# STRUCTURAL CHANGES IN HELA CELLS CULTIVATED IN SERUM-FREE MEDIUM

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In general, it is still impossible, except in a few instances (7, 9), to cultivate animal and human cells successfully in serum-free medium. When cultivated under such conditions, the cells proliferate for only a limited period of time and then stop. It seems reasonable to assume that differences in the constituents of the medium which have such a marked influence on patterns of cell growth might be reflected in the fine structure of the cells.

The role of serum in cell growth is one of the most interesting problems in the field of tissue culture. The importance of serum protein has been clarified recently by the observations of many investigators (5, 8, 11, 12, 14). However, so far the role of substances of low molecular weight in serum has not been made clear. The present investigation was designed to ascertain some of the changes in the ultrastructure of HeLa cells grown in serum-free medium and to clarify the role of substances of low molecular weight in serum in the growth of cells.

## MATERIALS AND METHODS

In this experiment, HeLa cells were used which had been cultivated previously for 6 days in 5 ml of the *standard medium*: 80 per cent balanced solution (0.4 gm of lactoalbumin hydrolysate dissolved in 100 ml of Earle's solution) and 20 per cent bovine serum. These HeLa cells ($4 \times 10^4$), added to each flask, were cultivated for 24 hours in the following test media: (*a*) the balanced solution alone; (*b*) the balanced solution plus the dialysate of bovine serum (dialyzed through cellulose tubing (Visking Company, Chicago) at 37°C for 24 hours) in which protein was not present as determined by sulfosalicylic acid; and (*c*) the balanced solution (100 ml) in which was dissolved 0.5 gm of polyvinylpyrrolidone. The HeLa cells grew into sheets of cells on the bottom of the flasks.

In preparation for electron microscopy, the test medium was decanted from each flask and the cells were fixed by adding Palade's buffered osmium tetroxide plus 0.25 M sucrose to the flask. The specimens were embedded in methacrylate. Sections were cut with a JUM-5 ultramicrotome. Electron micrographs were taken with a JEM-5G electron microscope.

## RESULTS AND DISCUSSION

The cells grown in a serum-free medium, with or without polyvinylpyrrolidone, were different in appearance from the normal cells grown *in standard medium*, while the cells grown in the presence of serum-dialysate were normal in appearance.

Structures which shall be called lamellar bodies, with and without limiting membranes, were notably abundant in the cells grown in serum-free medium (Figs. 1 and 2) but were not observed in normal cells. Their diameter was of the order of 0.2 to 0.8 $\mu$. They were characterized by profiles which showed a peripheral dense layer, consisting of concentric lamellae, and a central vacuolated area. In many instances, the configuration of the dense layer was very similar to that seen in myelin of peripheral nerve (4) (Fig. 2). In favorable sections, the most dense material in the dense layer displayed a highly organized ultrastructure (Fig. 3). Lamellar spacing was of the order of 80 to 100 A: the periodic dense bands were 30 to 40 A wide and the intermediate light bands were 50 to 60 A wide.

It has been suggested by previous investigators (1, 2, 6) that mitochondria may undergo conversion into lipid droplets. Evidence obtained in the present study suggests that mitochondria may be transformed into the lamellar bodies described above. Both organelles are similar in size and distribution. A structure has been observed which has the characteristics of both a mitochondrion and a lamellar body—one portion of this structure

