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CULTURE OF EPIDERMOCYTES ON A COLLAGEN SUBSTRATE

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A new trend has recently been formed in the treatment of burns, involving attempts to grow in culture, from a small piece of skin taken from a patient, much larger sheets of cells suitable for transplantation [1, 2, 4]. The development of techniques for transferring such an epidermal sheet to the wound surface is of great importance [1-3, 6]. To preserve the proliferative ability of the basal cells of the epithelium and to prevent retraction of the epidermal sheet thus obtained after its detachment from the floor of the culture vessel, various biological and synthetic substrates have been suggested (collagen lattice [1], stage I and stage II [2]).

In the investigation described below, the proliferative activity and rate of formation of the epidermal sheet during culture of epidermocytes on a collagen substrate were studied by modern methods.

EXPERIMENTAL METHOD

Pieces of skin obtained from patients during skin autografting and also split skin grafts obtained from cadavers served as the test material. Skin grafts 0.2-0.3 mm thick were taken with a DRM-60 dermatome and placed in a test tube containing 25 ml of Eagle's nutrient medium with antibiotics for 2 to 20 h. The nutrient medium with a high concentration of antibiotics was changed for fresh solution of the same composition, but containing only 1-20th of the concentration of antibodies. This solution was then poured off and the skin graft rinsed for 20 min in 0.02% EDTA and kept for 18-20 h in a 0.25% solution of trypsin at 4°C. After this stage the trypsin solution was poured off and the skin graft rinsed with Na-phosphate buffer, pH 7.2-7.4, and transferred to a Petri dish with medium containing calf serum to neutralize the enzyme, where the epidermis was separated from the dermis. The epidermocytes were isolated by repeated pipeting. After trypsinization by the method described above, the number of epidermocytes isolated from 1 cm^2 of skin graft was 10^5-10^6 cells. On supravital staining with a 1% solution of trypan blue, viable cells accounted for 90-95% of the total number of cells in the suspension, depending on the state of the initial skin. The cell suspension was harvested in a test tube and the cells were sedimented by centrifugation at 500 rpm for 10 min. The supernatant was poured off, then resuspended in hypocalcium medium (Ca^{++} concentration 0.14 mM) containing glutamine and Ultroser. The number of cells was counted in a Goryaev's

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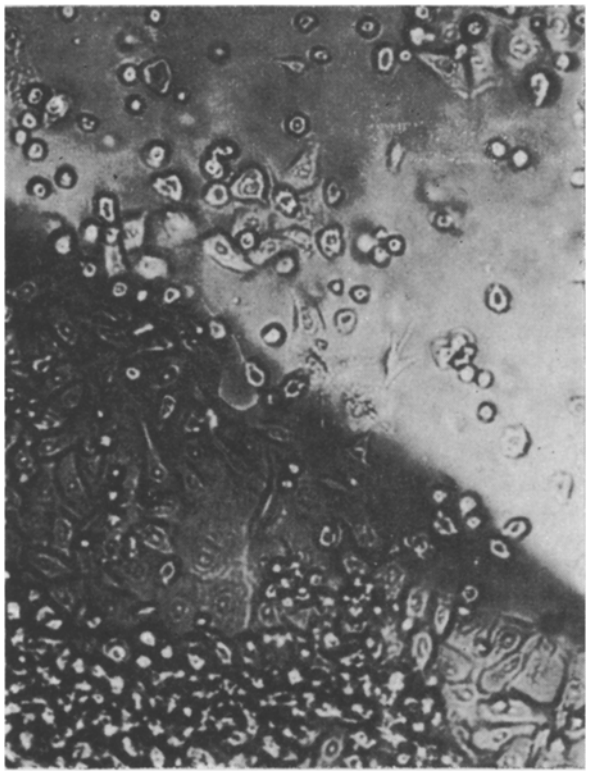

Fig. 1. Boundary region of bottom of culture dish. Top left — part of bottom of dish covered with collagen film on which epidermocyte monolayer was formed. Bottom right — uncovered region of bottom of plastic dish. Single, spread-out epidermocytes can be seen. 400 ×.

counting chamber and the cell suspension transferred into a Petri dish. The number of cells in the medium was (1-2.5) × $10^5/cm^2$. Before the cells were added to the nutrient medium, half of the bottom of the dish was covered with a solution of type R collagen in 0.1% acetic acid. The excess of solution was drawn off and the dish placed in a cupboard until the collagen film had dried. The half of the bottom of the dish not covered with collagen served as the control. The cells were incubated at 36-37°C in an atmosphere containing 5% CO_2 and 95% air, with a humidity of 80%. The nutrient medium was changed every 3-4 days. The time course of growth of the culture was monitored daily under the phase-contrast microscope. For electron-microscopic autoradiography, ^3H-thymidine was added to the medium on the 3rd-10th day of culture in a dose of 10 μCi/ml, and after incubation for 2 h, the culture was washed with buffer solution, and fixed with absolute ethanol for light-optical autoradiography or with a 1% solution of glutaraldehyde for electron-microscopic autoradiography.

EXPERIMENTAL RESULTS

The morphological control showed that 5-15 min after seeding the cells began to adhere to the collagen substrate, and after 2 h or more, to the bottom of the plastic Petri dish. Clear differences in cultures growing on the collagen substrate and without it could be observed after 24 h of culture. Epidermocytes growing on the collagen substrate adhered faster and better, and unlike the cultures on plastic, spreading out of the cells was observed. Between the 1st and 3rd days of culture colonies 0.5-1 mm in diameter (islets) were formed, with cellular connections (bridges) between them. Rapid proliferation of the epidermocytes took place later, and on the 3rd-5th day of culture a monolayer of epidermocytes suitable for transplantation to a burn wound had formed. No reports could be found in the literature on such rapid formation of the epidermal monolayers. Morphological investigation of cells growing on plastic revealed, in most cases (7 of 10) the same stages, but with rather later times of development. For example, colonies of epidermocytes were formed by the 3rd-5th day, and the monolayer was formed by the 5th-10th day of culture under the same conditions (Fig. 1).

