Tumor Res. 4 (1969) 1 - 28

LIVER TUMORS INDUCED IN RATS BY 3'-METHYL-4-DIMETHYL AMINOAZOBENZENE

III. Electron Microscopic Investigation -- Disdifferentiation of Cancer Cells --

Yusuke Fuse, Tomomi Iwasaki and Tamenori Onoé

Department of Pathology, Sapporo Medical College

Introduction

One of the basic characteristics of cancer cell is dedifferentiation. However, disdifferentiation and redifferentiation toward various cell types is also seen in the mass of cancer cells. The cancer cells in the various stages or types produced by the failure and disturbances of differentiation are seen in 3'-Me-DAB induced hepatoma. Such disdifferentiated and redifferentiated cancer cells is observed especially in undifferentiated carcinoma which consists of ductular cancer cells. This may have some relationship with the facts that ductular cell has a multiple differentiating potentiality (1) and it has less differentiated cells than hepatocytes and bile ductal cells.

Materials and methods were described previously (2).

Observations and discussion

As a result of disdifferentiation and redifferentiation of the cancer cells, the following cells of various types were observed in the 3'-Me-DAB induced hepatomas.

1. Goblet cell

In adenocarcinoma, many goblet cells were present (Fig. 5), whereas there were only a few goblet cells seen in undifferentiated carcinoma (Figs. 16 and 17) and in hepatocellular carcinoma. Goblet cells found in adenocarcinoma revealed a basement membrane and faced the glandular lumen. The cytoplasm was filled with mucus droplets of moderate electron density, and the nucleus was pressed down toward the basement membrane. These features of goblet cells are identical with that found in normal mucosa. Goblet cells in undifferentiated carcinoma revealed no basement membrane formation and no microvilli formation (Figs. 16 and 17). They appeared at random among the ductular cancer cells and did not show any cellular polarity. Some cancer cells contained a small number of mucous droplets in the cytoplasm (Figs. 3 and 4). These cells are considered to be in transition into goblet from adenocarcinoma cells.

2. Goblet cells containing needle-like crystals

Some goblet cells contained needle-like crystals in their mucous droplets. These cells were observed in adenocarcinoma (Figs. 6 and 7). In such goblet cells, two kinds of mucous droplets were observed, one of which was dark with a high electron density and the other was clear with

a low electron density (Fig. 6). The crystals were usually found in the clear mucous droplets. These crystals had a periodicity of 160 to 180 Å A (Fig. 9). In the glandular lumens of adenocarcinoma, a homogeneous and amorphous component of low electron density as well as crystals were observed in large amounts (Figs. 8 and 9). The nature of these crystals is unknown. However, judging from the fact that mucin is a complex of proteins and polysaccharides and the fact that the crystals are found only in the clear droplets, the unbalance of production between proteins and polysaccharides by goblet cells may occur. These goblet cells containing needle-like crystals have not been reported previously to our knowledge.

3. Argentaffin cell

Cells containing numerous tiny granules were present in adenocarcinoma (Figs. 12 - 15) as well as in undifferentiated carcinoma (Figs. 18 and 19). In adenocarcinoma, they were located closely to the basement membrane with a wide base (Figs. 12 and 13). Occasionally, they appeared as a star shape between neighboring cancer cells (Fig. 15). The granules measured from 200 to 300 mμ in diameter, and possessed a limiting membrane and an extremely high inner electron density. A translucent zone between the limiting membrane and its content was observed. The ultrastructures of these granules are similar to serotonin granules (3 - 5). The granules found in undifferentiated carcinoma showed a marked variability in size and the cells containing these granules were not attached to the basement membrane in most cases (Figs. 18 and 19).

4. Gastric surface mucous cell

Cells filled with large sized granules of high electron density measuring 0.5 to 1.0μ in diameter were rarely observed in adenocarcinoma. Such granules are characterized by the absence of a limiting membrane. The cells containing these dense granules had highly developed microvilli and were seen facing the glandular lumen (Fig. 10). These cells were similar to the grastric surface mucous cells (6,7). Concerning the appearance of gastric mucous cell in hepatoma, Flaks (13) reported the hepatoma cell with tubular invaginations resembling to gastric parietal cell, and he considered that both hepatocytes and gastric parietal cells are a common origin from the foregut of the embryo. Thus, the degree of disdifferentiation of hepatoma cells induced by 3'-Me-DAB was much higher than expected histologically.

5. Intestinal metaplastic cell

Some cells were observed facing the glandular space and possessed highly developed microvilli (Figs. 1 and 2). The axon of each microvilli elongated internally within the cytoplasm and appeared to be a terminal web like structure (Fig. 2). These cells possessed many mitochondria, RER and hypertrophic Golgi apparatus in the supranuclear regions. Many of these cells were observed in adenocarcinoma together with the goblet cells.

6. Transitional epithelial cell

In undifferentiated carcinoma, a group of cells which were spindle shaped and associated with many microvilli were observed. The inter-

cellular spaces among them were widely separated. These cells had many desmosome connections among one another and a paucity of cytoplasmic organelles such as mitochondria and ER, but they showed a rich amount of cytoplasmic fibriles. Svoboda (8) reported that DAB induced hepatoma cells contained compact bundles of fibriles. Firminger and Mulay (9) also reported that fibriles resembling keratin was observed in hepatoma cells which were interpreted as a subcellular stage of squamous metaplasia. In this observation, it is of interest to note that the argentaffin cells attached to the basement membrane were seen beneath these transitional epithelial cells.

7. Unclassified teratologic cancer cell

Large cells showing a similarity to the ultrastructural characteristics of hepatocellular cancer cells were seen in undifferentiated carcinoma (Figs. 17, 19, 20). RER in these cells was greatly distended, being filled with fine granular or fibrillar materials. Some of these cells showed a basement membrane formation and possessed a certain type of secretion granules of high electron density on one side of the cytoplasm (Fig. 20). Since RER is considered to be the site of exported protein synthesis, these cells were considered to be producing some kind of proteins. It may be possible that hepatoma containing these cells may represent some specific functional ability. It is not certain whether these ultrastructures indicate some new function in these cells which arises during disdifferentiation of cancer cell or whether it is the result of disturbances of exportation of synthesized protein.

