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Observations on cytoplasmic organelles in Langerhans cells of human epidermis

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INTRODUCTION

In a previous publication (Birbeck, Breathnach & Everall, 1961) describing the ultra-structure of the Langerhans cell, attention was drawn to a cytoplasmic organelle of characteristic shape and structure. This was thought to be a disc-shaped granule, sections of which appear as rod-like bodies with rounded ends and a striated line running down the centre (Pl. 1, fig. 3). Many of these sectional profiles showed what seemed to be a blowing out of the boundary membrane (Pl. 1, fig. 4), and it was suggested that this appearance might represent a formative stage of the granule. This question of the formation of the granules, and the wider question of their significance in relation to the nature and function of the Langerhans cell, was not pursued pending more extensive investigation of a larger number of cells.

The present study is based on electron microscopic observations of at least 1000 Langerhans cells in human epidermis. The cells have been identified by the presence of the organelles mentioned above, the indented nucleus and the absence of melanin or of any indication of tono-filaments or keratin formation (Pl. 1, fig. 1). As well as demonstrating new features of the characteristic granules, other types of cytoplasmic organelle, not seen in the previous study, have been found. Observations have also been made of the ultra-structural features of Langerhans cells at different levels in the epidermis. These are of interest in relation to the possibility that Langerhans cells undergo differentiation in their progress from basal to more superficial epidermal layers.

MATERIALS AND METHODS

Thin slices of forearm skin were removed by scalpel, divided into small pieces, and fixed for 2 hr. in buffered osmium tetroxide at 4° C. Dehydration was carried out in 70 %, 90 % and absolute alcohols, and the specimens were then embedded in Araldite according to the schedule given by Glauert (1961). Some specimens were stained in bulk by 1 % phosphotungstic acid in 90 % alcohol before embedding. Thin sections were cut on Porter–Blum or Huxley ultra-microtomes, and examined in a Siemens Elmiskop-I electron microscope. Sections of blocks not stained in bulk were stained on the grid by lead hydroxide (Karnovsky, 1961).

OBSERVATIONS

Rod-shaped profiles

Within the cytoplasm of most Langerhans cells there are vacuoles of varying diameter (100–200 mμ) and with a limiting membrane approximately 150 Å. thick (Pl. 1, fig. 2). Though more numerous in the Golgi region of the cell, they may be

encountered in all areas of the cytoplasm. In most micrographs profiles are seen which suggest that organelles exhibiting a partial blowing out of the boundary membrane represent stages in the transformation of these vacuoles into discs. The limiting membrane of the vacuoles is identical in appearance with that of the blown-out segments, and it seems that a collapse, or staged coming together of the walls of a vacuole could produce a rod-shaped profile on section in accordance with the sequence represented in Pl. 1, figs. 2, 5.

An appearance only very rarely observed (four instances), and illustrated in Pl. 2, figs. 6, 7, could suggest an entirely different mode of formation. In each case the boundary membrane of a rod-shaped organelle is seen to be directly continuous with the plasma membrane of the Langerhans cell within which it lies, suggesting that they are formed by an infolding and 'nipping-off' of a segment of plasma membrane.

Other cytoplasmic organelles

The micrographs (Pl. 2, fig. 8; Pl. 3, fig. 9; Pl. 4, fig. 12) illustrate a variety of organelles very occasionally seen in the cells of the present series. They include: (1) sacs, limited by a single electron-dense membrane, which may either be empty, or contain a few small rounded bodies (Pl. 1, fig. 5). Small vesicles resembling pinocytotic vesicles are occasionally seen just outside the limiting membrane (Pl. 2, fig. 8). (2) Similar structures filled with ill-defined inclusions some of which appear to be remains of the rod-shaped granules described in the previous section (Pl. 3, fig. 9). (3) Smaller rounded bodies with amorphous ground substance and lamellated or whorled internal membranes and small vesicles (Pl. 2, fig. 8; Pl. 4, fig. 12). Rod-shaped profiles are often seen in close proximity to this latter type of structure, and bent around it.

All these structures resemble one or other of the different varieties of lysosome described by various authors (see Novikoff, 1961), but the third type needs to be distinguished from another organelle much more frequently seen (*X*, Pl. 3, fig. 9, and other micrographs). The limiting membrane is similar in appearance, but the internal structure is of a uniform, finely granular nature. These organelles tend to be more numerous in peripheral areas of the cytoplasm, particularly in the region superficial to the nucleus. It might be thought that they are disc-shaped granules (which have a rod-shaped appearance in one plane of section) cut in a plane at right angles to their surface. However, it has already been shown (Birbeck *et al.* 1961) that in this circumstance, which is very rarely achieved, the disc-shaped granules exhibit a two-dimensional array of particles with a spacing of 90 Å.

