Changes in Peroxisomes and Catalase Activity of Morris Hepatoma 7316 A in Culture

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SUMMARY

Changes in peroxisomes of Morris hepatoma 7316 A in culture were studied cytochemically, electron microscopically and biochemically. In primary culture, peroxisomes were decreased rapidly in number and size, and crystalloid nucleoids of peroxisomes disappeared after one week of the cultivation. In the first subculture, the number of peroxisomes and catalase activity were further decreased eventuating in that the organelles were hardly recognized on the light microscopic cytochemistry. However, cytochemical electron microscopy revealed that the organelles were present, though in a considerably small number. In the second subculture, the number and size of peroxisomes appeared to be increased. Catalase activity was maintained at a 30% higher level during the second subculture than those of the cells used for primary culture.

The established cell line was obtained from the second subculture, and maintained in culture for more than 12 months. Catalase activity of the established cells was higher than those of the cells used for primary culture and was nearly constant irrespective of the phases of growth. Peroxisomes were devoid of nucleoids, smaller in size than those of the original hepatoma cells, and as numerous as those of the second subculture. In addition, peroxisomes of the established cell line showed a peculiar intracellular localization. Many peroxisomes localized in association with the concentric lamellar arrays of rough or smooth endoplasmic reticulum.

The tumor cells which were propagated in rats by back-transplantation of the cultured hepatoma cells in early cultures and the established cells were morphologically similar to the original tumor cells. Crystalloid nucleoids reappeared in peroxisomes of the back-transplanted tumor cells and peroxisome-associated lamellar bodies disappeared.

The abbraviations used are: BSS, balanced salt solution; 3,3′-DAB, 3,3′-diaminobenzidine; ER, endoplasmic reticulum; sER, smooth endoplasmic reticulum; rER, rough endoplasmic reticulum.

INTRODUCTION

Peroxisomes are of cytoplasmic organelles characteristic of hepatocytes, though the organelles are widely distributed in animal and plant kingdoms (5). Although hepatocytes in culture provide a profitable tool for studying various biological events at the cellular level and hepatocyte peroxisomes relate to differentiated function of the cells, there have been quite a few studies on the organelles of hepatocytes or hepatoma cells in culture (9, 24, 25). Karasaki *et al.* reported that peroxisomes disappeared in a tumorigenic cell line derived from a long-term culture of rat hepatocytes, but they reappeared when the cells were implanted into rats (9). In electron microscopic studies of newborn rat liver, and Reuber H-35 and Morris 5123 tc hepatomas, Watanabe and Essner failed to recognize peroxisomes in the cells in explant cultures (24, 25). Cytochemical electron microscopy using 3, 3'-DAB reaction is considered to be necessary for identification of peroxisomes, particularly when the organelles are altered entailing an appearance of microperoxisomes.

The present study was made to clarify the changes in peroxisomes after the transfer of Morris hepatoma 7316 A cells to the culture condition, and the changes in the organelles of an established cell line derived from the hepatoma.

MATERIALS AND METHODS

Isolation and culture procedures of the hepatoma cells

Isolation and primary cell cultures of the tumor cells were made according to the method previously reported (17). Cells were grown in plastic tissue culture dishes (60×15 mm, Corning Glass Works, U. S. A.). They were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Culture medium was Ham's F-12 nutrient mixture (GIBCO, U. S. A.) supplemented with 10% inactivated (56°C for 30 minutes) fetal calf serum (GIBCO, U. S. A.), 0.5 μg/ml insulin (24.9 IU/mg, Sigma Chemical Co., U. S. A), 0.04 mg/ml streptomycin sulfate and 40 IU/ml penicillin-G.

At the 7th day after inoculation, the cells on the dishes were incubated with 0.25% dispase II (300,000 Pronase Units/g, Godo Schusei Co., Japan) in Ca^{++}-free Hanks' BSS for 1 minute. The dispase solution was removed by aspiration and the cells were incubated for an additional 5 minutes. Cells were then detached by repeated pipetting after adding 6 ml of the culture medium, and the cell suspension was centrifuged at 50×g for 1 minute. The sedimented cells were resuspended in the culture medium and subcultured at a concentration of 10^3 viable cells per dish. Viability of the

cells examined by trypan blue-exclusion test was 95 to 98%.

Phase contrast microscopically, two distinct cell types attached on dishes were observed in primary cultures: large, polygonal-shaped, epithelial-like cells with granular cytoplasm, showing an elongation at the periphery of colonies, and small, fibroblast-like cells with clear cytoplasm, though the latter cells were seldom encountered and they did not proliferate for 7 days. After the first subculture, fibroblast-like cells decreased in number. The second passage was done from the dishes in which only epithelial-like cells proliferated in a period of 4 to 6 weeks. A cell line of the hepatoma (MHC-10) was established from the epithelial-like cells grown in the second subculture after 12 months of the culture with repeated subcultures. The culture medium was changed twice weekly and the cells were subcultured routinely in 8 to 10 weeks using 0.25% dispase II in Ca^{++}-free Hanks' BSS.

Back-transplantation of cultured cells

The cultured hepatoma cells were detached by the same method as mentioned above, and were collected in a centrifuge tube. After three washings in Ca^{++}-free Hanks' BSS by centrifugation at $50 \times g$ for 1 minute, the cells were resuspended in the same solution at a concentration of 7×10^6 cells/ml. Half milliliter of the cell suspension was injected into the thigh muscle of male Buflalo rats.

Measurement of cell growth of MHC-10 cells

At intervals of 7 days after cultivation of 10^4 cells/dish, the cells were collected by the subculture procedure and counted in a hemocytometer.

Determination of catalase activity and protein concentration

Replicate dishes were washed three times with ice-cold Ca^{++}-free Hanks' BSS without phenol red, and the cells were harvested with rubber policemen and were collected in ice-cold distilled water in a final volume of 3.0 ml. The cell suspension was homogenized with ten passes in a tight-fitting Potter-Elvehjem homogenizer.