In undifferentiated carcinoma there were also the cells which showed ultrastructures resembling hepatocellular cancer cells which were filled by granules of various electron densities measuring from 100 to 200 mμ without a limiting membrane on one side of the cytoplasm (Fig. 21). Such cells were considered to be showing multi-directed differentiation at the subcellular level in a single cell rather than in the cells in the transitional stage from blastic cells toward a certain definite cell.

The various kinds of cells mentioned above in addition to the basic three cancer cells, i.e. ductular, hepatocelluar and adenocarcinoma cells were observed. These various types of cancer cells appeared at random among the groups of cancer cells. From these observations, it may be said that the mechanism which organizes and controls the differentiation of the cell is lost or that such a mechanism is markedly unstable in cancer. It has been stressed that the presence of a marked degree of ultrastructural variability (2) is present in the cancer mainly due to disdifferentiation and redifferentiation rather than that expected from their histologic appearance. This marked variability was particularly noted in undifferentiated carcinoma induced by 3'-Me-DAB. This fact may be common in chemically induced tumors. From this point of view, it is of interest that the antigenic properties of chemically induced tumors are different from each other (10).

Cancers with little dedifferentiation and disdifferentiation are recently known as minimal deviation tumors (11). However, the cancers which are the targets in medical fields may have a more or less variable degree of dedifferentiation and disdifferentiation depending on each cancer,

even in those which arise from the same tissue and are classified as the same histologic entities.

The cancers containing an abnormally large number of cells deviated from differentiation may represent the possibility of specificity in its functional ability. This fact must be emphasized in immunological and biochemical research for cancer.

Summary

As a result of disdifferentiation and redifferentiation of cancer cells, the following cells of various types, i.e. goblet cells, intestinal metaplastic cells, transitional epithelial cells and even gastric surface mucous cells are found under an electron microscope in 3'-Me-DAB induced hepatomas. Varying disdifferentiated cancer cells are found in abundance in undifferentiated carcinomas. Disdifferentiation and re-differentiation of cancer cell are briefly discussed.

(Recieved March 15, 1969)

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Explanation of figures

Fig. 1 Intestinal metaplastic cancer cell
The intestinal metaplastic cancer cells (I) have well-developed microvilli and terminal web structures (arrows). These lumens are filled with dense minute particles. The adenocarcinoma cell (A) with several cytolysosomal inclusions is seen with rudeimentarly developed microvilli. BM: basement membrane L: glandular lumen x 6,000

Fig. 2 Intestinal metaplastic cancer cell
The intestinal metaplastic cell with well-developed microvilli and terminal web structure (I), and the adenocarcinoma cell (A) form a common narrow glandular lumen (L). The lumen contains a neutrophil and mucinous materials. The intestinal metaplastic cell is more abundant in an amount of cytoplasmic organelles than those of the adenocarcinoma cell. x 8,000

Fig. 3 The transitional cell into a goblet cell from adenocarcinoma cell is observed in the center of this figure. The cell contains several dense mucous droplets in the moderately condensed cytoplasm. BM: basement membrane L: glandular lumen x 6,000

Fig. 4 Several adenocarcinoma cells form a glandular lumen (L). Some of them reveal microvillus formation, but some are flattend without microvilli. The clusters of vacuoles (arrows) considered as mucous droplets are seen in one cell. BM: basement membrane x 5,000

Fig. 5 Goblet cells filled with mucin droplets are observed among the adenocarcinoma cells. The goblet cell is in contact with a basement membrane. BM: basement membrane N: neutrophil x 7,000

Fig. 6 A goblet cell is filled by mucous droplets. Two kinds of mucous droplets are seen. Ones are clear and the others are dark. Needle-like crystals are seen only in the clear mucous droplets (arrows). BM: basement membrane x 9,500

Fig. 7 Two goblet cells with the needle-like crystals are seen among the adenocarcinoma cells associated with abundant free ribosomes and polyribosomes. The goblet cells are connected by desmosomes with the surrounding adenocarcinoma cells. x 20,000

Fig. 8 The adenocarcinoma cells associated with poorly developed microvilli form a glandular lumen (L). The lumen are seen filled by mucinous materials of low density and numerous needle-like crystals. An arrow indicates the cilium formation. x 5,500

Fig. 9 The flattened adenocarcinoma cells having lost microvilli represent abundant fibriles in the apical surfaces of the cytoplasm. The areas condensed with these fibriles appear as dark spots. The glandular lumen contains numerous needle-like crystals and mucinous materials. By high magnification, the mucinous materials appear not to be homogeneous, but to be finely fibrillar. BM: basement membrane x 20,000

Fig. 10 Cells with large dense droplets and microvillus formation are observed among the adenocarcinoma cells. L: glandular lumen x 9,000

Fig. 11 Human gastric surface cells: Their mucin droplets are quite similar in size and density without a surrounding membrane to those in figure 10. x 6,000

Figs. 12, 13 and 14

Argentaffin cells

The cells filled with the numerous minute granules (arrows) are seen in contact with the basement membrans (BM). Most of the granules are round shaped with a surrounding membrane, but some are rod shaped. These granules are identical to the serotonin granules of the argentaffin cell. Figs. 12 and 14 x 9,000 Fig. 13 x 16,000

Fig. 15 The star-shaped argentaffin cell (arrows) is connected by desmosomes with the neighboring adenocarcinoma cells. x 8,000

Fig. 16 Undifferentiated carcinoma cells

The three kinds of cancer cells are seen in this figure. In the upper portion oval cell type cells are seen (O). In the lower portion intermediate cancer cells (H) between the oval cells and hepatocytes are noted. Moreover, the goblet cells (G) appear among them without cellular polarity. x 3,000

Fig. 17 Undifferentiated cancer cells
The majority of the cancer cells are of the oval cell type. Among them the Goblet cell (G) and cells resembling hepatocytes in which the rough endoplasmic reticulum is dilated and filled with dense granular materials (arrows) are seen. x 3,000