Langerhans cells at different epidermal levels

The features described above are characteristic of cells in the deeper and intermediate layers of the stratum spinosum. Features of cells at other levels are as follows:

Basal layer. Langerhans cells are rarely seen in this layer. Those present contain very few rod-shaped profiles or vacuoles, but small vesicles may be numerous and rounded granules are fairly constantly seen (*X*, Pl. 3, fig. 10). This relative paucity of rod-shaped profiles makes their identification as Langerhans cells somewhat uncertain. For example, the cell shown in Pl. 3, fig. 10, has the markedly indented

nucleus and 'clear' cytoplasm characteristic of Langerhans cells; one or two rounded granules are also present, but rod-shaped profiles are absent. Cells of similar appearance are sometimes seen closely associated with melanocytes (Pl. 4, fig. 11). Whether these can be regarded as relatively undifferentiated Langerhans cells, or an entirely different type of non-keratinizing cell, is problematical. In the absence of any positive evidence to the contrary, it seems reasonable to assume that they are, in fact, Langerhans cells.

More superficial layers. Cells at the superficial level of the stratum spinosum (Pl. 4, fig. 13) contain relatively few cytoplasmic organelles compared with those at deeper levels. R.N.P. granules are more obtrusive, perhaps as a consequence. No evidence of keratinization is found in cells at this level.

Nothing that could certainly be identified as a Langerhans cell has been seen in the stratum granulosum or stratum corneum. In the stratum granulosum, cells with indented nuclei separated by an extensive area of 'clear' cytoplasm from adjacent masses of keratin fibrils and keratohyalin granules were often seen. No definite plasma membrane could be identified separating these latter two areas. If the cells in question were indeed Langerhans cells, the plasma membrane must have disintegrated.

DISCUSSION

Two views are current concerning the nature of the Langerhans cell. Some authors (Ferreira-Marques, 1951; Richter, 1956) maintain it is an intra-epidermal neural or neuro-hormonal element, while others (Masson, 1948; Billingham & Medawar, 1953) regard it as an effete melanocyte in the process of being desquamated. The most characteristic feature of the cell is the presence of rod-shaped bodies in the cytoplasm. These, it has been concluded, are sectional profiles of disc-shaped organelles (Birbeck *et al.* 1961). Obviously, any view of the nature and function of the Langerhans cell must take account of these structures, and, in particular, of their mode of formation and ultimate fate. As already indicated, two possible methods of formation are suggested by the present findings.

If, as some micrographs suggest, they are formed by an infolding and nipping off of segments of plasma membrane, it is remarkable that this is not observed more frequently. It is difficult also to account for the presence of profiles with a blowing out of the boundary membrane on this basis. One possibility would be to suggest that, having been formed, the organelles move to the interior of the cell, and here, for whatever reason, become partially blown out. On this basis, vacuolar profiles (Pl. 1, fig. 2) could be explained as sections in various planes through blown out segments. On the other hand it could be that the vacuoles and the rod-shaped bodies are distinct entities which join to present the appearances illustrated.

The alternative method of formation suggested, i.e. that they may arise by a graded collapse of vacuoles, also raises some points of difficulty. The first concerns the nature of the vacuoles themselves. They are very similar in appearance to Golgi vacuoles as commonly described. If, however, discs presenting as rod-shaped profiles on section are produced by a collapse of vacuoles, it is evident from the micrographs that some increase in size would have to occur during or after the completion of this process. This would be necessary to account for the fact that the

length of many rod-shaped profiles is much greater than the diameter of any vacuole seen. Finally, if this method of formation is accepted, the conditions illustrated in Pl. 2, figs. 6 and 7, require explanation. They could represent organelles in process of being discharged or excreted from the cell.

One must conclude that the mode of formation of these highly characteristic organelles remains unsettled. Their ultimate fate is also uncertain. It should be noted that they are relatively abundant in cells from the deeper and mid levels of the stratum spinosum, but virtually absent from those at more superficial levels. There is no evidence that, like melanin granules, they are transferred to neighbouring keratinocytes, and the fact that structures resembling them are sometimes seen within lysosome-like bodies suggests that some at least may degenerate and be destroyed within the cell. The uncertainty concerning their mode of formation and fate makes speculation about their function unprofitable at present.

As regards the wider question of the nature and derivation of the Langerhans cell itself, the present observations are of some significance. No evidence has been found to support the view (Richter, 1956) that they are continually migrating from the dermis into the epidermis. Nothing that could be classed as a Langerhans cell has been seen among a large number of cells examined on the dermal side of the dermo-epidermal junction. In gold chloride preparations examined by light microscopy, dendritic cells seen in this situation have been taken to be Langerhans cells. However, preliminary observations on gold-impregnated material examined by electron microscopy indicate that the vast majority of these are fibroblasts. There is nothing in the ultra-structural appearance of the Langerhans cell which would suggest that it is neural, either in nature or function.

