FIBRIN AND SERUM AS A CULTURE MEDIUM.

By ALBERT H. EBELING, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, February 17, 1921.)

INTRODUCTION.

Various artificial media have been used so far in the cultivation of tissues. Lewis and Lewis used bouillon and agar,[1] salt solutions,[2] and other media of known chemical constitution,[3] but the growth was by no means equal to that in plasma or lymph, either in extent or duration. Swezy[4] obtained cell proliferation of chick embryo heart tissue in a medium composed of egg albumin and muscle extract. Carrel and Burrows[5] found homogenic as well as heterogenic serum a useful culture medium for embryonic chick tissue. Ingebrigtsen[6] studied the growth of tissue outside the organism in a medium composed of agar and serum. Smyth[7] devised a so called simplified medium composed of agar and trypsinized peptone solution for embryonic tissue culture. No attempt was made to undertake a comparative quantitative study of the amount of tissue produced in these different media. Burrows[8] pointed out that embryonic tissues grew as well in saline solution as in plasma. He thought that growth consisted in a spreading of the cells and not in any increase in the mass of tissue, and that it appeared to take place at the expense of the original fragment. It is certain that the tissues did not increase

[1] Lewis, M. R., and Lewis, W. H., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 126.

[2] Lewis, M. R., *Anat. Rec.*, 1915–16, x, 287. Lewis, M. R., and Lewis, W. H., *J. Am. Med. Assn.*, 1911, lvi, 1795.

[3] Lewis, W. H., and Lewis, M. R., *Anat. Rec.*, 1912, vi, 207.

[4] Swezy, O., *Biol. Bull.*, 1915, xxviii, 47.

[5] Carrel, A., and Burrows, M. T., *J. Exp. Med.*, 1911, xiv, 244.

[6] Ingebrigtsen, R., *J. Exp. Med.*, 1912, xv, 397–398.

[7] Smyth, H. F., *J. Med. Research*, 1914–15, xxxi, 255.

[8] Burrows, M. T., *Tr. Cong. Am. Phys. and Surg.*, 1913, ix, 77.

in mass in any of the artificial media, nor could they be kept alive after a certain time. Therefore, these media could not be used for a quantitative study of the problem of growth.

It is known that the presence of embryo juice in adult plasma allows an indefinite growth of the fibroblasts and an increase in the mass of tissue. But the composition of this medium is complex and cannot be modified easily. It would be useful to find a medium endowed with the same properties as plasma and embryo juice, and more adaptable to the nature of the experiment.

The purpose of this article is to describe a technique for preparing a medium composed of fibrinogen, serum, and tissue juice, and to compare the growth of fibroblasts in this medium with that obtained in a medium composed of plasma and embryo juice.

EXPERIMENTAL.

The technique of Mellanby[9] was used in the preparation of fibrinogen. 10 cc. of normal adult chicken plasma were diluted with 90 cc. of sterile distilled water, and thoroughly shaken in an Erlenmeyer flask; 1 cc. of a 1 per cent acetic acid solution was added, drop by drop, and at the same time the mixture was agitated. The precipitate was allowed to settle partially in the cold for about 1 hour. The contents of the flask were then shaken and poured into centrifuge tubes, 25 cc. in each tube. After 10 minutes centrifugation, the supernatant fluid was decanted. The tubes were inverted over a sterile piece of filter paper for complete drainage. The precipitate contained in each centrifuge tube was combined and made up to 2.5 cc. with sterile, distilled water. When thoroughly mixed, it had the appearance of rich milk; on standing, a heavy sediment settled, superimposed by a layer of turbid fluid. Equal volumes of this suspension and Ringer's solution formed a slightly hazy, firm homogeneous clot after about 4 minutes. With an equal volume of serum, the mixture formed a clear fluid which did not coagulate after 10 minutes, but on the addition of a trace of embryonic tissue juice, coagulation occurred rapidly. The hydrogen ion concentration of such a preparation of fibrinogen was between 6 and 6.3. A mixture

[9] Mellanby, J., *J. Physiol.*, 1917, li, 396.

of 12.5 per cent fibrinogen suspension, 37.5 per cent chicken serum, and 50 per cent embryonic tissue juice, had a pH of between 7 and 7.3. Such a preparation coagulated in about 1 minute.

The experiments were begun with 48 hour cultures, derived from a strain of connective tissue in its 9th year *in vitro*.[10] Each fragment of tissue was divided in two parts and washed in Ringer's solution for about 40 seconds. One fragment was then cultivated in equal volumes of normal chicken plasma and embryonic tissue juice. The other fragment was cultivated in the experimental medium composed of one-fourth volume of fibrinogen suspension, three-fourths volume of chicken serum, and one volume of embryonic tissue juice. The constituents of this medium were first mixed by drawing them up

TABLE I.

