LONG-TERM CULTURE OF THE AORTIC MEDIA OF RATS WITH PRESERVATION OF CHARACTERISTIC PROPERTIES OF DIFFERENTIATED SMOOTH-MUSCLE VASCULAR CELLS

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Smooth-muscle cells are known to take part in various pathological processes affecting the vascular system; they proliferate and dedifferentiate, to form either fibroblast-like or macrophage-like cells [2-4, 9].

Tissue culture, in which cells undergo analogous changes, is a convenient model with which to study these phenomena and the factors influencing them. However, it has been shown that the dynamics of these changes depends on the conditions and times of culture [9]. Accordingly it is important to obtain long-term cultures of smooth-muscle cells with definite growth characteristics, so that the character of dedifferentiation and proliferation can be studied under strictly controlled conditions.

This paper, which describes an investigation lasting 2 years, gives the results of long-term culture of smooth-muscle cells from the media of the rat aorta, during which the cells preserved their characteristic features of differentiation.

EXPERIMENTAL METHOD

Cells were isolated from the thoracic aorta of Wistar rats weighing 200 g. The middle part of the media was subjected to enzymic digestion by the method in [3], using a mixture of solutions of collagenase (1 mg/ml, from Worthington, USA) and elastase (0.5 mg/ml, from Serva, West Germany) in medium 199 (from Gibco, USA) containing 10% embryonic calf serum (from Gibco). Isolated cells thus obtained were transplanted into plastic flasks (from Falcon, USA) with a density of $1.5 \cdot 10^4$ to $3.5 \cdot 10^4$ cells/cm^2. The cells were cultured in an atmosphere of 95% air and 5% CO_2 at 37°C. On reaching the confluent state the cells were removed with 0.25% trypsin solution and transplanted in the ratios of 1:2 and 1:6.

The cultures were investigated by time-lapse photography, using a microscope with thermostatically controlled chamber (from Olympus, Japan). Cells for electron-microscopic analysis were grown on coverslips, fixed in 2.5% glutaraldehyde solution, and embedded in Epon. Ultrathin sections were studied in the IEM 100C electron microscope. The cytoskeletal proteins, isolated by the method in [5] during early (8th) and later (36th-44th) passages, were studied by SDS-electrophoresis. Cytoskeletal proteins from rat aorta were used for comparison [10]. Actin was isolated by ultracentrifugation from the cultured smooth-muscle cells and purified by affinity chromatography [7]. Multiple forms of actin were analyzed by isoelectric focusing in 9 M urea [8].

EXPERIMENTAL RESULTS

Smooth-muscle cells of the media of the rat aorta were studied in primary culture and during long-term culture (106 passages). The freshly isolated cells are round or slightly elongated in shape. Adhesion to the substrate takes place during the first 12 h of culture, and complete monolayer formation occurs by the 4th day of culture. Meanwhile the cells begin to divide and the peak of mitosis falls on the 8th-12th day. During long-term culture,

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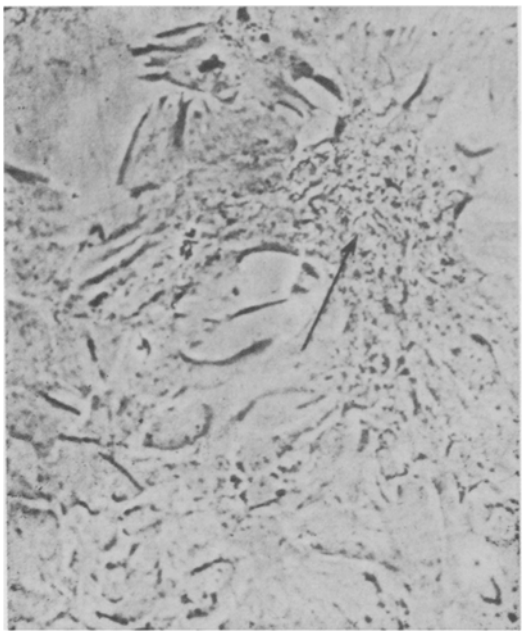

Fig. 1. Confluent culture of smooth-muscle cells of rat aortic media. Formation of characteristic structures of "hills" and "dales" type. Phase contrast. 215 ×.

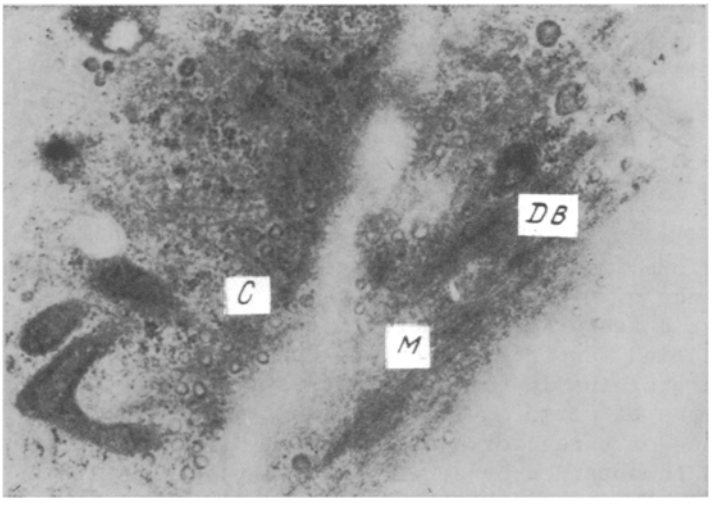

Fig. 2. Area of cytoplasm of smooth-muscle cell of rate aortic media (21st passage). C) Caveolae, M) microfilaments, DB) dense bodies. 30,000 ×.

at both early and later passages, structures of "hill" and "dale" type are formed, which is characteristic of smooth-muscle cells *in vitro*, by contrast with fibroblasts [9].

