CULTURE OF EPIDERMOCYTES FROM MAN AND EXPERIMENTAL ANIMALS

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The possibility of obtaining and culturing skin epidermocytes was studied in experiments on animals and on human biopsy material. To ensure sufficient proliferation of the cells, insulin and hydrocortisone were used as biological stimulators. Morphological investigation showed that the culture obtained is a monolayer of epidermocytes uncontaminated with other skin cells.

The problem of whether it is possible to culture epithelial cells from human skin was first posed more than 40 years ago [12]. In the subsequent time many methods have been suggested for studying the viability of human skin cells and skin cells from experimental animals in vitro, ranging from histological investigations to clinical transplantation [2, 4, 5, 6, 8, 14, 15, 20]. During these years the optimal culture media have been selected for skin epithelium: Eagle's medium in Dulbecco's modification [5, 6, 8, 14] and medium RPMI [1, 13], and methods of treatment of thin skin sections with proteolytic enzymes (trypsin, trypsin-versene [12, 17], and collagenase [11]) have been suggested and improved by several workers [2, 4, 16, 19, 21].

Investigations have led to shortening of the time required to obtain pure intensively growing cultures of epidermocytes from human skin, uncontaminated by fibroblasts. It has been shown that to improve growth of cultured epithelial cells from human skin, the nutrient medium must be enriched with hormones (hydrocortisone, dexamethasone, insulin) [14] and the concentration of embryonic calf serum must be increased to 20% [18]. To accelerate the process of cell differentiation, cholera toxin and the β-agonist isoproterenol [9, 10, 14] and epidermal growth factor [18] have been added to the nutrient medium.

The writers have attempted to determine optimal conditions for growth of epidermocytes from human skin and from the skin of experimental animals on the basis of existing data on culture.

EXPERIMENTAL METHOD

Epidermocytes were obtained from skin fragments obtained from various experimental animals and from human biopsy material. The skin of 10 guinea pigs, and of 29 newborn and seven mature albino rats was used. The skin fragment taken from the abdominal region measured 2 to 15 cm^2; after removal of the subcutaneous fatty areolar tissue the fragment was placed in nutrient medium 199 with a high concentration of antibiotics (penicillin 1000 U/ml, streptomycin 1000 U/ml, amphotericin B 5 μg/ml) for between 3 and 24 h.

Several versions of isolation of the epidermocytes were tested, with the aid of 0.25% standard trypsin solution (USSR, from Spofa, Czechoslovakia) and a mixture of 0.25% trypsin and EDTA (from Gibco). All the enzymes tested were found to function optimally at 4°C for 18 h. The epithelial layer was then removed mechanically, cut into small pieces, and subjected to further trypsinization on a magnetic mixer for 30-60 min at room temperature, or by pipeting. The action of trypsin was neutralized by the addition of 2% calf serum. After centrifugation at 1000 rpm for 10 min the residue was resuspended in 1.0 ml of Eagle's nutrient medium, containing α-glutamine, hydrocortisone, and antibiotics and seeded in plastic culture dishes.

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TABLE 1. Results of Trypsinization of Epithelial Cells from Skin by Different Methods in Animal Experiments

Species of animals	No. of animals	Method of obtaining epithelium	Concentration of cells in 1 ml	Number of living and dying cells in 1 ml of suspension				Yield of living cells from 1 cm^2 of skin
				living		dying		
				absolute	%	absolute	%	
Guinea pig	10	Pipeting	$4,2 \cdot 10^5$—$2,0 \cdot 10^6$	$6,1 \cdot 10^6$	91,5	$5,7 \cdot 10^5$	8,5	$1,7 \cdot 10^5$
		Magnetic mixer	$5,5 \cdot 10^5$—$2,9 \cdot 10^6$	$6,8 \cdot 10^6$	94,4	$4,3 \cdot 10^5$	5,6	$3,1 \cdot 10^5$
Rats: newborn	29	Pipeting	$2,2 \cdot 10^4$—$3,8 \cdot 10^5$					$6,9 \cdot 10^4$
		Magnetic mixer	$3,8 \cdot 10^5$—$4,5 \cdot 10^5$					$1,2 \cdot 10^5$
re	7	Same	$2,0 \cdot 10^4$—$4,5 \cdot 10^4$					$2,9 \cdot 10^4$

