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# AUTORADIOGRAPHIC STUDY OF HUMAN EPIDERMOCYTES CULTURED IN A LOW CALCIUM MEDIUM

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KEY WORDS: epidermocytes; calcium; multiplication; autoradiographic investigation.

Since the 1950s biologists and clinicians have displayed increased interest in the development of transplantation of tissues, especially the skin. It was Medawar who first showed that the epithelial cells of human skin can be cultured. However, growth of a large mass of epithelial cells, which would be necessary, for example, to cover burned areas, still presents great difficulties. These difficulties can be explained by the fact that, despite the evident therapeutic importance of transplantations of cultured epidermis, world wide there are only a few clinics in which this method of treatment of burns is used [3, 9]. The basic difficulty is that 4-5 days after seeding, epidermocytes grow more slowly and undergo differentiation. To intensify multiplication of epidermocytes, several stimulators and methods of culture have been suggested [4, 5, 7, 8], but none of them has solved the problem to a significant degree. The search for stimulators of cell division and inhibitors of differentiation therefore continues. Research by Hennings and co-workers [6] has shown that reducing the calcium ion concentration in the nutrient medium to 0.04-0.05 mM stimulates division of epidermocytes obtained from the skin of newborn mice. In the present experiments with epidermocytes isolated from adult human skin multiplication of the cells in medium with a higher calcium concentration was stimulated. The results of these experiments are described below.

## EXPERIMENTAL METHOD

Small pieces of skin pinch grafts obtained during autologous skin grafting operations on burned patients were used as material. The pieces were treated with 0.25% trypsin solu-

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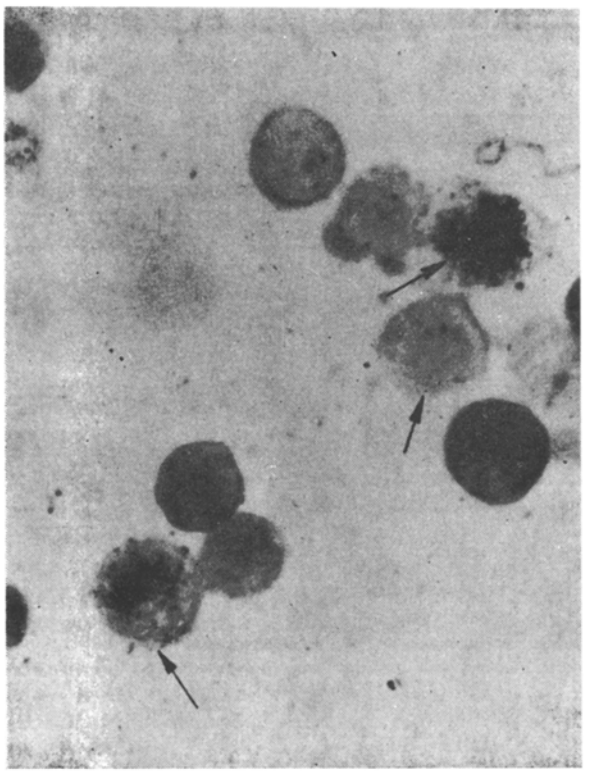

Fig. 1

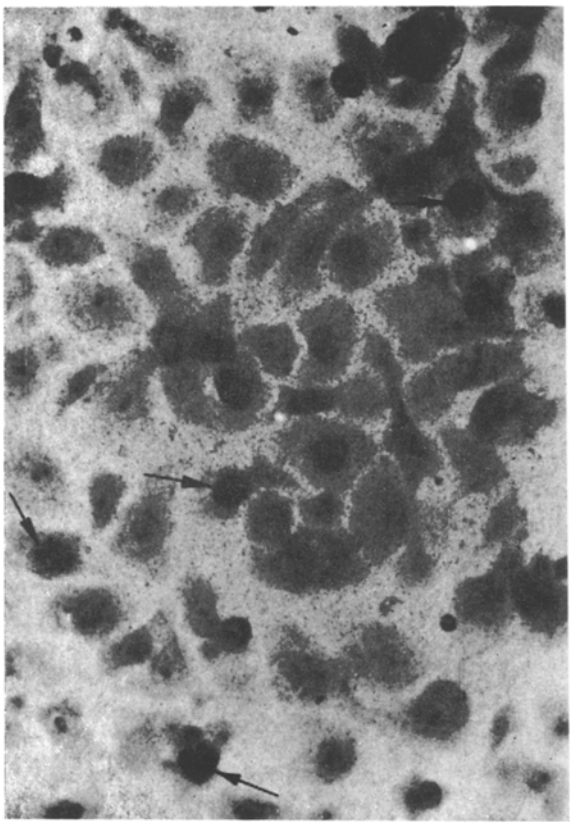

Fig. 2

Fig. 1. Suspension of epidermocytes isolated from skin. Basal cells are round (short arrow), and two of them are labeled (long arrows). 2000×.