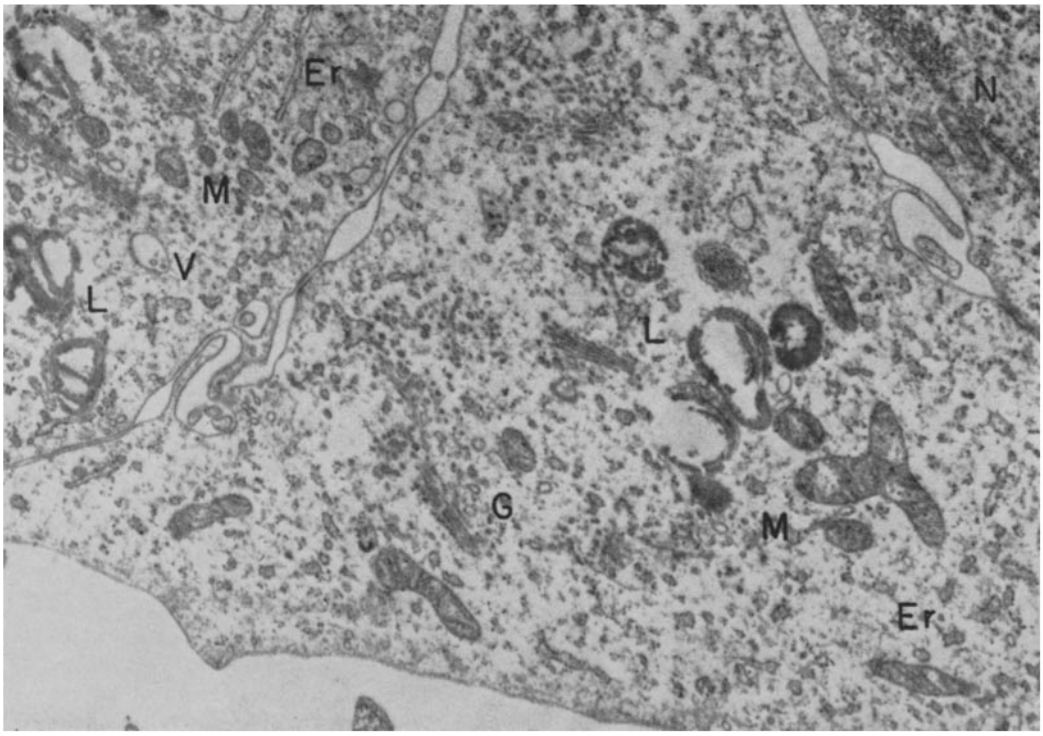


FIGURE 1

A micrograph showing cells grown in the balanced solution alone. Lamellar bodies (*L*) interpreted as being derived from mitochondria are seen in the cytoplasm. They are characterized by profiles which show a peripheral dense layer, consisting of concentric lamellae, and a central vacuolated area. The lamellae of the dense layer vary in width. Mitochondria (*M*) are seen which are round and swollen and contain irregular cristae. Endoplasmic reticulum (*Er*) is in the form of small vesicles. At the upper left, the endoplasmic reticulum retains its tubular pattern. A large vesicle (*V*) is seen within which are the remains of membranous cristae, showing its mitochondrial origin. *G*, Golgi complex; *N*, nucleus. × 20,000.

appears to be a lamellar body, the other a mitochondrion (Fig. 4). Furthermore, in some instances the lamellar bodies have retained their double limiting membranes and may represent early stages of the transformation (Fig. 2). Menefee's observation (10) that characteristic lamellar bodies were derived from mitochondria in cultured epithelial cells from human skin is in agreement with our findings. Since lamellar bodies are absent in normal HeLa cells, the proposed transformation of mitochondria into lamellar bodies can be considered to be a response to an unusual condition of the tissue culture.

The mitochondria of cells grown in serum-free medium appeared round and contained irregular cristae. In several places, large cytoplasmic vesicles were found in which membranous cristae could be seen (Figs. 1 and 2). Rough surfaced endoplasmic reticulum appeared in the form of small, irregularly shaped vesicles in most areas of the cytoplasm (Fig. 1). In normal HeLa cells the endoplasmic reticulum has been observed to be in the form of tubules or strings of small vesicles (3).

Several investigators (11, 12) have reported that serum protein protects cultured cells from the toxicity of salt solutions and that similar protection is afforded by the addition of other macromolecular substances, such as methylcellulose or polyvinylpyrrolidone, in place of protein. The importance of substances of low molecular weight present in serum has not been determined. As demonstrated in this paper, however, such sub-

