AN ADDITION TO THE TECHNIQUE OF THE CULTIVATION OF TISSUES IN VITRO.*

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Plates 25 to 27.

In a previous article,[1] we have described a technique which permits of the growth *in vitro* of adult and embryonal tissues of mammals and other warm and cold-blooded animals. In this article, we shall mention briefly some modifications or improvements of the technique, relating to the medium of growth and the manner of preparation of the tissues and the cultures.

THE MEDIUM.

We have employed two kinds of media; namely, natural and artificial, so called.

The natural media were solid or fluid, consisting either of coagulated plasma or fluid serum.

Coagulated plasma has always given the best cultural results. The plasma can be kept in a fluid condition in cold storage for a long time. We have found chicken plasma, after having been preserved for two months, to be still an excellent culture medium. The use of the plasmatic medium is, therefore, very convenient. Autogenic and homogenic plasma constitute the best media, but heterogenic plasma can also be used, as has been shown by Lambert and Hanes.[2] For instance, in our experiments fetal tissues of the chicken grew extensively in human, rabbit, and dog plasma. In general, however, it may be said that the development of the tissues

* Received for publication, June 14, 1911.

[1] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 387.

[2] Lambert and Hanes, *Jour. Am. Med. Assn.*, 1911, lvi, 587; *Jour. Exper. Med.*, 1911, xiv, 129.

is slighter, and often much less satisfactory in heterogenic than in autogenic and homogenic plasma.

We also employed normal plasma modified in different ways. Plasma diluted with two fifths of its volume of distilled water is more favorable to the growth of some tissues than is normal plasma. Ringer's solution, in the proportion of one part for one or two parts of plasma, or even in higher proportions, increases the rate and generally also the extent of the growth. A marked acceleration of growth has been observed when extracts of certain tissues, or heterogenic serum in which tissues have been allowed to autolyze, are used for diluting the normal plasma. The most extensive growths have been obtained in media of this type.

The natural fluid medium which we employed is serum. The growth is never as luxuriant in it as in the plasmatic media, for the reason that the cells find no support in the medium and hence must grow in a thin layer on the cover glass. Embryonal and tumor tissues grow well both in autogenic and homogenic sera; and an excellent growth may also be obtained in heterogenic serum. For example, the fetal tissues of the chicken grew extensively in rabbit serum, and equally well whether it was obtained from defibrinated blood or from coagulated plasma. Probably, therefore, the alexin is without essential effect on the growth of the cells.

The artificial media, so called, have given us positive results only as regards the cultivation of embryonal tissues or of tumors, and in these the growth was never as great as occurs in the natural media. The artificial media have been employed especially by M. R. and W. H. Lewis.[3] The medium advocated by them is composed of Locke's solution, agar, and bouillon. The one employed by us is composed of Locke's solution, to which 2 per cent. agar is added. We have not found that the addition of bouillon is an advantage. The proportion of salts can be altered in many ways, at least without necessary disadvantage. Tissues of the embryonal chick grow in .8 per cent. sodium chlorid solution, in Locke's and Ringer's solutions, and in various combinations of sodium chlorid, potassium chlorid, and calcium chlorid. In these media, the development goes

[3] Lewis, M. R., and Lewis, W. H., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 126; *Anat. Rec.*, 1911, v, 277.

on well for a short time; but neither the extent nor the duration of growth is nearly so great as in the plasmatic medium. Moreover, pure sodium chlorid solution is in itself harmful to the cells. This is shown by the fact that when fragments of chick embryos are well washed by maceration in large quantities of salt solution, they no longer grow even in plasma, while after a similar washing in Locke's solution, growth still takes place.

An important fact is the following: toluol can be added in the proportion of 4 per cent. to solid or fluid media, without apparent disturbance of the growth of tissues such as skin and nerve cells, while bacteria added in small quantities to the toluolized media do not multiply.

PREPARATION OF TISSUES AND CULTURES.

