Since our therapy simulates metrazol treatments it may be well to compare the two. The present procedure insures a greater depletion of the oxygen saturation of the blood than does the metrazol. With metrazol the hemoglobin saturation may decrease to 40%, while with our treatment the hemoglobin saturation was 30% or lower, going down to 15%. Despite a degree of anoxemia more profound than that attained with that of metrazol the patient is nevertheless under control of the anesthetist, thus insuring greater safety.

Though our experience is limited with this treatment of schizophrenia, the results are encouraging. Many more data must be accumulated before conclusions can be drawn. Nevertheless, the results with anoxia do suggest that a profound diminution of cerebral metabolism produced by this technic exerts favorable effect in the course of schizophrenia.

We are grateful to J. F. Fazekas, S. J. Martin, H. E. Doudna, and H. C. Slocum for their coöperation in this investigation.

10209 P

Causes of the Discontinuity of Growth of Fibroblasts Cultivated in Embryo-Juice.

LILLIAN E. BAKER.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

Fibroblasts that are cultivated according to the flask-technic in a plasma-coagulum with embryo-juice as nutrient fluid do not proliferate continuously. Generally after 2 or 2½ weeks' cultivation the cells stop multiplying, although only a small portion of the coagulum is covered with tissue. If a part of the tissue is transferred to a new coagulum, growth is resumed. If it is not so transferred the cells degenerate. Investigators who have attempted to find the cause for this cessation of growth and subsequent degeneration of the cells have attributed it either to changes that take place in the physical structure of the plasma-coagulum, or to the accumulation of toxic products therein.[1] The experiments reported here were

[1] Ephrussi, B., *Arch. d'Anat. Micros.*, 1933, **29**, 95; Fischer, A., *Cytologia*, 1930, **1**, 217, and *Virchow's Arch. Path. Anat. u. Physiol.*, 1930, **279**, 94; Mayer, E., *Arch. f. Entwich. Mech.*, 1933, **130**, 382; *Compt. Rend. Soc. Biol.*, 1935, **119**, 422; Olivo, O. M., *Arch. f. Exp. Zellforsch.*, 1931, **11**, 261.

designed to test another hypothesis, namely, that it might be the removal of serum from the coagulum that is responsible for the cessation of growth. Or, to express it in another way, it might be an inadequacy in the food supplied.

In the first experiment 2 fragments of tissue from a 23-year-old* strain of chick-heart fibroblasts were embedded in a flask 3½ cm in diameter in a coagulum containing 33% plasma. They were then cultivated for 9 days in 33% embryo-juice.† By this time their initially rapid growth had already decreased to a noticeable degree. Two drops of chicken-serum were then given every 48 hours in addition to the embryo-juice that had previously been supplied. The cultures immediately resumed active growth. But, after 9 more days of cultivation their growth-rate diminished again. The concentration of embryo-juice was then increased to 66%, and serum was given as before. Again, active growth was resumed. This time it continued until the edge of the colony reached the vertical side of the flask.

These results indicate that two factors are involved in the cessation of growth: (1) that serum is needed as well as embryo-juice as a nutrient for the cells, and (2) that the embryo-juice must be supplied in higher concentration than that usually given. To test these hypotheses further, 3 experiments were made. Cultures of chick-heart fibroblasts were divided into 2 equal parts. These were embedded in separate flasks, 3½ cm in diameter, in coagula containing 33% chicken-plasma. A single fragment of tissue was placed in each flask as near the center of the coagulum as possible. In the first experiment one-half of the original culture was cultivated in 33% embryo-juice, the other in 33% embryo-juice to which serum at either 4 or 8% concentration was added. In the second experiment one-half of the tissue was cultivated in 66% embryo-juice, the other in a mixture of 66% embryo-juice and 8% serum. And in the third experiment serum an 8% concentration was given to both tissues but one was supplied with embryo-juice at 33% and the other with embryo-juice at 66% concentration. In each case, a few days were allowed to elapse at the beginning of the experiment before the serum was added to the nutrient fluid, since there was at this time more than an adequate supply of serum in the coagulum. And, of course, each experiment was repeated a number of times.

In the first experiment the colony cultivated in 33% embryo-juice

* Experiments made in 1934 to 1935, but not published.

† Made by extracting one volume of pulp from 9-day embryos with 3 volumes of Tyrode's solution.

stopped its active proliferation after 2 weeks, *i. e.,* at just about the time that all of the serum was removed from the coagulum. The sister-culture which was given serum as well as embryo-juice proliferated for a longer time, and grew to be much thicker, but did not fill the flask. In the second experiment the colony that was cultivated in 66% embryo-juice grew at an exceedingly active rate for 14 days, but, then, while still proliferating actively suddenly liquefied the coagulum. The sister-colony that received serum with the embryo-juice continued its proliferation and eventually covered the entire coagulum, forming a colony 3¼ to 3½ cm in diameter, and many cell-layers thick. In the third experiment the colony receiving serum and embryo-juice at 33% concentration grew much more slowly than the sister-colony and stopped growing before the coagulum was covered with tissue, while the one which received serum and embryo-juice at 66% concentration grew as long as cultivation was continued, *i. e.,* until the colony reached the vertical side of the flask. In one of these experiments in which a strain of heart-tissue in its sixth passage *in vitro* was used the tissue covered the entire coagulum in 17 days. In other experiments continuous growth was observed for a period of 30 to 36 days.

It would seem, therefore, that the cessation of growth observed under the usual conditions of cultivation is not due to the change that occurs in the structure of the coagulum. Neither is it due to the accumulation of toxic substances in that coagulum, but is caused by the removal of serum which is required for proliferation in addition to embryo-juice.‡ Moreover, after the colony has reached a certain size a higher concentration of embryo-juice than that which is usually given must be supplied.

‡ Hanging-drop cultures of fibroblasts in a medium made by extracting embryo-pulp with serum have been reported by des Ligneris, M. J. A., *Arch. Exp. Zellforsch.,* 1936, **18**, 442, but the object of his work and the results obtained were quite different from those reported here.