Urological Research 3, 13-20 (1975)

Ultrastructural Characteristics of "Aging" of Human Prostate Cells in Monolayer Cell Culture*

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Received: November 1, 1974

Summary. Monolayer tissue culture cells from benign nodular hyperplasia of the prostate transferred at 1-2 weeks intervals were examined under the electron microscope after the 3rd, 9th, and 10th transfers. Changes seen after 9 to 10 transfers were interpreted as an "aging process" and consisted of the presence of lysosomes of various types and variations in the mitochondria profile. These changes were described in detail and illustrated and compared to the ultrastructural appearance of monolayer cell cultures in the early transfer stage.

Key words: Prostatic ultrastructure, monolayer culture, cell aging.

Some cells can be maintained in tissue culture for long periods of time (3, 6, 8). However, many primary cell strains isolated from chick or human tissues have a limited growth potential in culture (5). Limited time of cell survival and restricted numbers of potential doublings are indications of limited growth potential (10). Data from this laboratory (3, 4, 15) show that cell cultures from human prostatic tissue can be maintained for only a limited time period, usually not exceeding 3 to 4 months. Therefore aging changes should be expected in prostate cell cultures.

Ultrastructural changes due to aging of tissue culture cells have received little attention (5). Our ultrastructural study of prostatic cell aging after several transfers in tissue culture has therefore been undertaken in order to differentiate changes due to aging from changes possibly produced by various drugs being evaluated concerning their effect on prostatic tissue in monolayer cell culture.

Materials and Methods

Monolayer tissue cultures of human prostatic cells have been established and the employed methods reported previously (3, 4, 15). The culture medium used was basal medium Eagle (BME, Gibco). The culture medium overlying the primary cell culture was removed from the culture dish, leaving the cells attached to the bottom of the dish.

The cultures were then washed with a calcium and magnesium free phosphate buffered saline (PBS, Gibco). The cells were then treated with a 0.1% trypsin solution (diluted from a 0.25% solution (Gibco) with PBS) at 37 °C for about 2-3 min and then scraped off the bottom with a "rubber policeman".

An equal volume of culture medium supplemented with 10% fetal calf serum was then added, and the cell suspension was centrifuged at 1000 rpm for 4 min. The supernatant was discarded and the pellet of cells resuspended in culture medium. The cells from one dish covered by a confluent monolayer of cells for 1 to 2 weeks were transferred to two or three 65 mm Falco plastic dishes.

* This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft and Veterans Administration Research Funds.

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Cultured cells for examination by electron microscopy were prepared in two different ways: 1) The cells were fixed in the culture dish, still adherent to the bottom, according to a method described by Ross (16). 2) The cells were scraped from the bottom of the culture dish with a "rubber policemen". Both cell preparations were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4, dehydrated in ethanol and embedded in Epon 812. The embedded cells were sectioned on a Porter-Blum ultramicrotome MT2, stained with uranyl acetate and examined in a RCA-EMU-3-G electron microscope.

Cells after the 9th and 10th transfers were compared to cultures and cells after the 3rd transfer. All patients had benign nodular hyperplasia of the prostate.

