EFFECT OF SEEDING DENSITY ON GROWTH OF A CULTURE OF HUMAN EPIDERMOCYTES

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UDC 615.361.771.03:616-089.843].012.6

KEY WORDS: cell culture; epidermocytes; autoradiography.

Transplantation of sheets of epidermis grown in the laboratory may become one of the most effective methods of treatment of extensive burns. For this to happen, a number of problems connected with culture of epidermocytes must be solved and, in particular, we must learn how to grow epithelial sheets of a sufficiently large area in a short time. This accounts for the urgency of the study of all the conditions promoting more rapid growth of a culture. One such condition is the seeding density. Human epidermocytes are usually placed

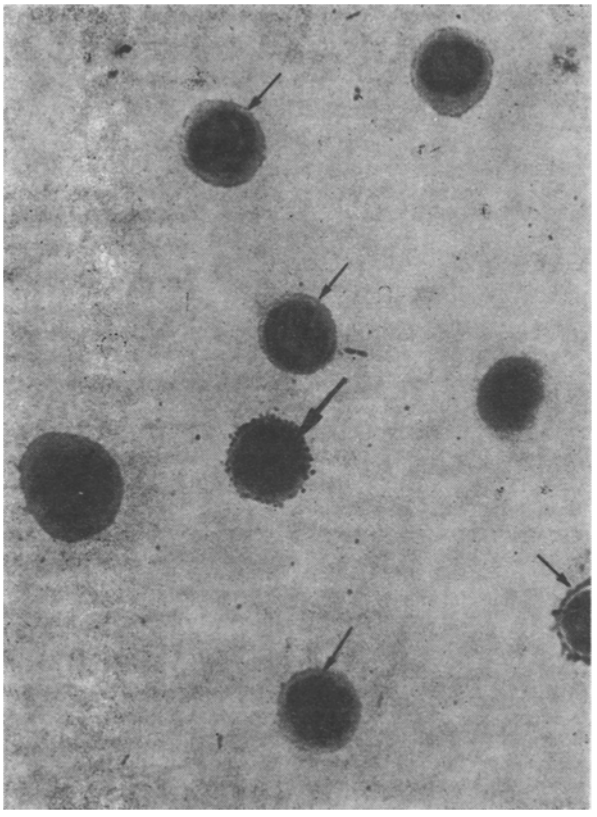

Fig. 1. Unlabeled (short arrows) and labeled (long arrows) basal cells in suspension of epidermocytes obtained after trypinization of skin. Magnification 1000.

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 12, pp. 724-727, December, 1989. Original article submitted May 20, 1989.

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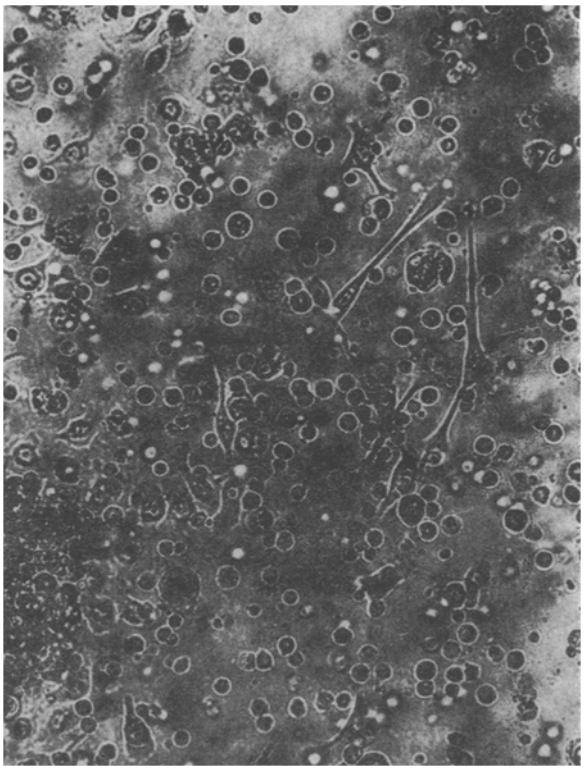

Fig. 2. Culture of epidermocytes photographed in phase contrast 24 h after seeding. Some basal cells, adherent to bottom of dish, have become polygonal in shape with a wide zone of cytoplasm (arrows). Magnification 100.

in dishes with a density of (1.5-2.5) × $10^5/cm^2$, without any consideration whether this density is optimal [1-5]. Observations described below show that the initial density of epidermocytes in a culture vessel may have a marked influence on the character of subsequent growth of the culture, and accordingly the density must be chosen, not by chance, but in strict accordance with the purpose of the culture. The effect of seeding density on growth of the culture is of great theoretical importance, for it sheds light on one of the factors regulating cell proliferation. The aim of this investigation was to analyze the time course of growth of cultures seeded with different densities.