TABLE 2. Results of Obtaining Epidermocytes from Human Skin Biopsy Material

Age of patients years	Number of skin fragments n	Mean area of skin, cm^2	Concentration of epidermocytes in 1 ml		Number of epidermocytes from skin fragment		Yield of epidermocytes from 1 cm^2 of skin	
			limits of variation	mean	limits of variation	mean	limits of variation	mean
From 10 to 20	6	2,51	$2,0 \cdot 10^4$—$6,0 \cdot 10^5$	$3,41 \cdot 10^5$	$2,0 \cdot 10^4$—$7,65 \cdot 10^5$	$4,1 \cdot 10^5$	$1,25 \cdot 10^4$—$3,0 \cdot 10^5$	$1,62 \cdot 10^5$
From 20 to 30	3	1,57	$3,5 \cdot 10^4$—$4,8 \cdot 10^5$	$2,61 \cdot 10^5$	$3,5 \cdot 10^4$—$5,0 \cdot 10^5$	$2,68 \cdot 10^5$	$3,5 \cdot 10^4$—$2,25 \cdot 10^5$	$1,53 \cdot 10^4$
From 30 to 40	3	3,67	$3,0 \cdot 10^5$—$4,0 \cdot 10^5$	$3,5 \cdot 10^5$	$3,0 \cdot 10^5$—$4,0 \cdot 10^5$	$3,5 \cdot 10^5$	$5,0 \cdot 10^4$—$2,0 \cdot 10^5$	$1,22 \cdot 10^5$
40 years or older	5	2,3	$2,0 \cdot 10^3$—$1,3 \cdot 10^6$	$5,05 \cdot 10^5$	$2,0 \cdot 10^3$—$9,9 \cdot 10^5$	$4,48 \cdot 10^5$	$1,0 \cdot 10^3$—$6,6 \cdot 10^5$	$2,31 \cdot 10^5$

To obtain a pure culture of human epidermocytes and to culture them in vitro, pieces of skin from various parts of the body were used for the next stage of the investigation, obtained during autologous skin grafting on 17 patients (15 men and two women) aged from 10 to 56 years. Pieces of skin measuring 1-2 cm^2 and 0.25-0.35 mm in thickness were cut in the operating theater with a manually driven dermatome, placed in medium 199 with a high concentration of antibotics, determined by reference to their activity profiles. After trypsinization in a 0.25% solution of trypsin for 18 h at 4°C the skin fragments were transferred into sterile Petri dishes. After removal of the stratum corneum mechanically and conservatively, the epidermocytes were washed off into nutrient medium. The number of living and dying cells was counted by the trypan blue test. A modified Eagle's medium with the addition of 20% fetal calf serum and α-glutamine was used. Insulin (10 mg/ml) and hydrocortisone (0.4 mg/ml) were used as growth stimulators. Epidermocytes obtained from animal and human skin were cultured in plastic culture dishes (Flow Laboratories) in an incubator in an atmosphere containing 5% CO_2 at 37°C. The nutrient medium was changed on the 4th day. Growth of the epidermocytes was studied over a period of time in the field of vision of an inverted microscope. Fixed cells were stained by the Giemsa method for morphological investigation.

EXPERIMENTAL RESULTS

The animal experiments showed that epithelial cells can be obtained in a high concentration, which varied from $2 \cdot 10^4$ to $2,9 \cdot 10^6$/ml (Table 1). The trypan blue test showed that 91.5-94.4% of the cells were viable, equivalent to $1.7 \cdot 10^5$-$3.1 \cdot 10^5$ cells from 1 cm^2 of skin. Cultures obtained during trypsinization by pipeting and by the use of a magnetic mixer were virtually identical. Morphological investigation of the culture showed that cells of epithelial character included cells from all layers of the epithelium from the stratum basale to the stratum corneum (large polygonal cells). Epithelial cells adhered to the walls of the dish 1 h after introduction, and after 6-7 days they formed islands of epithelial growth with marked differentiation of the cells. Experiments on newborn and adult rats showed that such a culture contains cells of both epithelial and connective-tissue origin. During culture on plastic culture dishes, growth of islands of epithelium was observed, but they were contaminated with fibroblasts.

In the experiments to obtain epidermocytes from a fragment of human skin, with a seeding density of $2.5 \cdot 10^5$-$7 \cdot 10^5$/cm^2 area of culture dish, a good yield of living cells capable of growth was obtained. The number of these cells in 1 ml varied from one experiment to another