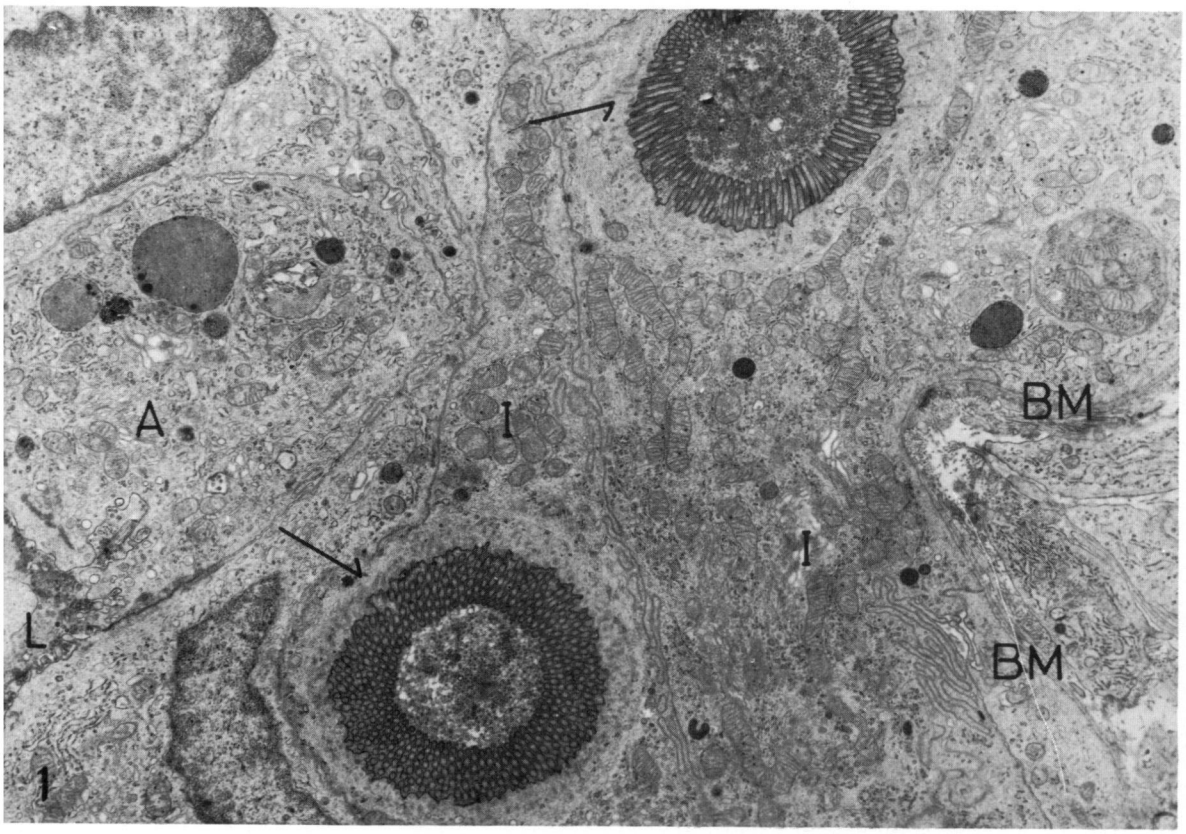
Fig. 18 Undifferentiated cancer cells
Among the cancer cells of oval cell type, various cells such as mitotic cells (M), goblet cells (G) argentaffin cells (arrow) and cells with microvillus formation are seen. x 3,000

Fig. 19 Undifferentiated cancer cells
Various cells such as goblet cells (G), argentaffin cells (A) and cells in which the rough endoplasmic reticulum is dilated and filled with dense materials (arrows) appear at random among the oval cell type cancer cells. x 3,000

Fig. 20 In the center of the figure unclassified teratologic cancer cells are noted among the cancer cells of oval cell type. The cell is widely in contact with the basement membrane (BM) and forms a bile canaliculus (BM) in the opposite direction. The cytoplasm is filled with rough endoplastic reticulum which is dilated and packed with dense granular materials. x 3,000

Fig. 21 Cancer cells which possessed well developed mitochondria and rough endoplasmic reticulum and resembles hepatocellular cancer cells hold poorly developed microvilli and numerous unidentified dense granules on one side of the cytoplasm. x 3,000