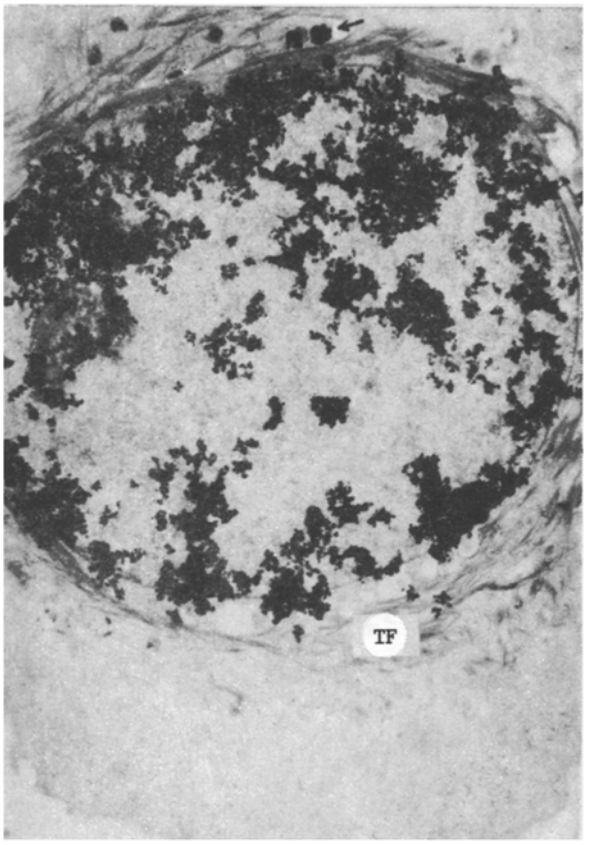

Fig. 2

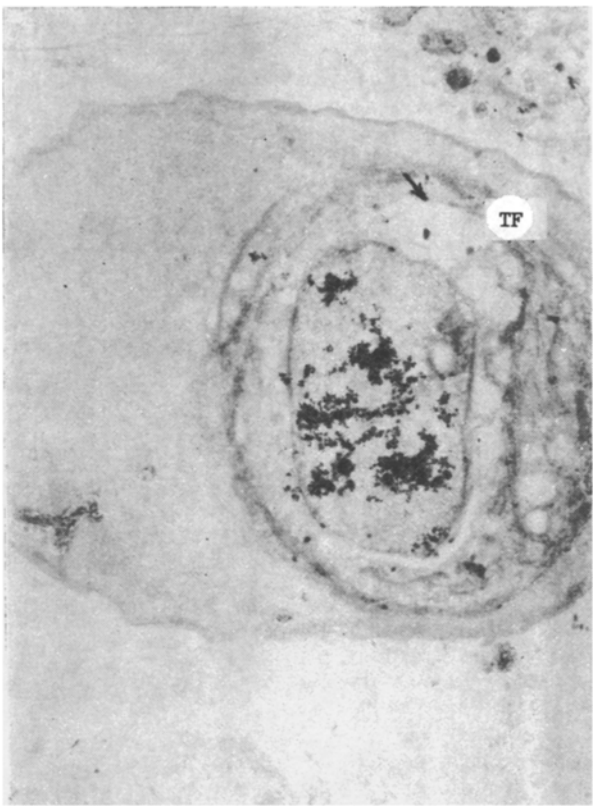

Fig. 3

Fig. 2. DNA synthesis in a keratinocyte, similar to cell of stratum basale. Nucleus surrounded by bundles of tonofibrils (TF). Melanosomes are present (arrow). 18,000 ×.

Fig. 3. DNA synthesis in a keratinocyte, similar to a cell of the stratum spinosum. Electron-translucent "cells" (arrow) in network of tonofibrils (TF). 7000 ×.

During subsequent culture of the epidermocytes, if not subjected to passage, stratification of the culture was observed. However, the stratum corneum as such was not formed. Keratinized epidermocytes were desquamated singly or in groups of three or four cells into the nutrient medium. The stratum corneum, according to data in the literature, is formed quickly after transplantation of an epidermal sheet on to a burn wound [5].

Autoradiographic investigation of the cultures in the early stages (2nd-4th day) after seeding revealed a larger number of dividing cells in the case of growth on the collagen substrate (10-30%) compared with cells growing on plastic (10-20%). In the later stages of culture these differences disappeared, because cells growing on a collagen substrate filled the free space more quickly and, correspondingly, the rate of incorporation of labeled thymidine was reduced. It was shown by electron-microscopic autoradiography that mainly cells which, in their ultrastructural features, could be classed as basal cells, proliferated. They very often contained melanosomes and the nucleus was always surrounded by a network of tonofibrils (Fig. 2). Much less frequently the label was incorporated by more highly differentiated cells with a wide-looped network of tonofibrils, resembling in structure the tonofibrillar apparatus of the cell of the stratum spinosum of the epidermis (Fig. 3).

To sum up, it is possible by morphological methods to determine the state of epidermocytes over a period of time and, on that basis, to indicate ways of improving the process of their culture. One way, as was shown above, is to use a collagen substrate as the feeder layer. This method speeds up adhesion of the cells to the substrate appreciably, stimulates the proliferative activity of the epidermocytes, and promotes the rapid formation of an epidermal sheet suitable for transplantation.

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