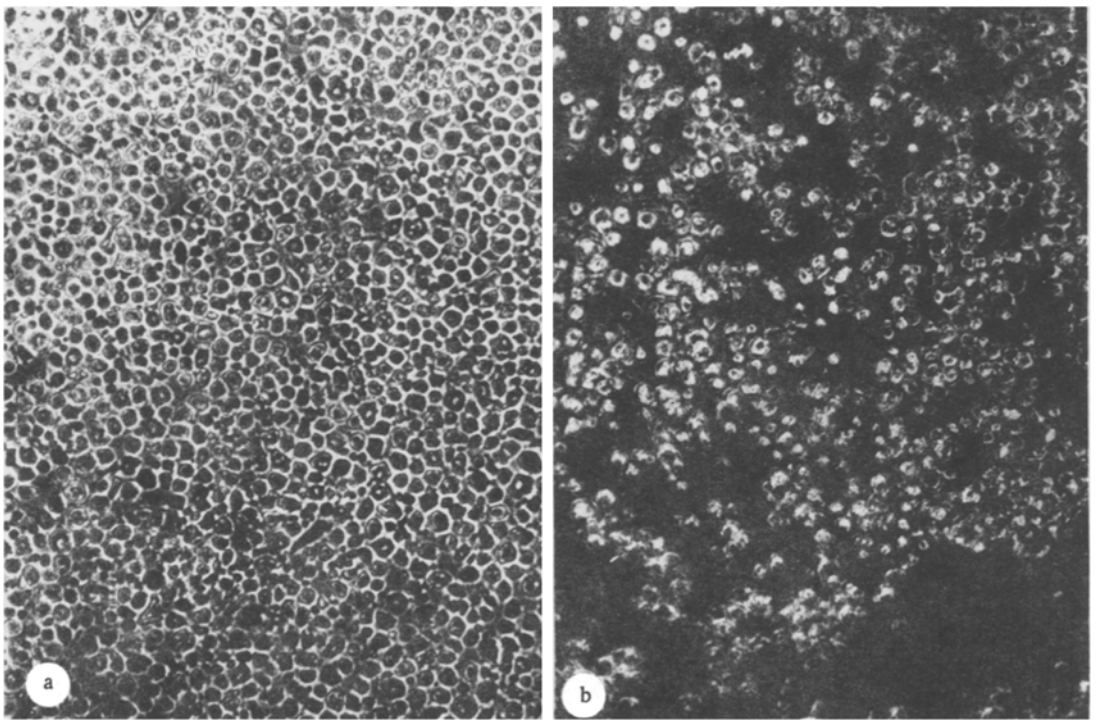

Fig. 1. Culture of epidermocytes from patient V. A. on the 24th day. 160 ×. a) With insulin; b) without insulin.

from $2.0 \cdot 10^3$ to $1.3 \cdot 10^6$ (Table 2). The yield of epidermocytes from 1 cm^2 of skin likewise varied, from $1.0 \cdot 10^3$ to $6.6 \cdot 10^5$. No correlation could be found between the paitent's ages and the size of the skin fragment taken from them. This may perhaps be explained by individual differences.

Further observation on growth and proliferation of the epidermocytes in culture continued for 45 days. It was found that after 72 h most of the epidermocytes were adherent to the surface of the plastic dish and formed a monolayer. A tendency was noted for the cells to aggregate. The cytoplasm of these cells contained vacuoles. Quite large, round nuclei were located in the center of the cells. In some cases islets of epithelial cells formed circumscribed colonies. With time the boundaries between the separate cells became indistinguishable and disappeared. The developing monolayer became finely granular in structure.

Morphologically, the whole layer of cells contained typical epidermocytes of polygonal and branching shape, uncontaminated by cells from the subjacent layers. Monolayer formation, according to our observations, took place after 20-28 days. In the presence of insulin the monolayer was formed after 20-22 days (Fig. 1a), compared with 26-28 days in the control (Fig. 1b). The use of hydrocortisone, as our results showed, did not accelerate proliferation.

The problem of mass culture of epidermocytes will evidently be solved by the successful search for and use of various growth factors and their combination with nutrient media.

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MORPHOLOGICAL MANIFESTATIONS OF ANTHRACYCLINE CARDIOMYOPATHY IN THE VENTRICULAR MYOCARDIUM OF RATS

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KEY WORDS: anthracycline cardiomyopathy; plastic insufficiency of the heart; right and left ventricular myocardium; cardiomyocyte ultrastructure; morphometry.

Anthracycline cardiomyopathy of laboratory animals is a convenient and promising model with which to study the general rules of development of plastic cardiac insufficiency [2, 5, 11]. During investigation of the left ventricular myocardium by light and electron microscopy, combined with stereologic analysis and the method of cell isolation by alkaline dissociation of the fixed myocardium, the writers discovered the fundamental rules governing involutional reorganization of cardiomyocyte ultrastructure and quantitative reduction of the population of these cells in the absence of any necrotic changes in the myocardium [9, 11]. However, contractile cardiac insufficiency has not been investigated when structural metabolism in the muscle tissue of the right ventricle is disturbed.

The working myocardium of the right ventricle, despite similarity in the ultrastructural organization of its cardiomyocytes with those of the left ventricular myocardium, presented considerable autonomy, manifested as greater resistance to metabolic damage, high sensitivity to hemodynamic overloads [6], and differences in the dynamics and rates of growth and of aging of the myocardium during postnatal development [1, 10]. The degree of involvement of the cardiomyocytes in the elimination process during anthracycline cardiomyopathy of the right ventricle is therefore an interesting problem.

The aim of this investigation was to discover whether any differences exist in the sensitivity of the right and left ventricular myocardium to the action of the anthracycline antibiotic rubomycin, which depresses DNA-dependent RNA synthesis in the cardiomyocytes, by concentrating on the study of "disappearance" of some cells from the population of ventricular cardiomyocytes [9].

EXPERIMENTAL METHOD

Experiments were carried out on 15 male Wistar rats weighing 180 ± 20 g, divided into two groups. The experimental animals (n = 10) received a single intraperitoneal injection

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