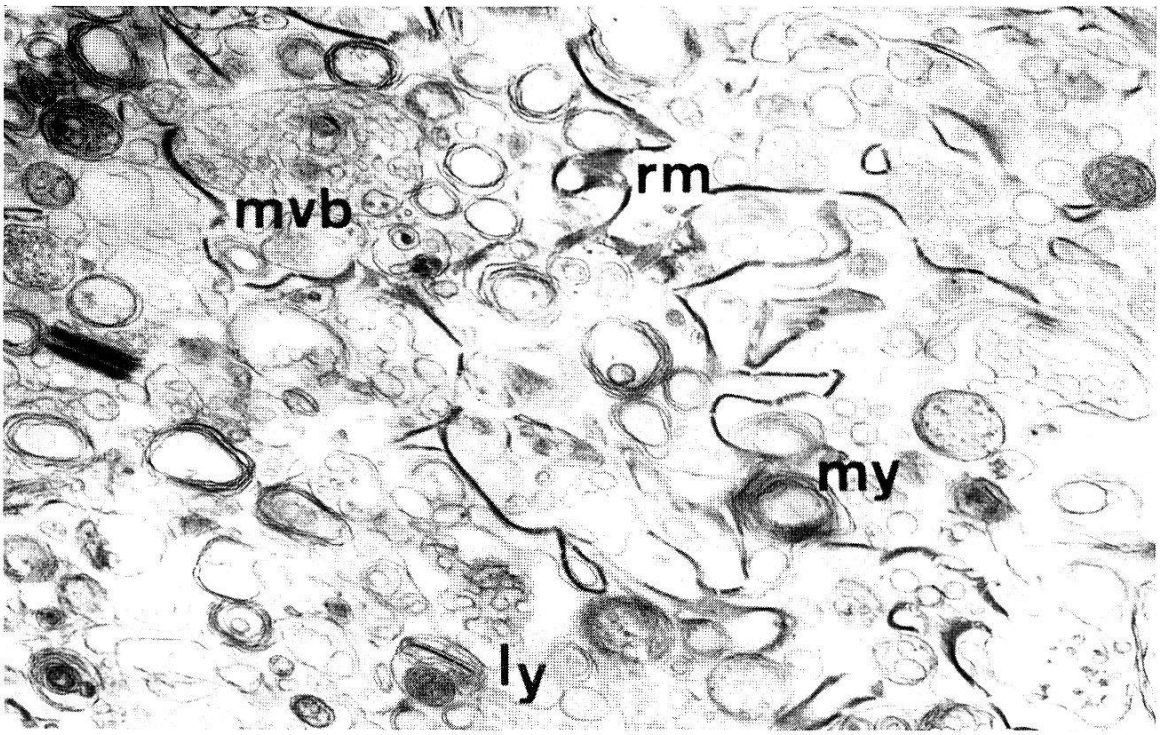
Fig. 28. 15 days: demonstration of acid phosphatases in lysosomes (ly); nucleus (nu), mitochondria (mi), microvilli (mv). 15,000 ×.

Fig. 29. 24 days: numerous and large lamellar bodies probably residual bodies (ly), short bits of *rer;* vesicular Golgi zone (go), mitochondria (mi). 15,000 ×.

Fig. 30. 6 hours: mitochondria (mi) with irregular surface and partly not parallelly aligned cristae of varying matrix density, small granules of mitochondria (→); nucleus (nu). 15,000 ×.

Fig. 31. 10 days: regenerative cell (re) in the zone of the basal labyrinth (lab) of epithelial cells, nucleus (nu) of epithelial cell, nucleus (nur) of regenerative cell; mitochondria (mi), muscle cells (mc), tracheole (tr). 7,000 ×.

Fig. 32. 4 days: vesicles (ve) of various density (possibly containing secretion products) in the basal part of a cell; mitochondria (mi), basal lamina (bl). 45,000 ×.



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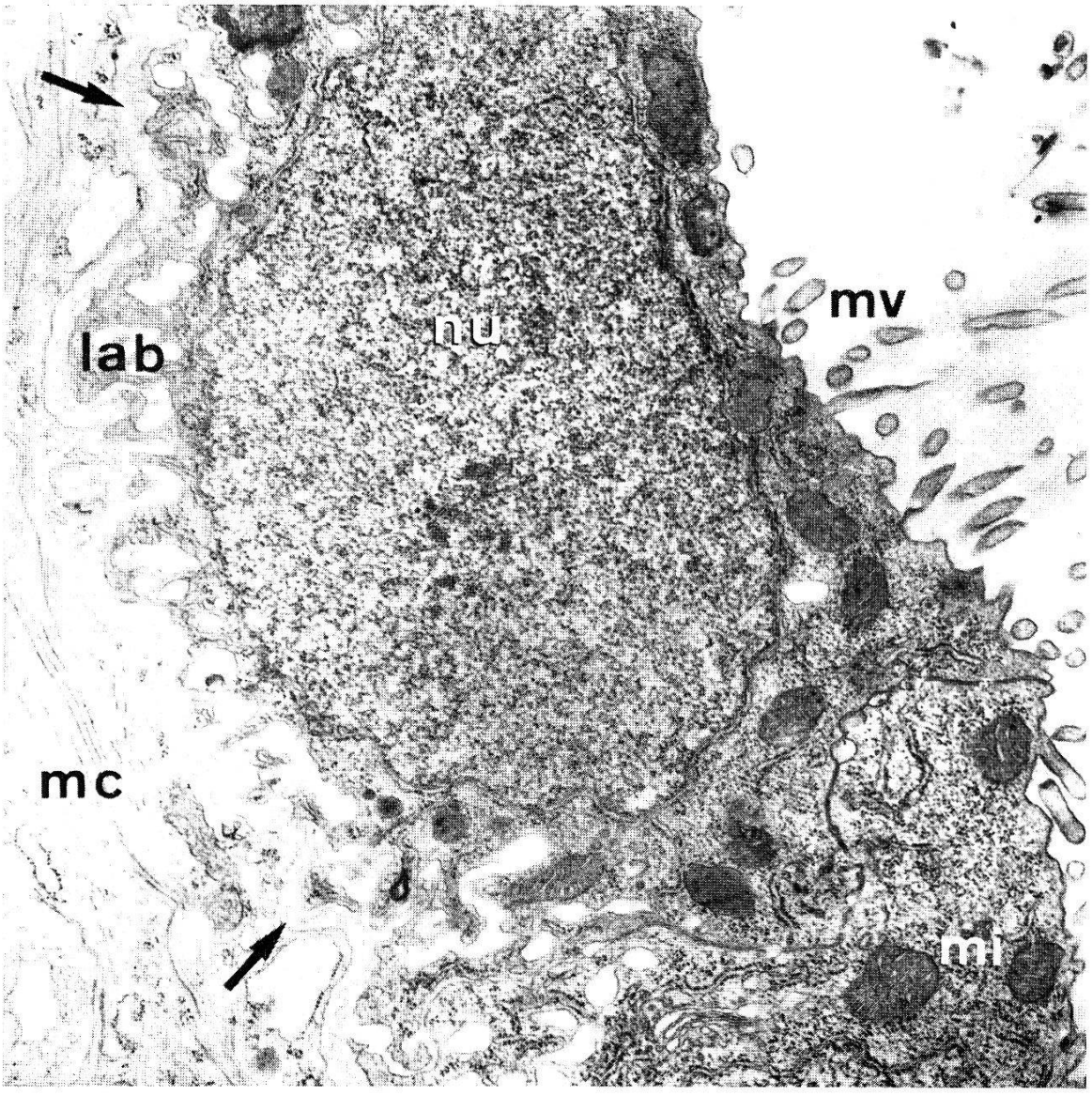

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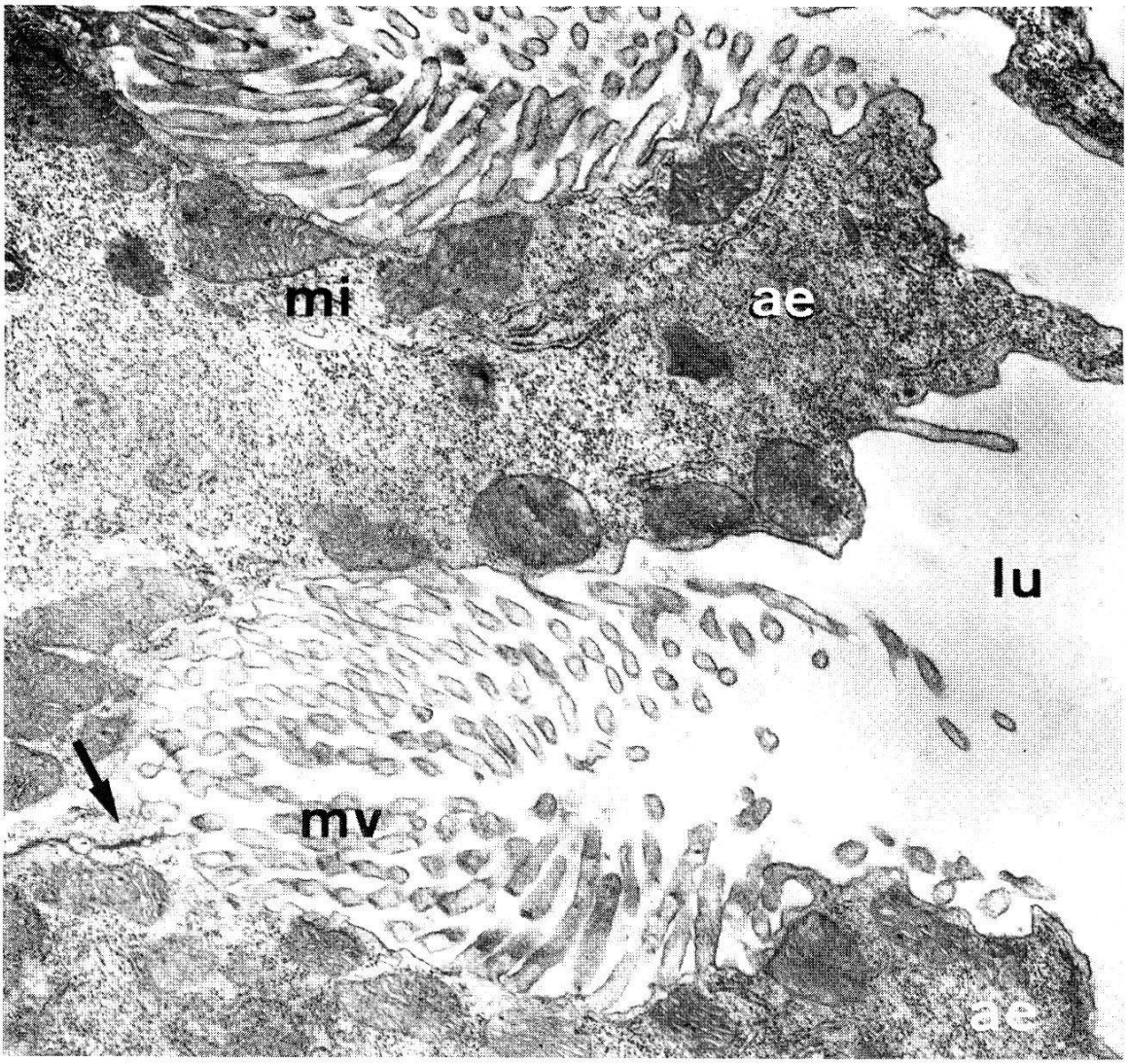