For the determination of catalase activity, an aliquot of homogenate was mixed with an equal volume of 1% Triton X-100, and diluted appropriately with distilled water. The activity was assayed spectrophotometrically at 25°C by the initial rate of disappearances of 240 nm absorption from a solution of hydrogen peroxide and 100 mM phosphate buffer, pH 7.0 (1). One unit of the enzyme activity was defined as the amount of catalase causing the decomposition of 1 μmol of hydrogen peroxide per minute. Concentration of protein of the homogenates was determined by the method of Lowry *et al.* (10).

Visualization of peroxisomes by cytochemistry

For light microscopy, the cultured cells on the dishes were fixed in 10% formalin solution for 24 hours at room temperature. After washing with distilled water, the cells were incubated with alkaline 3, 3'-DAB medium, pH 9.7, for 2 hours (14). They were then stained with hematoxylin.

For electron microscopy, the cells on the dishes were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 6 hours at 4°C and rinsed overnight with 3% sucrose in 0.1 M cacodylate buffer, pH 7.4. After the alkaline 3, 3'-DAB reaction for 1 hour, the cells were postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4. The cells were then dehydrated and embedded *in situ* in Epon 812 as reported Brinkley *et al.* (2). Parallel sectioning to the surface of the dishes were performed by Porter-Blum II Ultramicrotome. Sections were then stained with uranyl acetate and lead citrate.

RESULTS

Changes in peroxisomes during 19 weeks of culture

Peroxisomes of Morris hepatoma 7316 A were round or oval in shape, limited by a single membrane and were in close contact with sER and/or rER. Crystalloid nucleoids were prominent in these peroxisomes, however, the size of peroxisomes themselves distinctly smaller than that in normal hepatocytes (Fig. 2). At the 24th hour after cultivation, peroxisomes of the cells were easily detectable with light microscopic cytochemistry. Electron microscopically, the peroxisomes were smaller than those of the original hepatoma cells, but nucleoids were maintained (Fig. 3). At the 48th hour, the size of peroxisomes was further decreased and those containing nucleoids were considerably few (Fig. 4). At the 7th day, peroxisomes decreased strikingly in number and size, and crystalloid nucleoids were lost therein. The first subculture was done at 7th day after inoculation. It became difficult to detect peroxisomes by light microscopic cytochemistry from 1 to 3 weeks of the first subculture. Electron microscopically, it was difficult to distinguish peroxisomes from other small vesicular structures unless cytochemistry of catalase was made (Fig. 5). However, at the 6th week of the first subculture, peroxisomes in the cultured cells increased in number and became visibley by light microscopic cytochemistry. Electron microscopic cytochemically, peroxisomes were observed easily, though nucleoids thereof were difficult to be recognized (Fig. 6).

As shown in Table 1, catalase activity of the cultured cells was strikingly decreased within 24 hours of the primary culture, and the decreased values

Table 1. *Catalase Activity of Morris Hepatom 7316 A, Isolated Cell Suspension and Cultured Hepatoma Celles Following the first Cultivation*

		Catalase Activity
Morris hepatoma 7316 A tumor		64.67± 3.43
Isolated cell suspension		119.31± 2.78
24 hours after inoculation		64.71±15.68
7 days	(first subculture[a])	60.74± 1.27
3 weeks		49.47± 2.48
5 weeks		48.69± 7.20
7 weeks		76.00± 3.46
9 weeks		135.09± 4.55
11 weeks	(second subculture[b])	133.97±10.61
13 weeks		141.23± 7.96
15 weeks		143.89± 7.53
17 weeks		148.92± 4.21
19 weeks		143.44± 8.36

All activities were determined according to the procedure described in MATERIALS AND METHODS. Results are given as units/mg protein. Values are means±S. D. The tumor was excised and minced with scissors. After washing with Ca^{++}-free Hanks' BSS without phenol red, three aliquots of minced tumors were suspended in ice-cold distilled water and homogenized. For the measurements of activity of the isolated cells, three aliquots of the final cell suspension were centrifuged at 100×g for 1 mitute and homogenized following the resuspension in ice-cold distilled water. Results of 24 hours to weeks after inoculation were obtained from 5 dishes in which cells other than hepatoma cells were not found by phase contrast microscopy. a, the first subculture was done at 7th day after cultivation. b, the second subculture was done at 11th week after cultivation. Results of 7 days and 11 weeks were derived from pre-subculturing cells. Determinations were performed in each dish separately.

were continued until the 6th week of the first subculture. Then, the activity was increased on the 8th week of the subculture and also in the cells of the second subculture, showing the values even higher than that in the cell suspension.

The cultured cells were inoculated to the rats at the 7th, 10th and 15th week of the culture. The tumor growth was visible at 6th to 10th month after the inoculation. Electron microscopic features of these tumor cells were essentially the same as those of the original tumor cells (Fig. 7).

Morphology of MHC-10 cells

The cells were observed to grow forming colonies, with an elongation

of cytoplasm at the periphery of the colonies. The cells occupied only 70 to 80% of the available plastic surface even at the highest cell densities. Most of the cells grew in a monolayer, though there were small areas in "old" cultures (7th to 11th week of subculture) where the cells formed multiple layers. Individual cells were essentially polygonal in shape, and border lines between cell to cell were irregular and rugged. Occasionally, the intercellular spaces were seen by phase contrast microscopy with a considerable width. Each cell had one or two nuclei which were round or oval in shape and contained one, two, and occasionally more mucleoli (Fig. 8).