The general appearance of the cell is also difficult to reconcile with the view that it is a functionless, worn-out melanocyte (Masson, 1948; Billingham & Medawar, 1953). On the contrary, it presents a picture of considerable activity and appears to undergo some differentiation during its passage from the basal layer to more superficial levels. If it be accepted that the 'clear' cells in the basal layer figured in Pl. 3, fig. 10, and Pl. 4, fig. 11, are in fact Langerhans cells, an hypothesis recently advanced (Breathnach, 1963) appears to be confirmed to some extent. This postulates that the Langerhans cell is not a worn-out melanocyte, but one of the two products of division of a mature melanocyte, which, unlike its sibling which remains on the basal layer, never engages in melanogenesis and is ultimately exfoliated. If this be so, the two cells illustrated in Pl. 4, fig. 11, could be looked upon as daughter cells of a melanocyte which had recently divided, and which are still in intimate association. The one on the left remains on the basal layer to form melanin (and maintain the 'germ line') while the other ascends to become a fully differentiated Langerhans cell. According to this explanation, which must be regarded at present as highly speculative, the relation between melanocyte and Langerhans cell would be similar to that obtaining between the basal keratinocytes and the cells of the stratum spinosum. This interpretation differs from the one advanced by Masson (1948) and Billingham & Medawar (1953), who regarded the Langerhans cell as a melanocyte which becomes 'worn-out' while still in the basal layer, and then ascends to the supra-basal layers without dividing. Such worn-out cells, according to these authors, are replaced by division products of neighbouring basal melanocytes.

SUMMARY

1. Examination of a large number of Langerhans cells suggests that the characteristic disc-shaped cytoplasmic organelles are formed in one or other of two different ways. They may be formed by an infolding and nipping off of segments of plasma membrane, or they may arise from collapse of vacuoles which are probably Golgi vacuoles.

2. Lysosome-like structures are occasionally found in Langerhans cells, and there is some evidence that they may engulf the disc-shaped organelles.

3. Differences between Langerhans cells at different epidermal levels indicate that they undergo differentiation in their passage from the basal layer to the surface.

4. No evidence has been found of Langerhans cells in the dermis, or for the suggestion that they migrate from the dermis into the epidermis.

5. The general ultra-structural appearance of the Langerhans cell is difficult to reconcile with the view that it is a 'worn-out' or 'effete' melanocyte. In line with this it is suggested that it may be one of the products of division of a mature melanocyte which never engages in melanogenesis, but which is exfoliated.

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EXPLANATION OF PLATES

PLATE 1

With the exception of fig. 1, which is from a section stained with P.T.A., all micrographs are from sections stained with lead hydroxide.

Fig. 1. Langerhans cell to show general features. Note indented nucleus, 'clear' cytoplasm due to absence of keratin fibrils abundantly present in adjacent keratinocytes (*K*), and vesicles, vacuoles and rod-shaped profiles in the cytoplasm. × 16,000.

Fig. 2. Portion of cytoplasm of Langerhans cell. *m*, Mitochondrion; *va*, vacuoles. The numerals 1, 2,3 indicate a possible sequence of transformation of vacuoles into discs which are rod-shaped on section. × 40,000.

Fig. 3. A cluster of rod-shaped profiles from the cytoplasm of a Langerhans cell. × 66,000.

Fig. 4. Rod-shaped profile showing partial blowing-out of boundary membrane. × 66,000.

Fig. 5. Portion of cytoplasm of Langerhans cell to show sac (*S*) with rounded inclusions. The numerals 1, 2, 3 indicate a possible sequence of transformation of vacuoles into discs which are rod-shaped on section. × 54,000.

PLATE 2

Fig. 6. Langerhans cell to show rod-shaped profile (arrowed) in continuity with plasma membrane (*pl*). *K*, Keratinocyte; *n*, nucleus of Langerhans cell. × 36,000.

Fig. 7. Portion of another cell at a higher magnification showing direct continuity between rod-shaped profile and plasma membrane (*pl*). *K*, Adjacent keratinocyte. × 50,000.

Fig. 8. Lysosome-like organelles (*L*) in cytoplasm of Langerhans cell. A number contain small vesicles and whorled membranes. Note small vesicles resembling pinocytotic vesicles close to limiting membrane of a sac (*S*). × 28,000.

PLATE 3

Fig. 9. Cytoplasm of Langerhans cell to show lysosome-like organelles (*L*) containing apparent remnants of rod-shaped profiles. *K*, Keratinocyte; *n*, nucleus; *X*, rounded organelles with granular internal structure. × 40,000.