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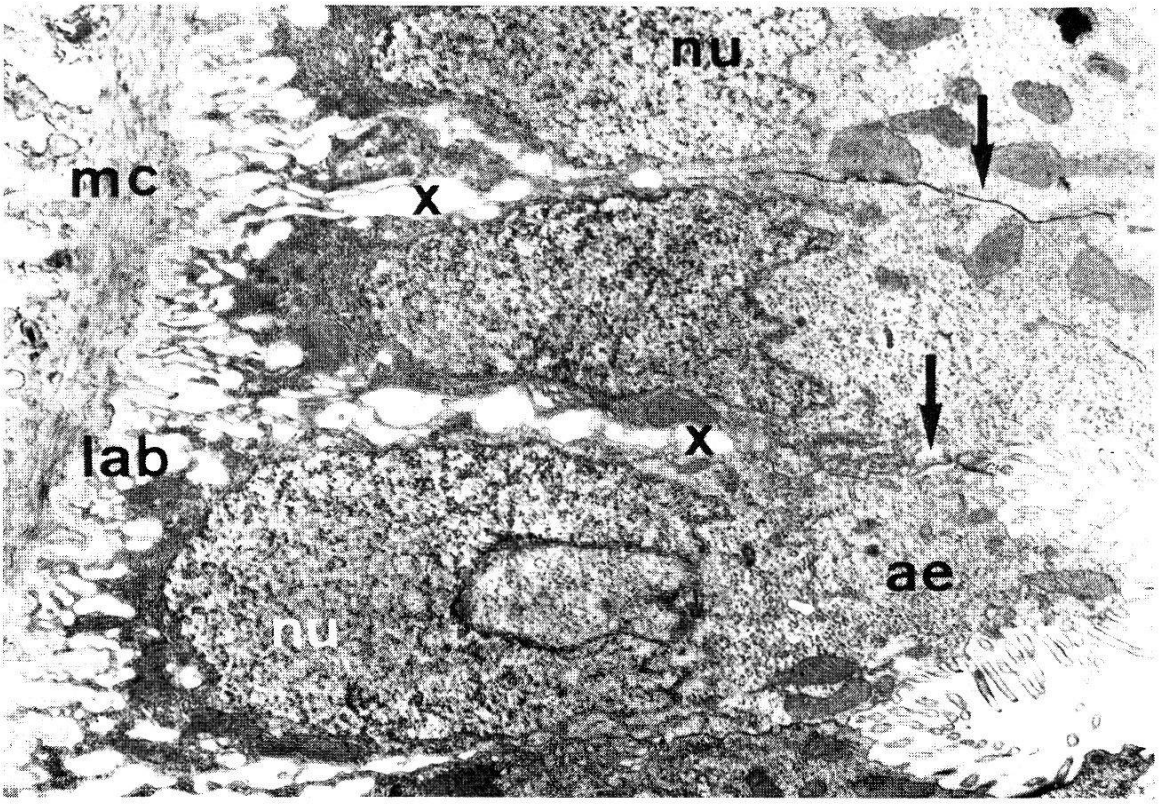

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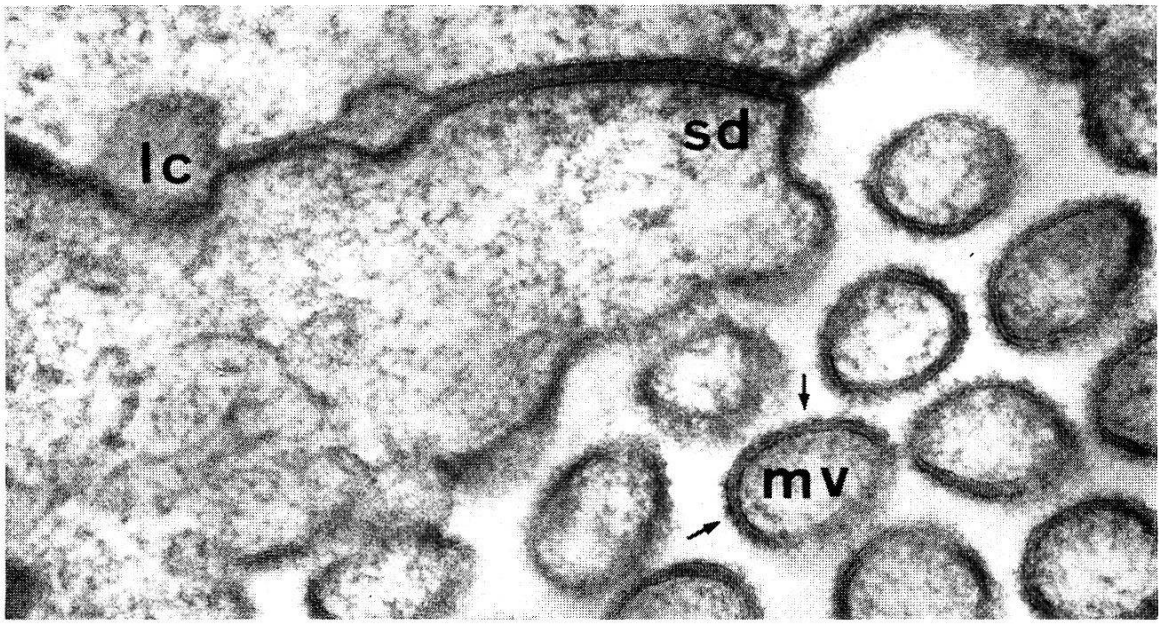

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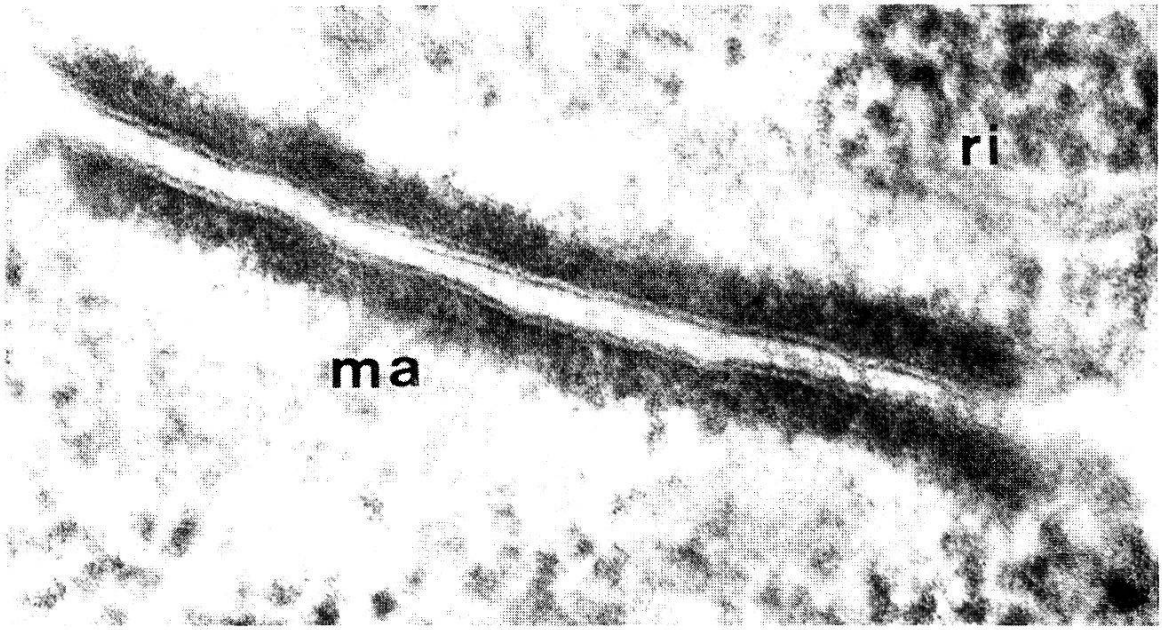