Growth characteristics and catalase activity of MHC-10 cells

Following subculture, the cells entered a phase of exponential growth (Fig. 1). The population doubling time was 3 to 4 days. Although the

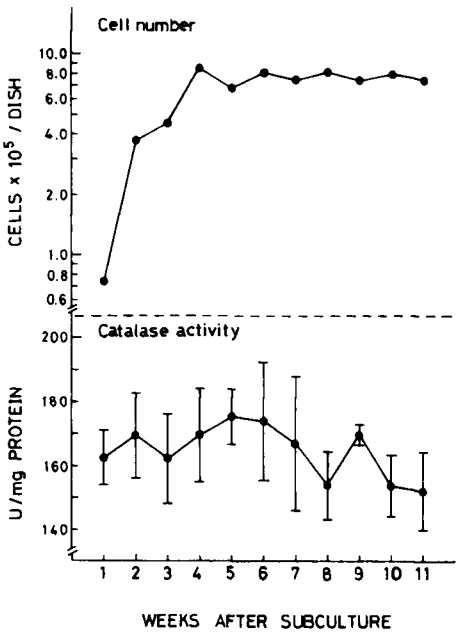

Fig. 1. *Growth Curve and Catalase Activity of MHC-10 Cells After Subculture*

The growth curve of MHC-10 cells after subculture are represented in the upper part of the figure by hemilogarithmic scale. Results are derived from three experiments and are given as mean.

The lower part of the figure shows catalase activity of MHC-10 cells after subculture. Determinations were performed simultaneously with examination of cell number for growth curve of MHC-10 cells. Results are given as mean± S. D.

growth of the cells was not terminated, the cell number reached a plateau at the 4th week of subculture. The cells were lost from the surface of the dishes and were replaced by newly divided cells, thus keeping the population of the attached cells nearly constant at 7 to 8×10^5 cells/dish as long as the culture was maintained. Changes in catalase activity following subculture are illustrated also in Figure 1. Differences between young and old cultures were not statistically significant, ranging from 140 to 190 units/mg protein.

Peroxisomes in MHC-10 cells

By light microscopic cytochemistry with alkaline 3, 3'-DAB reaction, MHC-10 cells showed the reaction-positive bodies larger than peroxisomes, in addition to the peroxisomes in the sizes of those seen in the cells of the second subculture (Fig. 9). At high magnifications, these bodies exhibited various shapes and were recognized as complex bodies which consisted of many small positive granules (Fig. 10). Electron microscopically, small peroxisomes lacking nucleoids were observed in clusters. In particular, many of small peroxisomes were wrapped in concentric lamellar bodies (3). The membranes arranged in the lamellar bodies were predominantly sER, though occasionally were rER. Peroxisomes associated with sER-lamellar bodies were localized mainly in the central areas of the bodies, and they were also seen , though in a small number, associated with the outside membranes of the bodies. Few peroxisomes were seen between the membranes as well (Fig. 11). Occasionally, few other organelles, such as mitochondria or lipid droplets, were also included in the central areas of sER-lamellar bodies. sER in these bodies were meandering. It was dilated in some parts, forming small vacuoles on occasion. There were narrow cytoplasmic matrices between the concentric arrays of sER-membranes consisting of the bodies. Peroxisomes in the rER-lamellar bodies were arranged both in the central areas of the bodies and between the membranes. Dilation of the cisternae was seldom seen in this case. In the rER-lamellar bodies, ribosomes attached on the membranes were fewer than those of usual rER (Fig. 12). Both types of concentric lamellar bodies showed no positive reaction products of acid phosphatase cytochemically (data is not shown). Although concentric lamellar bodies were also seen in the cultured cells during 19 weeks after inoculation (Fig. 6) and in the original hepatoma cells (Fig. 2), peroxisome-associated concentric lamellar bodies were seldom encountered.

Focal accumulation of peroxisomes in close contact with sER were also found in the cytoplasm of MHC-10 cells as shown in Figure 13, though it was infrequent. Some of peroxisomes in MHC-10 cells showed club-like appearances suggesting the occurrence of funsion of two or more peroxisomes

(Fig. 12 and 13). These characteristic patterns of the distribution and shape of peroxisomes were maintained constantly irrespective of the growth and density of the cells. The tumor cells formed by back-transplantation of MHC-10 cells showed morphological characteristics similar to the original tumor cells, including those of peroxisomes.

DISCUSSION

Since peroxisomes of hepatocytes are one of differentiated phenotypes of the cells, the organelles are considered to be altered according to the states of the cells related to growth, differentiation and cancerization (4, 7, 8, 18-21). Furthermore, it is suggested that differentiated function of the cells is suppressed when the cells are cultivated *in vitro*.

As to peroxisomes of hepatoma cells in culture, Watanabe and Essner reported that the organelles of Morris 5123 tc and Reuber H-35 hepatomas disappeared during cultivation (24, 25), and Karasaki *et al.* also reported that a tumorigenic cell line derived from a long-term culture of adult rat hepatocytes did not show peroxisomes *in vitro* (9). However, the present experiments showed clearly that peroxisomes of cultured Morris hepatoma 7316 A cells did not disappear throughout the considerably long periods of the culture. They were always detected by electron microscopic cytochemistry with 3, 3'-DAB reaction. Major differences between the observations by Watanabe and Essner, and Karasaki *et al.* and of the present experiments might lie in the methods of the culture, the source of tumors, and the methods for observation of peroxisomes. As to the first possibility, in the present experiments, the tumor cells were dispersed with collagenase and dispase II and then the cells were subcultured using dispase. In our previous study on glucose-6-phosphatase of the cultured hepatoma cells, the use of dispase II was suggested to be favorable for preservation of regulatory mechanisms of liver function particularly in a long-term culture (17). Furthermore, it is well known that a collagenase-perfusion method is suitable for the isolated hepatocytes to maintain the specific functions, even though within a relatively short period (11, 22, 23, 26). It is conceivably assumed that the cell-isolation and subculture methods used in the present experiments are suitable for maintaining peroxisomes in the cultured cells. As to the second possibility, Morris 5123 C tumor, the original tumor of 5123 tc, contains peroxisomes of electron microscopic features similar to the organelles of Morris 7316 A tumor. Thus, a contrast between the findings of Watanabe and Essner and of the present experiments might not be ascribed to a difference between the tumor lines. In the present experiments, ultrastructural features of peroxisomes were altered giving rise to an appearance of

microperoxisomes within relatively a short period of the cultivation. 3, 3′-DAB cytochemistry is considered to be necessary to identify microperoxisomes, thus, persistence of peroxisomes was recognized in the present experiments. However, after back-transplantation of the cultured tumor cells into rats, typical peroxisomes containing crystalloid nucleoids reappeared, suggesting that changing of peroxisomes into microperoxisomes is appropriately due to a phenotypic alteration caused by a culture condition. Alteration of the tumor cells as indicated by a numerical decrease in peroxisomes accompanying a decrease in catalase activity in early periods of the cultivation may be attributed to a state of the cells not adapted to the culture conditions.