Fig. 22 Cells possessing numerous microvilli and which are connected by desmosomes with each other are seen. These cells contain a large amount of fibriles in the cytoplasm resembling the transitional epithelium. Argentaffin cells (A) attached to the basement membrane (BM) are also observed. In this photograph the tumor corresponding to the area appearing in figure 18 of Part I is shown. x 10,000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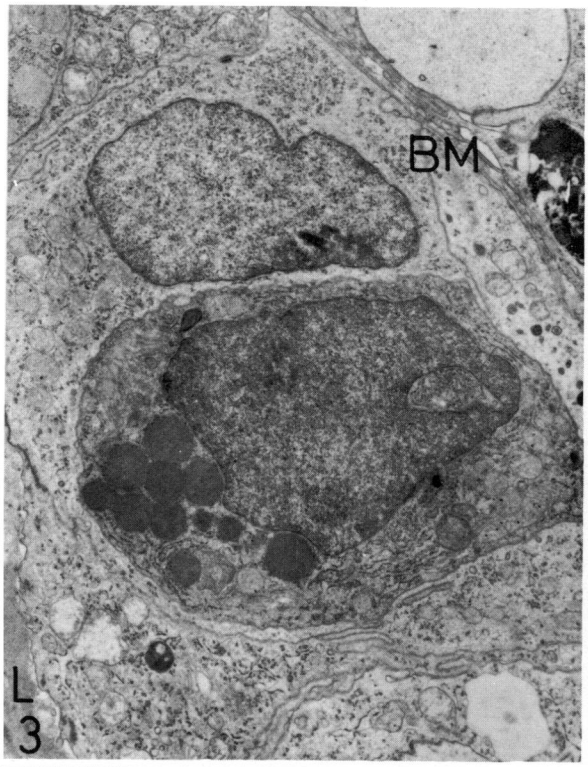
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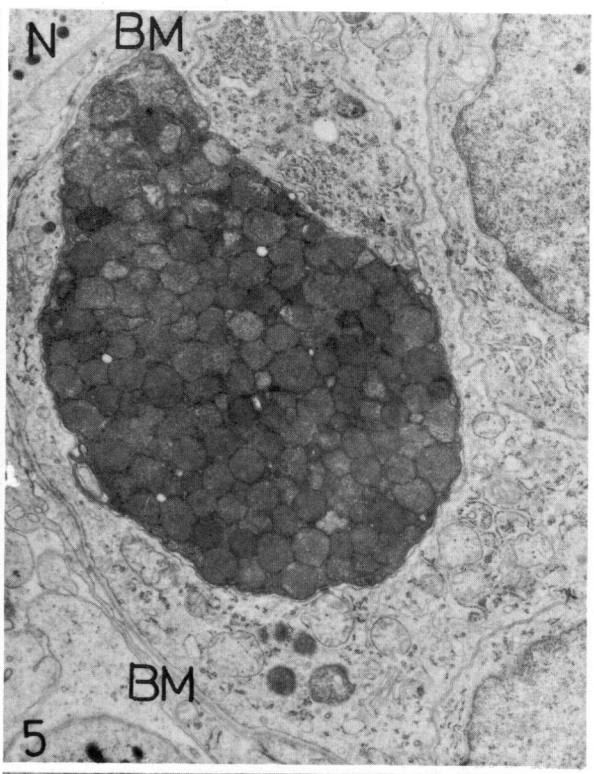
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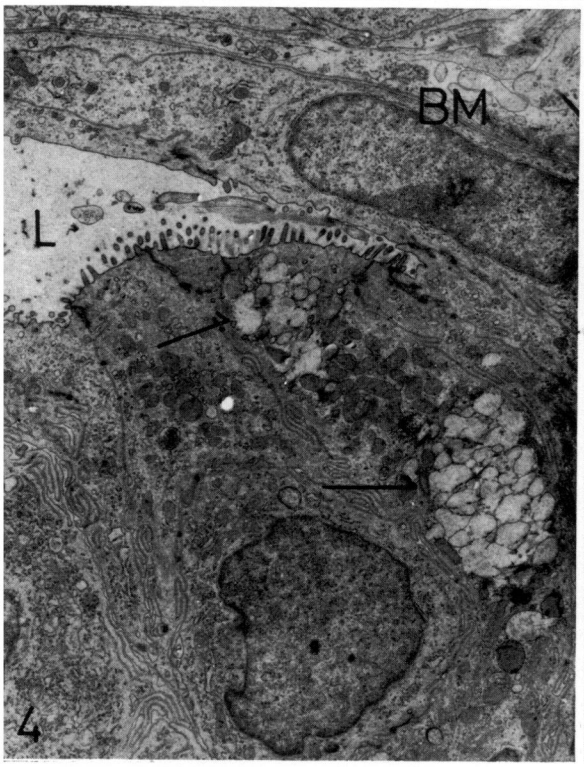
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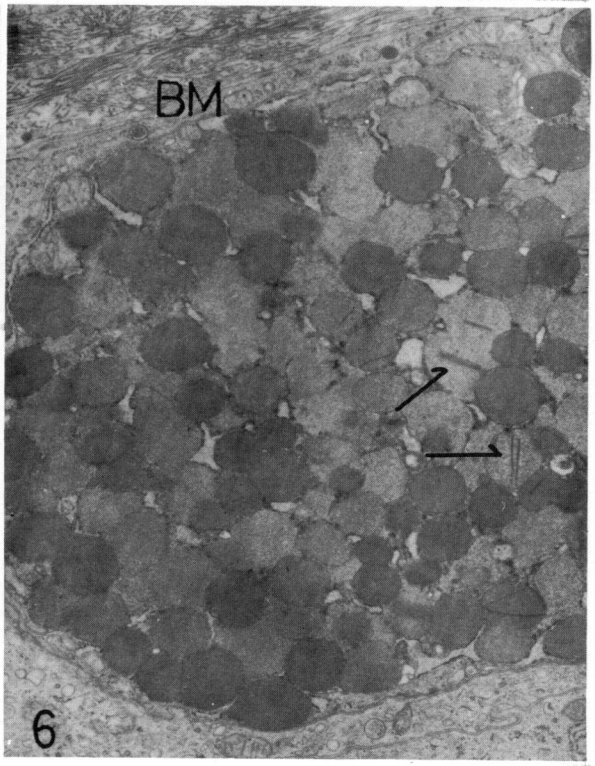