EXPERIMENTAL METHOD

Material was obtained in the form of pieces of skin taken with a dermatome, during operations of skin autografting. The fragments were immediately placed in nutrient medium with antibiotics (1000 U/ml of penicillin, 500 U/ml of streptomycin) for 3-24 h at 4°C. The material was then treated for 20 min with a 0.02% solution of versene and a 0.25% solution of trypsin for 18 h at 4°C. After trypsinization, the skin fragments were transferred into a sterile Petri dish containing a small volume (5-10 ml) of nutrient medium and calf serum to neutralize the trypsin. The stratum corneum was removed with forceps, as a rule coming away in a continuous sheet, and the epidermocytes were removed by rinsing the piece of skin in nutrient medium. Cells were sedimented by centrifugation (500 rpm for 10 min) and resuspended in culture medium. Minimal Eagle's medium with a calcium concentration of 0.15 mM (determined by an ionized calcium analyzer, from "Radiometer," Denmark) plus 2% by volume of Ultroser medium (LKB) plus 2% by volume of the medium of 3% glutamine solution was used. The cells were cultured in plastic dishes in an incubator with an atmosphere containing 5% CO_2 and with humidity of 90%. The cultures were examined daily in phase contrast. The medium was changed twice a week. The seeding density in the dishes was (4-5) × $10^4/cm^2$ or (2.5-2.7) × 10^5 cm^2. For autoradiography, ^3H-thymidine was added to the culture medium in a dose of 10 μCi/ml and, after incubation for 2 h, the material was fixed in 100% ethanol. Autoradiographs were prepared in situ with the aid of M emulsion. The exposure was 3 days and the D-19 developer was used. The number of labeled cells was counted. The significance of differences in the

