membrane was omitted. On the contrary, in many cases there was a definite improvement of differentiation. Our findings indicate that the cellophane membrane can be omitted, when dissociated cerebral cortical cells from young chick embryos are cultivated, thus simplifying the technique of culture in ROSE chambers.

The ROSE chamber culture system affords advantages for testing the effect of growth factors. It is therefore necessary to be able to cultivate the nerve cells without cellophane membrane when some factors are unable to dialyze through this membrane.

The system of cultivating dissociated nerve cells offers new possibilities for electro-physiological studies. These studies, as previously performed by CRAIN, PETERSON and BORNSTEIN[12] on explants, were done in Maximow assembly. ROSE chambers with cellophane were used for this purpose with some difficulties. Stripping out the cellophane, what is necessary in these experiments, could evoke a 'mechanical shock' of the cells and influence their membrane potential and functional abilities (LODIN, personal communication)[13].

Zusammenfassung. Dissoziierte Nervenzellen der Gehirnhemisphären von Hühnerembryonen wurden in ROSE-Kammern mit und ohne Cellophanmembran kultiviert. Die Differenzierung der Neurone wurde mit Phasenkontrast und histochemischen Methoden untersucht.

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[13] Acknowledgments. This work was supported in part by the 'Actions Thématiques sur Programmes: Différenciation Cellulaire' No. 4112, and the South African Medical Research Council. We are particularly grateful to Dr. Z. LODIN for helpful discussions. We thank J. BOOHER for reviewing the manuscript. We are grateful to Mrs. M. F. KNOETGEN for her expert technical assistance.

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A Simple Method for the Determination of the Growth Rate in Human Fibroblast Cell Cultures

A variety of methods are available for measuring the rate of multiplication of cells in culture, the most common among them being based on cell counts after trypsinization and resuspension, or protein determinations at regular intervals[1,2]. Each of these methods has its advantages and disadvantages, but we found that neither was suitable for comparative growth studies of a large number of human fibroblast cell lines with chromosome anomalies[3].

The method finally chosen was based on the technique originally used by PUCK, MARCUS, and CIECURA[4] to measure the clonal growth of HeLa cells. They deposited single cell suspensions in nutrient medium in Petri dishes and counted the number of cells in some of the resulting clones at various time intervals thereafter. However, unlike heteroploid cells such as HeLa cells which are epithelial in nature and which usually remain together after cell division to form a discrete colony, human fibroblasts have a pronounced tendency to migrate, and after the two- or three-celled stage it is impossible to recognize individual colonies at the concentrations needed for optimal growth. Therefore, instead of counting the cells in a single colony, we counted the number of cells in the same delimited area daily, thus measuring the number of cells in colonies initiated from single cells less those cells which migrated out of the delimited area. We made the

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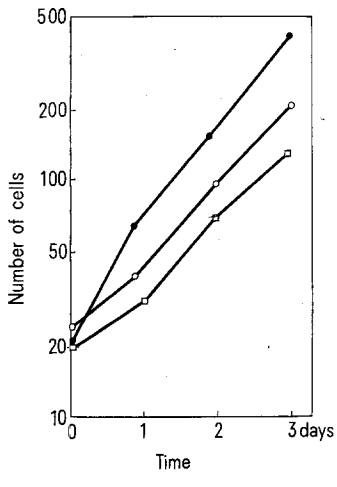

Fig. 1. Representative growth curves, L 205 cells. Open circles: 9th passage, 20,000 cell inoculum. Closed circles: 17th passage, 10,000 cell inoculum. Open squares: 13th passage, 15,000 cell inoculum.

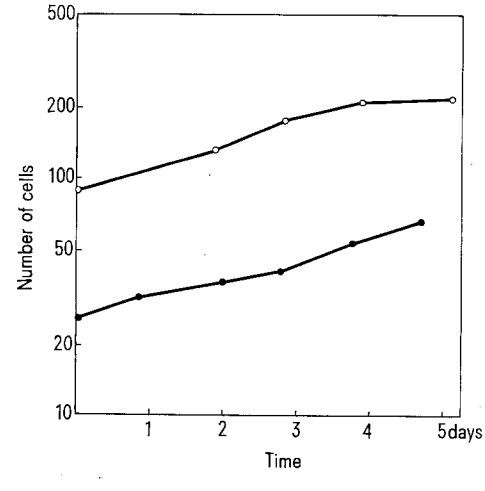

Fig. 2. Growth of L1116 cells. Closed circles: 13th passage, 10,000 cell inoculum. Open circles, 14th passage, 20,000 cell inoculum.

