CULTIVATION OF ADULT HUMAN SKIN IN VITRO FOR CYTOGENETIC INVESTIGATIONS

Yu. E. Blok

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Cultivation of the adult human skin in vitro is widely used for the study of congenital anomalies of development and metabolism of genic or chromosomal origin [2, 6, 8, 9]. Chromosomal analysis is important in practice, for it may often be a decisive measure in the diagnosis of extensive developmental defects and of certain chronic diseases [4, 5, 10]. The advantages of the cultivation of skin over brief cultivation of the leukocytes of the peripheral blood lie in the possibility of making prolonged observations on the metabolic properties of the cells in vitro.

The methods of cultivation of adult human skin for cytological investigations which have been described are associated with the use of special synthetic mixtures stimulating proloferation of fibroblasts [11, 12] or with the production of subcultures [7].

The object of this investigation was to develop a simple and effective method of cultivating adult human skin in vitro for various cytogenetic investigations. The morphological characteristics of skin cultures are also described.

EXPERIMENTAL METHOD

This paper presents the results of cultivation of 15 skin biopsy specimens obtained from donors aged 24-56 years.

An area of skin in the region of the medial palmar surface of the upper third of the forearm or the superolateral surface of the arm was rubbed repeatedly with 96° alcohol and ether. Local anesthesia was produced by the

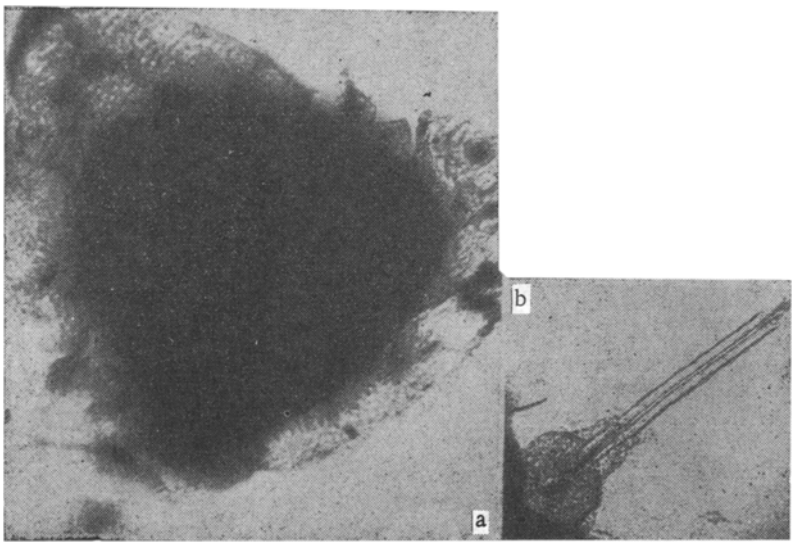

Fig. 1. Zone of growth of skin cultures in the initial period of cultivation. a) Epithelial membrane surrounding the explant in a culture of the skin of patient T., male, aged 27 years. The outlines of the individual cells of cubical epithelium forming the membrane are almost indistinguishable; b) a hair sac in a culture from patient S., male, age 56 years. Photomicrograph. Natural photograph. 45×.

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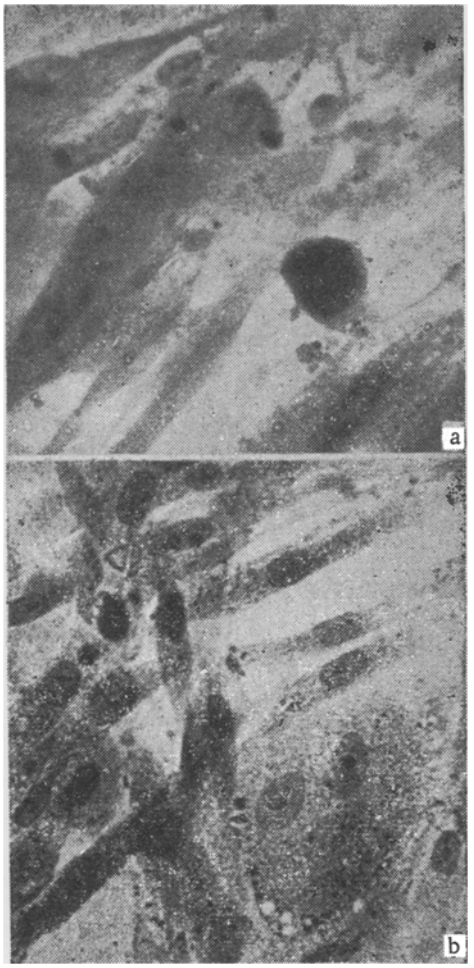

Fig. 2. Skin cultures in the period of proliferation of connective-tissue cells. a) Large fibroblast-like cells in a culture of the skin of patient K., aged 47 years. Stained with hematoxylin-eosin. b) Culture of skin of patient S., aged 50 years. Stained with Sudan black on the 40th day of cultivation, revealing a very slight degree of lipoidosis of the cells. On the right and left cells of endothelial type can be seen. Photomicrograph, 280 ×.

subcutaneous injection of 1 ml of 0.5% procaine solution. A piece of skin about 3 mm in diameter was lifted with forceps and excised with sharp-pointed ophthalmic scissors with curved blades, and placed in a test tube. A sterile dressing was applied to the region of the resected area. Epithelization of the wound surface was complete after 2-3 days.