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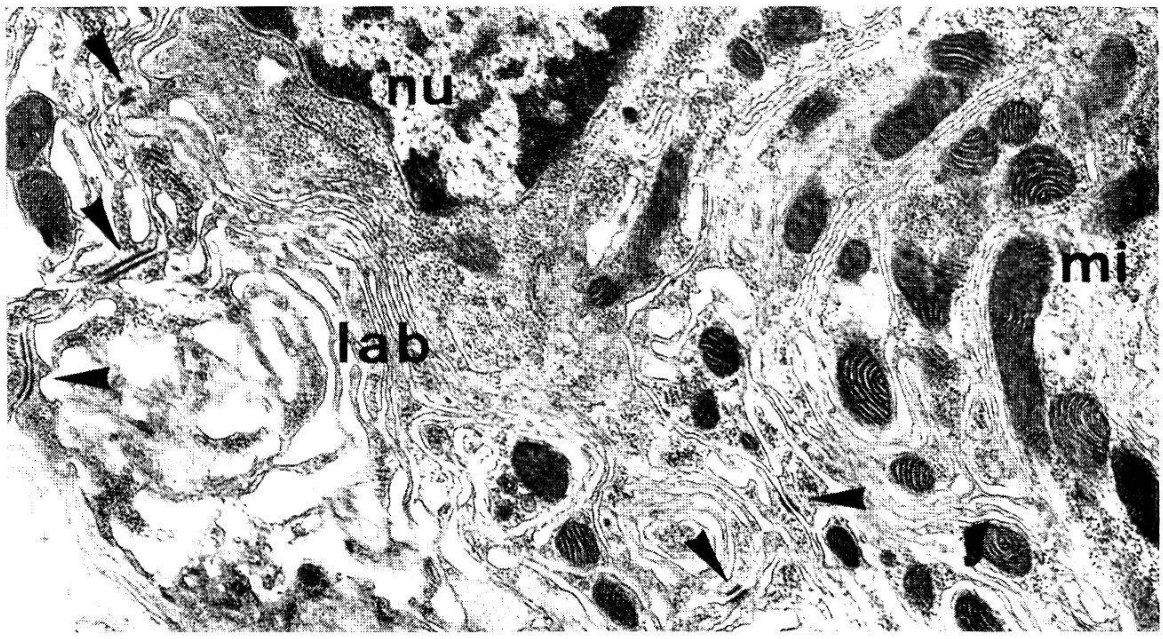

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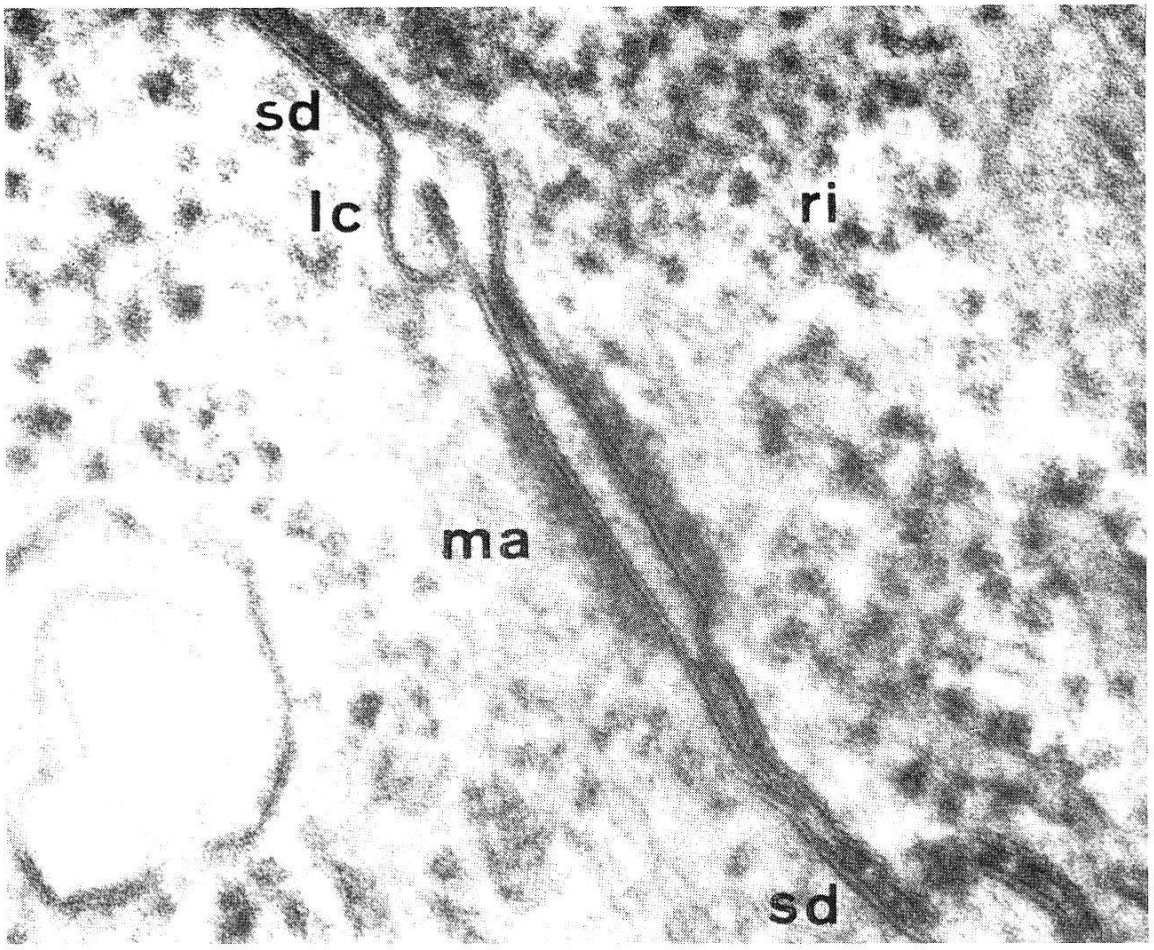

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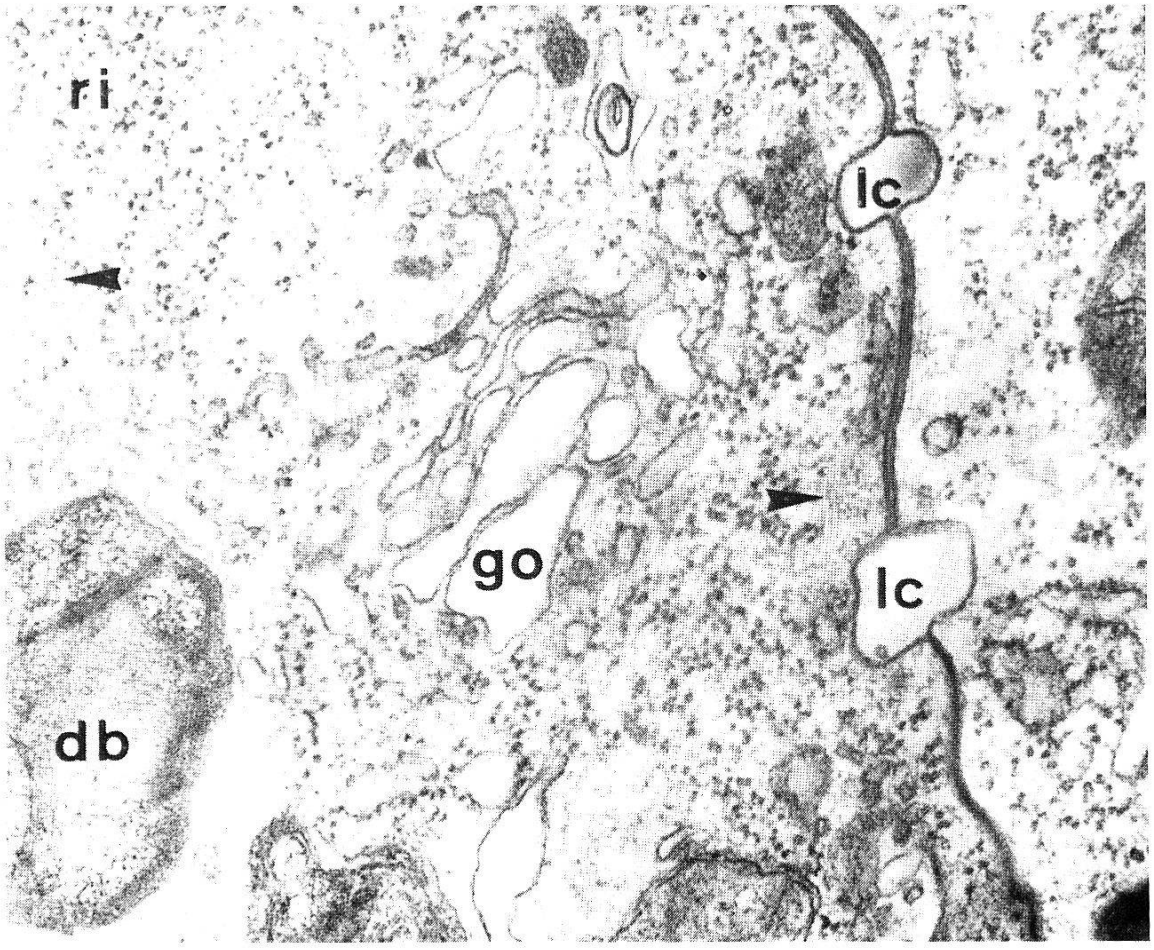