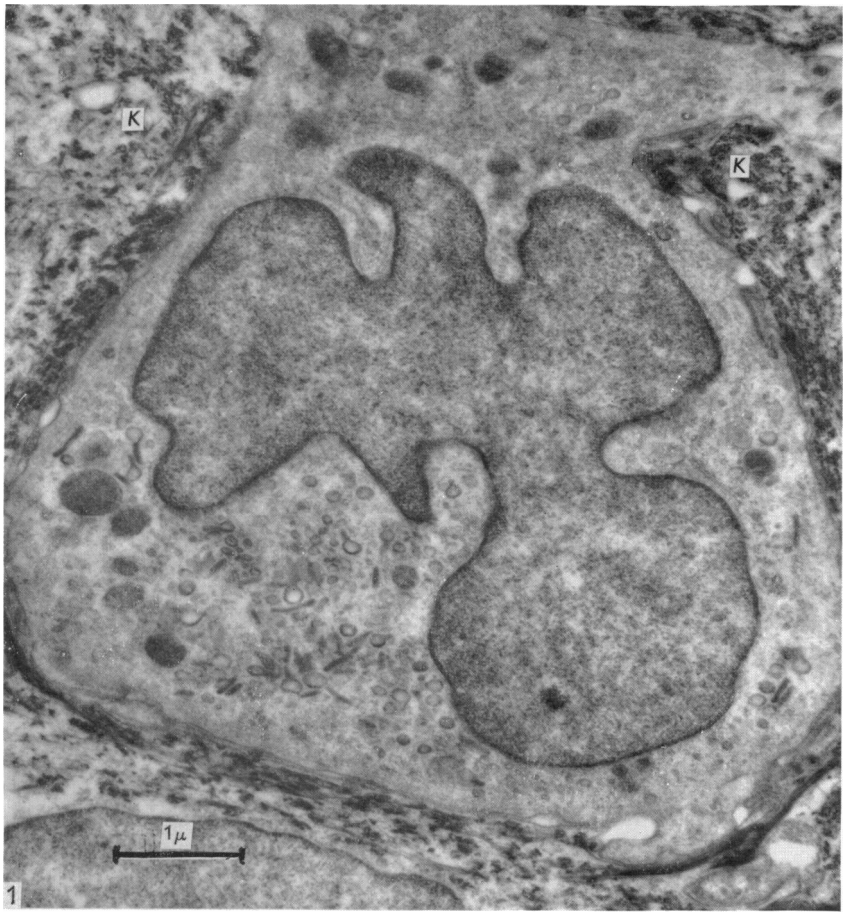
Fig. 10. A 'clear' cell, probably a Langerhans cell, in the basal layer of the epidermis. Arrows point to the basement membrane. *D*, Dermis; *K*, keratinocytes of basal layer; *m*, mitochondria; *X*, rounded organelles. × 12,000.

PLATE 4

Fig. 11. To show close association between basal melanocyte (*M*) and probable Langerhans cell (*C*). *g*, Melanin granules in melanocyte; *K*, basal layer keratinocyte. Arrows point to the basement membrane. × 15,000.

Fig. 12. Lysosome (*L*) in the cytoplasm of a Langerhans cell. *m*, Mitochondrion; *X*, rounded organelle with finely granular internal structure. × 60,000.

