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CULTURE OF STRAIN L MOUSE FIBROBLASTS ON SILICA-GEL SLIDES

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A method of culturing strain L mouse fibroblasts on silica-gel slides, prepared from KSK silica gel, has been developed. Sterile silica-gel slides, free from organic impurities, suitable for petri dishes or test tubes, can be used successfully as supporting media for the normal development of this culture.

KEY WORDS: culture of mouse fibroblasts; silica-gel slides.

Culture of fibroblasts, especially from strain L mice, is carried out on very complex liquid media containing many components. As they develop, the cells of this culture become attached to the smooth surface of the slide, flask, or tube, to form a continuous monolayer. On a solid water-repellant surface or a soft hydrophilic surface the cells do not form a monolayer and, consequently, they do not develop. Maroudas [7] considers that in such cases it is the structure of the medium on which the fibroblasts develop which is mainly responsible.

Rovenskii et al. [3, 4, 9] describe a series of investigations which indicate that cultures of strain L mouse fibroblasts can be grown on various supporting substrates with an oriented structure. They include fibrous gels, fish scales, and also slides prepared from various chemically inert polymers, such as polyvinyl chloride, polymethacrylate, and polyethylene. By means of the scanning electron microscope, these workers observed the dynamics of development and attachment of mouse fibroblast cells to a polyvinyl chloride slide 40 μ thick. They found that cells can attach themselves to such slides, on which intensive growth of the culture was possible.

In the course of work connected with selection of the mold Asp. terricola strain No. 5, the writer found it necessary to devise a method of culturing strain L mouse fibroblasts on a solid sterile medium that would promote its normal development. Furthermore, the supporting medium must be free from organic impurities and suitable for direct microscopic examination of the growing culture by means of an ordinary, and not an inverted, microscope.

The results of these investigations are described below.

EXPERIMENTAL METHOD

Experiments were carried out with a well-developing 3-day culture of strain L mouse fibroblasts grown on liquid medium No. 199 [8]. In view of the demands with respect to the medium listed above, and in the attempt to create conditions for growth of the culture similar to those usually obtaining when grown on glass, an attempt was made to grow this culture on thoroughly washed cellophane and on silica-gel slides. The experiments with cellophane were unsuccessful. For the work with silica-gel slides, the KSK brand was used. The silica sol for these slides was obtained by Kryukov by Gal'chenko's method* [1].

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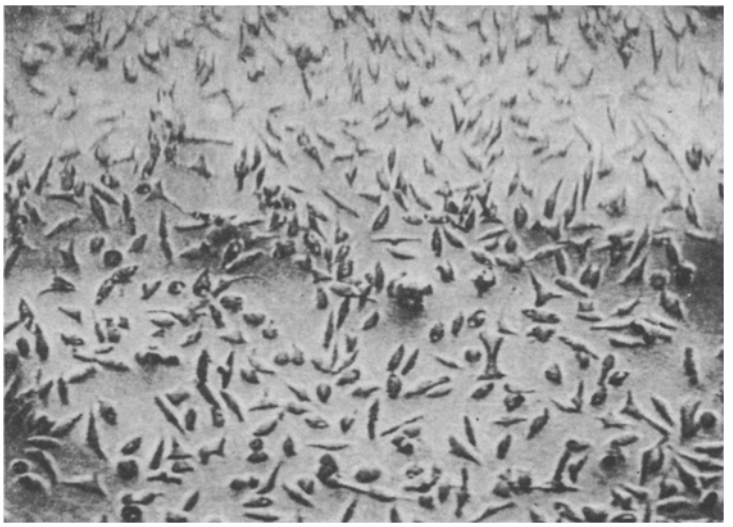

Fig. 1. Control. Cells of 48-h culture of strain L mouse fibroblasts developing on glass wall of tube. Here and in Figs. 2 and 3, 100×.