The "hills" are areas consisting of five to ten layers of cells, the "dales" areas consisting of one to three layers (Fig. 1). Both primary and transplanted cultures consist of cells of different shapes: rectangular and polygonal, small and large. Investigation of the cultures by time-lapse photography reveals transitions between the forms described above. This morphologic plasticity is evidently connected with the behavior of the cells *in vitro* and does not reflect their functional heterogeneity. This is confirmed by the results of electron-microscopic analysis. A study of the cells at the 21st and 64th passages showed that cells of different shape have a similar ultrastructure. In the cytoplasm of the cells in culture structures characteristic of differentiated smooth-muscle cells are preserved: invaginations of the plasmalemma (caveolae), often arranged in long rows, microfilaments (4-6 nm), intermediate filaments (10 nm) forming bundles oriented along the long axis of the cells at the periphery, and finally, dense bodies (Fig. 2).

Cells in the cultural layer are joined together by junctions with gaps among them (gap junctions). The results of electron-microscopic investigation agree with data obtained by workers [6] who studied smooth-muscle venous cells of rats during long-term culture, and they indicate that the principal morphological properties of the differentiated smooth-muscle cells are preserved in culture. Preservation of specific properties for smooth-muscle cells during long-term culture also is supported by the results obtained by time-lapse photography, by

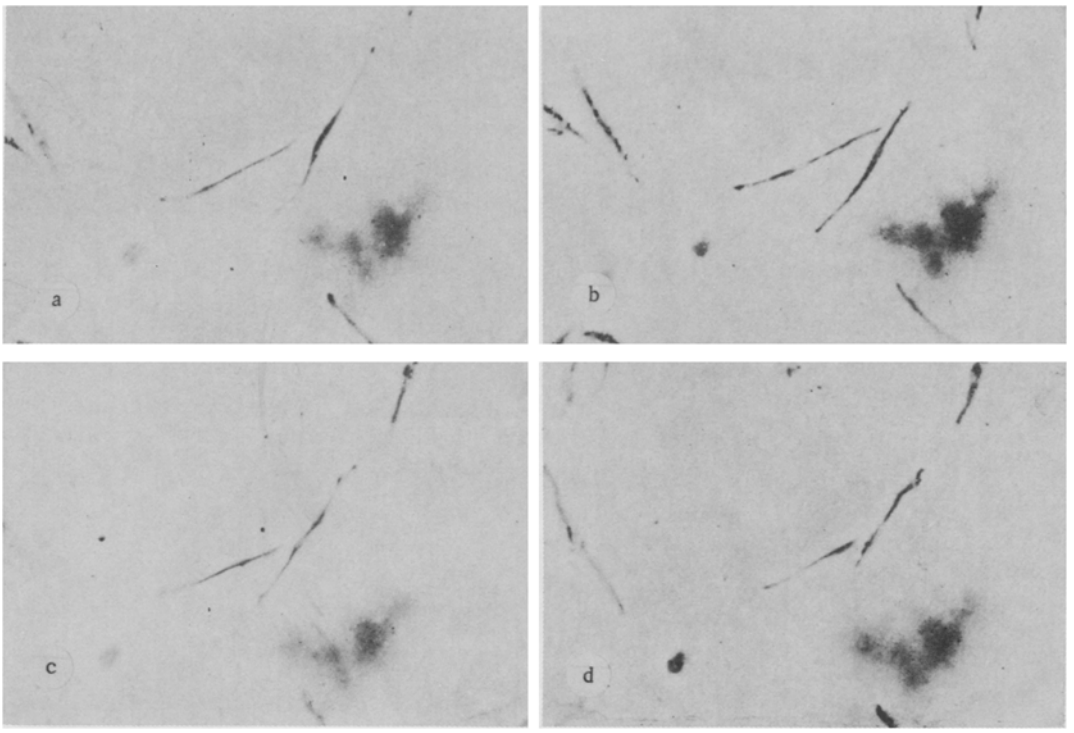

Fig. 3. Contraction of smooth-muscle cell of rat aortic media (21st passage). a, b, c, d) Interval between frames 1 h. Time-lapse photography. 180 ×.

means of which spontaneous contractions of smooth-muscle cells were recorded not only in primary culture, but also in certain cases at late (21st) passages (Fig. 3).

Preservation of elements of the contractile apparatus in smooth-muscle cells in culture also is demonstrated by the results of a previous immunomorphologic study [1]. Using antiserum against smooth-muscle myosin, the presence of myosin was demonstated in cells at the 6th and later passages. The most characteristic feature of differentiated smooth-muscle cells is that they contain the specific α-form of actin. Experiments by the SDS electrophoresis method showed that the principal cytoskeletal proteins in culture, just as in rat aorta, are actin and vimentin. The study of multiple forms of actin in transplantable smooth-muscle cells (79th-83rd passages) reveals the presence of three forms of actin (α, β, γ) in the approximate ratio of 3:4:1, in agreement with data obtained by other workers [6]; it indicates that smooth-muscle cells preserve the basic smooth-muscle α-form of actin during long-term culture.

Long-term cultures thus preserve the basic properties characteristic of differentiated vascular smooth-muscle cells and the method can be used as a model with which to study factors influencing proliferation, differentiation, and dedifferentiation of smooth-muscle cells, which is unquestionably important as a means of understanding the pathological processes which these phenomena accompany.

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