The growth characteristics of MHC-10 cells are unique with respect to that they did not reach to confluence and occupied only about 70 to 80% of the available surface of the dishes. This growth pattern is similar to that of two clonal hepatoma cell strains established from Morris 7795 and 7800 hepatomas by Richardson *et al.* (15, 16). However, mechanisms involved in this phenomenon were not elucidated in the present experiments.

It is known that there are three types of concentric lamellar bodies which may occur in hepatocytes under a wide variety of conditions. These are the bodies consisting of membranes studded with ribosomes (fingerprints or ellipsoidal bodies), the bodies containing glycogen particles in cytoplasmic matrix between the layers of the membranes (glycogen bodies), and the bodies composed of sER (3, 12). Although few peroxisomes were found within such concentric whorls in Morris hepatomas (6) and hepatocytes of the hamster treated with phenobarbitone (4), there have been no reports that numerous peroxisomes were included in the bodies as those of MHC-10 cells. In MHC-10 cells, it was shown that there were two types of concentric lamellar bodies which contain many peroxisomes. These were the bodies consisting of sER and of rER. Hepatocyte peroxisomes shows frequently a close spatial relationship with the membranes of both sER and rER. This relationship was outstanding in the morris hepatomas (13) (Fig. 2 and 7). Accordingly, it may be conceived that peroxisomes are wrapped in concentric lamellar bodies when the ER arranged to form the bodies.

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REFERENCES

1. BEERS, R. F., Jr. and SIZER, I. W.: J. Biol. Chem. **195**, 133–140 (1952).
2. BRINKLEY, B. R., MURPHY, P. and RICHARDSON, L. C.: J. Cell Biol. **35**, 279–283 (1976).
3. GHADIALLY, F. N.: In Ultrastructural Pathologyof the Cell, pp. 266–271, Butterworths, England (1975).
4. GOTOH, M., FURUKAWA, K. and TSUKADA, H.: Tumor Res. **13**, 20–30 (1978).
5. HRUBAN, Z. and RECHCIGL, M., Jr.: Microbodies and Related Particles. Int. Rev. Cytol. **Suppl. 1** (1969).
6. HRUBAN, Z., MOCHIZUKI, Y., MORRIS, H. P. and SLESERS, A.: Lab. Invest. **26**, 86–99 (1972).
7. ITABASHI, M., MOCHIZUKI, Y. and TSUKADA, H.: GANN **66**, 463–472 (1975).
8. ITABASHI, M., MOCHIZUKI, Y. and TSUKADA, H.: Cancer Res. **37**, 1035–1043 (1977).
9. KARASAKI, S., SIMARD, A. and DE LAMIRANDE, G.: Europ. J. Cancer **12**, 527–534 (1976).
10. LOWRY, O. H., ROSENBROUGH, N. F., FARR, A. L. and RANDALL, R. J.: J. Biol. Chem. **193**, 256–275 (1951).
11. MICHALOPOULOS, G. and PITOT, H. C.: Exp. Cell Res. **94**, 70–78 (1975).
12. MIYAI, K.: In Toxic Injury of the Liver, edited by FARBER, E. and FISHER, M. M., pp. 80–83, Marcel Dekker, Inc., New York and Basal, (1979).
13. MOCHIZUKI, Y., HRUBAN, Z., MORRIS, H. P., SLESERS, A. and VIGIL, E. L.: Cancer Res. **31**, 763–773 (1971).
14. NOVIKOFF, A. B., NOVIKOFF, P. M., DAVIS, C. and QUINTANA, N.: J. Histochem. Cytochem. **20**, 1006–1023 (1972).
15. RICHARDSON, U. I., TASHJIAN, A. H., Jr. and LEVINE, L.: J. Cell Biol. **40**, 236–247 (1969).
16. RICHARDSON, U. I., SNODGRASS, P. J., NUZUM, C. T. and TASHJIAN, A. H., Jr.: J. Cell. Physiol. **83**, 141–150 (1974).
17. SAWADA, N., FURUKAWA, K., MOCHIZUKI, Y. GOTOH, M. and TSUKADA, H.: Tumor Res. **14**, 1–8 (1979).
18. TSUKADA, H., MOCHIZUKI, Y. and GOTOH, M.: J. Natl. Cancer Inst. **54**, 519–523 (1975).
19. TSUKADA, H., MOCHIZUKI, Y., ITABASHI, M., GOTOH, M. and MORRIS, H. P.: J. Natl. Cancer Inst. **55**, 153–158 (1975).
20. TSUKADA, H., MOCHIZUKI, Y. and GOTOH, M.: Adv. Exp. Med. Biol. **92**, 331–362 (1977).
21. TSUKADA, H., GOTOH, M., MOCHIZUKI, Y. and FURUKAWA, K.: Cancer Res. **39**, 1628–1634 (1979).
22. WALKER, P. R.: J. Cell. Physiol. **91**, 169–180 (1976).
23. WANSON, J.-C., DROCHMANS, P., MOSSELMANS, R. and RONVEAUX, M.-F.: J. Cell Biol. **74**, 858–877 (1977).

24. WATANABE, H.: Exp. Cell Res. **42**, 685–699 (1966).
25. WATANABE, H. and ESSNER, E.: Cancer Res. **29**, 631–644 (1969).
26. WILLIAMS, G. M.: Cancer Res. **37**, 1845–1851 (1977).

Figure legends

Fig. 2. Electron micrograph of Morris hepatoma 7316A *in vivo*. ×17,280.

Fig. 3. Electron micrograph of cultured Morris hepatoma 7316A cell at the 24th hour after inoculation. ×15,600.