Fig. 2. Culture of epidermocytes in calcium-free medium. Large spaces can be seen between the cells composing the monolayer. Many labeled cells composing the monolayer. Many labeled cells (arrows). 1000×.

tion for 18 h at 2-4°C. The stratum corneum was separated from the dermis with forceps and both layers were repeatedly washed with medium. A concentrated suspension of basal and other differentiated skin cells was obtained. After the cells had been counted in a Goryaev counting chamber the suspension was seeded in plastic Petri dishes in a concentration adjusted to $10^4$ cells/cm$^2$. The cells were cultured in an incubator with absolute humidity and with a $CO_2$ concentration of 5%. The following nutrient media were used: in the experiments of series I – Eagle's minimal medium (EMM) with the addition of embryonic calf serum (10% of the volume of the medium) and 3% glutamine solution (2% of the volume of the medium). The calcium concentration in this combination was 1.5 mM. In series II, EMM with Ultroser (LKB, Sweden; 2% of the volume of the medium), and glutamine was used. The calcium concentration in the medium was 1.46 mM. In series III, low-calcium EMM with the addition of 10% embryonic calf serum and glutamine was used. The calcium concentration in the medium was 0.28 mM. Low-calcium EMM with 2% Ultroser and glutamine was used in series IV. The calcium concentration in this medium was 0.15 mM and determined by means of an ionized calcium analyzer (Radiometer, Denmark). The cultures were examined daily in the phase-contrast microscope. The medium was changed twice a week. For electron-autoradiographic investigation of the cultures, $^3$H-thymidine was added to the nutrient medium in a dose of 10 μCi/ml 2 h before fixation. The cells were fixed with 2.5% glutaraldehyde solution and 1% osmium tetroxide solution, and embedded in Epon. The autoradiographic study of the original cell suspension, 0.5 ml of the suspension immediately after isolation was incubated for 2 h in test tubes with $^3$H-thymidine in the same concentration. The suspension was then washed off with nutrient medium and films were made on a slide and fixed in ethanol. Light-microscopic and electron-microscopic autoradiographs of films and cultures were prepared with the aid of M emulsion by the method of Sarkisov and coworkers [1, 2].