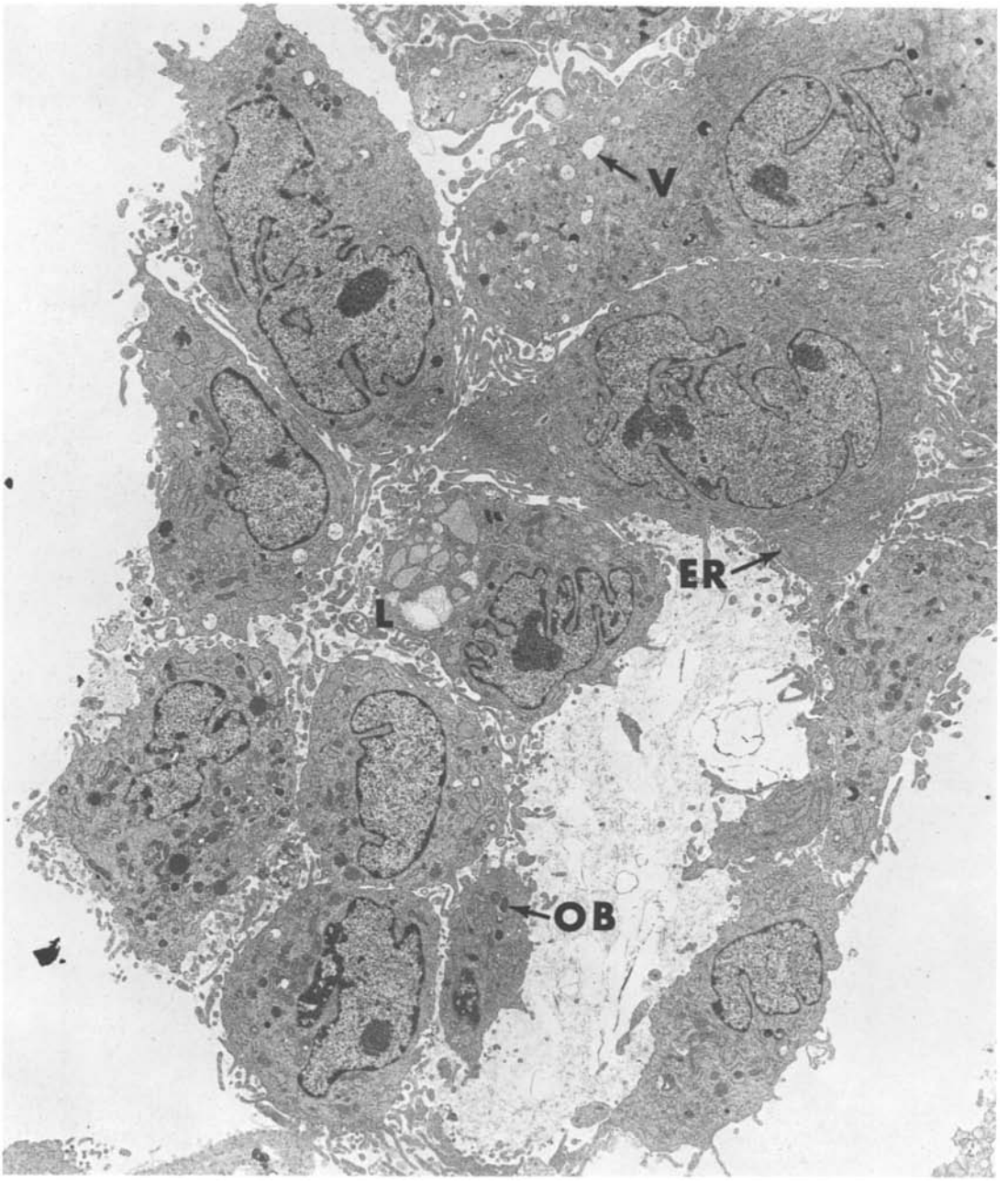

Fig. 1. Electron micrograph of cells from benign nodular hyperplasia of prostate in tissue culture after three transfers. The cytoplasm of the cells contains a moderately, sometimes tubular rough surfaced endoplasmic reticulum (ER), a small number of vacuoles (V) and osmiophilic dense bodies (OB). Lysosomes (L) are rare.
Magnification: x 3.750