Fig. 4. Cytochemistry of peroxisomes of cultured Morris hepatoma 7316A cell at the second day after inoculation. 3,3′-DAB reaction. ×15,600.

Fig. 5. Cytochemistry of peroxisomes of cultured Morris hepatoma 7316A cell at the 5th week after inoculation. 3,3-DAB reaction. ×15,600.

Fig. 6. Cytochemistry of peroxisomes of cultured Morris hepatoma 7316A cell at the 17th week after inoculation. 3,3′-DAB reaction. 15,600.

Fig. 7. Electron micrograph of the tumor which was formed in the femoral region of the male Buffalo rat by back-transplantation of cultured Morris hepatoma 7316A cells 7 weeks after inoculation. ×17,640.

Fig. 8. Appearance of MHC-10 cells established from cultured Morris hepatoma 7316A cells. This photograph show the cells 15 months (6 subcultures) after inoculation. Phase contrast microscopic photograph. ×223.

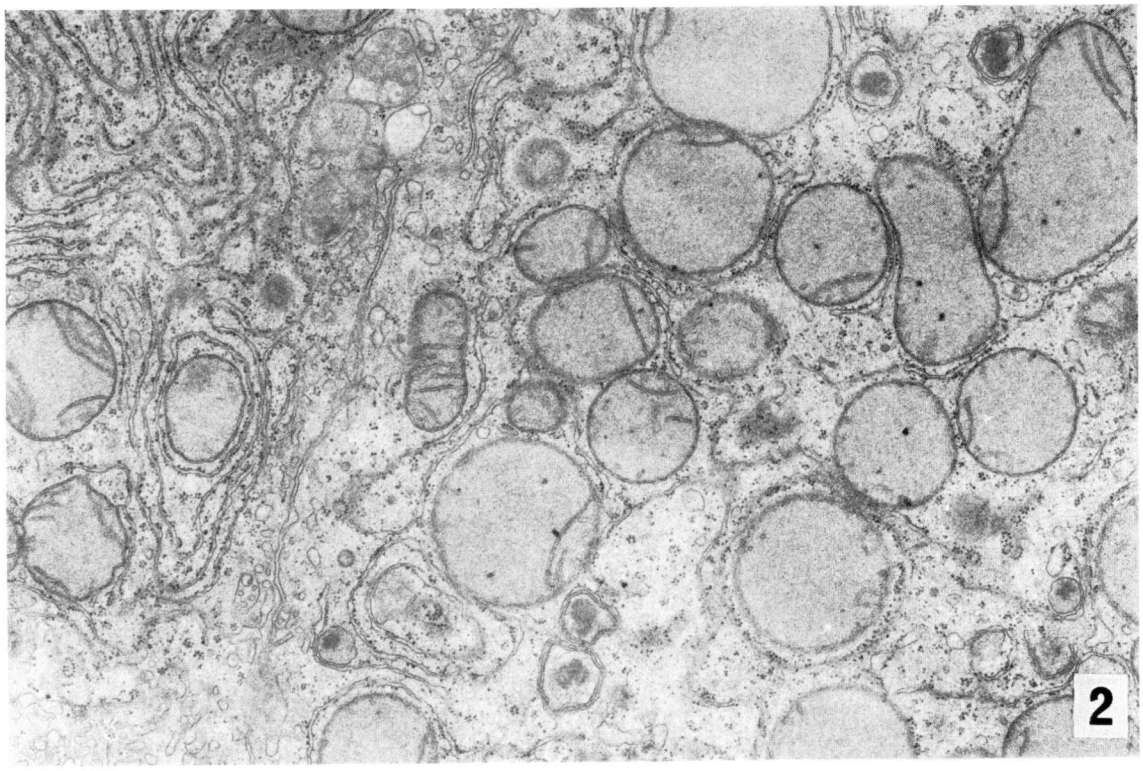
Fig. 9. Light microscopic cytochemistry of MHC-10 cells. Arrows indicate the "ghost" cells and/or the spaces which were made by dettachment of dead cells. 3,3′-DAB reaction and hematoxylin. ×128.

Fig. 10. Higher magnification photograph of light microscopic cytochemistry of MHC-10 cells. Arrows indicate large reaction-positive granules. Nucleoli are indicated by arrow heads. 3,3′-DAB reaction and hematoxylin. ×630.

Fig. 11. Cytochemistry of MHC-10 cells in which sER-concentric lamellar bodies were contained. In the core of, and/or in the surrounding part of the lamellar bodies, peroxisomes are detected. Electron micrograph. 3,3′-DAB reaction. ×9,400.

Fig. 12. The concentric lamellar body which consisted of rER and peroxisomes. Peroxisomes are also seen existing between the membranes. Electron microscopic cytochemistry of HMC-10 cell by 3,3′-DAB reaction. ×14,700.