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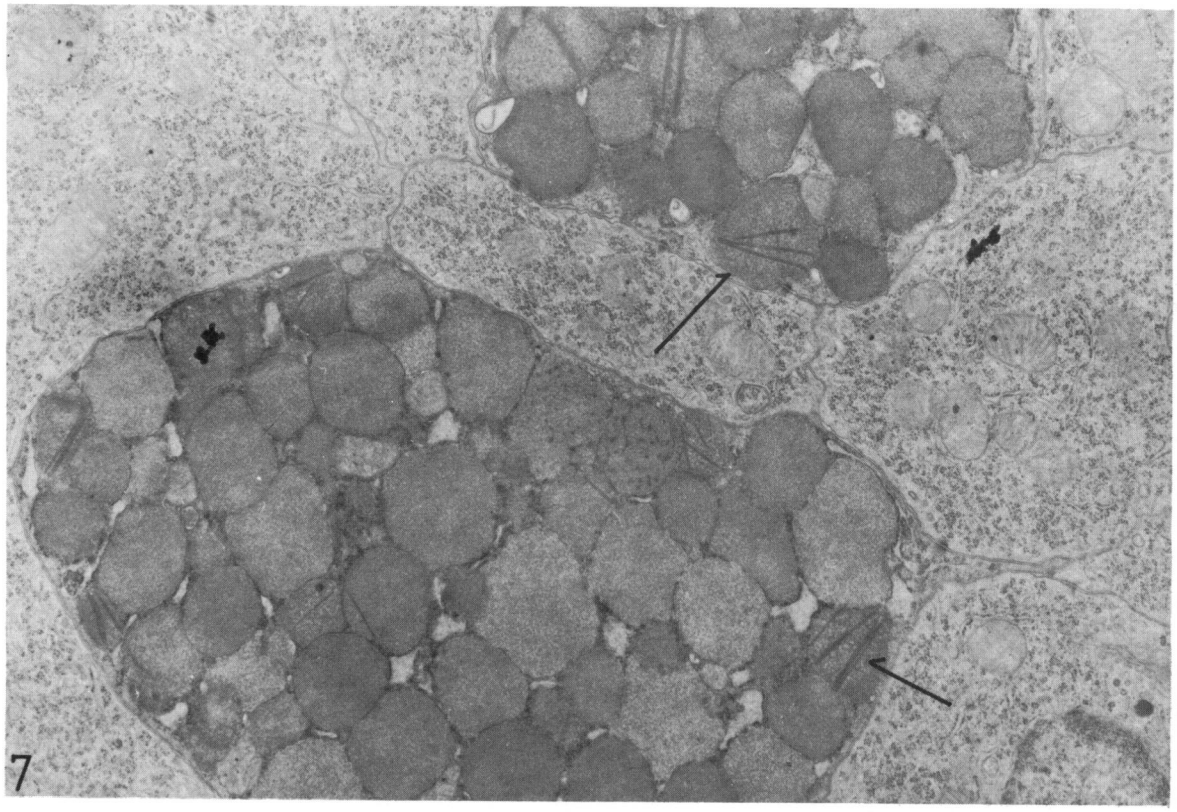
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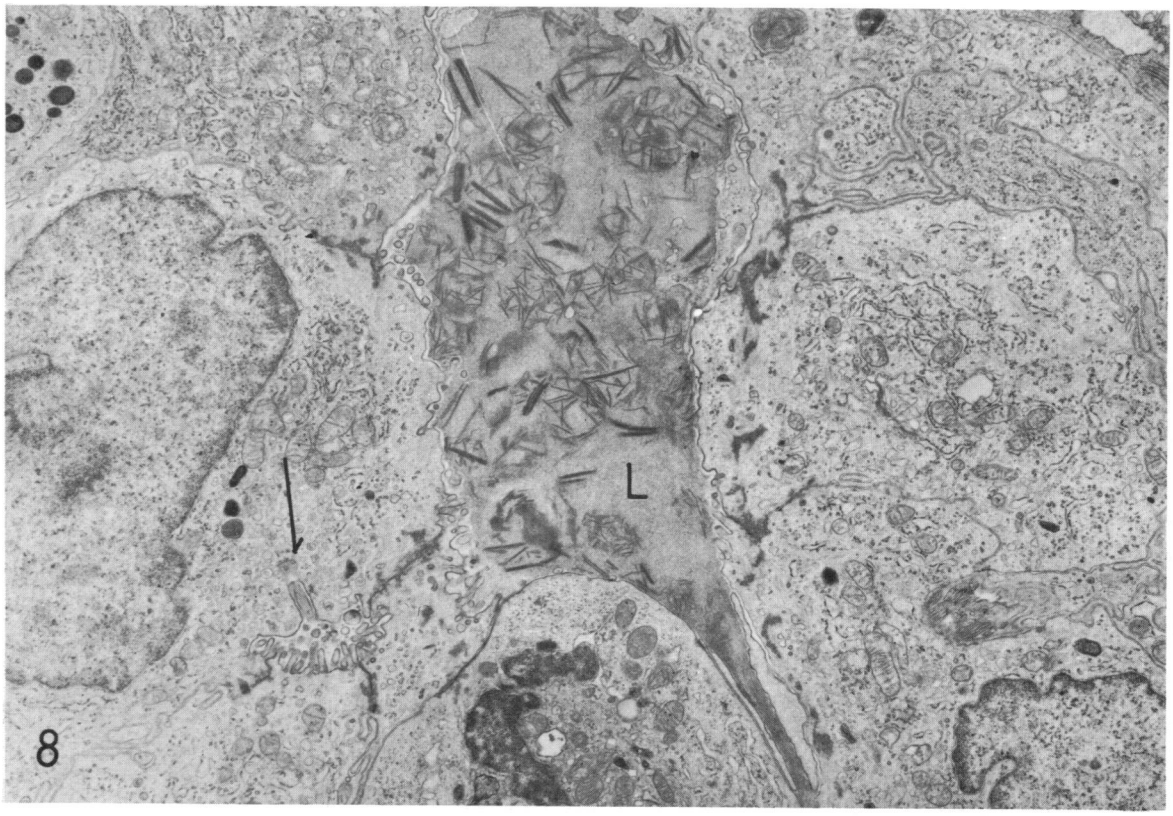
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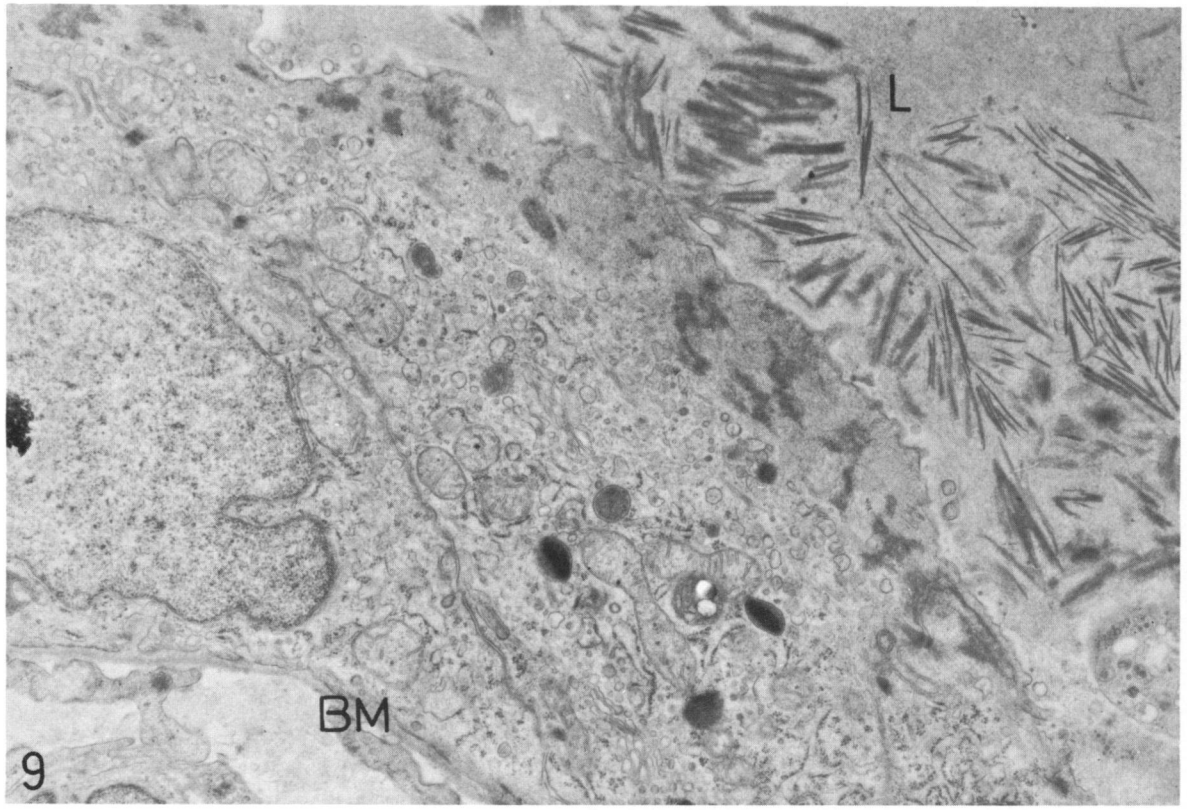
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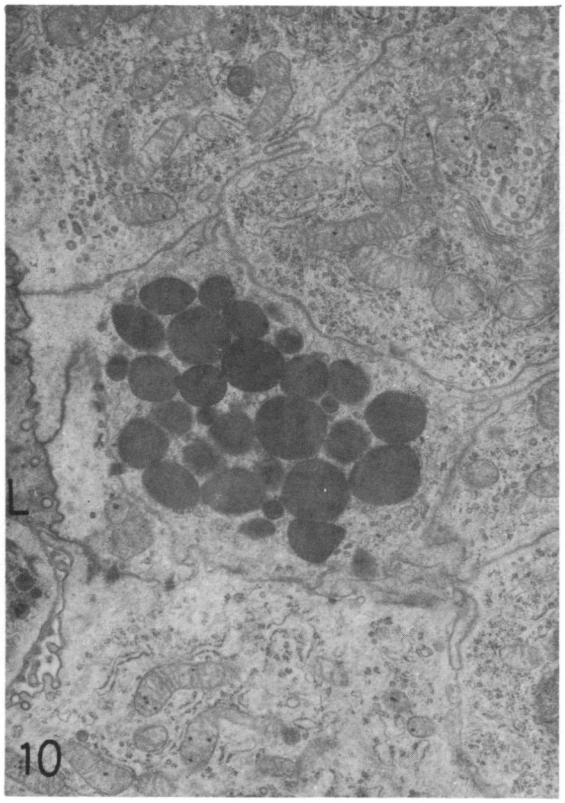
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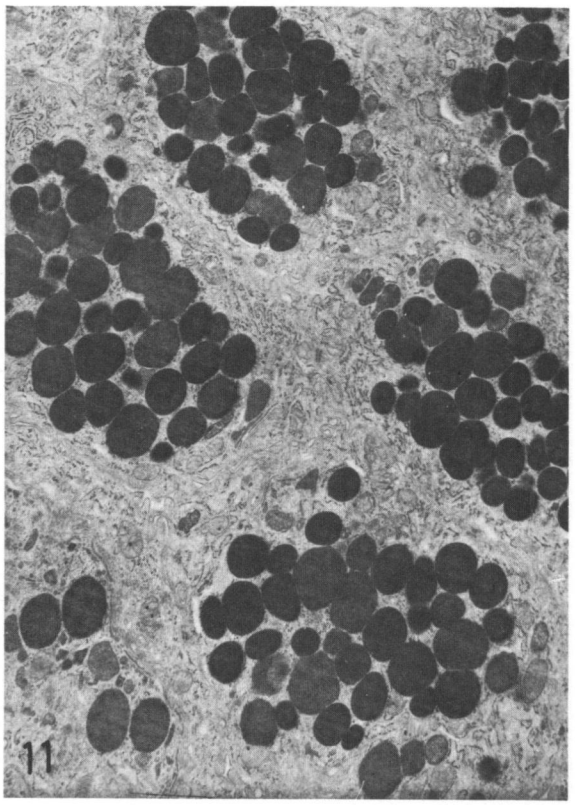
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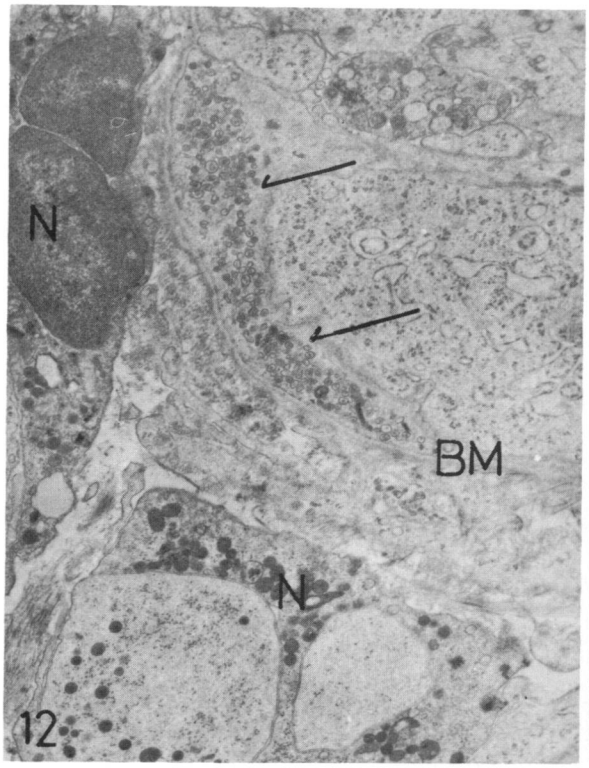
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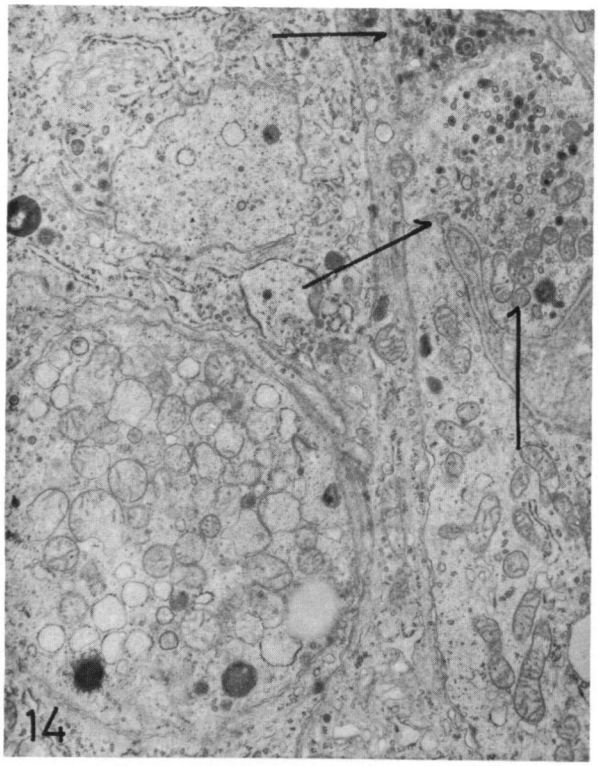
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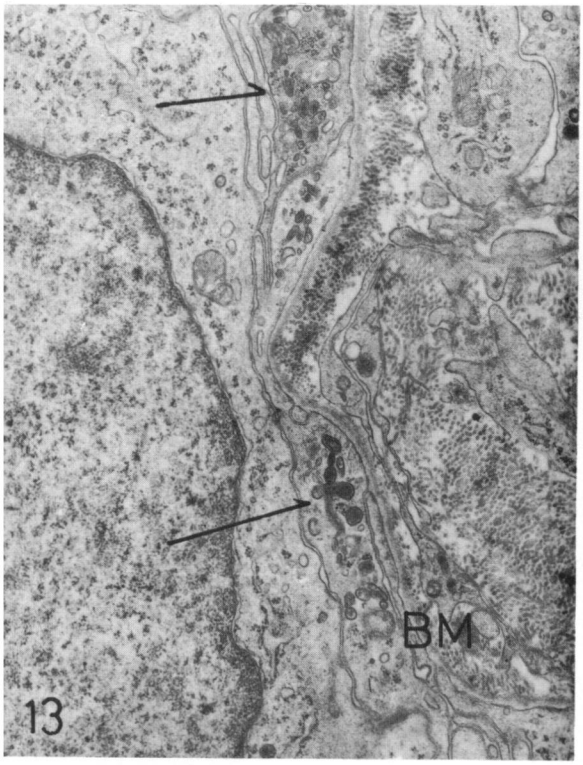
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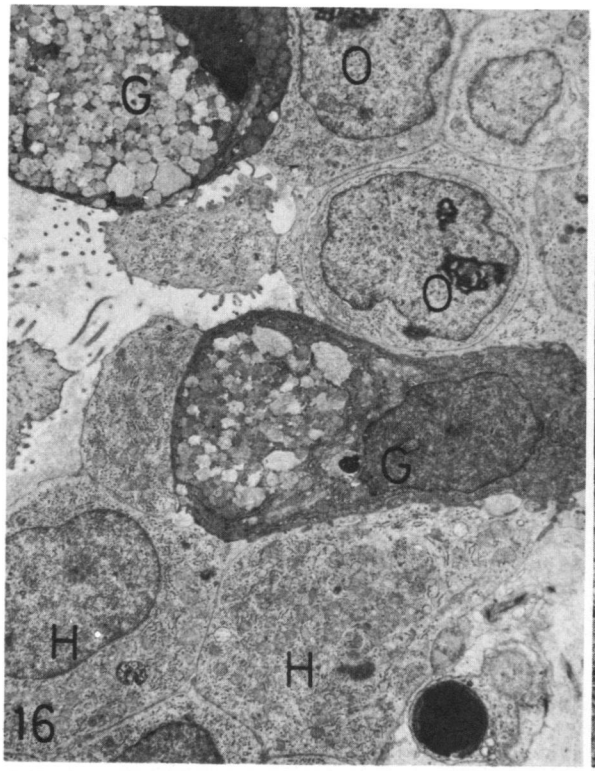

