USE OF AN ARTIFICIAL LIPID MEMBRANE TO OBTAIN CELL CULTURES OF A SPECIFIED FORM

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An artificial thin lipid membrane applied to a slide is nonadhesive for normal fibroblasts and cells of various lines. If narrow grooves are scratched in the film, cells entering them become attached, increase in length, and incorporate thymidine-H^3. If the film is removed from part of the slide, and after a monolayer has formed on it, grooves are made in the film the cells will migrate along them from the monolayer. Interaction between the cells and phospholipid film can simulate interaction between the cell and the surface of another cell. The suggested method can be used to study intercellular contacts, multiplication of cells in islets, and grooves, and their collision with each other.

To study many of the problems connected with interaction between cells in tissue culture (contact inhibition of movement and multiplication, electrical contacts, and so on) it is essential to be able to arrange the cells in such a culture beforehand in a particular manner, i.e., to obtain a culture of specified shape and size. For this purpose the surface of an adhesive substrate (for example, a glass slide) must be covered with a substance nonadhesive to cells, leaving only certain areas of the required shape and size uncovered. In that case the cells will adhere and proliferate only on the areas of the uncovered glass. The substance covering the substrate must evidently possess the following properties: 1) it must be nonadhesive for the cells studied; 2) it must not dissolve in the medium; 3) it must be firmly attached to the slide so that the shape and size of the uncovered areas do not change in the course of the experiment; and 4) it must be nontoxic for the cells. The writers have used several substrates, but most did not satisfy all these requirements. For example, paraffin wax does not stick firmly to the glass; teflon and films of fatty acids, cholesterol, and other substances possessed varied degrees of adhesiveness. Lipids applied as membranes (a thin uniform film) to the slide alone possessed all the properties listed above.

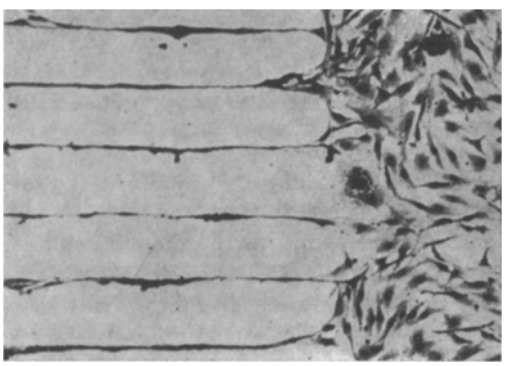

Fig. 1. Fibroblasts migrating from monolayer into grooves (100 ×).

The object of this investigation was to study whether a thin lipid film can be used to obtain cell cultures of a preassigned form.

EXPERIMENTAL METHOD

Lipids were isolated from the white matter of the bovine brain with a chloroform–methanol mixture [6, 9]. The material was washed twice or three times with 0.1 M KCl solution, yielding lipids giving a transparent solution in chloroform. The solution was dried on a rotary evaporator and the dry residue dissolved in decane or benzene to give a concentration of 10 mg/ml.

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Fig. 2. Fibroblasts labeled with thymidine-H^3 in a groove. Mayer's hematoxylin, 400×.

To apply the film a cover slip was placed in the center of a stage revolving at 2000-2500 rpm. A drop of solution (~0.02 ml), falling in the center of the cover slip, spread out as a uniform layer and formed a thin, glossy, transparent film [4]. Areas of the cover slip of the required shape and size could be freed from film. In the present experiment, half of the cover slip was freed from film with benzene or decane. On the half of the cover slip covered with the film, straight grooves were scratched by means of a razor blade or an electrolytically sharpened tungsten electrode. The cutting instrument and the cover slip with the lipid film were secured to a microtome so that straight grooves could be formed at specified intervals. In this way grooves 10 μ (±2 μ) in width could be drawn.

The following types of cells were used: 1) normal embryonic fibroblasts from trypsinized ASn mouse embryos, 2) line L cells [5], 3) line KHIM cells obtained at the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, from a tumor induced by subcutaneous implantation of a plastic disc, and 4) MTR cells, obtained at the same Institute and derived from epithelioid cells of the mouse kidney transformed with SV-40 virus. The cells were grown in penicillin flasks. The composition of the medium and the method of obtaining autoradiographs and carrying out time-lapse microfilming were described previously [1, 2].

EXPERIMENTAL RESULTS

The experimental results show that the lipid film is nonadhesive for all types of cells used: when grown on a cover slip half of which was covered by lipids the cells spread out and proliferated only on the uncovered glass; cells in contact with the edge of the lipid film became elongated and arranged themselves along its border. Analysis of the frames of the time-lapse motion pictures showed that the undulating membrane of a fibroblast, when it came into contact with the edge of the phospholipid film, did not cease to undulate and did not adhere to the surface of the lipids. Meanwhile, a new membrane began to develop at the side, leading to a change in orientation of the cell. If the lipid solution was not completely removed, particles to which the cells could adhere could be seen on the surface of the film.

The lipids were firmly adherent to the color slip; measurements showed that the widths of the groove scratched in the film were unchanged after incubation for many days in nutrient medium and medium with serum, with or without cells.

The phospholipid film is nontoxic; if grooves are scratched in it beforehand, only the cells which fall in them will spread out. Because of the nonadehesiveness of the remainder of the substrate, the cells are stretched out along the grooves to form a monoline. If the grooves are scratched after the monolayer has formed on the cleaned half of the cover slip, the cells migrate along the groove (Fig. 1). Cells compressed in narrow (20 μ wide) grooves divide; if thymidine-H^3 is added to the medium the cells in the grooves incorporate the label (Fig. 2) just as in the control monolayer. (On the 4th day of cultivation the percentage of labeled cells in the grooves was 15 ± 1; in the monolayer it was 16 ± 1.) If the cells migrate from a formed monolayer, just as in the case of migration into a wound [2] the labeling index in the grooves is considerably higher than in the monolayer.

Cells falling on the upper surface of the lipid film did not spread although analysis of the frames of the motion-picture film showed that their membrane was in motion; small appendages appeared and disappeared quickly. Special experiments showed that 3 days after seeding the normal mouse fibroblasts falling on the lipid film remained viable and capable of spreading and proliferating when applied to the cleaned cover slip.

The phospholipid film thus satisfies the requirements for a nonadhesive substrate. The causes of differences in the adhesiveness of substrates for cells are not clear. Taylor [11], who investigated various substrates, showed that adhesion of fibroblasts is unconnected with the hydrophilic or hydrophobic character of the substrate. This fact has previously been established for platelets [7]. Rosenberg [10] reported that the degree of spread of human connective-tissue cells on the boundary between two liquid phases (hydrophobic and hydrophilic) is considerably reduced if ovolecithin or its synthetic derivative is added to the partition boundary.

On the basis of their investigations of interaction between artificial phospholipid bimolecular membranes, Liberman and Nenashev [3] postulated that because of the greater electrostatic force of repulsion, the linking of cells to the phospholipic membrane may be less strong than the linking of cells to the glass. Interaction between cells and the artificial phospholipid membrane is also interesting from the point of view that it may be used as a simplified model of interaction between the cell surface and the membrane of another cell.

The method suggested in this paper can be used, for example, to study intercellular contacts and proliferation of cells in grooves and islands and also to analyze the movements of the surfaces of cells on impact.

When this investigation had been completed, the writers learned of the results of work by Lieberman et al. [8], who cultivated myoblasts in grooves drawn in semidried agar applied to collagen. This method also can evidently be used for the purposes indicated above.

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