Fig. 13. Peroxisomes localized in close contact with sER. Electron microscopic cytochemistry of MHC-10 cell by 3,3′-DAB reaction. ×10,800.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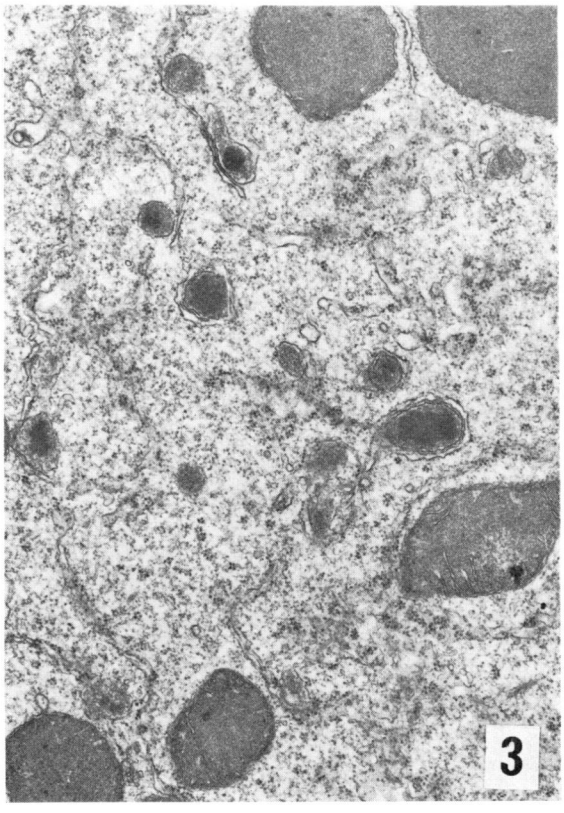
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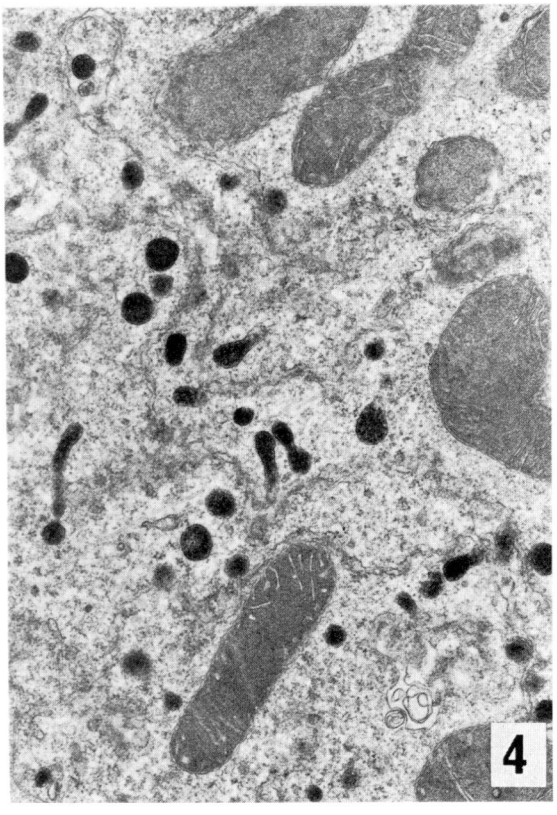
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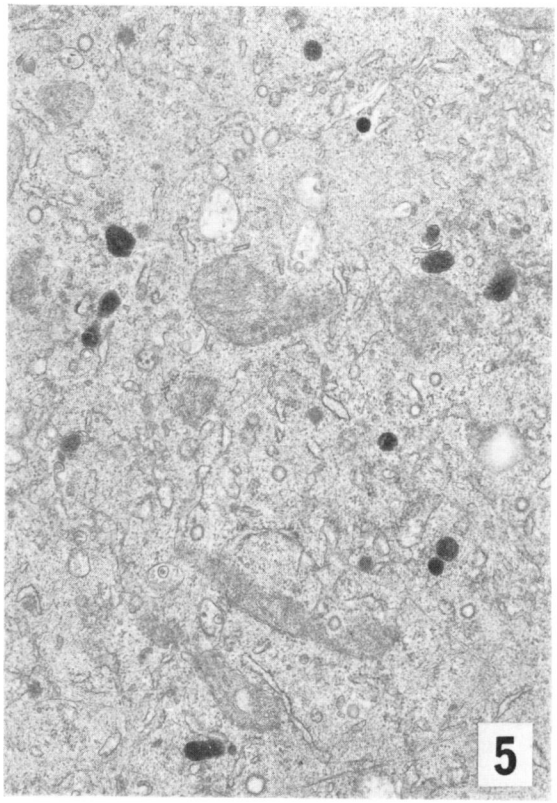

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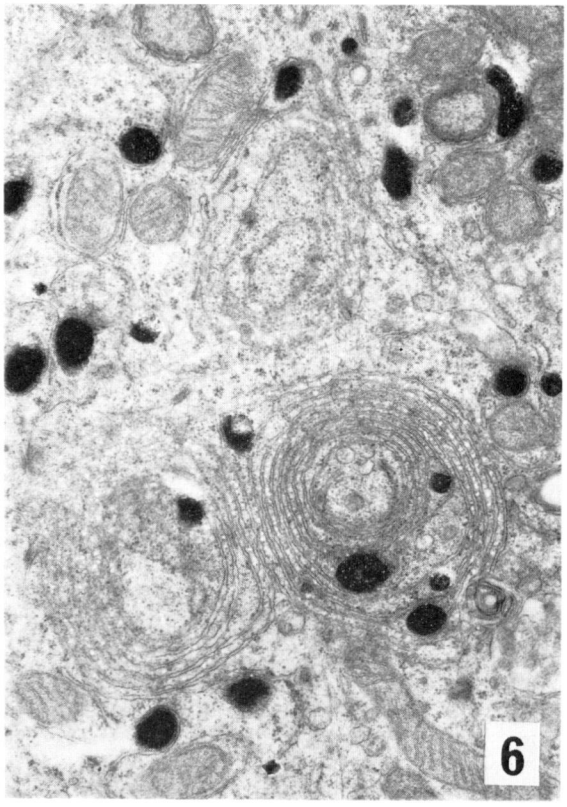