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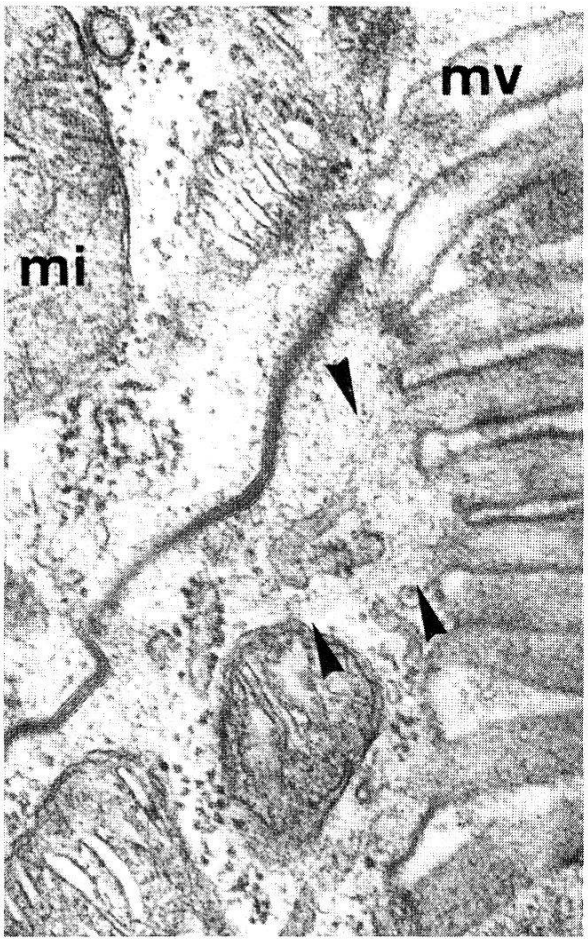

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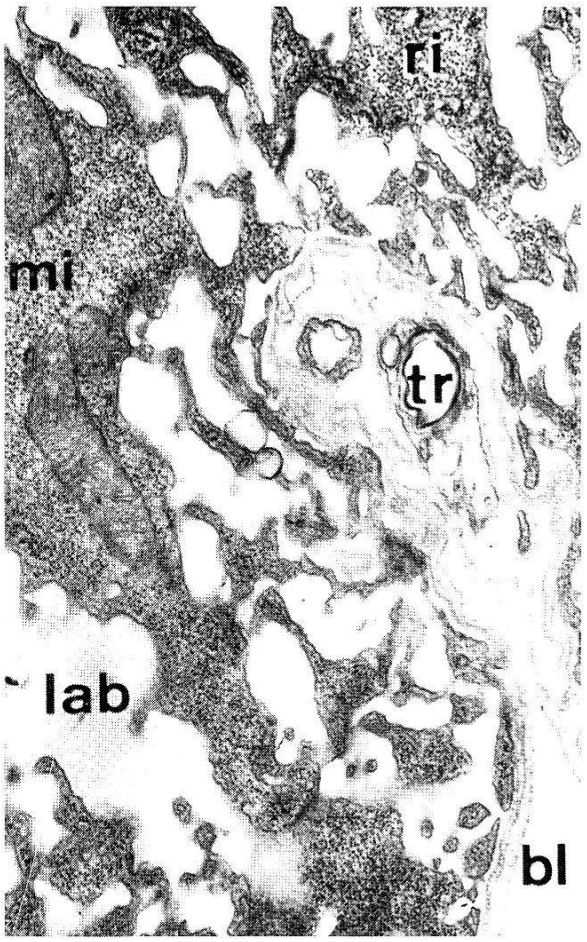

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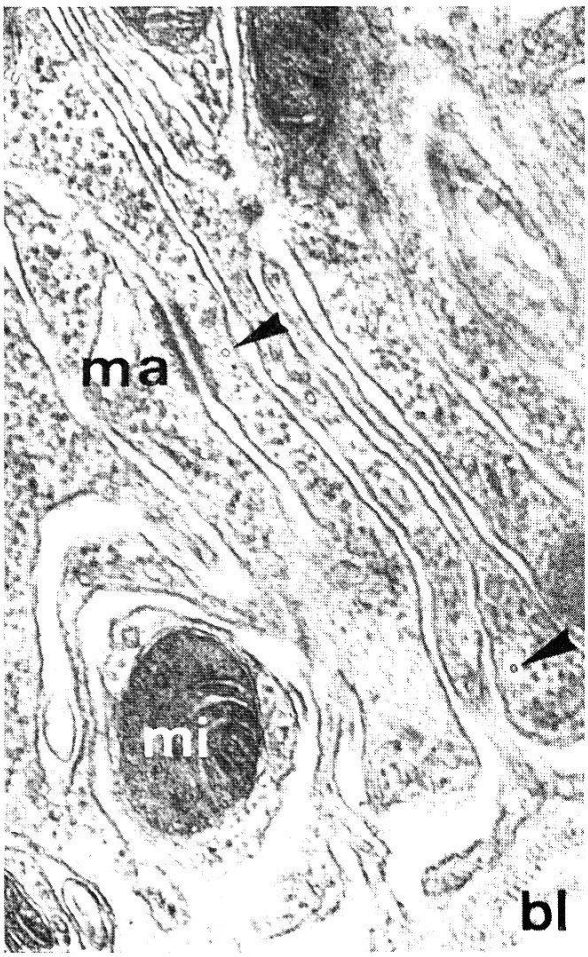

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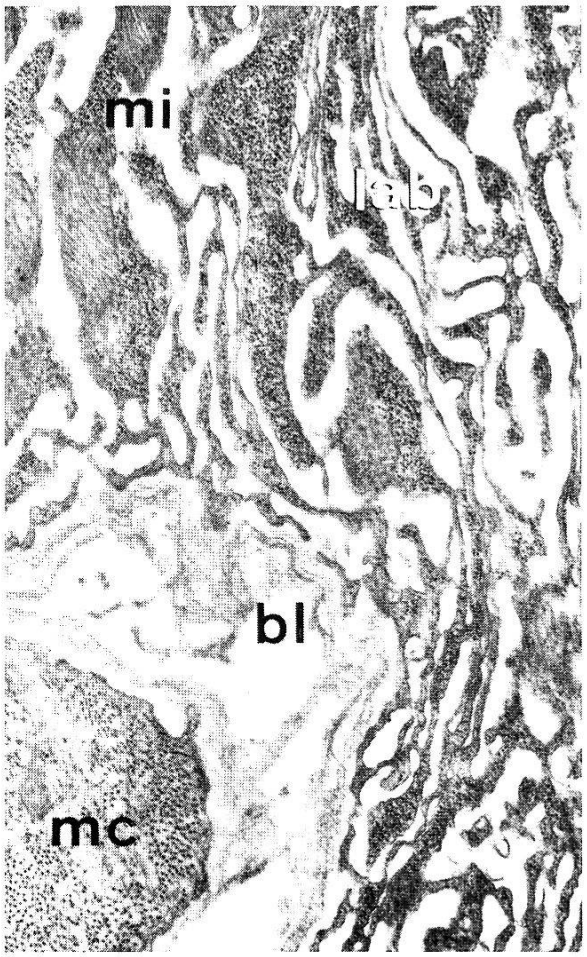

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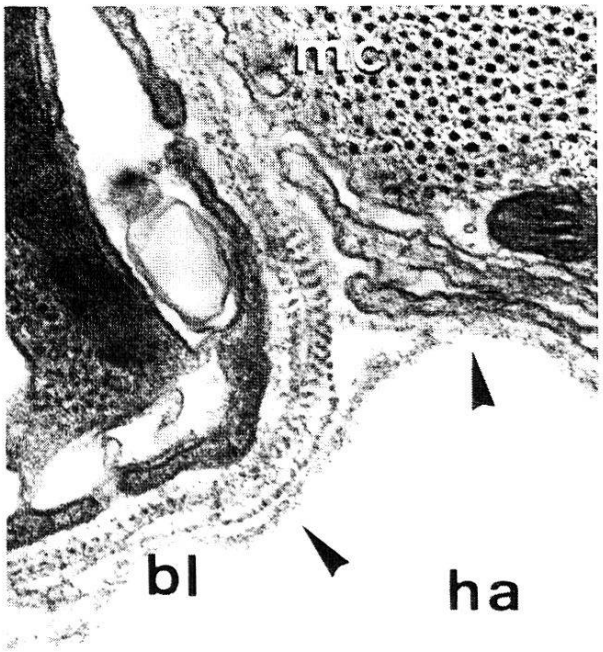

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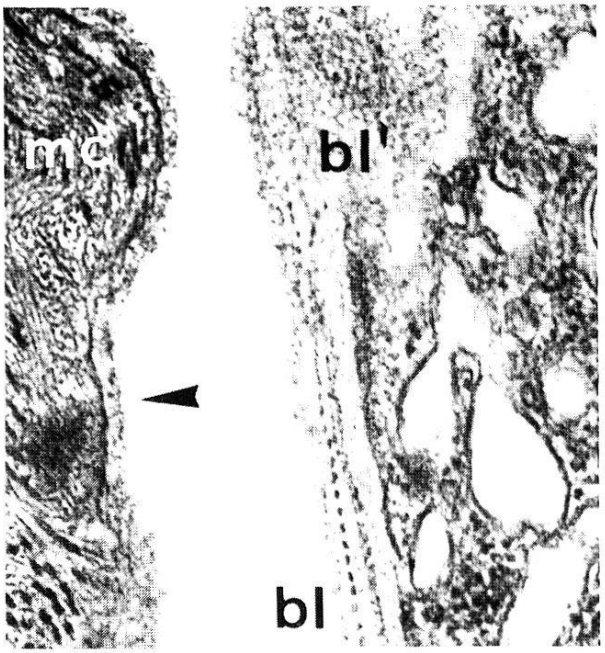