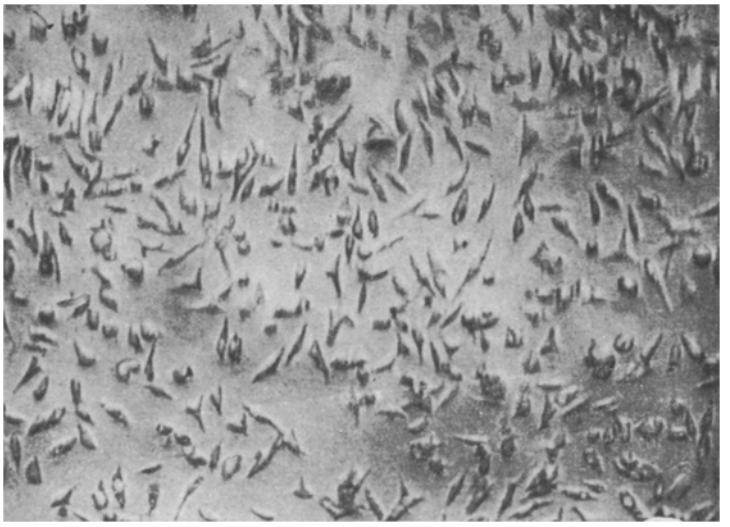

Fig. 2. Experiment. Cells of 48-h culture of strain L mouse fibroblasts growing on silica-gel slides at pH 7.0-7.8.

According to data given by Gal'chenko in his paper, silica-gel slides for Petri dishes and coverslips for test tubes are obtained by mixing sterile silica-sol with sterile mineral medium in the ratio of 4:1. Under these circumstances the salt composition of the test medium must be of fivefold concentration.

This technique was somewhat modified. The complex media used to grow the cultures of mouse fibroblasts contained Earle's [5] or Hanks' [6] balanced salt solutions, of which the main component is NaCl in a concentration of 0.8-0.68%.

According to Paul [2], the Na ion is extremely essential for maintenance of the osmotic pressure of the growth and supporting medium for this culture.

With these considerations in mind, in the present experiments a 0.8% solution of NaCl was added to the silica-sol in the ratio of 1:1 in accordance with the following scheme to obtain the silica-gel slides.

Silica sol was poured in a volume of 1 ml into sterile, specially washed test tubes and they were sterilized at 1 atm. A sterile 0.8% solution of NaCl was prepared separately. Next, under aseptic conditions, 1 ml of NaCl solution was added to each tube containing silica-sol. After rapid and thorough mixing (by rotatory

Fig. 3. Experiment. Deformed cells of 48-h culture of strain L mouse fibroblasts developing on silica-gel slides at pH 6.7-6.8.

movements) of the fluids the tubes were racked in a sloping position. After 6-10 h completely translucent solid slopes convenient for microscopic examination were formed. In this stage special attention must be paid to the pH of the mixed sol and NaCl solution, which must lie between 7.0 and 7.3. At more alkaline pH values the sol does not gel, whereas weakly acid pH values are unfavorable for development of the fibroblast culture. To obtain the optimal pH it is best to equilibrate the mixed fluids after they have been sterilized by the careful addition of a sterile 1% solution of $NaHCO_3$.

To determine whether the culture could grow on silica-gel, 2 ml of sterile medium No. 199 and 0.15 ml of a suspension of cells of a mouse fibroblast culture were added to each experimental test tube containing a silica-gel slide. Tubes without silica-gel slides, to which the medium and seeding material also were added, were used as the control. After seeding, the experimental and control tubes were closed with specially prepared sterile rubber stoppers in order to prevent volatilization of the CO_2 formed, which is essential for growth of this culture. The tubes were then racked at an angle of 3° to the horizontal so that the medium added during seeding covered the silica-gel slides. The experimental temperature was maintained at 37°C. After 24 h, a continuous monolayer of a well developed culture, indistinguishable in every respect from the monolayer formed on the wall of the control tubes, was observed on the surface of the slide. A 48-h culture from the control and experimental series is illustrated in Figs. 1 and 2. The photomicrographs clearly reveal normal development of the cells on the silica-gel slide. They are firmly attached to its surface and spread out at both ends just as on the glass in the control. However, such good development of the fibroblast culture on these slides can be obtained only if the pH of the supporting medium is close to neutral. If the pH falls to 6.7-6.8, a monolayer is produced but the cells forming it have an obviously degenerative appearance. Their membrane is condensed, they are reduced in size, and they become vacuolated and very granular. One such 48-h culture is illustrated in Fig. 3. A reduction in the pH of the slide to 6.5 leads to complete absence of growth and death of the cells.

The good development of the test culture on silica-gel which was observed can evidently be attributed to the fact that the conditions of growth on this substrate are very close to those created when grown in the usual way on glass.

If sterile, free from organic impurities, and with a smooth and even surface, this supporting medium enables the cells of the fibroblast culture to adhere to it and to develop normally. Silica-gel slides can thus be used successfully as a solid support for media during microbiological investigations with cultures of strain L mouse fibroblasts.

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