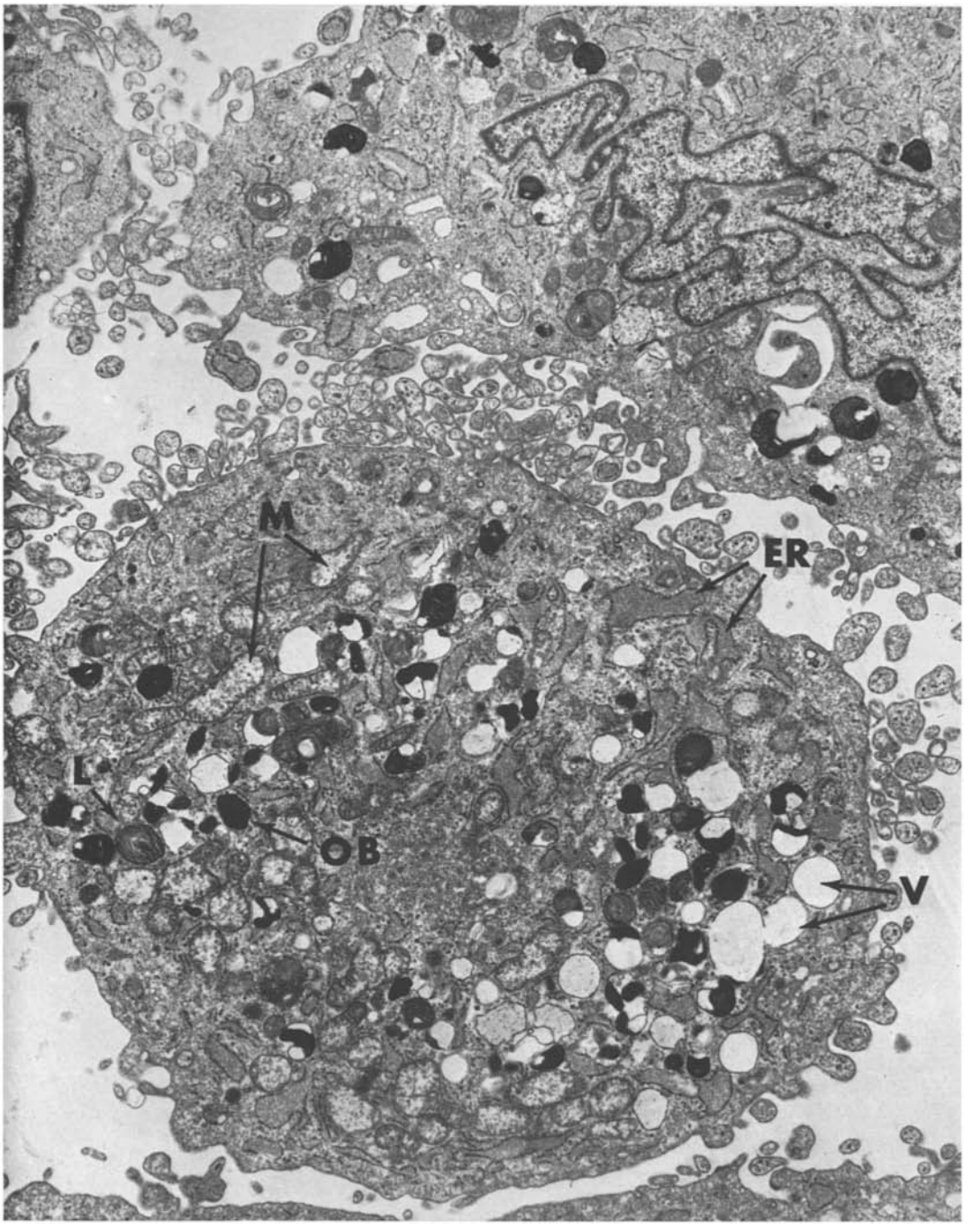

Fig. 2. Electron micrograph of a human prostate cell in tissue culture after 9 transfers. The cytoplasm contains numerous profiles of dilated rough surfaced endoplasmic reticulum (ER) containing grey, granular material; numerous vacuoles (V) and osmiophilic bodies (OB), some of which are lysosomes (L). The mitochondria (M) appear to have lost part of their internal structure and some areas appear as a granular mass surrounded by the double mitochondrial membrane.
Magnification: x 8.775

Results

Tissue culture cells originating from patients with benign nodular hyperplasia of the prostate were examined after the 3rd, 9th and 10th transfers under the electron microscope. There were marked differences between cells from early transfers and the degenerating cells found in the 9th and 10th transfers. In the early transfer (Fig. 1) the cytoplasm of the cells contains moderately dilated endoplasmic reticulum, only a few osmiophilic bodies, thought to be lipid droplets, and a small number of vacuoles and lysosomes. The mitochondria are of normal structure (15).

