HUMAN FIBROBLASTS GROWN FOR A YEAR IN A MEDIUM OF SHEEP PLASMA AND TWO SOLUTIONS OF KNOWN COMPOSITION [1]

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In a previous article, published in this journal, it was shown that human fibroblasts can be grown for a considerable length of time in heterogeneous plasma without using any tissue extract (3). It was possible to keep such tissue growing for a period of three months, using as a medium a mixture of beef plasma and a feeding solution, the main components of which were Witte peptone, glucose, insulin, hemin, thyroxin, and cysteine.

The purpose of this article is to report upon a strain of human fibroblasts which have been kept alive and actively growing for a period of twelve months. Since these cultures were controls in a series of experiments in which human fibroblasts were grown in sheep plasma, the latter was used instead of beef plasma in the preparation of the culture medium. The plasma was prepared from blood taken aseptically from a sheep and therefore did not require sterilization either by treatment with ultra-violet light or by filtering through a Chamberland-Pasteur candle, as is now done regularly in the preparation of sterile beef plasma for tissue culture work. The citrated sheep plasma, to which equal amounts of calcium Ringer solution and the feeding solution, as formerly described (3), had been added, proved to be an excellent culture medium for human fibroblasts. The latter were grown from several thyroid specimens obtained from the operating room. The tissue grew out as vigorously as when beef plasma is used, showing the same latent period of two or three days. Subcultures were made every fourteen days.

Among several series of fibroblast cultures grown in sheep plasma, one strain was obtained from the thyroid gland of a twenty-seven-year-old woman who was operated upon for a non-toxic nodular goiter. The tissue, grown in Gabritschewsky dishes, showed a vigorous growth of fibroblasts. Subcultures were made at regular intervals of fourteen days by either one of the three following methods: (*a*) the cultures were cut out from the plasma and placed in fresh culture medium; (*b*) the trypsin method previously described (4) was used; (*c*) the cultures were cut into two halves, and each placed in the mixture of plasma and feeding solution. The third method is used generally in the cultivation of animal tissues. However, cultures from the adult human body are more difficult to handle than cultures from embryonic tissue,

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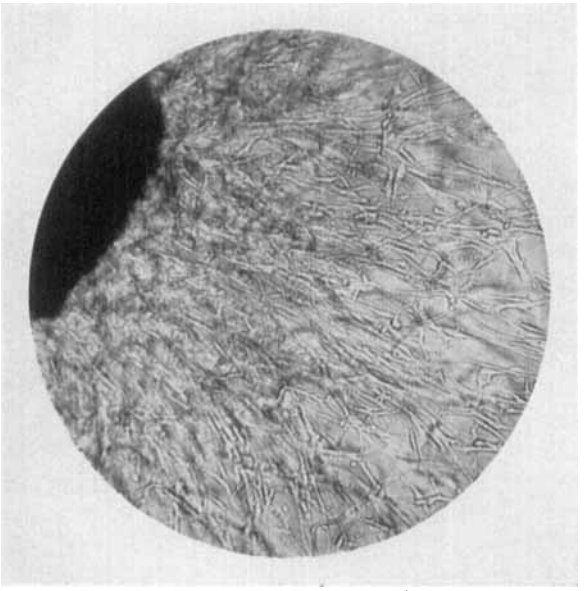