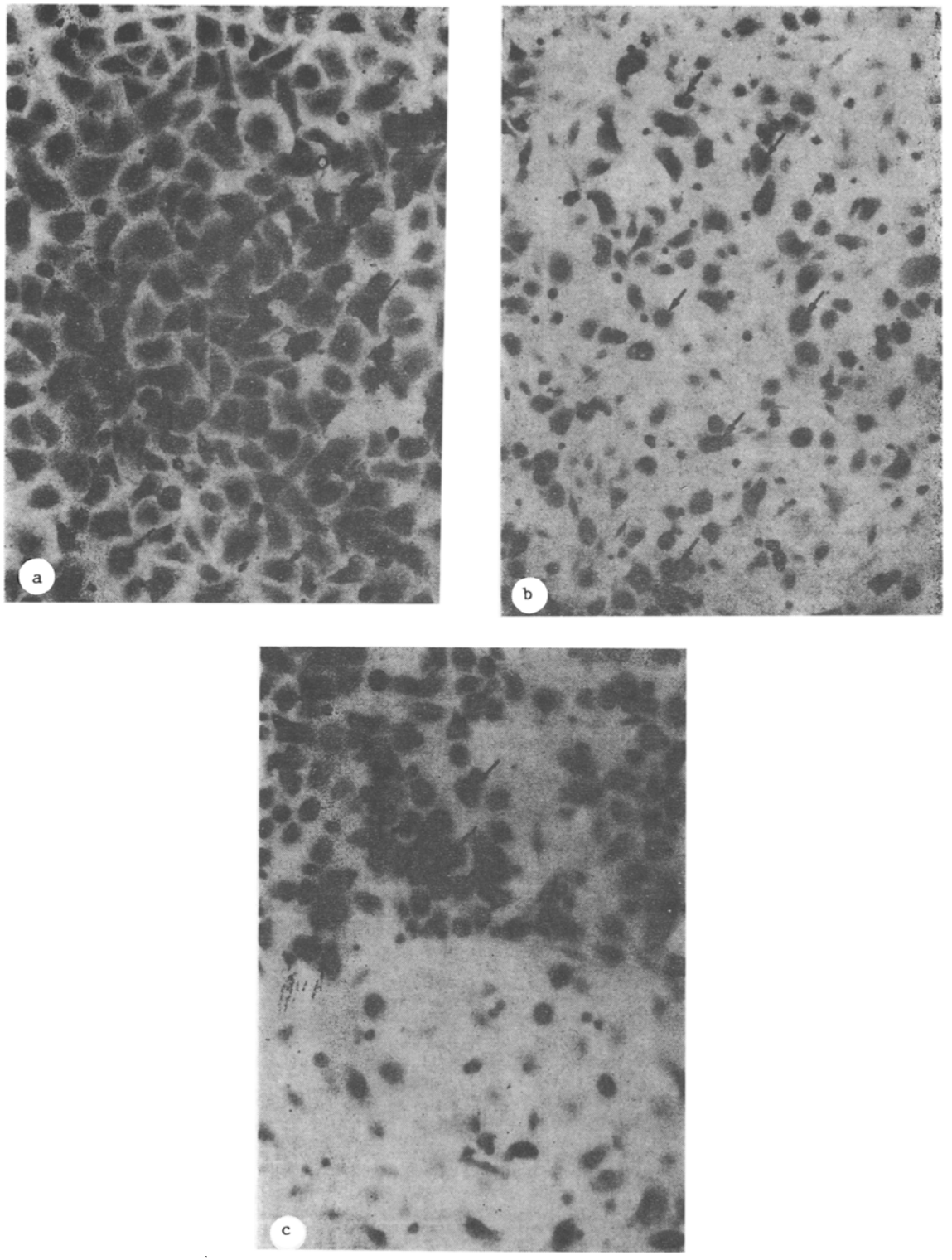

Fig. 3. Structure of cell layer and character of proliferation of epidermocytes in culture. a) Part of monolayer containing many cells labeled with ^{3}H-thymidine (arrows); b) part of less dense arrangement of epidermocytes, containing many cells labeled with ^{3}H-thymidine (arrows); c) cells labeled with ^{3}H-thymidine present (arrows) only in parts of monolayer (top left corner), zone of less dense arrangement of epidermocytes (rest of figure) contains no labeled cells. Magnification 200 ×.

rate of proliferation of the cultures using the chosen seeding densities was determined by Student's test.

EXPERIMENTAL RESULTS

Suspensions of epidermocytes obtained after trypsinization of the skin fragments contained mainly basal cells, although some differentiated cells and even keratin scales were found. Basal cells were identified by their ability to incorporate ^{3}H-thymidine (Fig. 1); the number of basal cells labeled with ^{3}H-thymidine varied from 1 to 5%. Basal cells in suspensions were small and round, with a narrow rim of cytoplasm, staining palely with toluidine blue (Fig. 1). Many of the basal cells adhered to the bottom of the dish 24 h after seeding. The morphology of these cells showed significant changes, for they were appreciably larger in size, polygonal in shape, and they were surrounded by a wide zone of cytoplasm (Fig. 2).

The autoradiographic study showed that individual, as yet not clearly determined features of cells from a given patient are a very important factor influencing the rate of growth of the epidermocyte culture. Other conditions being the same, cells from different patients grew at different rates and could cover the bottom of the dish with a more or less uniform layer after 4 to 13 days.

Analysis of the autoradiographs constantly revealed a marked difference in the structure of the cell layer and the rate of proliferation of the cells in different zones of the same dish. The main difference was in the number of cells on parts of the bottom of the dish of equal area. Some parts were covered comparatively quickly (in 3-4 days) with a layer of cells arranged in a single row vertically, separated by intervals that were appreciably smaller than the diameter of the cells. In other parts the cells were arranged also in a single row vertically, but at much wider distances apart, much greater than the diameter of the cells. The number of examples of these rarefied regions decreased with growth of the culture, but they remained on small parts of the bottom of the dish in all dishes and at all times of observation.