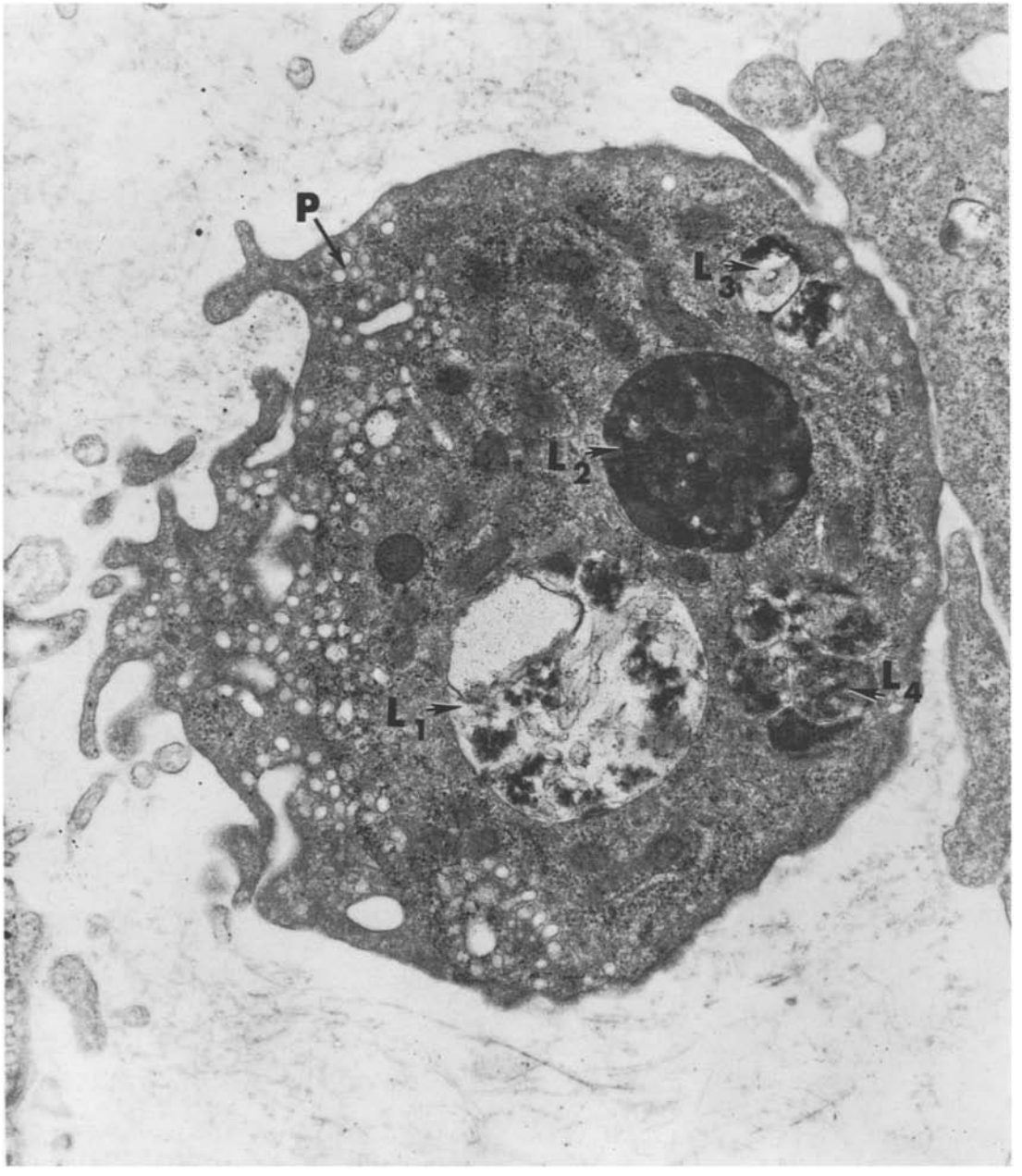

Fig. 3. Electron micrograph of a human prostate cell in tissue culture after 10 transfers. The cytoplasm contains four lysosomes (L). $L_{1,3,4}$ contain cellular components, resembling membranes, and ground substance; L_2 contains a homogenous black material. The higher number of pinocytic vesicles (P) suggest that this is a fibroblast.
Magnification: x 22.837