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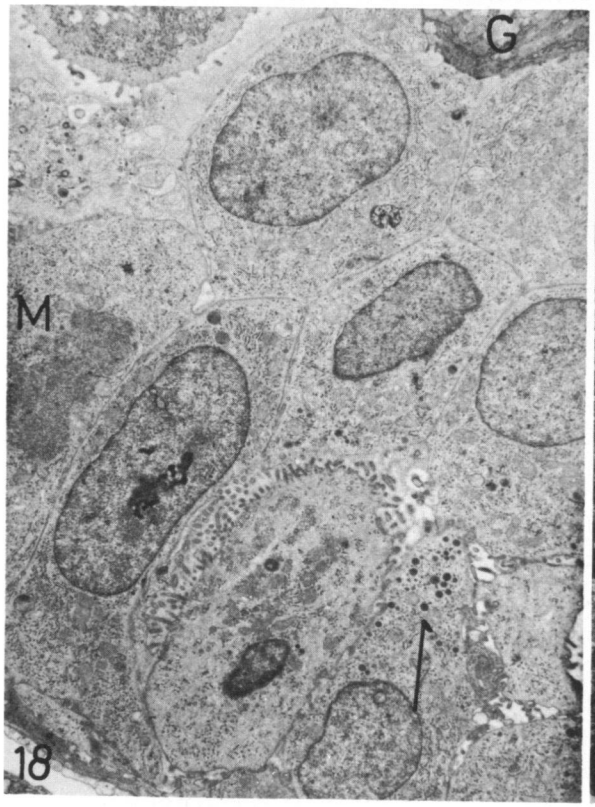