Fig. 13. A Langerhans cell from the upper level of the stratum spinosum. Relatively few cytoplasmic organelles of any type are present. *K*, surrounding keratinocytes. × 26,000.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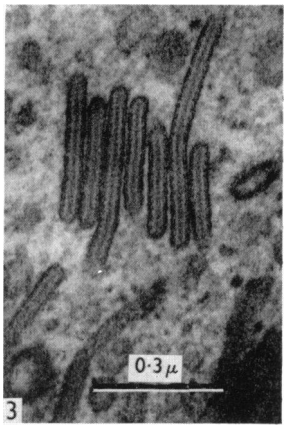
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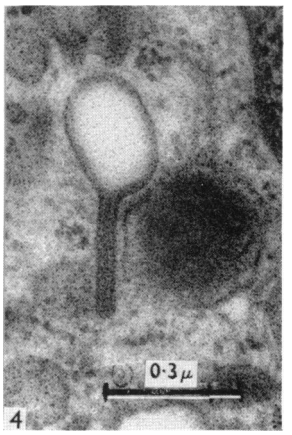
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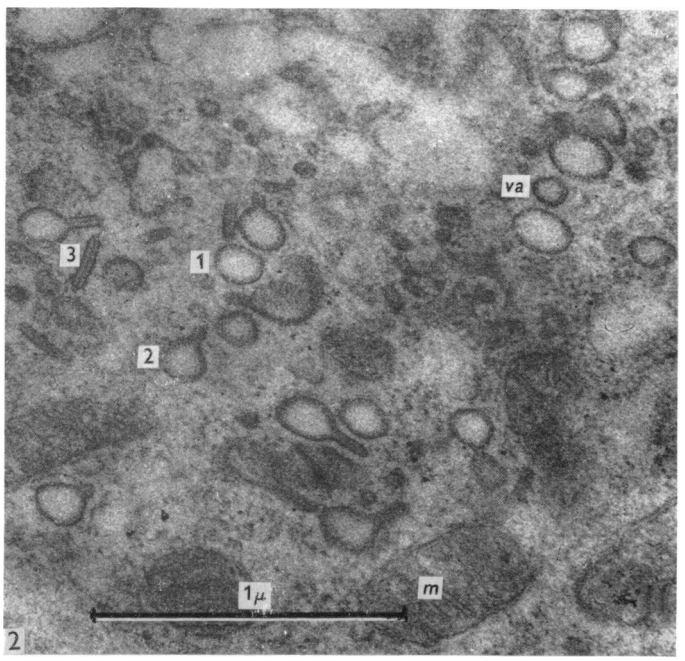
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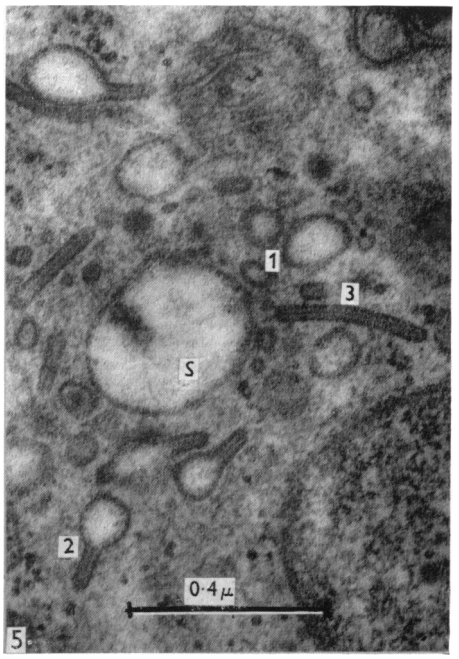
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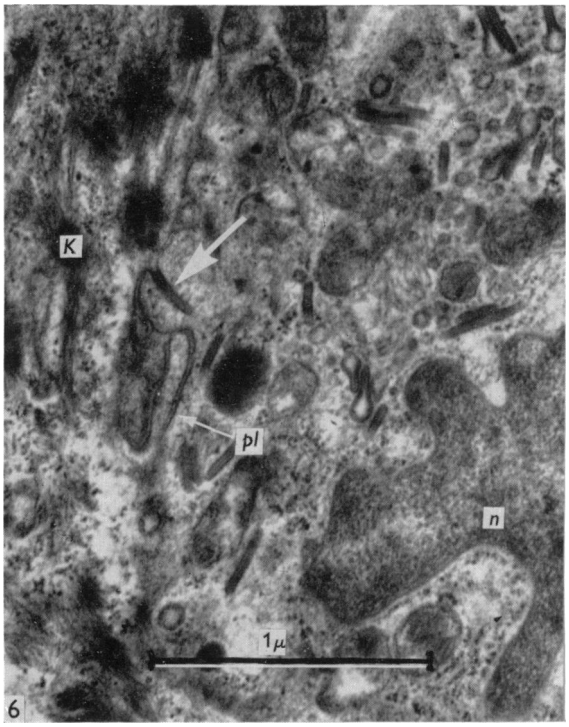
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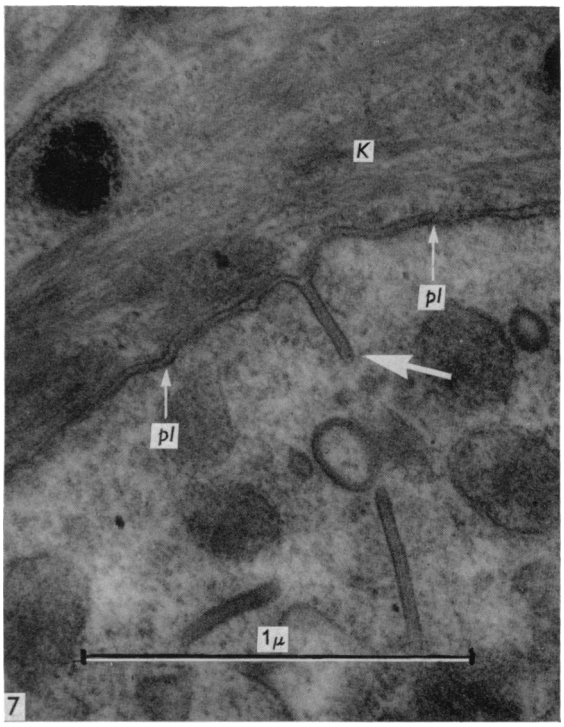