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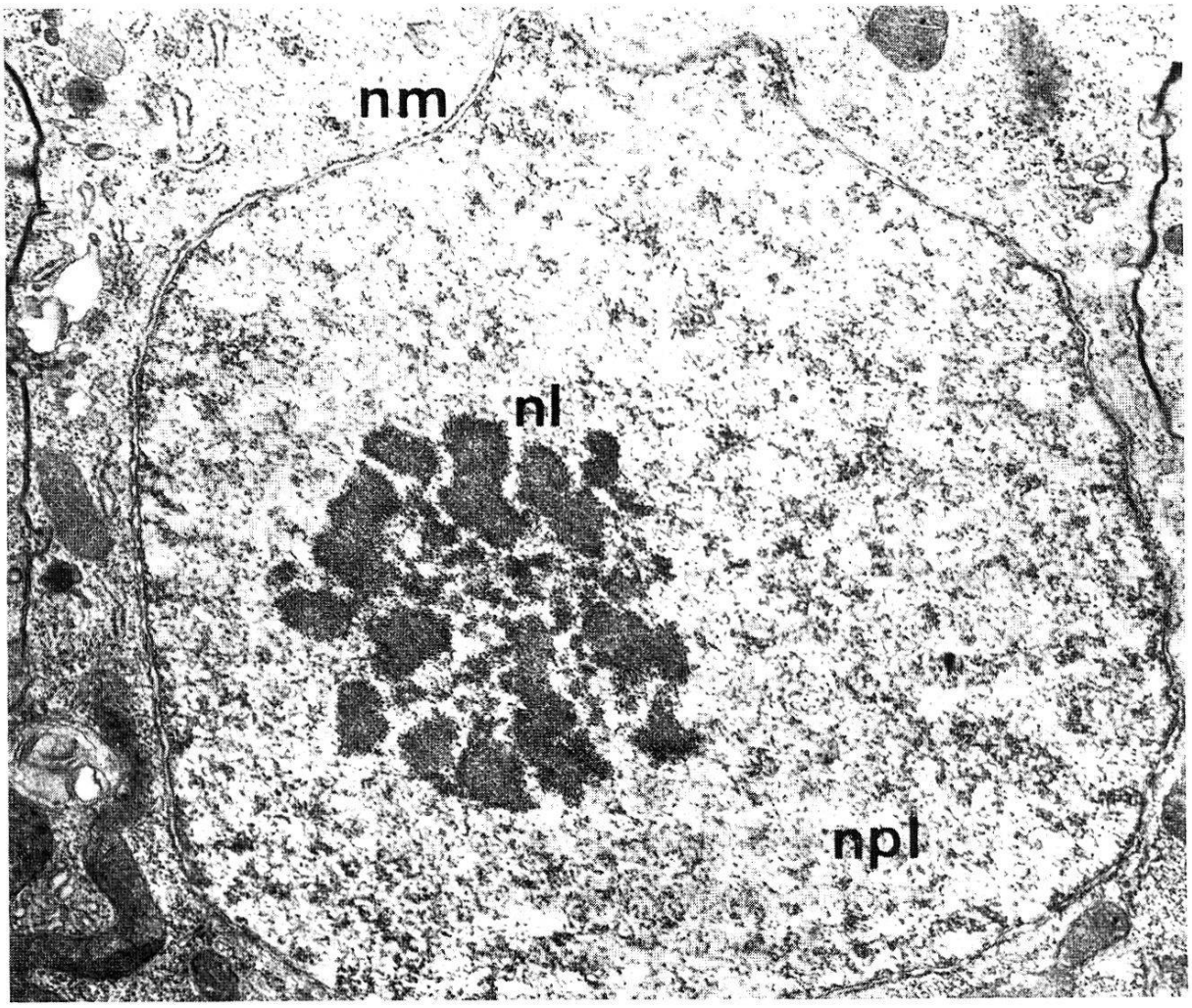

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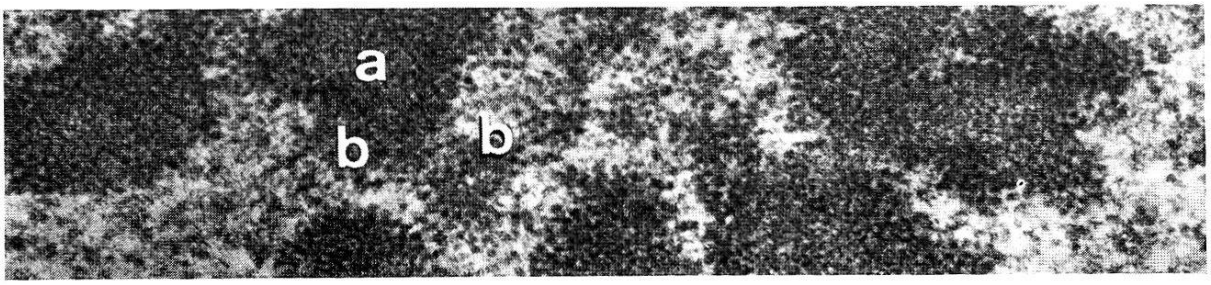

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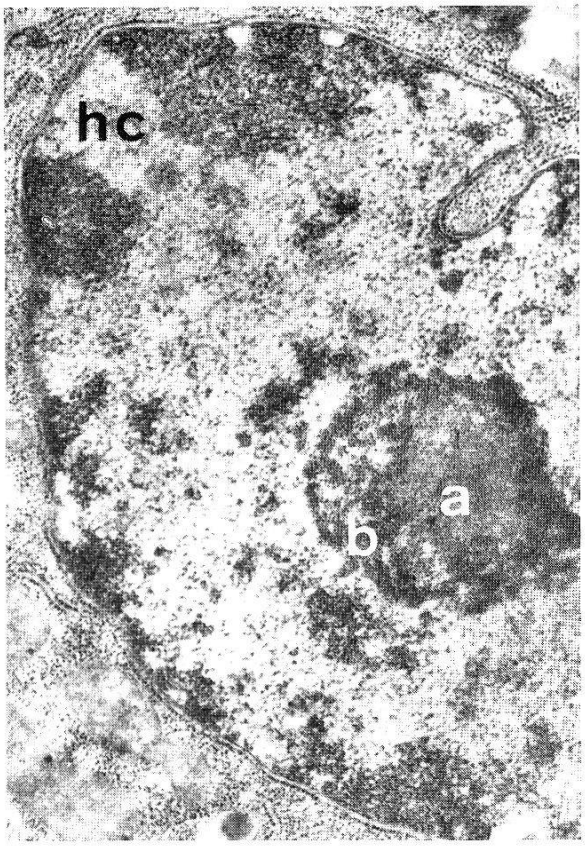

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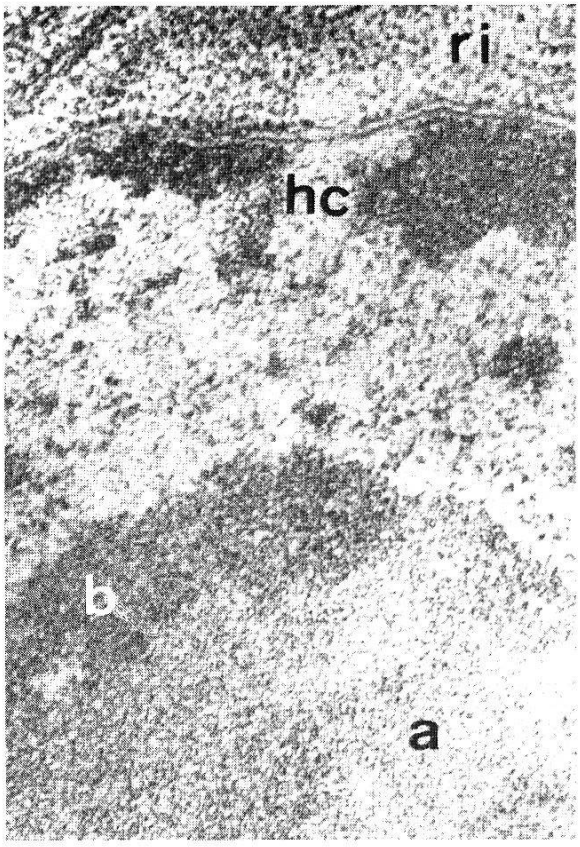

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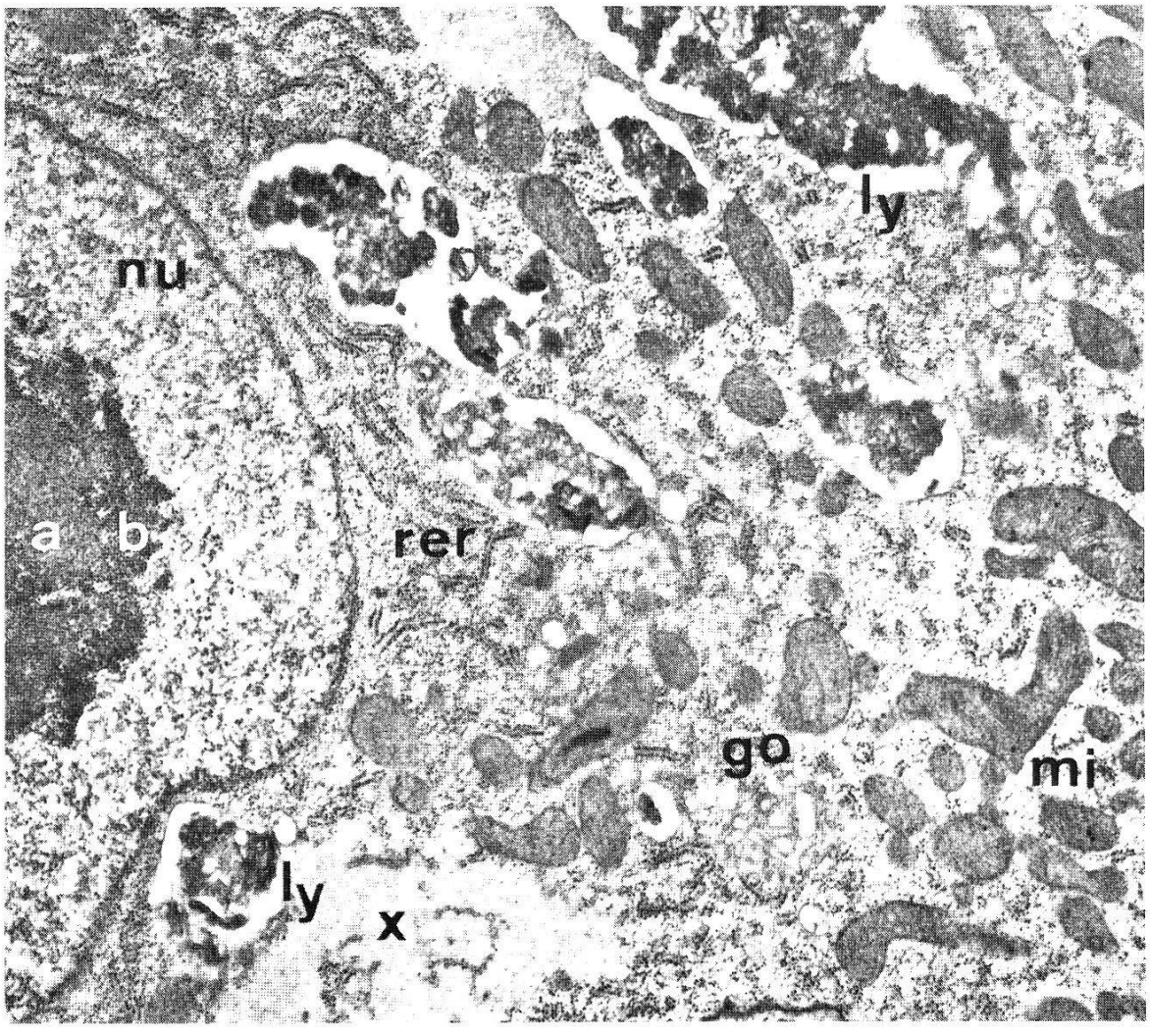