assumption that, on the average, for each cell which migrated out of our circumscribed area, another cell would migrate into it. The establishment and maintenance of the cell lines have been described[3]. For growth studies, well-dispersed cells in about 6 ml of growth medium were placed in small plastic flasks (Falcon plastics) at a concentration of 10,000 to 20,000 cells per flask and incubated in a humidified CO_2 incubator. A pattern of squares was scratched on the bottom of the flask, and the number of cells in each square was counted 4 to 6 h after subcultivation and daily thereafter at $\times 100$ magnification under an inverted microscope with the illumination and diaphragm regulated to obtain maximum contrast. Each cell line's growth rate was determined in duplicate during at least 3 different passage levels.

Data from typical growth experiments are presented in Figures 1 and 2. Cells were randomly dispersed over the surface of the flask except at the edges where cells became confluent sooner. Although early experiments indicated that the location or nature of the pattern of squares did not make any difference, we always used a pattern of 3 to 6 squares, side-by-side, half way between the middle and edge of the flask and parallel to its long axis. Duplicate growth curves obtained with inocula of 10,000 to 20,000 cells were parallel; larger inocula sometimes led to areas of confluency on day 3 which caused difficulties in numeration.

Comparisons between results obtained from standard growth studies performed by trypsinization-resuspension methods and those obtained by counts of growing cells in plastic flasks showed no significant differences in doubling times. There were no variations at different passage levels during phase II[5], nor when experiments were performed by different individuals. It was initially feared that debris from cells which did not attach to the plastic surface might exert a toxic effect and inhibit the multiplication of attached cells, but replacing the medium in the flasks after 6 h incubation with fresh medium had no effect on the growth rate. This experiment also indicated that none of the increase in cell numbers after the first counts were performed was due to attachment of cells formerly in suspension to the plastic surface.

Discussion. This method makes possible the accurate determination of growth curves and doubling times of human fibroblasts in cell culture. The technique is simple, fast and economic. Only 20,000 to 40,000 cells and a few plastic flasks suffice for the whole experiment versus several 100,000 cells for trypsinization-resuspension methods which also entail more manipulation of cells with concomitant introduction of errors.

We have found this technique particularly useful for our comparative studies of abnormal human cell lines, but it could also be adapted to screening of various chemical and biological agents for their effects on cell multiplication, or to other routine growth studies.

Résumé. Description d'une technique simple permettant de mesurer le rythme de croissance in vitro des fibroblastes humains. Les suspensions cellulaires sont cultivées en flacons plastiques et les cellules présentes dans des surfaces délimitées sont dénombrées à des temps réguliers. Les applications de cette technique sont discutées.

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CONGRESSUS

Israel

1st International Congress for Bacteriology

in Jerusalem, 2–7 September 1973.

This will be the first international congress of the newly formed Bacteriology Section of the International Association of Microbiological Societies.
Further information about the congress may be obtained from the Congress Secretariat, P.O. Box 16271, Tel Aviv, Israel.

Austria

First International Congress for Aerosols in Medicine

in Vienna, 19–21 September 1973

Aerosols in Medicine' (Advantages and Dangers). Main Topics: 19 September: Environmental Aerosols (Air Pollution), Hygienic Aspects of Aerosols.

Secretary of the Congress: Mrs. E. Weidenhaus, Wiener Medizinische Akademie, Stadiongasse 6–8, A–1010 Vienna, Austria.

Turkey

IAEA Symposium on Radioimmunoassay and Related Procedures in Clinical Medicine and Research

in Istanbul, 10–14 September 1973.

Further information by the scientific Secretaries: Dr. E. J. Garcia and Dr. E. H. Belcher, International Atomic Energy Agency, Kärntner Ring 11–13, A–1010 Wien (Austria).

Switzerland

Symposium on Thirst organized by the Swiss Union of Societies of Experimental Biology

in Lugano, 20 and 21 October 1973.

The symposium will be held under the auspices of the International Commission 'Regulation of Food and Water Intake' of the International Union of the Physiological Sciences. Further Information by: Prof. Georges Peters, Institut de Pharmacologie de l'Université, 21, rue du Bugnon, CH–1011 Lausanne/Switzerland.