In our early experiments we employed tissues taken directly from the living animal. We found afterwards that tissues preserved in cold storage would grow. Spleen, skin, and heart of fifteen or sixteen day old chick embryos were kept for one day, two days, and even six days in cold storage before imbedding in the plasma and incubating. Although the latent period was markedly increased, the growth was characteristic.

For storing tissues, Ringer's solution may be employed, but not sodium chlorid solution, as it is toxic. Serum also is a less favorable medium of storage than Ringer's solution.

We first used the hanging drop culture and the larger cultures on plates; but more recently we have modified the technique with the view of obtaining better support for the cells and better nutrition for the cultures.

For the cultures in the plasmatic medium, watch glasses, sealed on glass plates, have been substituted for the ordinary cover glass. They are filled with a large quantity of plasma, or their internal surface is covered by a thin plasmatic layer. A fragment of spleen cultivated in plasma and serum has thus been kept alive for one month.

Fragments of tissue have been cultivated on pieces of silk veil impregnated with plasma (figure 1). It is a simple matter to cut the artificial tissue composed of living cells, plasma, and silk into

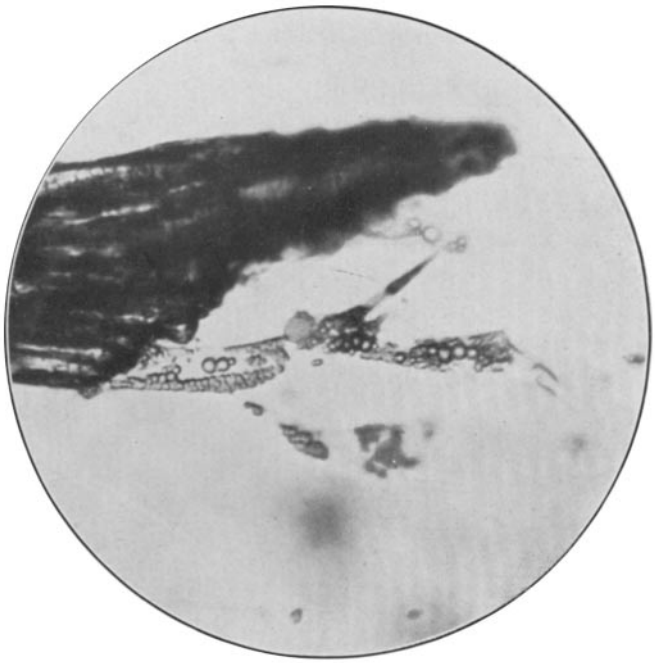

FIG. 1.

FIG. 2.