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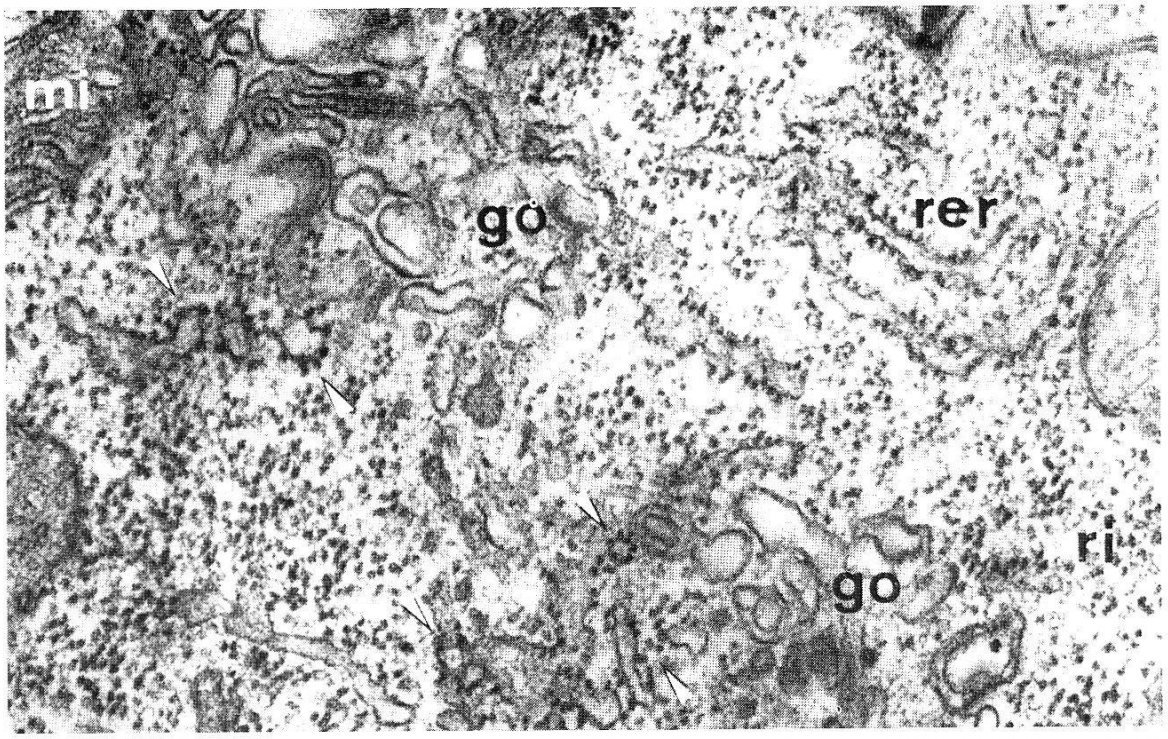

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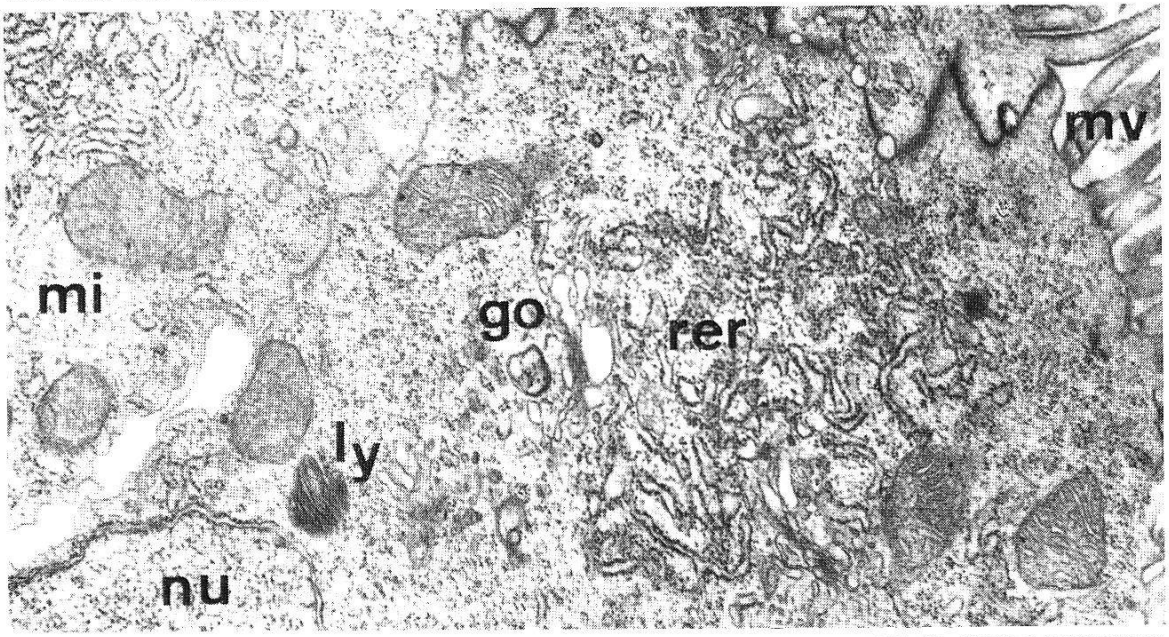

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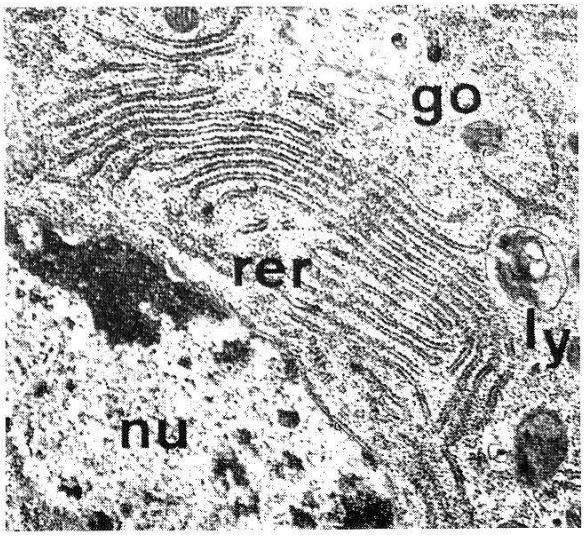

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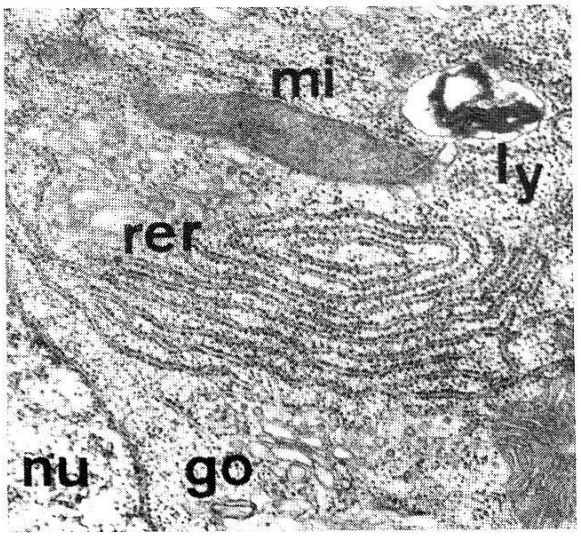