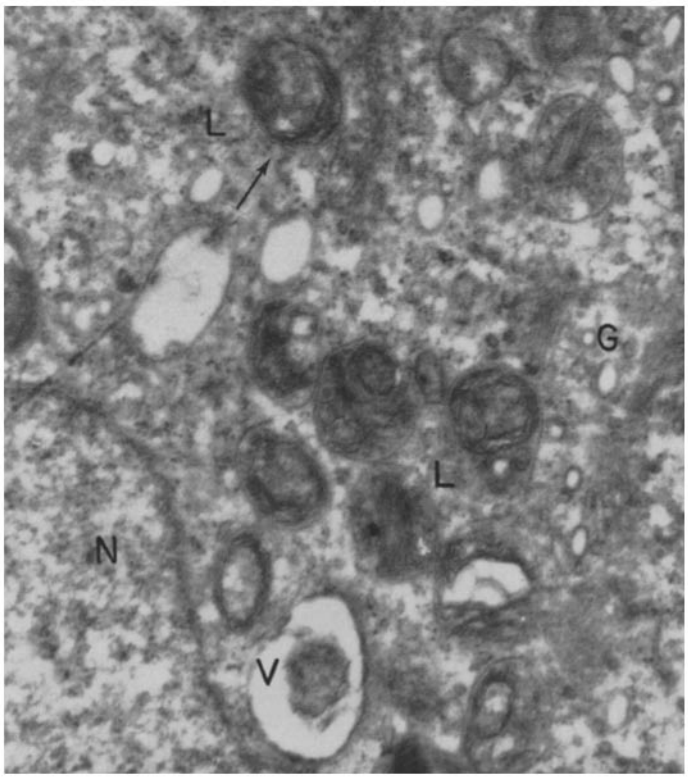


FIGURE 2

Portion of cytoplasm of a cell grown in the balanced solution alone. Lamellar bodies (*L*) resembling myelin are seen abundantly in the cytoplasm. One body (arrow) retains its double limiting membranes. *N*, nucleus; *G*, Golgi complex; *V*, vesicle. × 31,000.

stances in the serum can prevent, at least over 24 hours, the occurrence of structural changes which appear in cells grown in serum-free medium, whereas a macromolecular substance such as polyvinylpyrrolidone in place of serum protein can not. These findings indicate that substances of low molecular weight in serum may be important for normal growth of cultured cells, especially for their respiratory function, since mitochondria seem the most affected of all constituents of the cells. The chemical nature of these substances is unknown. Of interest in this connection is Sato's finding that serum protein on continued dialysis loses its ability to promote cell growth but recovers its competence when additional supplements of low molecular weight are added (13). The clarification of the mechanism of action of the dialysate of serum will represent a further important step in the improvement of the synthetic medium in which cells can grow more favorably.

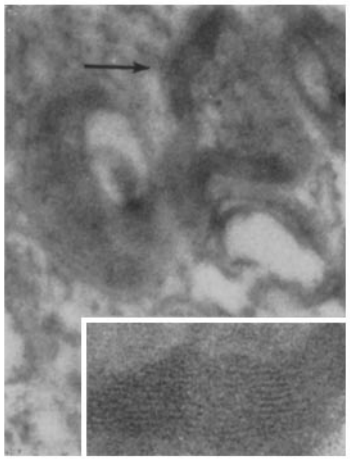

FIGURE 3

Arrangement of the dense material in the lamellar body. In the most dense portion (indicated by arrow), fine parallel lamellae are seen. × 33,000. Inset illustrates a high power magnification of the lamellae. × 105,000.

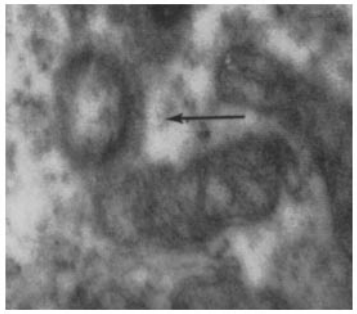

FIGURE 4

Structure showing characteristics of both a lamellar body and a mitochondrion—one portion shows a concentric arrangement of dense material and a central vacuole (arrow), and the other a normal-appearing mitochondrion. × 40,000.


## SUMMARY

A comparative study was made of the fine structure of HeLa cells cultivated for 24 hours in the following media: (*a*) balanced solution alone (0.4 per cent lactoalbumin hydrolysate in Earle's solution); (*b*) balanced solution plus dialysate of serum (dialyzed through cellulose tubing); and (*c*) balanced solution containing 0.5 per cent polyvinylpyrrolidone.

In the cells grown in balanced solution alone, characteristic lamellar bodies were observed which appeared to be derived from mitochondria. Vesiculation of rough surfaced endoplasmic reticulum was also noted. These changes in fine structure were prevented temporarily by the addition of serum-dialysate containing substances of low molecular weight, but not by the addition of polyvinylpyrrolidone.



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