THE INFLUENCE OF HEAT ON DIFFERENT SERA AS CULTURE MEDIA FOR GROWING TISSUES.*

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PLATES 59-61.

In the cultivation of tissues outside of the organism, heterogenic plasma has proved a useful culture medium. In it, cells of mammals grow rather rapidly for several days at least. Carrel and Burrows 1 have shown that embryonic chick tissues develop extensively in plasma from rabbits, dogs, and human beings. Lambert and Hanes, 2 who cultivated rat sarcoma in heterogenic media, were able to keep this tissue alive in guinea pig plasma for thirty days by transferring it to fresh medium every seventh day. Of the media examined by them, only goat plasma proved unsuitable as a culture medium. In goat plasma no growth occurred. All these authors emphasize the fact that the growth of tissue is more rapid and extensive in homogenic than in heterogenic media.

In order to understand more thoroughly the laws governing life and growth of tissues outside of the organism, and possibly to find an explanation of the variations in growth in different media, it seemed to me to be of importance (1) to make an exact comparative investigation of the quantitative growth in autogenic, homogenic, and heterogenic media, and (2) to investigate the growth in heated as well as in unheated media. I have begun a study of these problems, and the present paper gives a preliminary report of my results.

In this series of experiments, I have used as a culture medium a mixture of serum and 2 per cent. agar. 3 Serum was chosen instead

* Received for publication, February 20, 1912.
1 Jour. Exper. Med., 1911, xiv, 244.
3 Fluid serum has been used as a culture medium by Carrel and Burrows (loc. cit.), who applied fine cotton threads as a scaffold for the growing cells. They found homogenic as well as heterogenic serum a useful culture medium for embryonic chick tissue.
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of plasma, in order that the medium might be exposed to heat without altering its physical qualities. Heating the medium exerts a marked influence on it, as will be shown by the results of this investigation.

Technique.—A 2 per cent. solution of agar in distilled water was kept fluid in a water bath at 60° to 65° C. It was quickly taken up in a pipette, and when the temperature had fallen to 38° to 40° C., it was thoroughly mixed with serum in the proper proportions. The

mixture of serum and agar was then placed upon the piece of tissue on the cover-glass where it coagulated, forming a transparent homogenic jelly, which covered completely the small fragment of tissue. It is important that the preparation of the serum agar mixture be made very quickly, to prevent coagulation from taking place before the mixture covers the fragment of tissue. It is also important that the coagulation be complete, so that the presence of small pools of serum between clumps of agar may be prevented.
The serum and agar were mixed in the ratio of 3 to 1 or 4 to 1, and this proportion gave a fairly good culture medium. Agar dissolved in distilled water was slightly hypotonic. This should have been an advantage, for, according to Carrel and Burrows, plasma diluted with two fifths of its volume of distilled water is a better culture medium than normal plasma.

In this medium I have cultivated the bone marrow of adult guinea pigs. The bone marrow was taken from the femur, was placed in Ringer solution, and was then cut into small pieces the size of a pin-head. Each piece was placed on a cover-glass and covered by the culture medium. After the medium coagulated, the cover-glass was turned over, so as to have the tissue on its under surface. The cover-glass was then placed on a hollow slide, and was incubated at 38° C.

The appearance of living cells in the serum agar medium is the same as that of cells in plasma, and during the first few days there are only very slight differences, quantitatively, in the growth in these two media.

**Bone Marrow in Plasma.**—As a control on the results with serum agar, the following description of the cultivation of bone marrow in plasma is given: A short time after the incubation of the culture, leucocytes start their emigration from the fragment by active ameboid movements, so that the tissue, after two or three hours, is surrounded by a dense area of living cells. This area rapidly increases in size during the following twenty-four hours, but the increase is less rapid on the second and third days. After three days, the ameboid movements decrease, and after four or five days, movement stops almost completely, the leucocytes become globular, and disintegration begins. It is difficult to keep leucocytes alive for more than five days, but by this time the non-ameboid connective tissue cells of the fragment have started to grow, and have formed an area of new cells around the tissue. The description of the later development of these cells, however, is of no importance for this investigation, and will be omitted.

In serum agar, during the first few days of incubation, the same appearance is shown. The same area of living ameboid cells sur-

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rounds the tissue after a few hours, and increases rapidly in size during the first day. But the length of life of the cells is shorter in the serum agar medium than in plasma, and I have usually been unable to keep the leucocytes alive for more than three days, while life for four days was exceptional.

By living cells, I mean leucocytes with active ameboid movements. The term is possibly inadequate, the cells perhaps being alive for some time after they have ceased to move. Ameboid movements are, however, an indisputable sign of life. Moreover, the cessation of movement can be easily determined, while beginning degenerative alterations are not always very evident. As for the life of the leucocytes outside of the organism, there may be some question as to whether we are dealing with real growth and multiplication of cells, or only with an active emigration. It is probable that the latter assumption is the correct one, for there is no evidence of the multiplication of the leucocytes.

