THE INFLUENCE OF TEMPERATURE AND FLUID MEDIUM ON THE SURVIVAL OF EMBRYONIC TISSUES IN VITRO.*

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The object of this report is to present briefly the results of studies upon the duration of life of embryonic tissues preserved in vitro at varying temperatures and in several fluid media, the method of tissue cultivation being used to determine the viability of the tissue.1

There are many reports in the literature concerning the survival of tissues and organs after removal from the body, especially of the tissues which are frequently transplanted surgically in human beings. Wentzchcr (1), for example, was able to graft pieces of human skin preserved for twenty-two days in vitro; Ljunggren (2) obtained positive results with skin kept for thirty days in tubes of ascitic fluid; while Carrel (3) reported recently that he had been able to transplant to other individuals pieces of skin removed from a child, after two to seven weeks' preservation in cold storage; the pieces preserved for the longer period, however, were not uniformly successful.

Similar reports have been made regarding the preservation of other tissues. Tuffier (4) transplanted omentum kept for one month in cold storage; Magitot (5) grafted corneas which he had preserved for eight days; and Davis (6) obtained good results with fascia transplanted after being stored for forty days at 32° C.

In all these instances the survival of the organs or tissues was indicated by the success of the transplantation. A clinically successful transplantation does not, however, prove that the transplanted cells were living, for Levin and Larkin (7) and Carrel (8)

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have shown that segments of killed blood vessel may be successfully
grafted, the dead tissue affording a temporary but competent tube
which is gradually replaced by the tissues of the host. It may
nevertheless be assumed that many adult tissues, especially skin and
structures of a purely connective tissue type, are able to survive,
under suitable conditions, for days or even weeks after removal
from the body. But this may not be true of embryonic tissues.
Indeed, Askanazy (9) found in his transplantation experiments that
the tissues of rat embryos kept in the ice box for longer than eight
days gave regularly negative results.

In our investigations, in which the tissues of chick and rat
embryos have been used, the results as regards the possible duration
of life in vitro and the influence of certain factors (temperature
and fluid medium) on this period of survival have been so definite
that it seems probable that the points brought out may have a more
general application. It is rather on this account than because of
the interest of the results themselves that this report is made.

EFFECT OF TEMPERATURE.

The technique used in these studies was quite simple. Small
pieces of the tissue (chick or rat embryo) were preserved generally
in hanging drop plasma preparations, which were carefully sealed
with vaselin. The slides were divided into groups and stored at
different temperatures. After varying periods of time the slides
were removed, the pieces of tissue were transferred to fresh drops
of plasma, and the new preparations were incubated at 37° C. This
method of preserving the tissue in hanging drop slides is especially
convenient and reduces to a minimum the opportunity for infecting
the tissue in handling.

Two refrigerator ice boxes were used in the majority of the ex-
periments. The temperature in them varied somewhat in different
seasons, but for periods of one to three weeks the variations were
not, as a rule, greater than 2° to 3° C.

The first series of experiments was carried out in midwinter
when the temperature of one of the ice boxes was as low as —6°
to —7° C. A series of slides containing pieces of chick embryo was
stored at this temperature; another series was placed in a north room without a radiator, in which the temperature during the course of the experiment ranged between $+12^\circ$ and $+14^\circ$ C. After periods of from two to sixteen days, pieces of tissue from both series were transferred to fresh drops of plasma and incubated at $37.5^\circ$ C. Since the freezing point of chick tissue is about $-7^\circ$ C., some of the ice box specimens were found to be frozen. The tissue from none of these grew upon incubation. Tissue from the unfrozen preparations preserved as long as six days at this temperature exhibited active growth upon incubation. Eight day preparations gave only occasional growths, while tissue preserved ten days or longer at this temperature gave uniformly negative results. Tissue kept eight and ten days at room temperature, however, showed active growth, as did also, though less regularly, twelve day specimens; sixteen day preparations were uniformly negative. Repetitions of this experiment gave practically the same results.

It was concluded, therefore, that chick tissues may be preserved longer at cool room temperature than at a temperature just above the freezing point of the tissues, and, further that the limit of survival even at the more favorable temperature is relatively short (twelve to fourteen days).