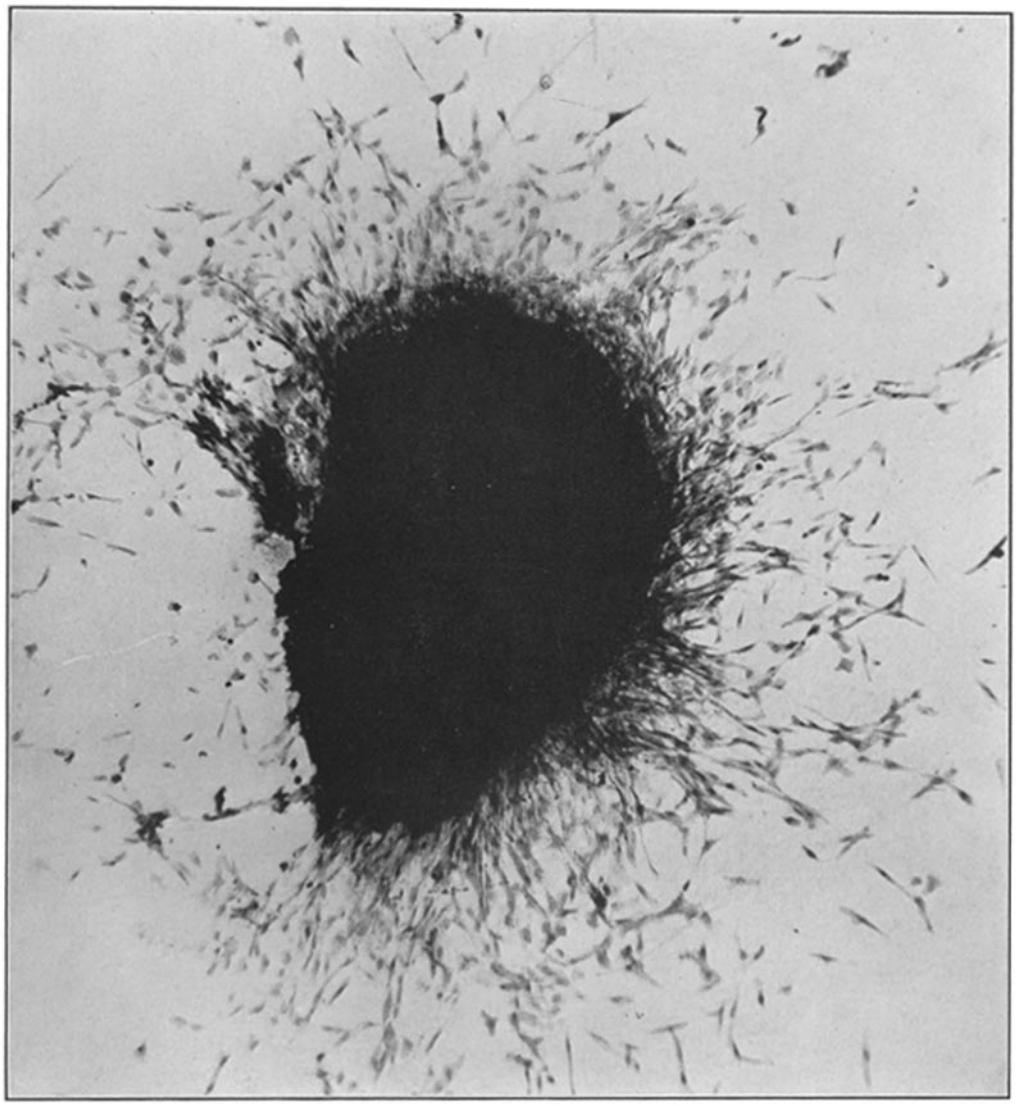

FIG. 3.