Many cells from the 9th and 10th transfer, thought to be epithelial cells, reveal an irregular rounded shape. Their cytoplasm contain varying numbers of vacuoles and numerous lysosomes (Fig. 2). The single cell shows a generally dilated endoplasmic reticulum with a fine granular electron dense material. Mitochondria which are present throughout the cell reveal striking differences. Most of them have lost many of their plate-like cristae and are filled with a sometimes coarsely granular, sometimes amorphous material (Fig. 3). Occasionally the mitochondrial membrane is interrupted.

Another distinct feature of cells after multiple transfers is the high number of lysosomes. They are predominantly located in the periphery of the cytoplasm. As part of many lysosomes it is possible to recognize structures resembling mitochondria or fragments of these (Fig. 4). Other material in-

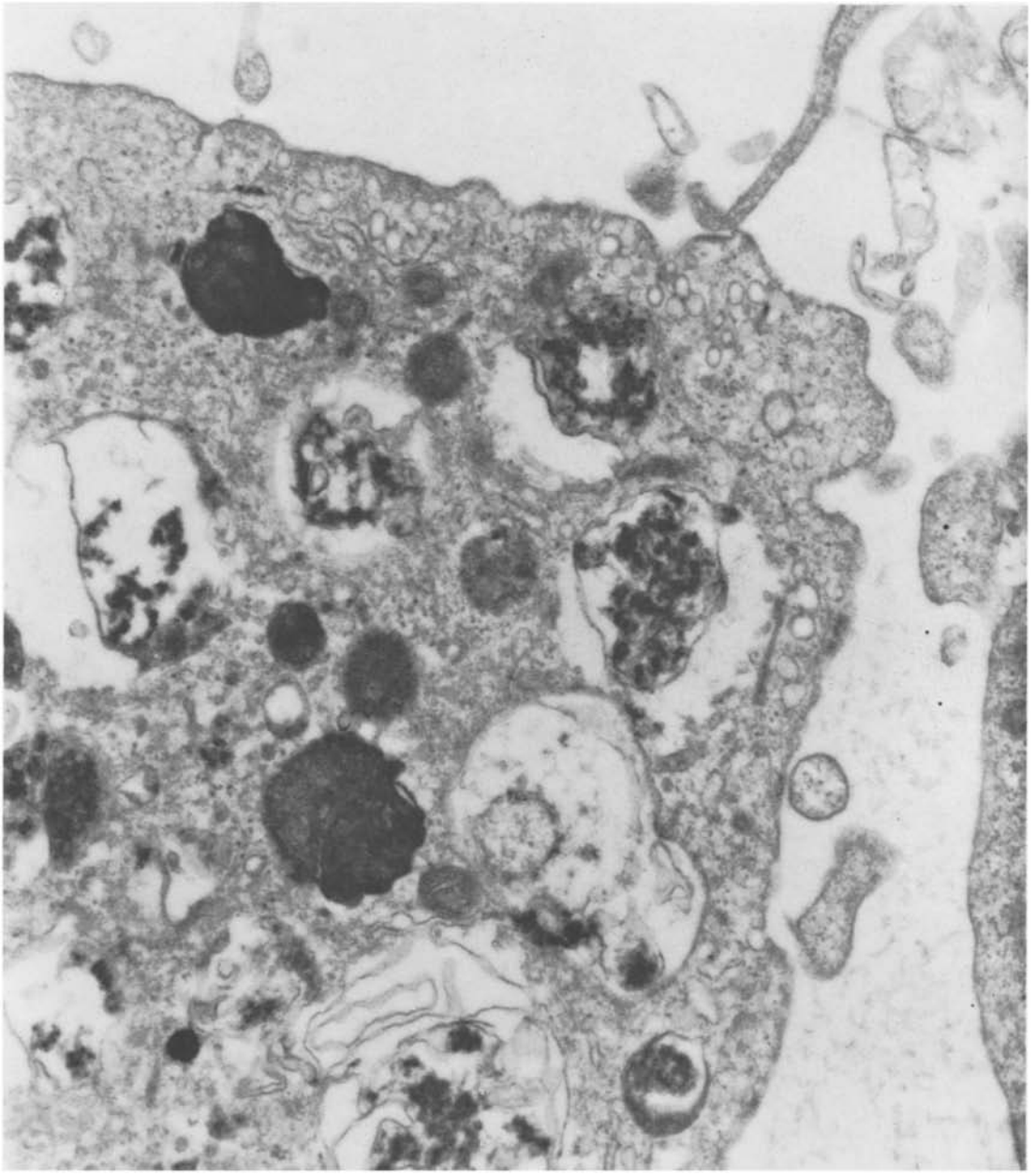

Fig. 4. Higher magnification of a portion of a human prostate cell and tissue culture after 10 transfers. The cytoplasm contains many lysosomes.
Magnification: x 32.400

cluded within the lysosomes can be identified as representing other cytoplasmic components, small vesicles, ribosomes and even cisternae of the endoplasmic reticulum. The exact identification of the contents however, is impossible in many cases. Most of the lysosomes appear to be surrounded by a membrane (Fig. 5). Numerous small vesicles apparently involved in a rapid process of pinocytosis are seen near the cell membranes of cells probably representing fibroblasts. A minority of cells has