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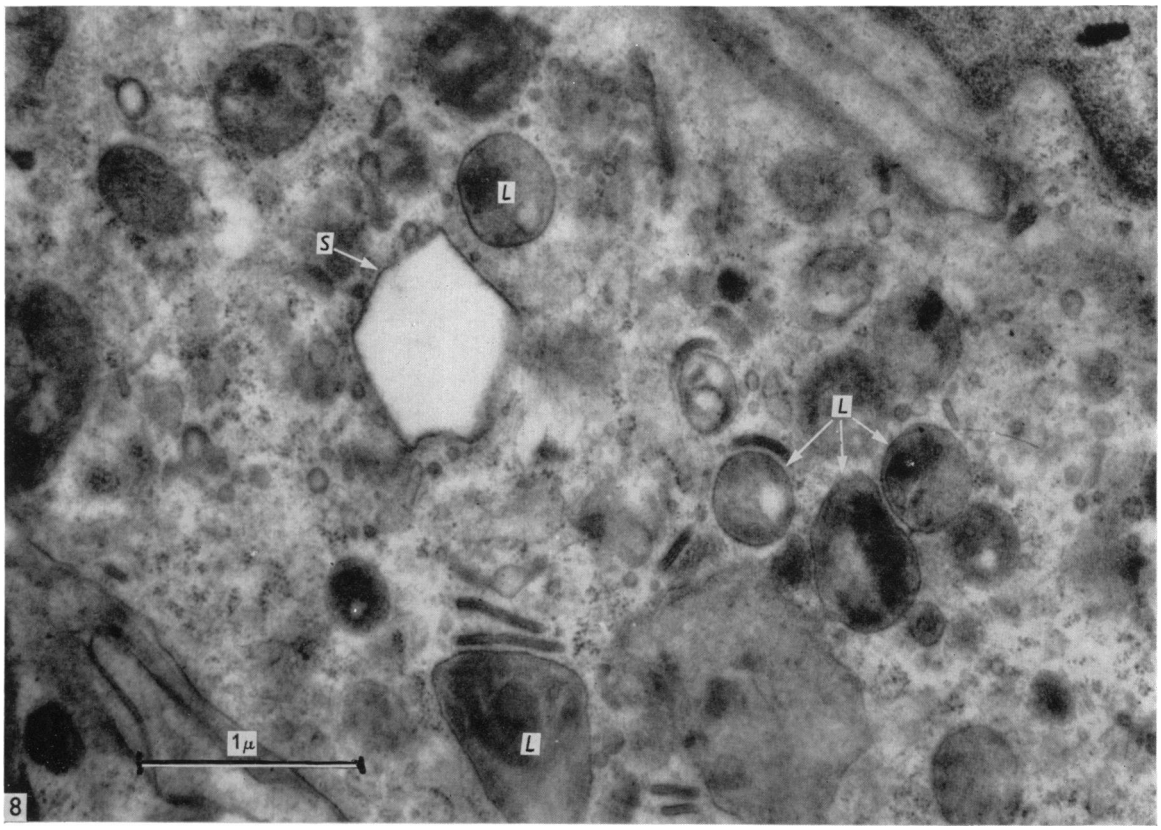
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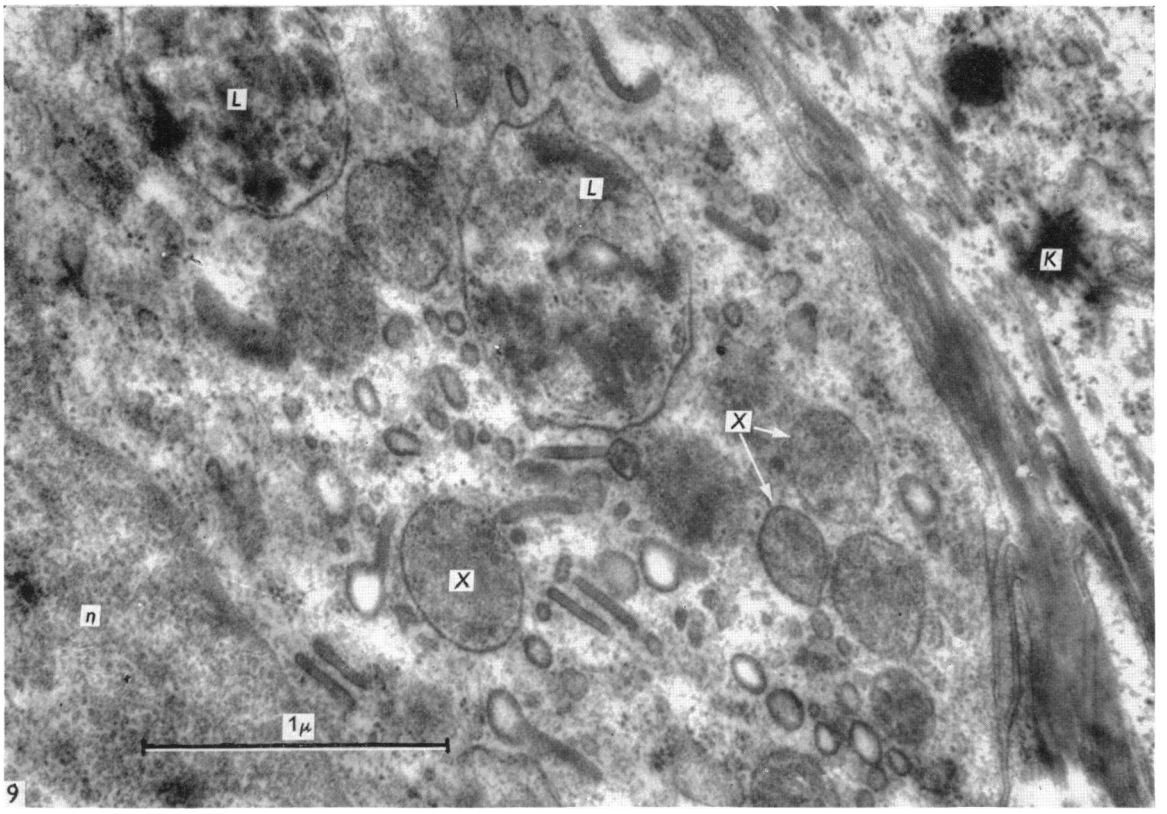
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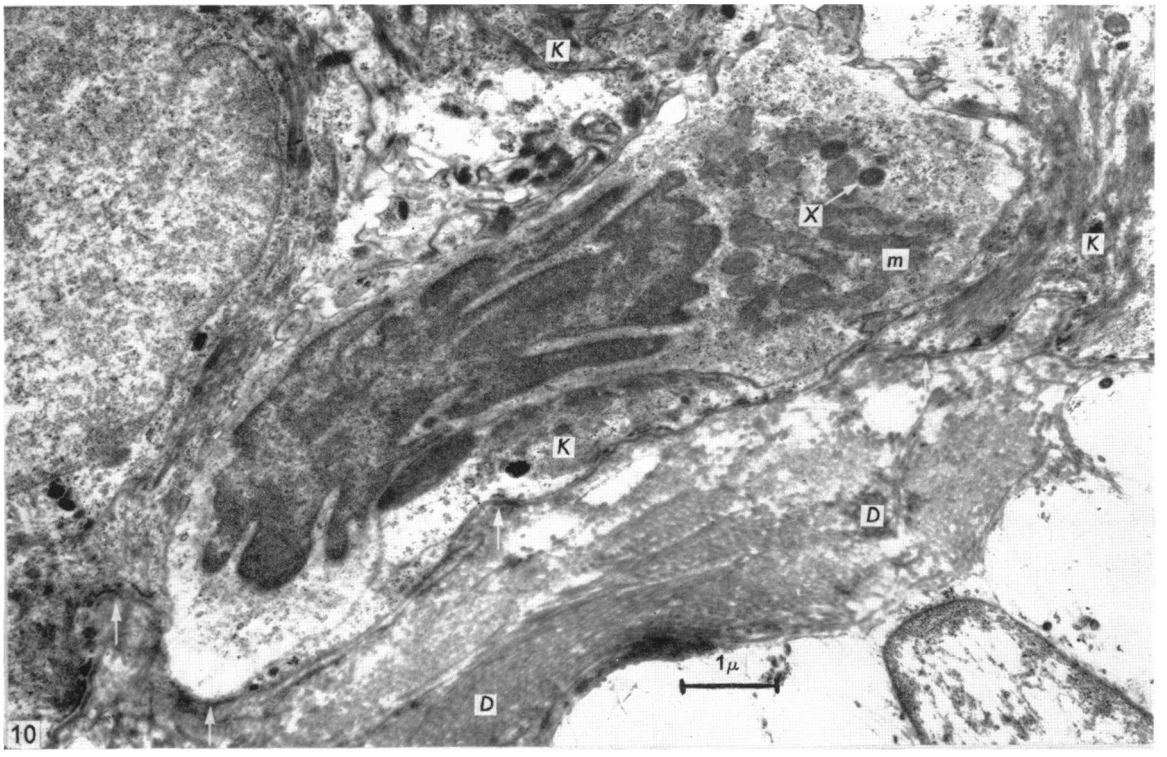
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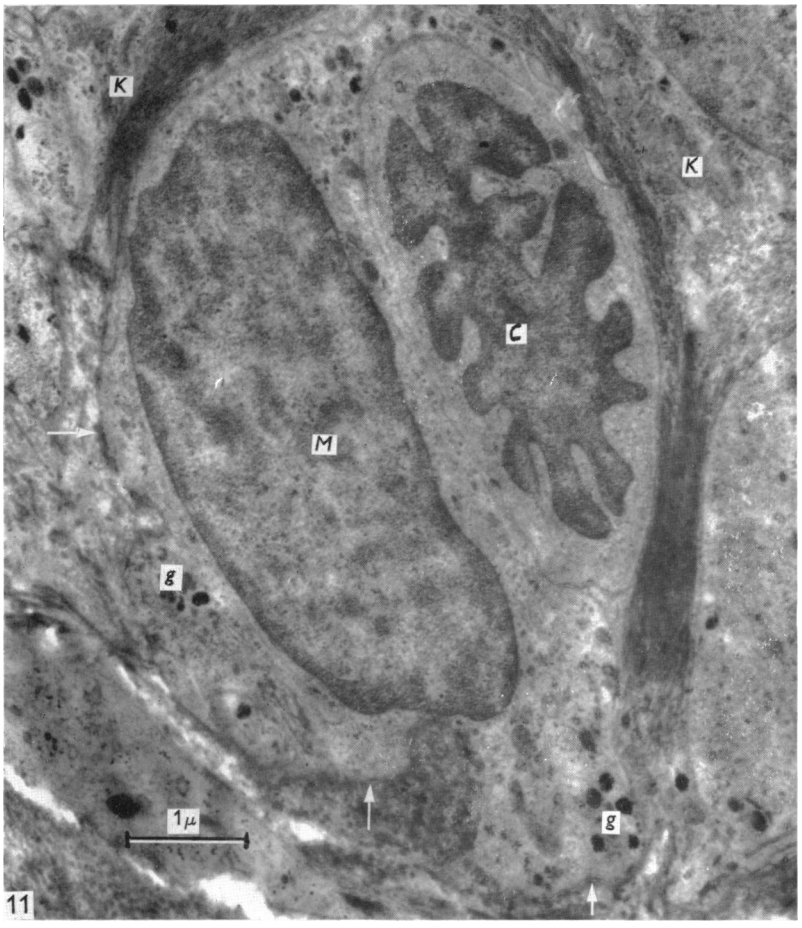