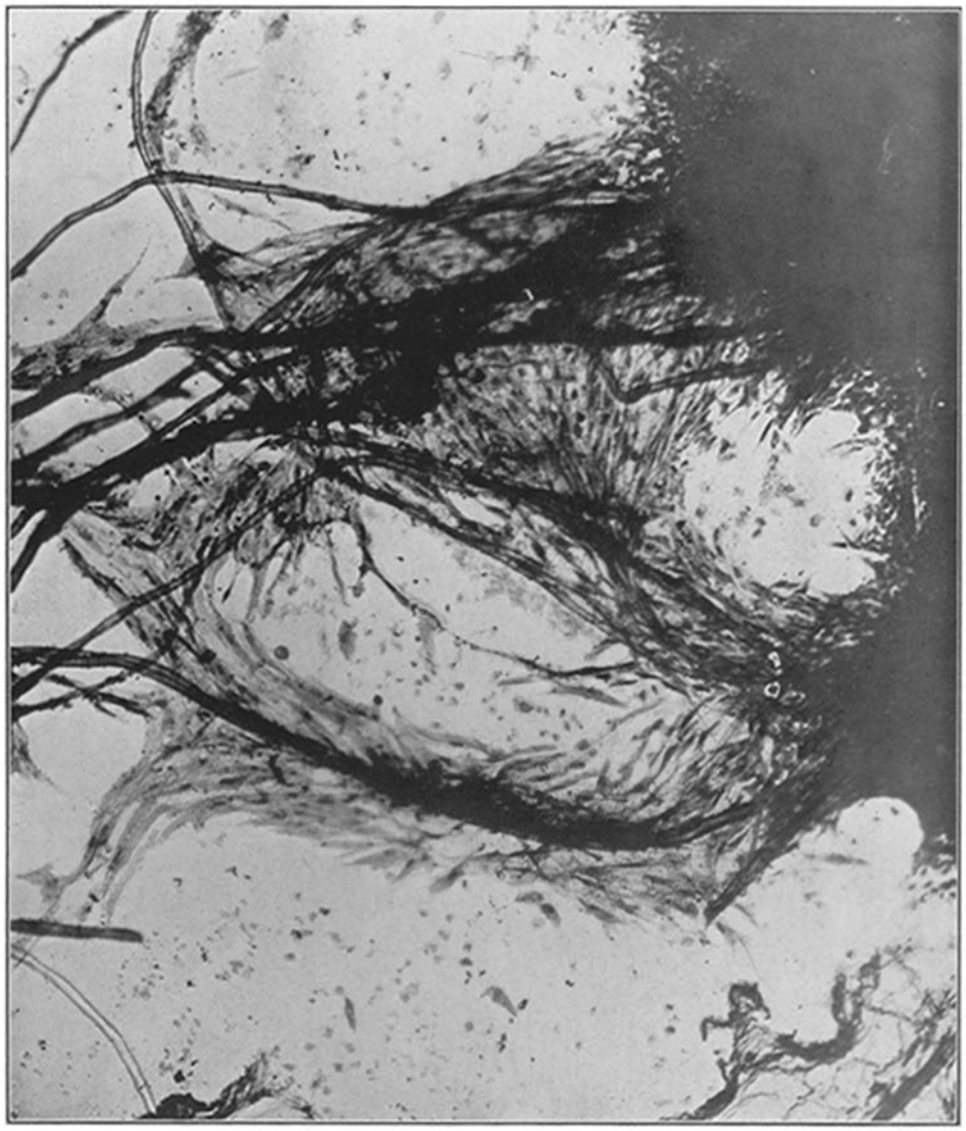

FIG. 4.

small pieces with the scissors, so that transplantation into a new culture medium can be effected without injury to the growing cells. The cells can also be seen growing from the silk veil into the culture medium (figure 2).

In the preparation of a culture in fluid medium, an important requisite is a suitable support for the growing cells. Although fibrin is an efficient support, it can be replaced by other bodies, such as cover glass, cotton, silk, etc. The cells grow in the fluid only if they meet with this condition of support. Therefore, the fragments of tissue must be very small in order to remain adherent to the cover glass, and the fluid must be spread out in a very thin layer about them. The growing cells cling to and spread over the surface of the glass (figure 3). But if cotton threads are placed in the fluid, the cells have a better support and grow luxuriantly around them (figure 4).

The conclusion is that the plasmatic medium is, beyond comparison, the optimum medium thus far studied for the growth of tissues. But cells can also grow in other media and under many different conditions. The technique of the cultivation of tissues *in vitro* is far from being worked out and a great many improvements will surely be developed.

EXPLANATION OF PLATES.

PLATE 25.

FIG. 1. Skin of fetal chicken. Cells growing on silk veil and plasma. Peripheral part of a living culture, three days old.

FIG. 2. Skin of fetal chicken. Cells growing from a silk thread into a new medium. Living secondary culture, three days old.

PLATE 26.

FIG. 3. Culture of fetal chicken skin grown in dog serum. Cells are clinging to the cover glass. Forty-eight hour culture. Stain, hemotoxylin.

PLATE 27.

FIG. 4. A small part of a peripheral growth of a culture of fetal chicken skin grown in rabbit serum. Cells are clinging in part to the cotton thread and cover glass. Fifty hour growth. Stain, hemotoxylin.