The resected fragment was washed in two batches of nutrient medium (see below), cut up into pieces not more than 0.5 mm in diameter, and placed in a hollow in a thick glass slide into which 0.5 ml of a 0.3% solution of trypsin (Difco) had previously been poured. The pieces along with the solution were aspirated into a pipet and transferred to a centrifuge tube, and after the addition of 2 ml of trypsin solution, the skin was incubated for 15 min at 37°. Next, a large part of the solution was carefully aspirated from the test tube and replaced by 1.5 ml of a nutrient medium of the following composition: 30% human serum obtained from one donor, 20% extract of a 9-day chick embryo, and 50% medium No. 199. The medium No. 199 and the embryonic extract contained antibiotics – penicillin and streptomycin in a dose of 100 units/ml fluid each. The pieces were resuspended 4-5 times in the nutrient medium and the suspension was collected in a pipet and distributed in two Carrel's flasks, 5 cm in diameter, containing mica disks. A gas mixture containing 5% CO_2 was passed into the flasks, which were incubated at 37°. The nutrient medium was changed twice a week.

Similar results were obtained by a method using a plasma clot, described previously by the author for cultivation of aortic tissue [1]. However, in the primary plasma cultures, incubation with a hypotonic solution usually did not cause separation of the chromosomes.

The cultures were periodically fixed in a 10% solution of neutral formalin and stained with Delafield's hematoxylin and eosin, and also with Sudan black.

To detect chromosome complexes, an aqueous solution of colchicine in a final concentration of 0.5 μg/ml medium was added to the flask containing the culture 72 h after changing the nutrient medium. After 18-20 h the medium with the colchicine was removed, 5 drops of fresh nutrient medium was added to the flask, and this was followed by 15 drops of distilled water. After incubation for 10 min in the flask with hypotonic medium at 37° the mica disks with the cultures were fixed and stained with orcein acetate, by the method described by Tijo and Puck [13].

EXPERIMENTAL RESULTS

Along the edge of many of the pieces, 24 h after explantation, a rim had formed, consisting of 3-4 rows of cells of cubical epithelium. During the next 5-8 days the sheet of epithelium grew wider on account of the formation of new layers of tightly packed cubical or polygonal cells.

The cytoplasm of these cells was a glistening yellowish color and it contained numerous vacuoles. The fairly large, round nuclei were situated in the center of the cells. In some cases islets of epithelial cells were separated from the main zone of growth and formed circumscribed epithelial membranes at a considerable distance from the explant. At the same period hair sacs with the root part of the hair were differentiated at the edge of the pieces (Fig. 1b). With time, growth of the epethelial membranes ceased, the borders of the individual cells became indistinguishable (Fig. 1a), and later they disappeared altogether, the outlines of the cell nuclei faded, the sheet of cells was reduced in volume, and its structure became finely granular. A similar process of "keratinization" of the epithelium in vitro was first described in detail by N. G. Khlopin [2] in cultures of human embryonic skin.

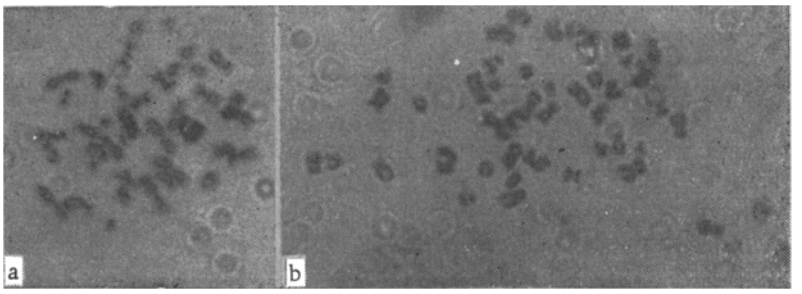

Fig. 3. Chromosomal complexes in cultures of the skin of patient B., male, aged 40 years. a) Early metaphase; b) late. Photomicrograph. Stained with orcein acetate. 700×.

Involution of the epithelium coincided in time with active proliferation of the connective-tissue cells. The first fibroblast-like cells appeared near the explant (outside the borders of the epithelial membranes) not before the 5th-6th day of cultivation. At first these cells were isolated, and in shape they were long, often fusiform, and sometimes triangular. With each day the number of connective-tissue cells increased, they anastomosed with long processes, and at the end of the 3rd week they formed a typically connective-tissue zone of growth, with a dense interlacement of many layers of elongated cells, especially near the explant. The dimensions of the fibroblast-like cells varied considerably (Fig. 2a, b). The oval nuclei, slightly stretched along the cell axis, were homogeneous in structure and often contained large numbers of nucleoli; the cytoplasm of the connective-tissue cells formed an endoplasmic zone near the nucleus staining more intensively with eosin. Tiny cytoplasmic vacuoles and granules were situated here, but the degree of vacuolation of the cytoplasm was usually very slight.

Besides the elongated cells, large polygonal cells of endothelial type with short, thick processes and an oval nucleus were seen at the periphery of the zone of growth, and sometimes in its more central areas (see Fig. 2b). These cells, arising evidently from the endothelium of the cutaneous vessels, were isolated or formed small groups of polygonal cells in contact at their edges, much larger than the cells of the cubical epithelium. Multinuclear giant cells of this type also were often seen.

Staining the skin cultures with Sudan black revealed extremely slight traces of lipid inclusions in the cells, even after prolonged cultivation (see Fig. 2b).

An adequate number of mitoses was found in the skin cultures during the 3rd week of cultivation. As Fig. 3a, b shows, according to the results obtained this is a favorable time for detecting chromosomal complexes.

SUMMARY

The method described is used for the cultivation of the skin from 15 donors, aged from 24 to 56 years. Skin explants about 0.5 mm in diameter were treated with a 0.3% trypsin solution and cultivated in Carrel's flasks in a nutrient medium with 30% of the human serum, 20% of a chicken embryo extract and 50% of medium No. 199. Following a brief proliferation of epithelial membranes one could observe the formation of a typical connective tissue zone of growth with an admixture of endothelial-type polygonal cells. The isolation of chromosome complexes in the cultures was carried out in the third week of cultivation.

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