FIG. 1. SEVEN-MONTHS-OLD STRAIN OF FIBROBLASTS OBTAINED FROM THYROID OF WOMAN TWENTY-SEVEN YEARS OLD

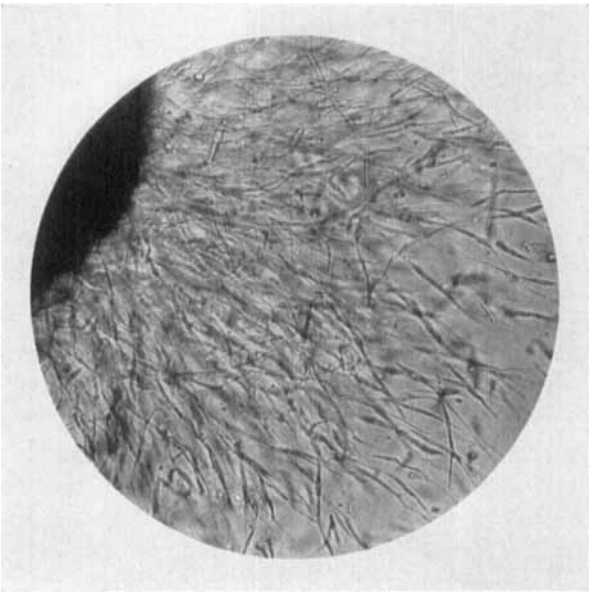

FIG. 2. SAME STRAIN AS SHOWN IN FIG. 1, EIGHT AND A HALF MONTHS OLD

which is used for most tissue culture experiments. It may happen that some of the cultures, after having been cut in half, show a decrease in activity. In such cases the cultures were allowed to grow for fourteen days and then either cut out as a whole or treated with trypsin. The trypsin treatment, which was applied only once to the strain of cultures here described, frequently has a very favorable influence on the activity of the tissue. All the old plasma is dissolved, making it possible for the cells to grow immediately into the fresh medium. When interpreting the apparent revival of the tissue activity after the trypsin treatment, one should not only consider the fact that the dissimilation

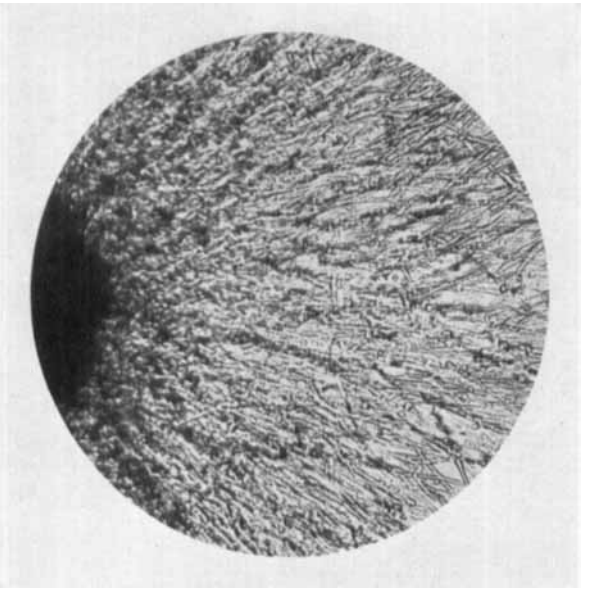

FIG. 3. THYROID CULTURE FROM WOMAN FIFTY-TWO YEARS OLD

products which have accumulated in the plasma are removed, but also take into account that, during the dissolution of the plasma, numerous protein digestion products are formed which may have a beneficial influence on the cells. The accompanying physical and physico-chemical changes also should be taken into account. Carrel and Baker (2) proved that cleavage products of fibrin form an excellent material for the growth of chicken fibroblasts. However, they used peptic digests in their experiments. Later it was shown by the same authors that normal rat fibroblasts proliferate rapidly in both peptic and tryptic digests of fibrin (1).

During the period of twelve months that the above mentioned strain of fibroblasts has been under observation, a decrease in growth activity occurred in the last month. This was probably due to technical difficulties encountered at that time. It seems very probable that human fibroblasts can be grown for an indefinite period in the mixture of sheep plasma and feeding solution. This presumably also holds true for beef

plasma, since no essential differences were observed between cultures grown in sheep plasma and those in beef plasma. As previously stated (3), cultures of human fibroblasts do not show any decrease in growth activity after having been grown for three months in a medium made up with beef plasma.

Figs. 1 and 2 show cultures from the same strain of human fibroblasts grown *in vitro* for several months. Cultures like these can be obtained easily from various thyroid specimens. The above strain, as has been said, was procured from a twenty-seven-year-old patient. However, a similar growth activity is shown by tissue from persons of a much greater age. A very good growth has been noted, for example, with thyroid specimens from patients over fifty years of age (Fig. 3).

A permanent strain of cultures is significant, because a more uniform material is available for various experiments than when cultures are prepared from tissues of different patients. It is an essential condition for the study of the action of certain factors on cellular activity over a long period of time. It may be very useful, for example, in the study of the action of carcinogenic agents on human tissue.

The composition of the medium used in the work reported in this article is only partly known, since plasma was used in its preparation. Efforts are now being made to replace the plasma by a substance of more accurately known composition.

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