Method for Obtaining Several Microcultures in the Same Petri Dish

The large volume of cell cultures in virology laboratories imposed the necessity of finding rapid and economical micromethods for obtaining these cultures. Thus, the cultivation of cells necessary for following up the viral cytopathic effect, in cups of glass[1] or plastic pannel[2], has been proposed instead of the classic method in glass tubes. A drawback in comparison to the classic method is that an inverted microscope is necessary for studying the cultures in the cups. The existing micromethods and tube cultures do not permit fine microscopic examination of the cell with high magnification objective lenses. The method proposed avoids these drawbacks and in addition makes possible simultaneous treatment of the microcultures in the same Petri dish.

Material and Method. Droplets of cell suspensions in growth medium are pipetted on to the glass surface, forming spots of desired size, isolated from one another by a hydrofobe material, non-toxic for the cells.

The following material was used: Petri dishes (Jena glass 80 mm diam.), sterilized by autoclavation, and paraffined paper bands or discs (60°–65° paraffin and thin writing paper) in which 3 or 4 mm diam. holes are punched (PP).

KB, HeLa and L cell suspensions (2–3 × 10^5 cells/ml) obtained from trypsin dispersed monolayers and human embryo fibroblast suspensions (4–5 × 10^5 cells/ml) obtained by trypsinization. The same growth medium was used on Hanks and Earle's solution, 0.5% lactalbumin hydrolysate and 10% calf serum[3]. The maintenance medium was prepared with 0.5% lactalbumin hydrolysate in Earle's solution and 2.5% calf serum. Both media contained penicillin (200 U/m) and streptomycin (50 μg/ml).

A cytopathic effect was obtained with poliovirus (type 1 Sabin), vaccinia virus or adenovirus (type 3) cultivated on KB cells and inoculated into the same type of cells, after 1:10 dilution in the maintenance medium.

The same cells cultivated in tubes were used as controls for the microcultures (MC).

The PP disc is introduced into a Petri dish, which is gradually heated until the paraffin melts, so that by cooling perfect adhesion of the PP to the bottom of the dish is obtained. After 1 h irradiation with a Hanau type germicidal lamp, a droplet of 0.02–0.025 ml cell suspension in growth medium is pipetted in each hole. The Petri dish is kept at 36 °C (±1°) in a CO_2 (5%) incubator. Growth of the cells is followed up by placing the overturned Petri dish on the microscopic stage. The medium droplets are not dislodged when the dish is turned downwards (Figure 1).

The growth medium is changed on the 3rd day separately for each MC or at the same time for 24 MC in the same Petri dish, by washing the MC with 2–3 ml new medium, which is then drawn off with a pipette. A draw of new medium is added to each MC. All the MC in one Petri dish were simultaneously stained by hematoxylin-eosin according to the usual technique.

Results and discussion. By this method several uniform, monolayered MC can be obtained in a single Petri dish within 24–48 h (Figure 2A). The surface of a MC represents 40–50% of the corresponding punched hole. The aspect of the KB, HeLa and human embryo fibroblast MC, followed up for 10 days was similar to that of the controls. The hazard of infection of the MC was minimal.

Inoculation of a MC with poliovirus (type 1 Sabin), vaccinia virus or adenovirus (type 3) did not produce

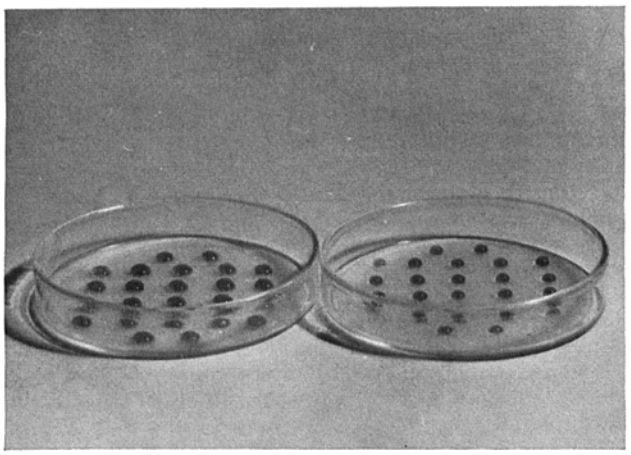

Fig. 1. Cell microcultures in droplets separated by a perforated paraffined paper disc adhering to a Petri dish.