A second series of experiments was carried out later when the temperature in the two refrigerator ice boxes was respectively $-1^\circ$ to $0^\circ$ C. and $+6^\circ$ to $+7^\circ$ C. The temperature at this time in the room used varied from $+18^\circ$ to $+21^\circ$ C. The tissue was preserved, as before, in the form of fairly small fragments in plasma sealed in hanging drop slides. Transfers were made after varying intervals up to twenty days as in the previous experiment. The results may be briefly stated as follows: The tissue preserved for eight days at $-6^\circ$ to $-1^\circ$ C. showed active growth upon incubation; ten day preparations were viable although many of the transfers were negative, and the positive cultures exhibited a relatively long latent period (two to three days). Tissue preserved for a longer time at this temperature failed to grow. Tissue preserved at $+6^\circ$ to $+7^\circ$ C., on the other hand, was regularly found to be viable after twelve to fifteen days, and some of the sixteen and eighteen day preparations gave a fair growth upon incubation.
Tissue preserved at \( +18^\circ \) to \( +21^\circ \) C. survived for from nine to eleven days; the tissue kept for a longer time failed to grow.

Stated summarily, then, it was found that of specimens of chick tissue kept at temperatures around \(-6^\circ\), \(0^\circ\), \(+6^\circ\), \(+14^\circ\), and \(+20^\circ\), those at \(+6^\circ\) survived longest; temperatures around \(0^\circ\) and \(+14^\circ\) were about equally favorable. Since chick tissues survive freezing at \(-8^\circ\) to \(-10^\circ\) for a few hours at most, and exposure to \(-20^\circ\) for a few minutes only, and since at incubator temperatures (\(30^\circ\) to \(37^\circ\)) the same tissue may be preserved in a state of active life for many weeks or even months, a curve plotted to show these variations in length of life at different temperatures would present first almost a horizontal line, then a sharp rise, then a gradual rise, then a slow decline, followed by a second rise (text-figure 1).

The experiments upon the effect of temperature were repeated with rat embryonic tissues instead of chick tissues. The findings were in general the same as with chick tissue. At \(0^\circ\) the period of survival was about ten days; at \(+6^\circ\), a few days longer. Rat tissues were more readily killed by freezing.
It occurred to us that in regard to the medium used there might be at least two factors influencing the duration of life of the tissue: first the kind of medium, and secondly the quantity of fluid used in proportion to the amount of tissue. Four isotonic fluids were used as media: plasma, unmodified or diluted blood serum, Ringer solution, and physiological salt solution (0.85 per cent. sodium chloride). The same technique was used as in the temperature experiments, except that in some instances instead of making hanging drop preparations, the tissue and fluid were placed in the bottom of the cavity slides and sealed as before. The specimens were kept at 0° and ± 6° C.

It was found, in brief, that the duration of life was not appreciably influenced by the use of the different fluids. It seemed conceivable, however, that although the kind of isotonic medium might be without effect, the relative quantity of fluid used and the size of the tissue fragments might exert an important influence. It seemed logical to suppose that a larger quantity would allow the dilution of the waste products of tissue metabolism, thus favoring a longer period of survival. To test this hypothesis small pieces of embryo were preserved in dishes containing 50 to 100 cubic centimeters of Ringer solution or diluted blood serum, and entire embryos were placed in another dish containing only enough fluid to cover the surface of the embryo. Pieces of tissue were also placed in dishes wrapped in gauze moistened with Ringer solution.

Subsequent transfers of the tissue showed little or no difference in the duration of life under the several conditions. In one experiment the tissue preserved in the form of entire embryos with very little fluid survived three days longer than the tissue divided into small fragments in a large dish of fluid, a result contrary to what our working hypothesis led us to expect. It seemed probable that the better preservation in the first case was not due to the smaller amount of fluid used but to the slighter mechanical injury which the larger piece of tissue suffered in handling.
CONCLUSIONS.

1. Embryonic chick and rat tissues preserved at temperatures ranging from $-7^\circ$ to $+20^\circ$ C. live longest at about $+6^\circ$ C. The duration of life under the most favorable conditions is less than twenty days.

2. The kind of isotonic medium used,—plasma, serum, Ringer solution, or normal salt solution,—does not appreciably influence the period of survival. The quantity of medium in proportion to tissue is similarly without marked effect.

BIBLIOGRAPHY.