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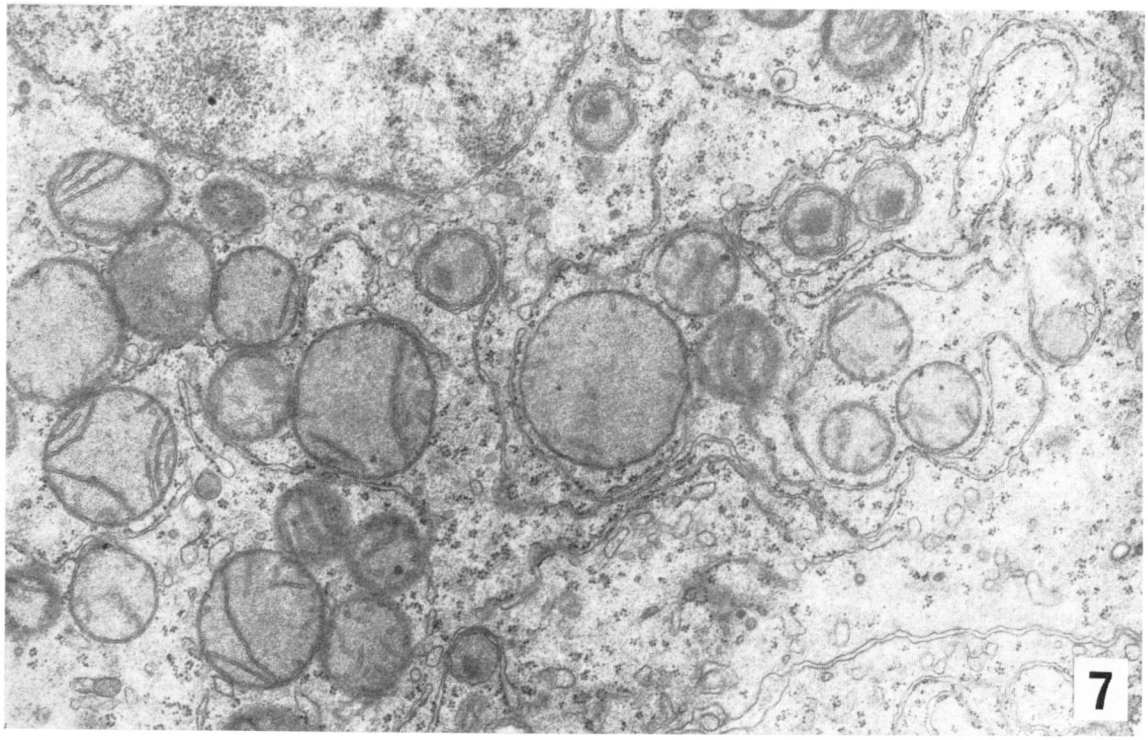

