Physiological Studies on Tissue in vitro.

I. Influence of Temperature upon the Growth of Fibroblasts in Coverglass Cultures

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The following experiments were undertaken from a practical technical point of view to determine the longest time during which fibroblasts in coverglass cultures could be left at certain temperatures in the unaltered medium, keeping the activity to grow further at a usual rate.

NEMOTO (1929) determined the duration of life of the fibroblast in vitro in various temperatures, but he did not pay much attention to the activity of the tissue after such treatment. But it is just this point that is necessary for the practice as we know thus the time during which we can escape from the trouble of changing the medium when the tissue is only to be stored.

The experiments were performed by Mr. OGATA, technical assistant in the laboratory.

Method

The hanging-drop method was used. The culture medium was composed of one drop of embryonic tissue juice and of plasma of the chicken. The fibroblast to be experimented upon was derived in the usual way from the heart muscle of the chicken embryo and was used after at least 6 passages. After the final preparation of the cultures, they were placed in the incubator of 39° for one day in order to examine whether they were in good condition, and if they were found to be good, they were distributed into the thermostats at temperatures to be experimented upon.
Experiments

1. In the refrigerator

Nemoto found that at 5°C the longest time during which all the cultures remained alive in an unaltered medium was 3 days. His method of ascertaining the life duration was to change the medium of the tissue, to put it into the incubator of 39°C and to observe after one day whether any new growing cells appeared. We examined the activity of the tissue by transferring it into 39°C without changing the medium, after having kept it for certain days in the refrigerator, and by observing the growth after one or two days. In the case where only a poor growth was observed, we changed the medium of that tissue as usual and continued the observation as to whether the tissue would recover the former activity of the growth gradually. In this paper we have remarked this only when the tissue, which had shown a poor growth, recovered its activity at the latest in the second passage of the medium.

The experiment was performed during January and February, 1930. The temperature of the refrigerator was from 1°C to -2°C, mostly at -1°C. We found no freezing of the medium or the tissue.

In 19 pairs of the cultures, two members of each pair being two halves of one tissue fragment, when it had stayed one day in the refrigerator, the tissue, after having been transferred into the incubator of 39°C, showed some retardation of the growth on the first day, but on the second the growth was almost as good as usual. After 2 days stay in the refrigerator the activity showed much variation. Of 30 pairs of the cultures, 13 pairs showed almost the same activity as in the case of one day stay in the refrigerator; 7 pairs showed the same in one member of each, while in the other members one showed no growth and the others poor growth, of which one showed a good growth at the second passage; 6 pairs showed poor growth, of which one pair in both members of it and 2 pairs in one member of each showed good growth in the later period of the culture; 2 pairs showed poor growth in one member of each and no growth in the other; and the remaining 2 pairs showed no growth at all.

On the whole the 2 days stay in the refrigerator affected the activity of the tissue much more than the one day stay.

BuccianTe (1929) observed that tissues of a 7–8 days chicken embryo in the shell could survive at the freezing temperature for 7–8
days. The result cannot be compared with ours, however, as the method is quite different.

2. At 25°C. and 30°C.

The temperature at which all the tissues could survive for the longest time was found by NEMOTO to be 30°C and the next to the latter to be 20°C. We compared the effects of 25°C and 30°C during certain days in regard to the activity of the tissue to grow. Two halves of a tissue were cultivated separately as a pair, and after one day incubation at 39°C were distributed into 25°C and 30°C. After certain days their media were changed and they were kept at 39°C for 2 days. Then their activities to grow were compared. When only a poor growth was found we changed the medium and continued the observation as in the case of the refrigerator.

The experiments were performed during March and July. The temperature variation was mostly smaller than ±1°C, but for a few days in July when the room temperature was over 30°C in day time and we did not succeed in controlling the thermostats, it was about ±2°C.

8 days stay (13 pairs of experiments) and 9 days stay (16 pairs) showed no apparent effect at both 25°C and 30°C.

In the case of 10 days stay (16 pairs of experiments), the growth after the first changing of the medium showed a distinct retardation at both 25°C and 30°C, but in the second passage the tissues recovered their former activities.

In the case of 11 days stay 17 pairs were experimented upon. At 30°C 2 cultures showed no growth at all, and another 2 only poor sprouts of cells in the first passage, the latter showing no recovery in the second passage. The other 13 cultures showed a distinct retardation of the growth in the first passage, but recovered their activities in the second passage. At 25°C all the 17 cultures showed some retardation of the growth in the first passage, but recovered in the second quite well.

On the whole staying at 30°C for 11 days was not so favourable as at 25°C.

In the case of 12 days stay 14 pairs were experimented on. At 30°C 5 cultures showed no growth, 4 very poor growth in the first passage, of which one no growth in the second passage, one showed a poor growth still in the second passage but no growth in the third, and the other two showed good growth in the second. The remaining 5 cultures showed a distinct retardation in the first passage, but recovered
the usual growth in the second. At 25° the results were not essentially different from those in the case of 11 days stay.

Here too it was shown that the condition at 25° was on the whole more favourable for the activity to grow than at 30°.

In the case of 13 days stay 18 pairs were used. At 30° 14 cultures showed no growth in the first passage, one a very poor growth and the other 3 a little better growth, the latter 4 recovering the activities in the second passage. At 25° 13 cultures showed much retardation of the growth in the first passage, of which 4 showed a poor growth in the second and the third passage (no further observation), and 6 recovered their activities in the second passage (3 infected), and 5 showed a better growth in the first passage and regained their former activities in the second.

On the whole, while 25° in this case retarded the growth of the tissue much more than in the former case, and the tissues could still mostly recover their activities, 30° in this case was very much more unfavourable for the growth.

The tissue showed a very poor but distinct growth at 25°; a quantitative comparison of the latter with that at 30° will be made on another occasion.

**Result**

The longest time during which all the tissues in the hanging drop cultures retain their activities to grow in unaltered media was one day at −1°C., 12 days at 25°C. and 10 days at 30°C.

The present experiments may be regarded as the continuation of Dr. NEMOTO's above cited experiment which I suggested to him when I was working in the Physiological Laboratory of Prof. SATAKE at Sendai. It is a great pleasure for me to acknowledge on this occasion my indebtedness to Dr. ALBERT FISCHER formerly of the Institute of General Pathology at Copenhagen, who gave me during my stay in his laboratory, facilities to carry out my experiments in tissue culture and suggested to me several problems, among which was the one dealt with in this paper.

**References**