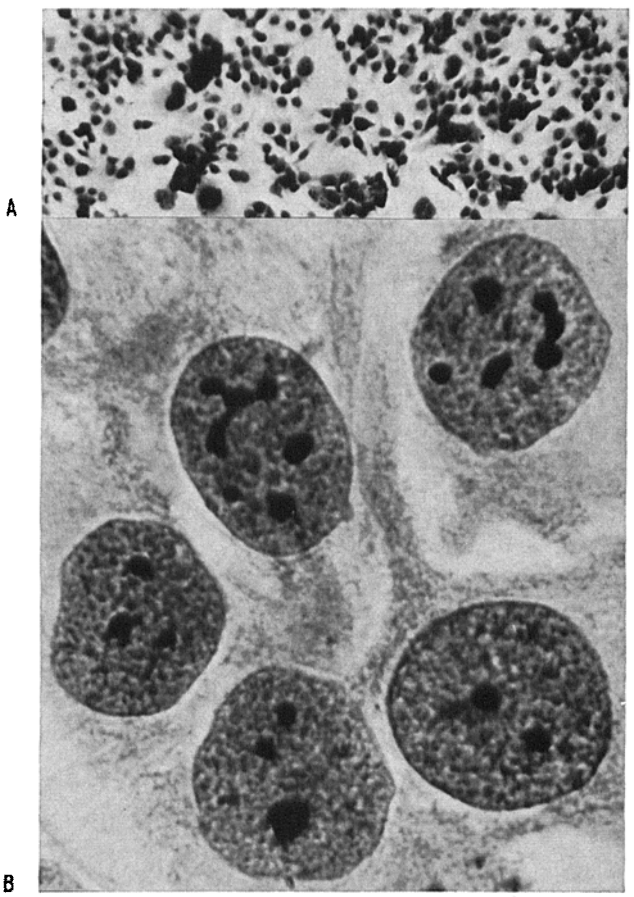

Fig. 2. Aspect of a KB cell microculture stained with hematoxylin-eosin. (A) normal monolayer (oc. 10, obj. 7); (B) detail (oc. 10, obj. 90).

[1] E. Mann-Weilep, Arch. ges. Virusforsch. *14*, 263 (1964).
[2] M. J. Rosenbaum, J. A. Phillips, Elisabeth I. Sullivan, A. E. Earle and L. F. Miller, Proc. Soc. exp. Biol. Med. *113*, 224 (1963).
[3] I. Aderca, M. Ianconescu and M. Nachtigal, Studii Cerc. Inframicrobiol. *10*, 187 (1959).

contamination of this neighbouring MC at 8 mm distance after 8 days microscopic follow-up.

Evidence of the destructive cytopathic effect was observed in the microscope or by simultaneous staining of the intact MC in a Petri dish with 3 ml of a 1:5000 neutral red solution. Hematoxylin-eosin staining permitted fine microscopic study of the cultivated cells with the immersion objective lens (Figure 2B). The method has certain evident advantages. The cells can be studied with any of the objective lenses of the common light microscope. The necessity of an inverted microscope and of special methods for fine cytological studies (cultivation on glass slides in Barki or Leighton tubes) are thus eliminated.

The cell MC, being placed in the same recipient, can be treated simultaneously, thus ensuring uniformity of the experimental conditions and rapid execution.

The amount of medium and cells used is reduced fifty-fold, e.g. from the content in cells of a 12/120 mm culture tube over 50 MC can be obtained. All the operations can be done by a single person within a shorter time, using the common facilities of any laboratory. The results obtained and the advantages of this method show its utility for laboratories using cell cultures.

Zusammenfassung. Die gleichzeitige Züchtung vieler Mikrokulturen in ein und derselben Petri-Schale wird beschrieben. Simultane Behandlung der Einzelkulturen und der mikroskopischen Kulturobjektive ist möglich.

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