The question of whether the leucocytes actually multiply or only emigrate from the bone marrow is of no consequence in our present study, for we are interested not in the multiplication of the leucocytes, but in the growth of the bone marrow. The ameboid movements and the behavior of the leucocytes are regarded as being of great importance, for under the most favorable conditions (growth in plasma), it has been found that the emigration of the leucocytes is always the first stage of the growth of bone marrow. As the growth of the non-ameboid cells of the bone marrow is too slow to make direct measurement practicable, the intensity of the ameboid movements of the leucocytes has been used in these experiments as an index to the influence exerted upon bone marrow by the various culture media.

Guinea pig bone marrow has been cultivated in serum from the same animal (autogenic serum), in that from another guinea pig (homogenic serum), and in that from other mammals (heterogenic serum); e. g., in serum of the rabbit, cat, dog, mouse, goat, and man. In each experiment, three series of cultures were prepared; one in an autogenic, one in a homogenic, and one in a heterogenic medium. In each series, moreover, three or four cultures of bone marrow were made in agar plus unheated normal serum, and three
or four cultures in agar plus serum which had been previously heated to 56° C. for half an hour. Only absolutely good cultures were used. Cultures were always discarded if there was liquefaction of the medium or an uneven mixture of agar and serum that might give rise to passive movements of leucocytes due to currents in the medium. Moreover, only those experiments were utilized in which all the results in each given series corresponded.

The cultures were examined and the results recorded after six, eighteen, twenty-four, and forty-eight hours. Camera lucida drawings were then prepared, which give a quite exact comparison between the growths in different cultures.

RESULTS.

Even after six hours' incubation, the differences between the emigration of cells in autogenic and homogenic serum were obvious. The area of cells invading the surrounding medium was larger and denser in autogenic than in homogenic serum, a difference which was evident during the two following days (figures 1 and 2).

Autogenic and homogenic serum heated to 56° C. for half an hour proved to be of less value, for in these the area of emigrated cells was smaller than in the corresponding unheated autogenic and homogenic serum media. The difference was most marked between the unheated and the heated autogenic serum, but heat seemed to be a real inactivator for the homogenic serum also. The difference between the growth in unheated and heated sera increased during the first twenty-four hours, and reached a maximum during the second day. At that time, the unheated culture medium was almost totally pervaded by leucocytes. On the third day, however, the growth in the inactivated medium overtook that in the unheated serum mixture.

The length of life of the cells was about the same in autogenic and homogenic sera, whether these were heated or unheated.

The normal heterogenic sera gave rise to a growth of cells which was far less rapid and extensive than that in the autogenic and homogenic sera.

There were, moreover, differences in the growth between different heterogenic sera (figures 2 and 3).
In normal rabbit serum, after eighteen hours, the piece of bone marrow which was originally red was quite pale, and in the small area of emigrated cells, no ameboid movements were seen. Moreover, after the eighteenth hour, no increase in the area of the emigrated cells was detected.

The growths in the other normal heterogenic sera had the same appearance, but in these death of the cells took place earlier. The sera employed, in the order of the most to the least favorable, are as follows: rabbit, mouse, dog, man, cat, and goat. Goat serum was unsuitable as a culture medium. In goat serum, a very insignificant emigration of leucocytes was seen. After six hours, the leucocytes in goat serum formed a small and irregularly shaped area of cells. Only a few of the leucocytes had ameboid movements, and after a short time, those that showed it ceased altogether to move. No increase of the area of emigration was seen after six hours.

Quite different from the results in autogenic and homogenic sera were those obtained by heating the heterogenic sera, for heating these sera increases their value as culture media. The area of emigrated cells was much larger in heated than in unheated heterogenic serum, and the length of life of the cells that varied from six hours (goat) to eighteen hours (rabbit) in unheated serum, was increased to thirty-six to forty-eight hours in heated heterogenic sera. In heated goat serum, we can see very active ameboid movements after twenty-four hours; and in heated rabbit serum, ameboid movements may be seen even on the third, fourth, and fifth days.

Further experiments similar to those here described are in progress.

In closing, I wish to thank Dr. Carrel, whose technique I have studied, for many valuable suggestions.

CONCLUSIONS.

1. Bone marrow of guinea pigs grows better in autogenic than in homogenic serum.

2. Bone marrow of guinea pigs grows more extensively and rapidly in unheated than in heated autogenic and homogenic serum (56° C. for half an hour).
Fig. 1.
3. In different heterogenic sera, there are differences in the growth of the tissue. In none of the sera examined, was the growth so extensive as in homogenic and autogenic serum.

4. Heated heterogenic serum is a better culture medium and shows more extensive tissue growth than normal unheated heterogenic serum.

EXPLANATION OF PLATES.

Figures 1, 2, and 3 are camera lucida drawings of cultures of guinea pig bone marrow in different sera, heated and unheated. The black spot in the center represents the original piece of tissue, and the surrounding dark grey and light grey areas represent the emigrated leucocytes. The magnification is the same in all the figures.

PLATE 59.

Fig. 1. A six hour old culture of bone marrow in (a) unheated autogenic serum, in (b) heated autogenic serum, in (c) unheated homogenic serum, in (d) heated homogenic serum.

PLATE 60.

Fig. 2. A twenty-four hour old culture of bone marrow in (a) unheated autogenic serum, in (b) heated autogenic serum, in (c) unheated homogenic serum, in (d) heated homogenic serum.

PLATE 61.

Fig. 3. An eighteen hour old culture of bone marrow in heterogenic sera, heated and unheated.