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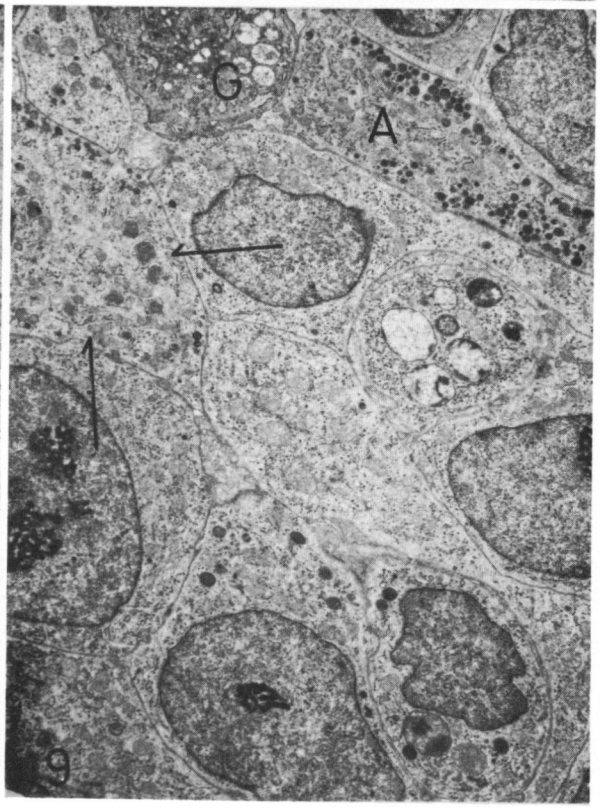