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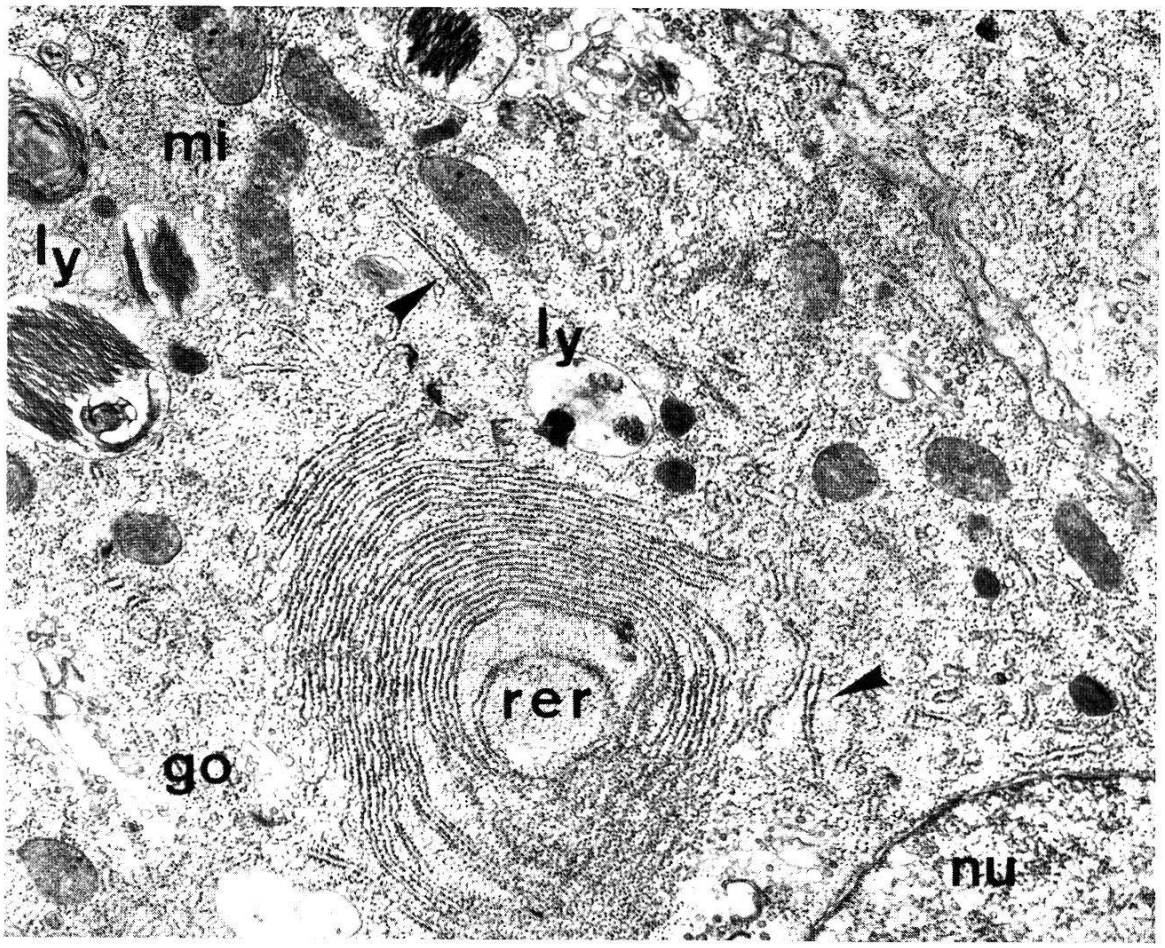

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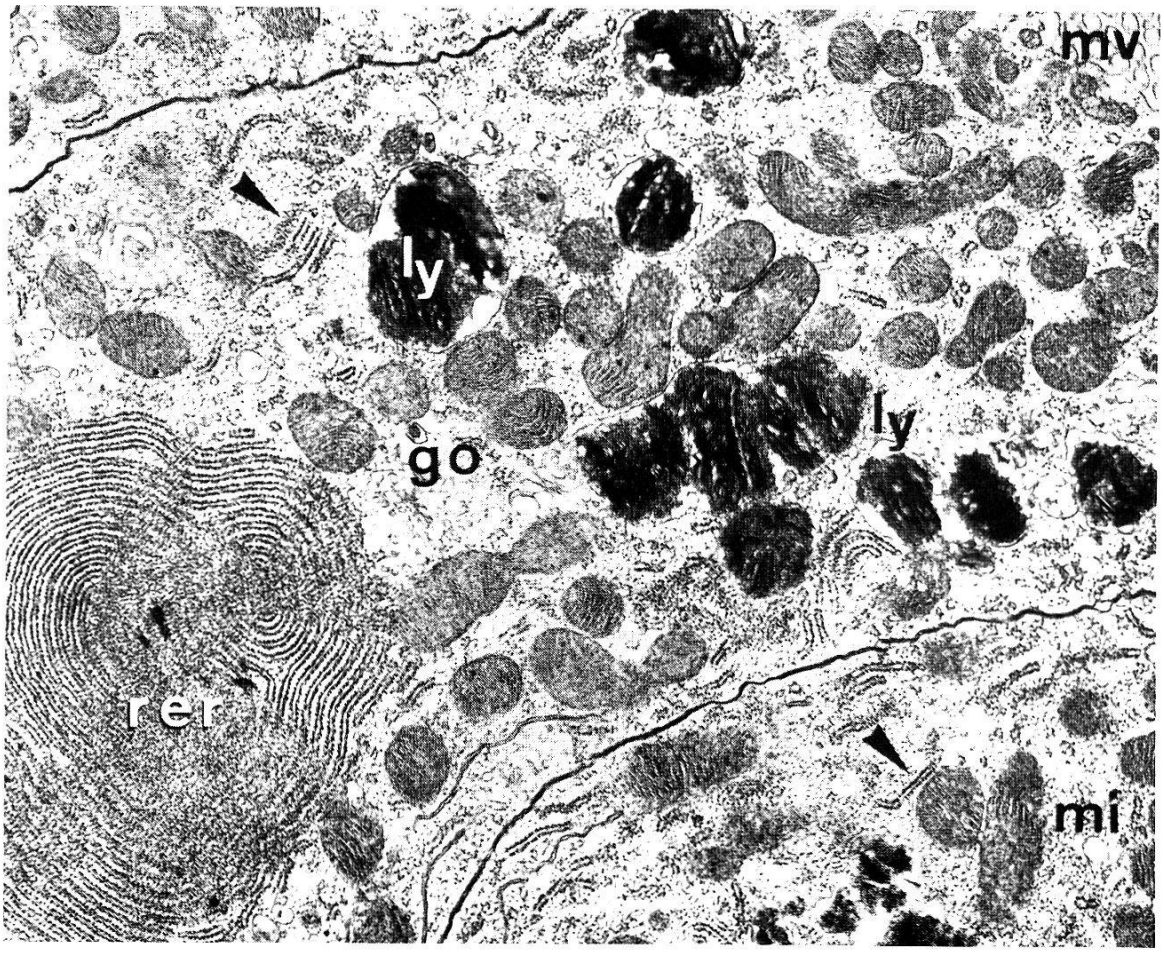

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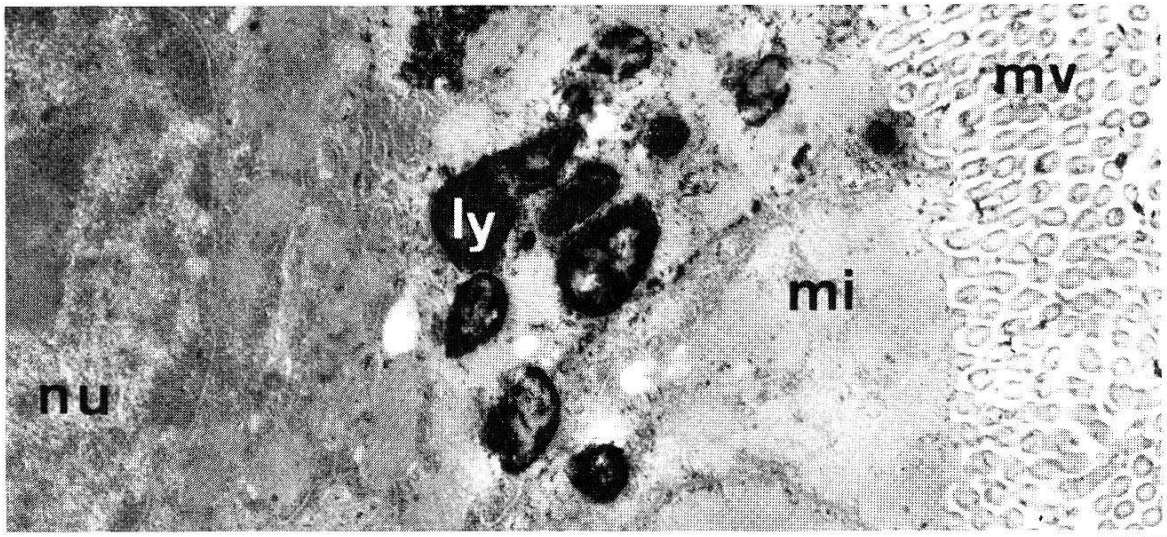

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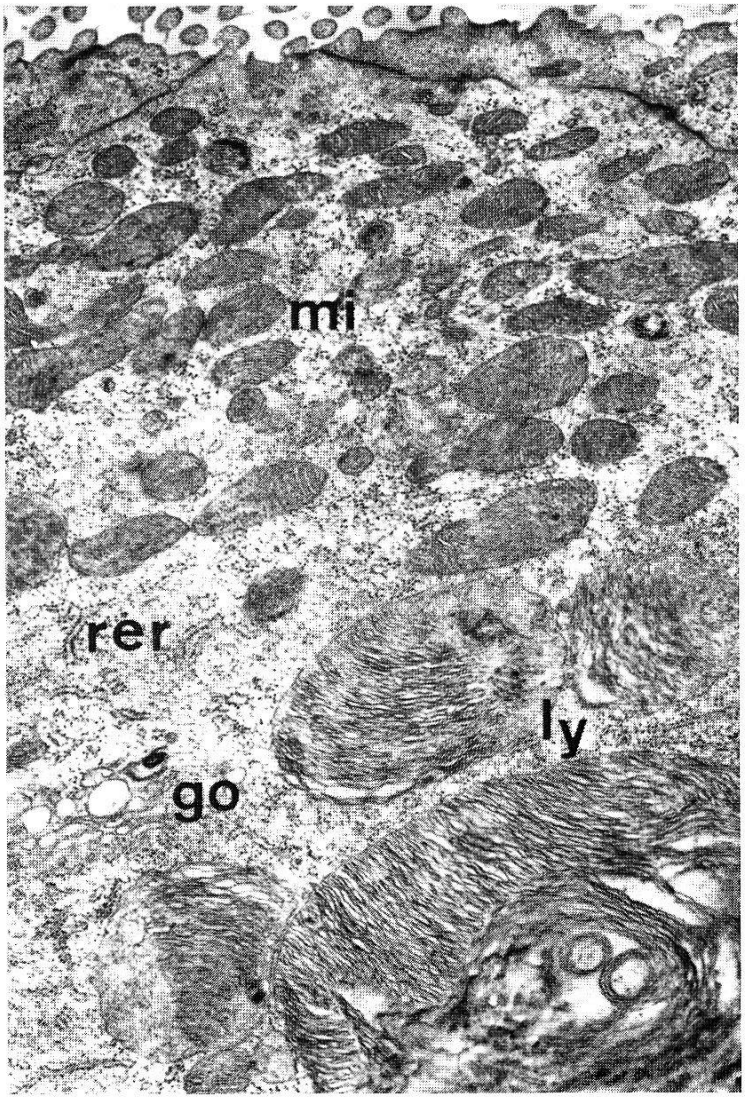