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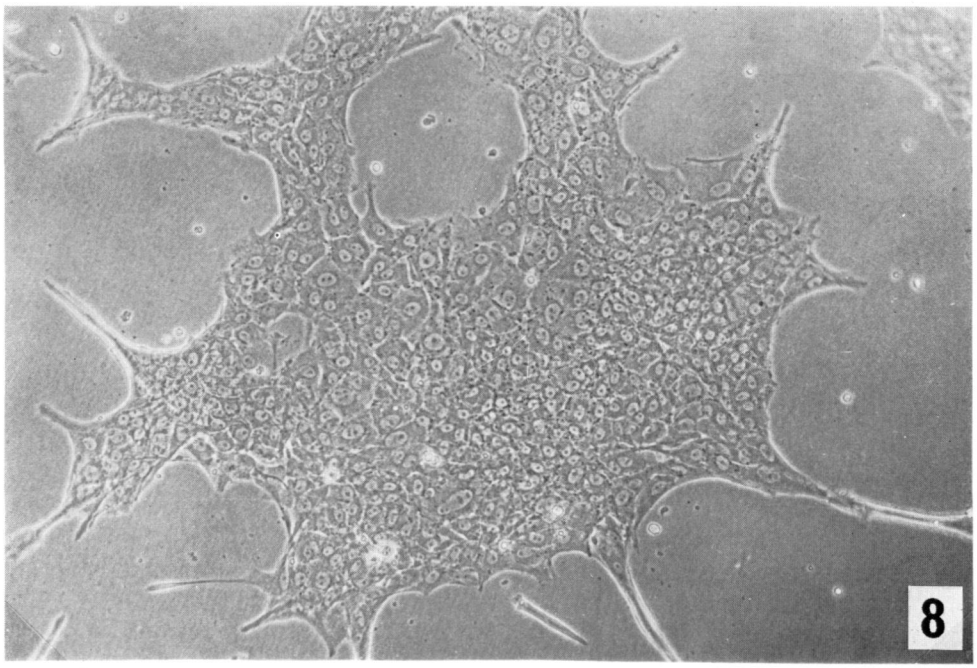
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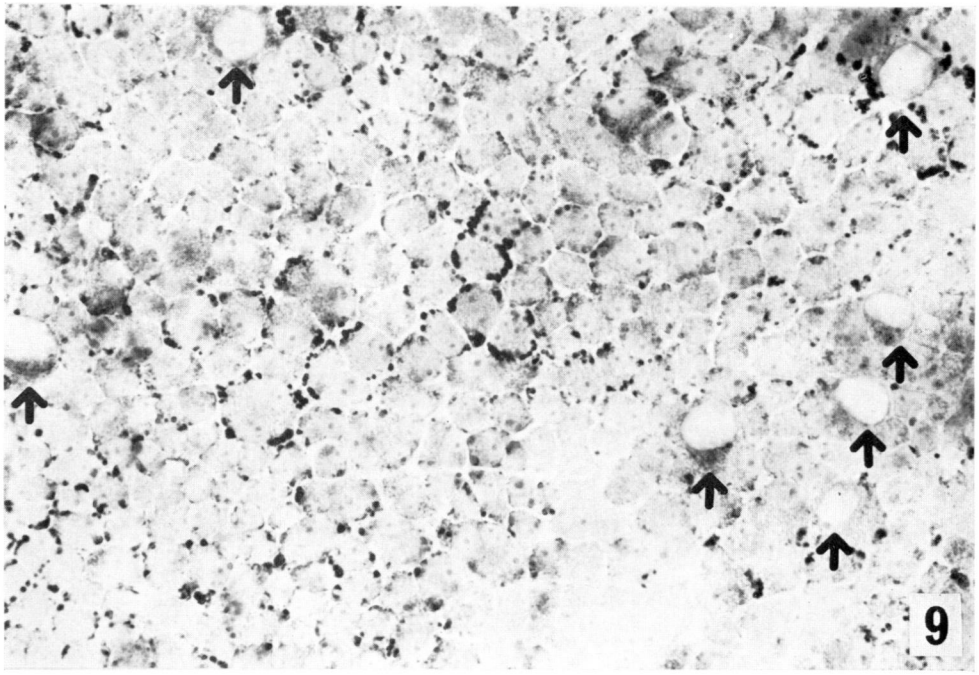
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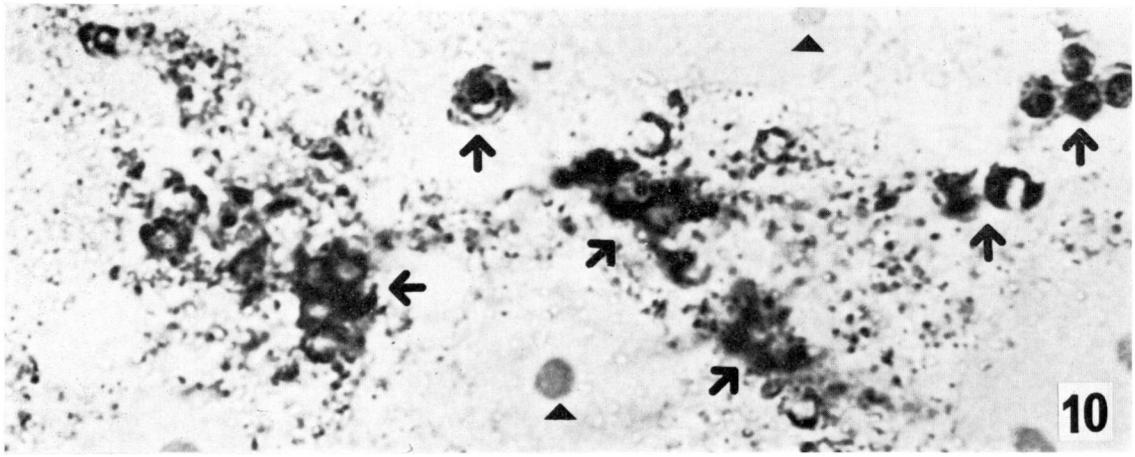
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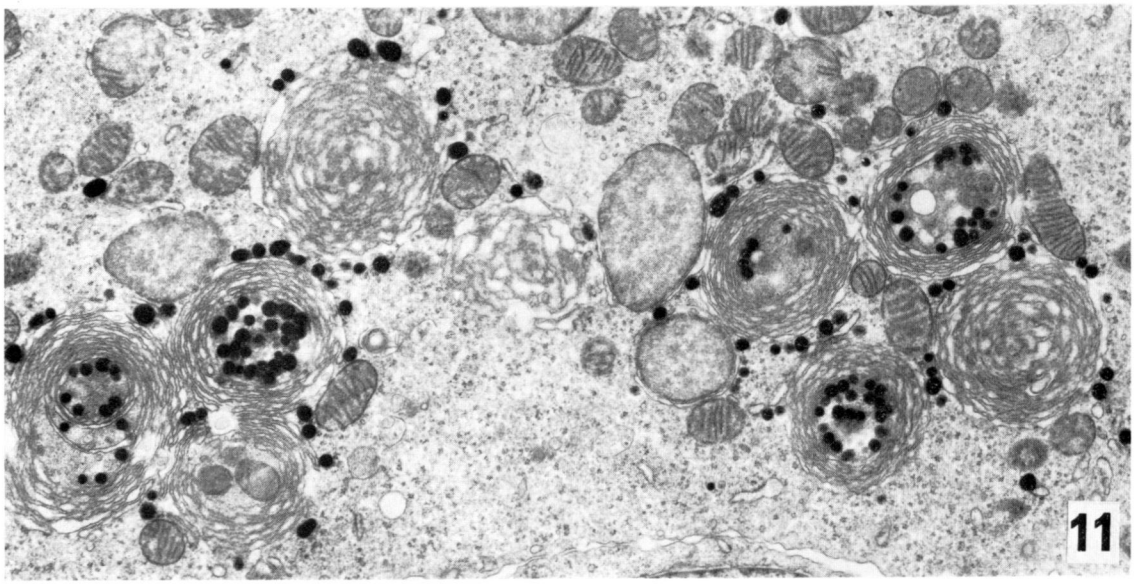
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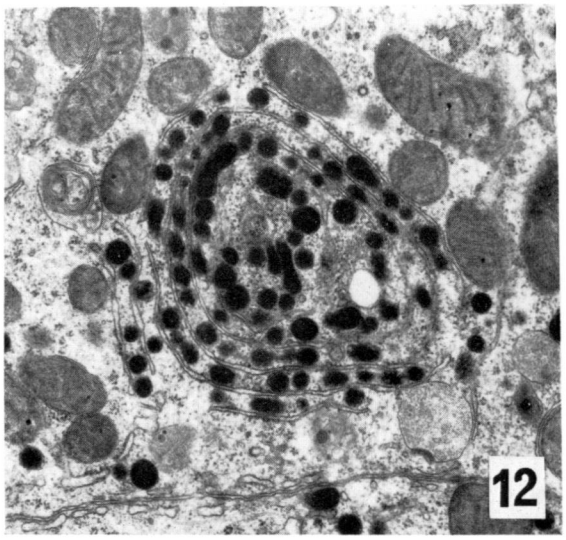
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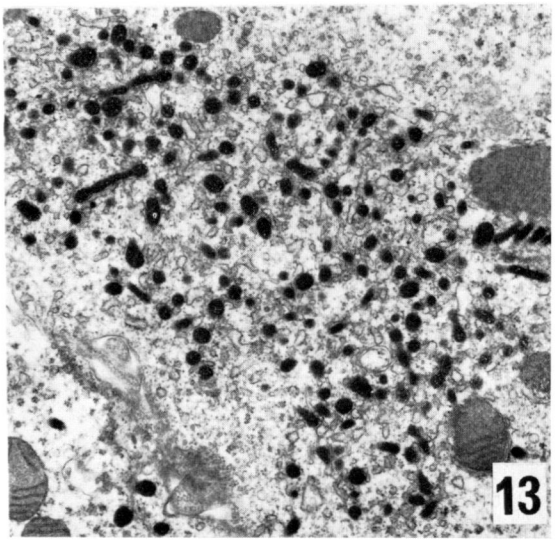
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