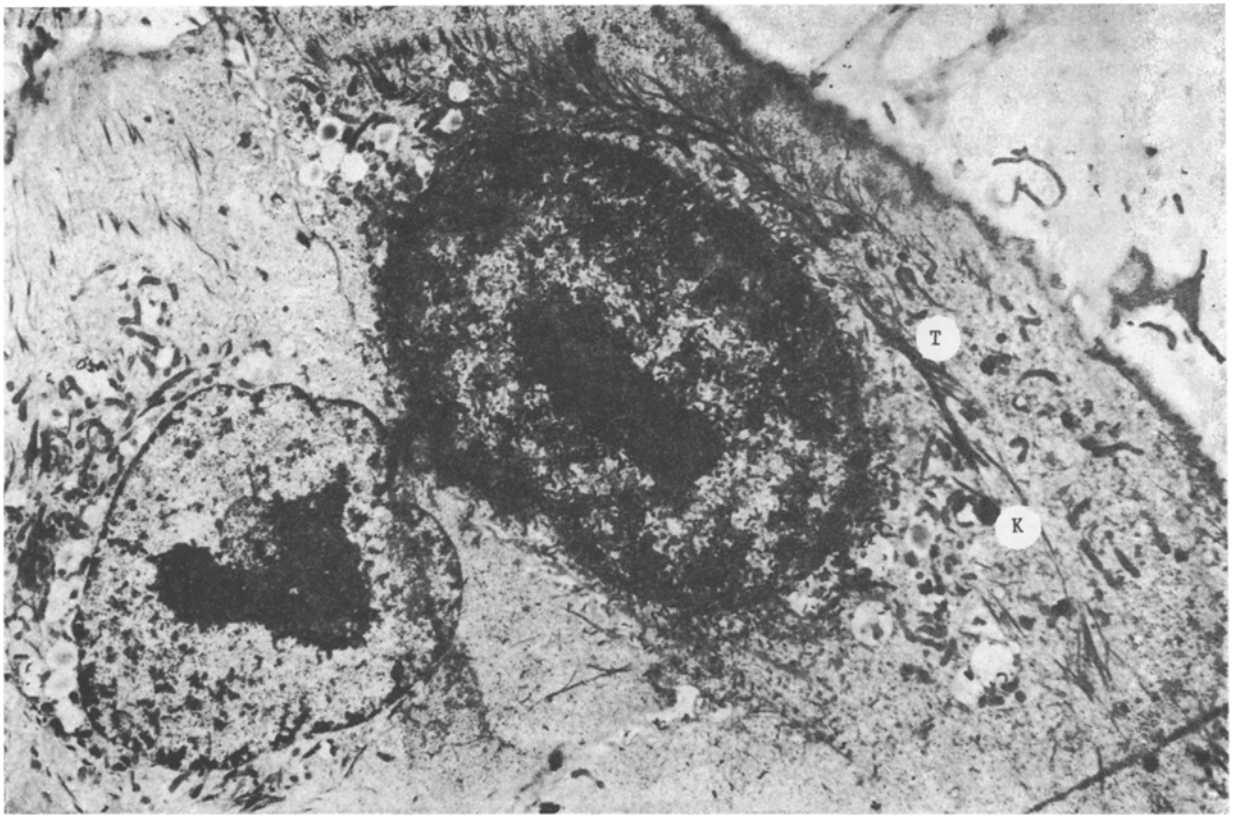


Fig. 3. DNA synthesis in epidermocyte in culture. Differentiation has begun in the cell, as shown by the presence of tonofilaments (T) in the perinuclear region and very few keratohyalin granules (K). 15,000×.

## EXPERIMENTAL RESULTS

Suspensions of cells isolated from skin for seeding contained chiefly basal cells, although differentiating cells and even keratin scales were found. The number of $^{3}$H-thymidine-labeled basal cells varied from 1 to 5% (Fig. 1). Morphologically, the cultures were almost indistinguishable from one another 24 h after seeding in media with different calcium concentrations. Many basal cells adhered to the bottom of the dish. The morphology of these cells was significantly altered. Whereas in films the basal cells were found with a narrow border of cytoplasm, the adherent cells were appreciably larger, polygonal in shape, and had a wide zone of cytoplasm. Many keratinizing cells floated on the surface of the medium and were removed from the dish when it was changed.

On further culture, morphological differences were observed between cultures growing on the nutrient media with low and high calcium concentration, and the differences gradually increased. After 5-6 days, cultures growing in media used in series I and II of the experiments had the appearance of separate colonies, unconnected with one another, or connected by narrow cell "bridges." The colonies in series I were large. The central areas of the colonies were covered with a layer of keratinizing cells. Labeled cells were extremely rare and were always located at the periphery of a colony. In many cultures examination of the whole surface of the dish failed to reveal a single labeled cell. At this time of culture in the experiments of series III, extensive colonies, confluent in many areas, were formed. Many isolated cells were present in the space between them. The colonies consisted of large cells, frequently binuclear. Cells composing the colonies incorporated $^{3}$H-thymidine frequently (4-11%), and the labeled cells could be present in any part of the colony. Isolated cells between colonies were never labeled. Keratinization was expressed by the formation of small concentrations of horn cells above some areas of the colonies (not always in the center).

In the experiments of series IV there were no colonies and large areas of the dish were occupied by a monolayer of large cells, separated by wider intercellular spaces than in the experiments of series III (Fig. 2). The number of labeled cells in these areas varied from 12 to 22% (Fig. 2). The electron-autoradiographic investigation showed that cells which had already passed through the initial stages of differentiation could be labeled with $^3$H-thymidine; in the perinuclear zone they formed a layer of tonofilaments, and only few keratohyalin granules were found (Fig. 3). In some smaller areas of the cultures described above the cells were smaller, did not form a monolayer, and did not take up $^3$H-thymidine. Keratinization was expressed by the presence of a certain number of isolated horn cells.

Culture of adult human epidermocytes in medium containing 0.15 mM calcium thus inhibits differentiation of the cells and stimulates cell division. These cultures largely fill the dish 1 week after seeding. The large number of dividing cells in the cultures enables them to be used both for transplantation to a wound surface and also for subsequent culture.

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# A METHOD OF OBTAINING A LONG-LIVING INTESTINAL TISSUE CULTURE


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KEY WORDS: malabsorption; tissue culture.


Interest in tissue culture research has increased considerably in recent years [3, 5, 6]. A special place is occupied by the study of the properties of intestinal tissue. The small intestine (SI) has been studied by various methods: the "everted pouch," a culture of isolated intestinal cells, and the organ culture method. The "everted pouch" method is applicable under experimental conditions. Some results have been obtained with respect to isolation and culture of single intestinal cells: enterocytes in culture preserve their viability for 4 h [1, 4]. However, in such a short time interval, opportunities for solving problems of clinical importance are limited. The viability of the human intestine in organ cultures, with preservation of the synthetic and secretory functions of intestinal tissue during explant culture for 24 h have been reported [3, 7]. However, no such practical studies have been described in the Soviet literature.

The method of small intestinal tissue culture has been used to study pathological states of the intestine included under the general term of "malabsorption syndrome." Disturbance

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