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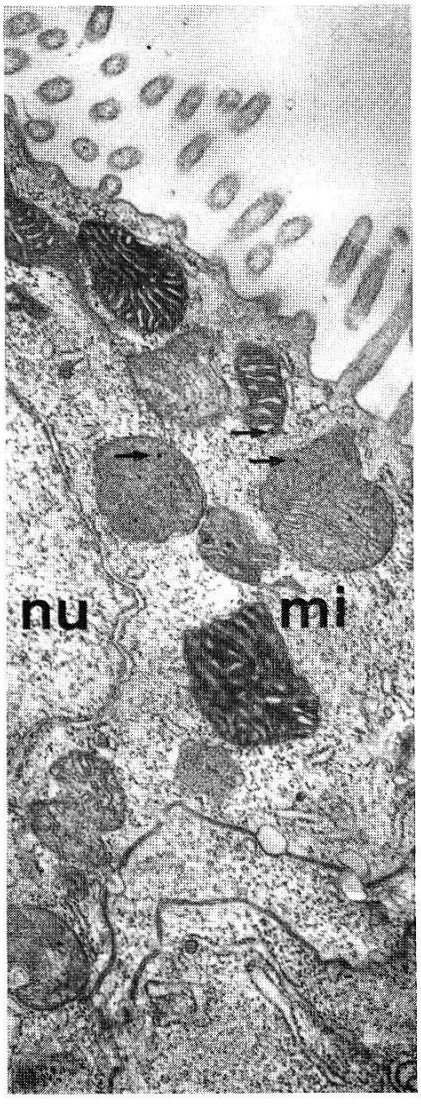

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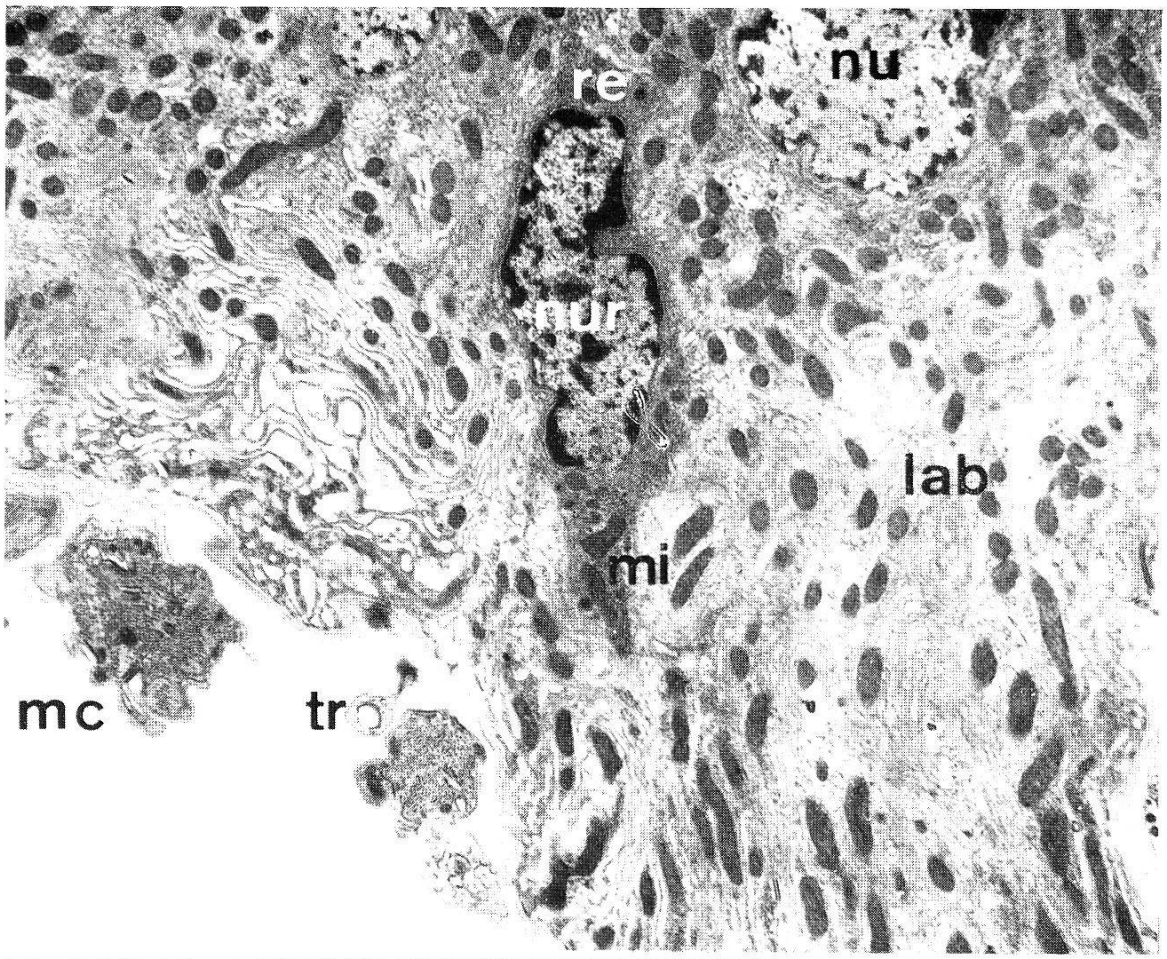

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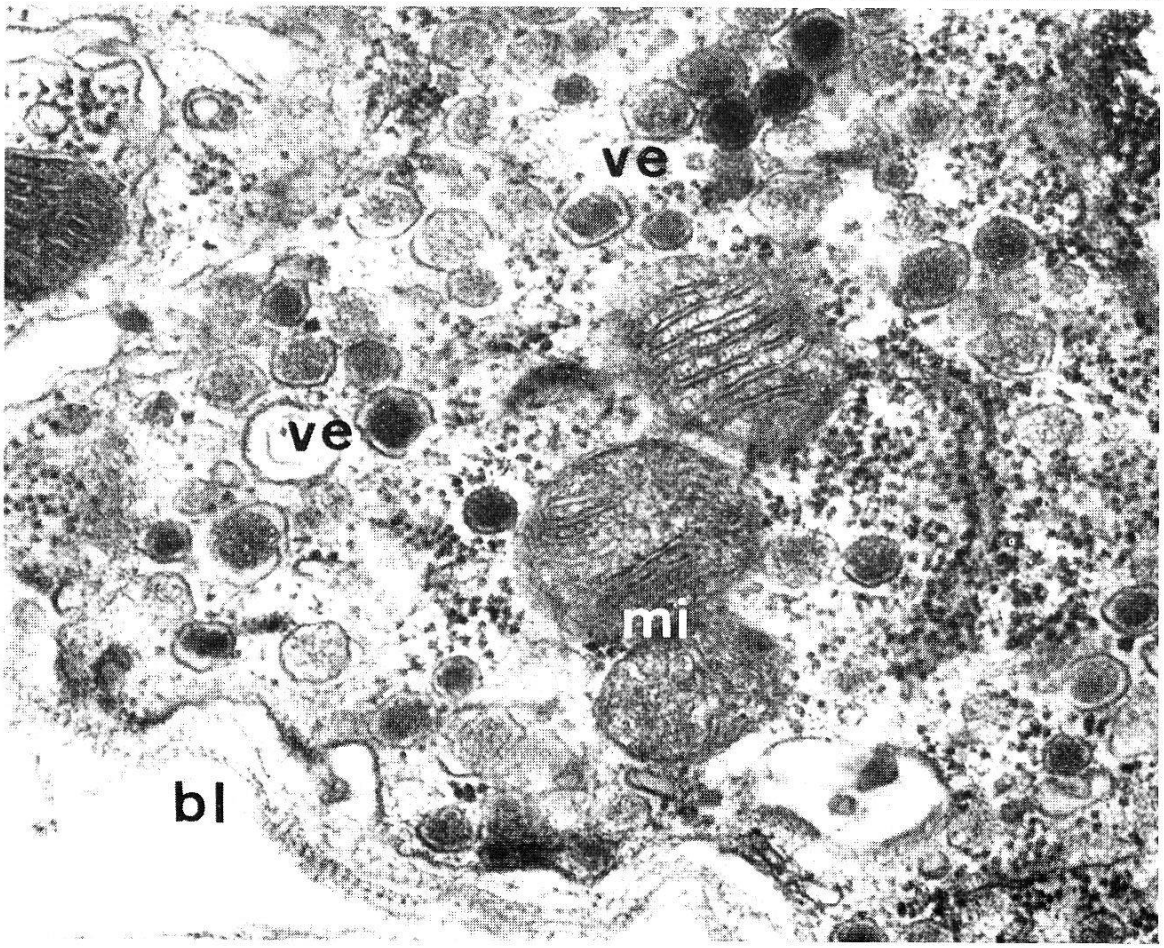

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