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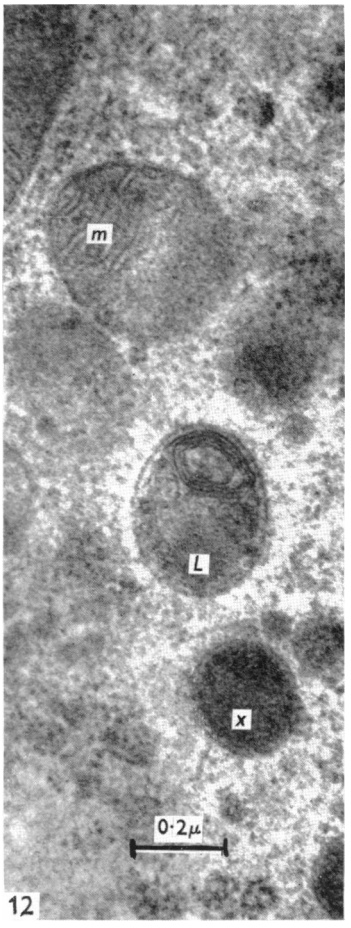

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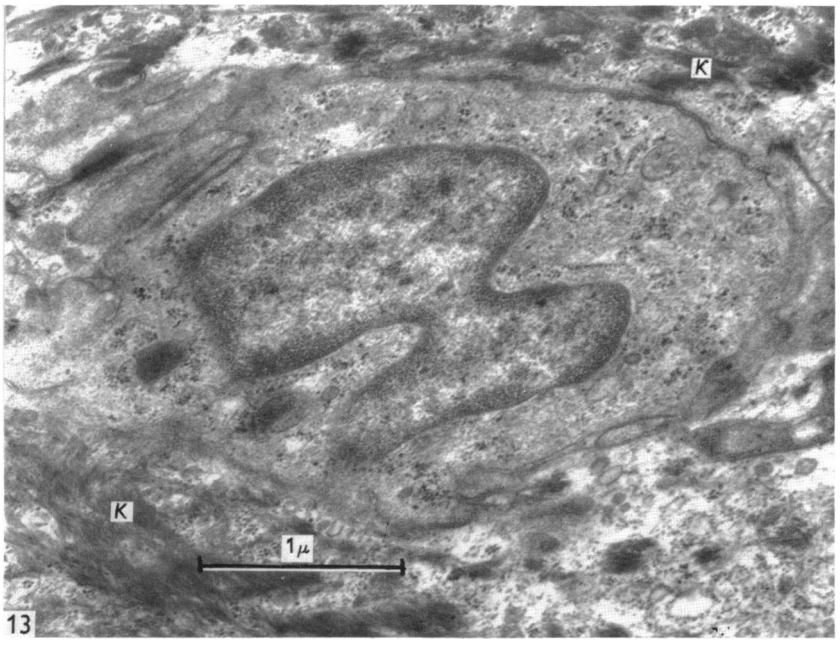

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