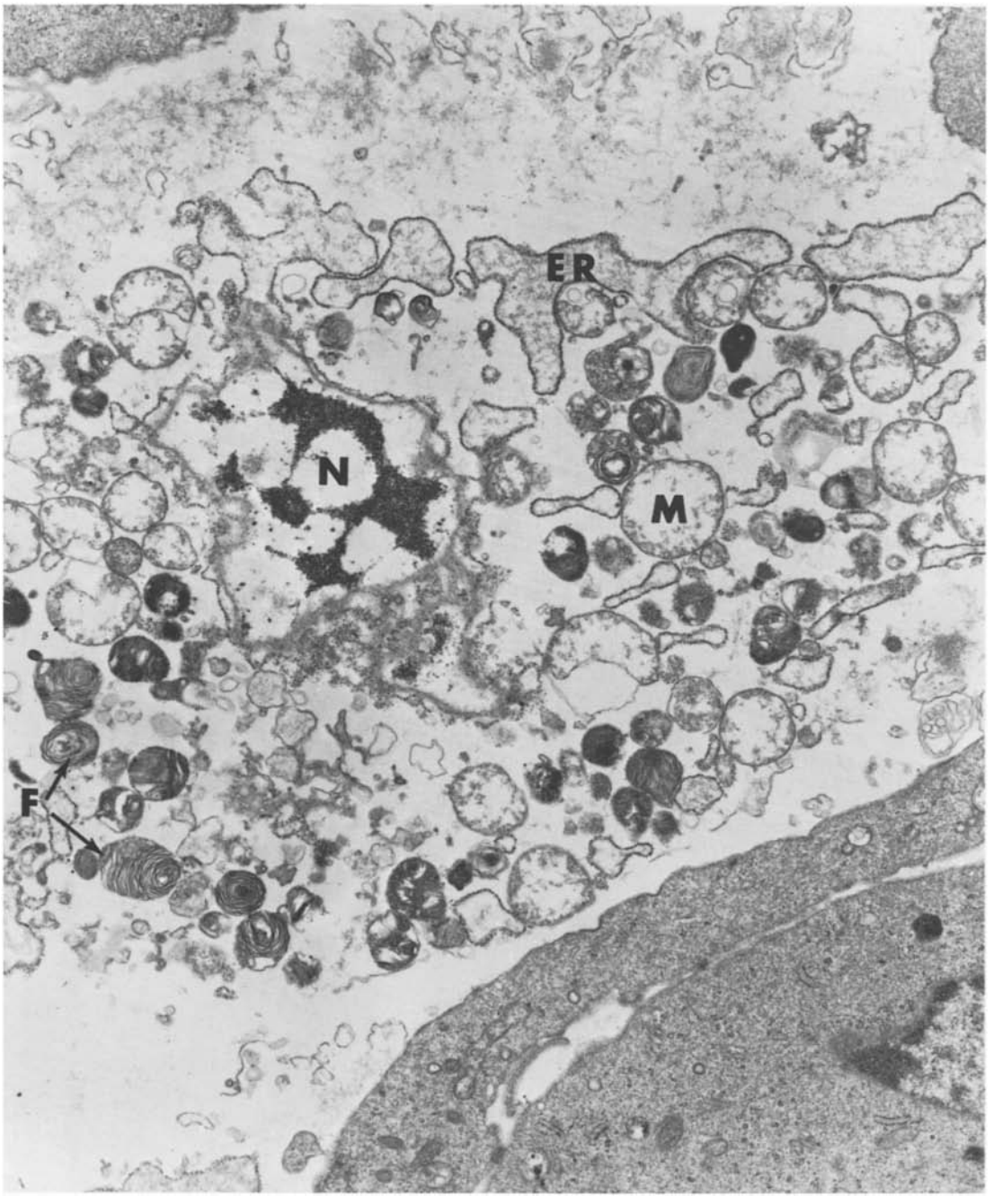

Fig. 5. Electron micrograph of the final stage of aging of a human prostate cell in tissue culture after 10 transfers. The plasma membrane of the cell and the cytoplasmic ground substance have disappeared. Dilated endoplasmic reticulum (ER) with fibrillar material is mainly located peripherally. Numerous degenerating mitochondria (M) and myelin figures (F) are present. The nucleus (N) demonstrates karyorrhexis.
Magnification: x 11,962

lost its vital function demonstrated by the lack of cell and nucleus membrane, the absence of cytoplasmic ground substance, multiple autolysosomes (myelin figures) occasionally disrupted endoplasmic reticulum filled with fibrillar material and almost completely destroyed mitochondria

Discussion

Little ultrastructural information is available concerning the aging process of cells in tissue culture. Several investigators have reported that the ultrastructural characteristics of cells do not show aging changes during the early transfers in tissue culture (14, 16). Chromosomal abnormalities and morphological changes, however, could be detected by light microscopy in cultured cells, grown under unstable environmental conditions (13). In a recent paper cultured chick fibroblasts were compared at early and late stages during their growth span and found to be significantly different in cell morphology (5). Examination of mammalian tissue culture cells by electron microscopy in a stationary phase ("aging phase") after multiple transfers has been reported to show a marked increase in vacuoles, multivesicular bodies and dense bodies (9). Cells presented a complex appearance and contained myelin figures and other membranous or tubular compounds.