The rate of proliferation of the epidermocytes differed in parts with different types of structure of the cell layer. Some parts of the monolayer (Fig. 3a) had the largest number of cells labeled with ^{3}H-thymidine. In the early period of observation (3-5 days) the proportion of these cells was between 12 and 22%; in the later stages (13-15 days) between 0 and 2%. Regions of the less dense cellular layer sometimes could not be distinguished in the early period of observation from parts of the monolayer by their content of labeled cells (Fig. 3b). In most cases, however, the number of labeled cells in the less dense parts was considerably less (by 2-5 times) than in the monolayer. Often no labeled cells were presented at all in such parts (Fig. 3c), whereas in parts of the monolayer in the same dishes there was a high percentage of labeled cells.

The average rate of proliferation of the cultures after seeding dilute suspensions was a little higher (12 $\pm$ 3% of labeled cells) than after seeding concentrated suspensions (10 $\pm$ 3.1% of labeled cells). In other words, differences between groups, differing in their seeding density, were not significant and were appreciably less than differences between individual zones of the same dish.

Spreading of the monolayer over a large part of the surface of the bottom of the dish coincided with a sharp fall in the level of thymidine uptake. The percentage of labeled cells at this stage of development of the culture did not exceed 5, and often cells of this kind could not be found at al. Further incubation of these cultures as a rule did not lead to covering of the whole surface of the bottom of the dish with the monolayer, and evidently served no useful purpose. At this stage the cultures should be used for transplantation or passage. The cell density on seeding affected the rate of covering of the bottom of the dish with the monolayer and cessation of growth of the culture. With a density of $(2.5\text{-}2.7) \times 10^5/\text{cm}^2$ cessation of growth was observed on the 7th-9th day, whereas with a density of $(4\text{-}5) \times 10^4/\text{cm}^2$ it was observed on the 9th-11th day.

It can be recommended on the basis of these results that it is more rational to use comparatively dilute cell suspensions for seeding. In this case it is possible to obtain, with a delay of only 2 days, cell layer 5 times larger in surface area for transplantation or for subculture.

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EFFECT OF CRYOCONSERVATION AND TRANSPLANTATION FACTORS ON MORPHOLOGY AND FUNCTION OF THE CANINE THYROID GLAND

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UDC 616.441-089.843-089.168-076-092.9

KEY WORDS: thyroid gland; cryoconservation; transplantation.

During transplantation of any organ, surgeons have to contend with the effects of nonspecific transplantation factors such as denervation, delymphatization, ischemia, operative trauma, and changes in blood supply. During conservation, the organ is additionally exposed to factors accompanying it, and in particular, during cryoconservation: very low temperatures and cryoprotectors.

The aim of this investigation was to study the effect of nonspecific transplantation and low-temperature conservation factors on the ability of the thyroid gland to secrete thyroid hormones and on its morphology.

EXPERIMENTAL METHOD

Experiments were carried out on 30 mongrel dogs weighing from 15 to 25 kg. A model of extracorporeal biological perfusion of the isolated dog thyroid gland was used [6]. In the control group the right lobe of the thyroid gland was removed together with a segment of the carotid artery and perfused after connection to the femoral artery with blood from the same dog, in a thermostatically controlled chamber. By using this approach it was possible to study the effect of nonspecific transplantation factors on the morphology and function of the thyroid gland and to compare it with those of the lobe perfused in situ. In the experimental group, the extirpated lobe of the thyroid gland was subjected to deep freezing before perfusion, which was carried out in the same way, by the method developed at the Institute for Problems in Cryobiology and Cryomedicine, Academy of Sciences of the Uzbek SSR [2]. Saturation of the gland with cryoprotector (a 10% solution of DMSO in Hanks' solution) was carried out by perfusion using a "Peripump" (Hungary) pump; the temperature of the cryoprotector was lowered after 90 min to −2°C. The duration of cryoconservation (−196°C) was between 45 h and 16 days. The organs were thawed in a water bath at 37°C for 5 min. The duration of perfusion in the control and experimental groups was 6 h. Levels of thyroxine and tri-iodothyronine were measured in blood flowing from the lobes perfused in situ and in the constant-temperature chamber by means of commercial kits from "Amersham" and "Corning." At the end of perfusion the thyroid gland tissue was fixed in a 10% solution of neutral formalin and, after histological treatment, sections were stained with hematoxylin and eosin.

Research Institute of Regional Medicine, Ministry of the Health of the Uzbek SSR, Tashkent. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 2, pp. 727-730, December, 1989. Original article submitted April 28, 1988.

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