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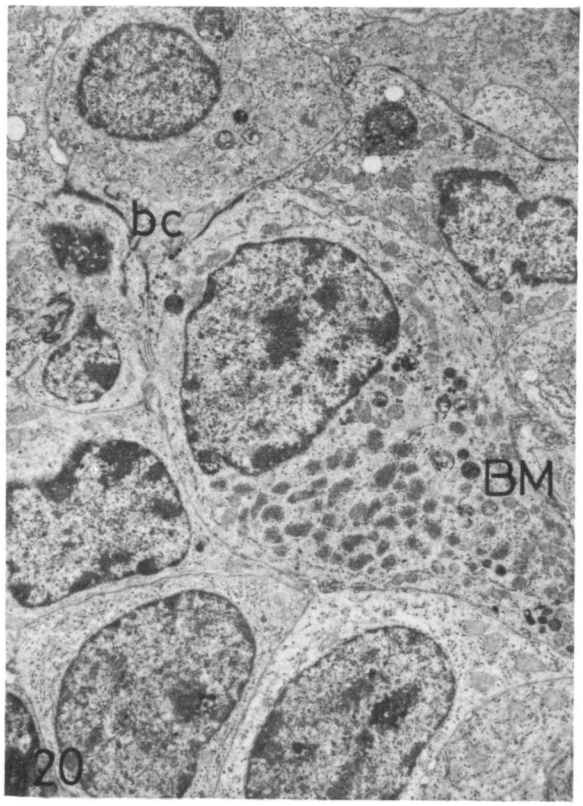
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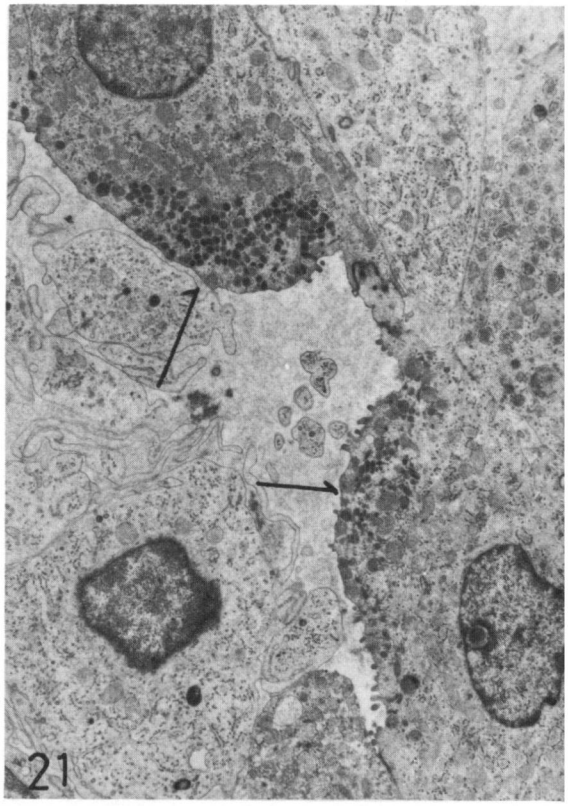
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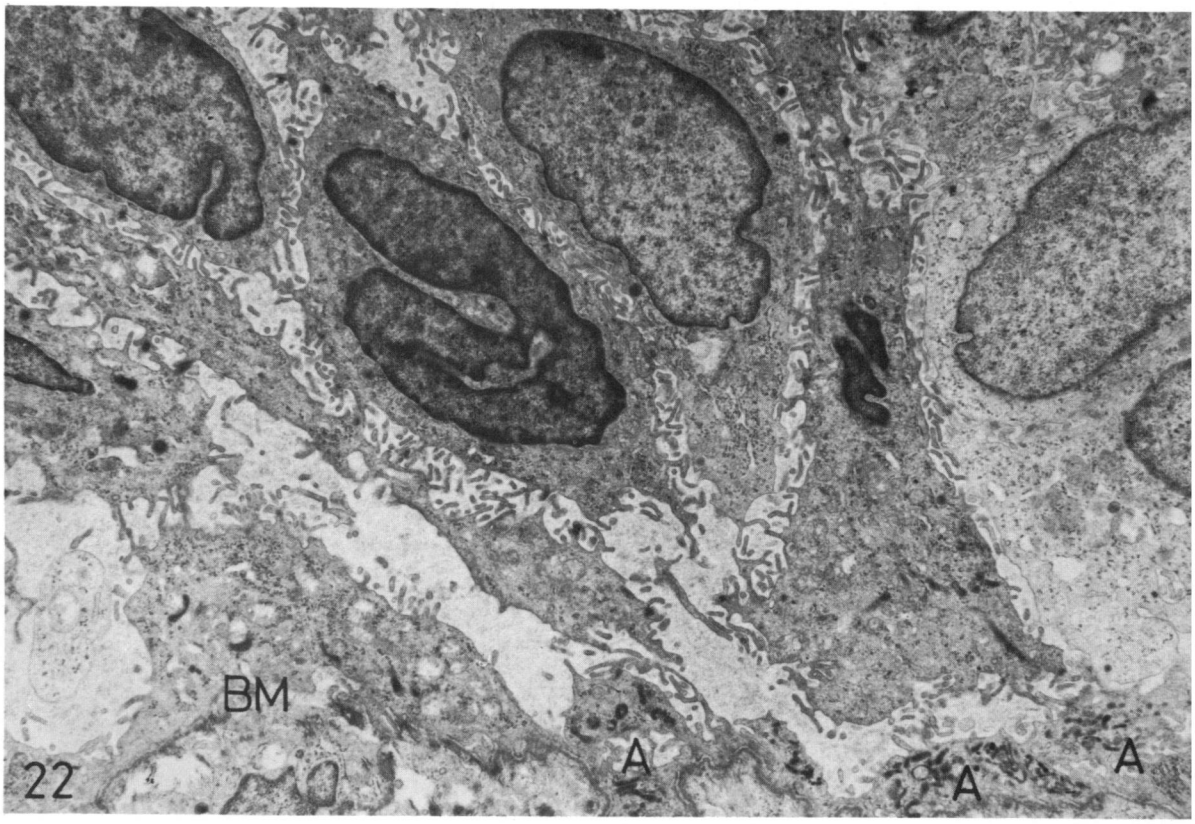
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