In this laboratory the ultrastructural appearance of prostatic tissue cells in culture from patients with benign nodular hyperplasia of the prostate examined after the 9th and 10th passages revealed marked alterations in the structure of the cell organelles when compared to cells from early culture transfers. As indicated by the almost complete loss of functioning mitochondria and the presence of numerous lysosomes filled mainly with cellular components, the majority of the cells appear to be markedly reduced in vital functions.

Lysosomes, membrane bound structures and rich in acid hydrolases, were first described by de Duve (7). Combined cytochemical and electron microscopic studies have shown that different morphological entities contain the acid hydrolase activity such as digestive vacuoles, multivesicular bodies, dense bodies and complex structures containing fragments of cell organelles (2, 12). The last mentioned structures have been termed autophagic vacuoles or phagolysosomes (11). They are presumed to occur as a result of autodigestion either during degenerative stages or in tissues undergoing physiological lysis (1, 2).

In our observations the aging process of cells in tissue culture was correlated with an increase in the number of lysosomes of various types involved in the process of intracellular digestion. The progressive morphological alterations and the appearance of numerous dead cells suggest that prostatic tissue culture cells are in a stage of gradual degeneration after the 9th and 10th transfers.

The changes may be a consequence of the length of cellular life in the culture as well as compensatory changes necessary to maintain cellular homeostasis in an artificial medium (5).

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