Experiment No.	Culture No.	First passage.			Second passage.			Third passage.			Fourth passage.		
		Relative increase.		Ratio, $\frac{E}{C}$.	Relative increase.		Ratio, $\frac{E}{C}$.	Relative increase.		Ratio, $\frac{E}{C}$.	Relative increase.		Ratio, $\frac{E}{C}$.
		Control.	Experiment.		Control.	Experiment.		Control.	Experiment.		Control.	Experiment.	
1	17555	17.5	16.2	0.93	12.5	11.6	0.93	10.3	9.2	0.89	11.0	8.9	0.81
											9.7	8.0	0.83
2	17536	21.5	18.3	0.85	21.7	19.3	0.88	10.5	8.0	0.76	10.0	9.2	0.92
					21.0	14.6	0.70	10.7	8.8	0.82	8.4	Liquefied.	

and expelling them from a bulb pipette, after they had been dropped into the hollow of a deep, concave slide. After 48 hours the tissues were washed and cultivated in the same medium.

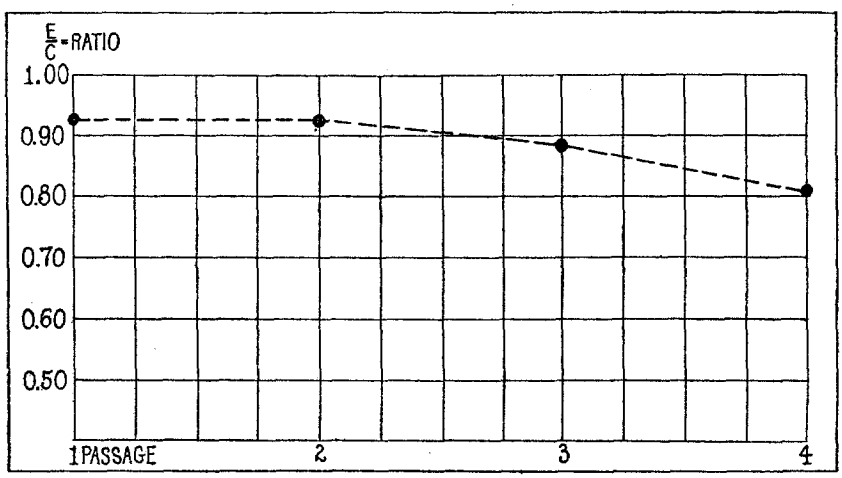
The cultures were incubated at 39°C. Observations were made on the coagulation time, consistency, duration of the coagulum, and character of growth as compared with the controls. The cultures were traced, measured, and the relative increase was calculated, according to the technique mentioned in a previous article.[11]

The technique was developed in the course of thirty-five experiments. The results do not require detailed description because the

[10] Ebeling, A. H., *J. Exp. Med.*, 1919, xxx, 531.

[11] Ebeling, A. H., *J. Exp. Med.*, 1919, xxx, 533–534.

appearance of the coagulum and of the growth of the strain of fibroblasts was about the same as that of the control in plasma and embryo juice. Generally, the width of the zone of new tissue and its density were slightly less in the experiment than in the control; the difference in most instances was approximately 10 per cent. In other experiments the tissues were allowed to grow for 48 hours several times, and then transplanted into a medium of the same composition. The results of two of these experiments are given in Table I and in Textfigs. 1 and 2. In the table the figures for the relative growth of the tissue obtained in the control and experiment represent the amount

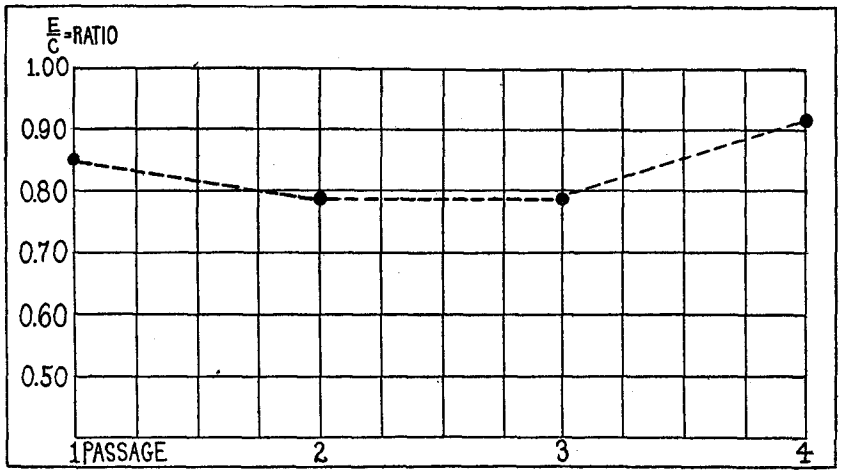


TEXT-FIG. 1. Experiment 1. Ratio between the two relative growths, experiment and control.

of growth which was obtained during the time interval chosen (48 hours), expressed in function of the area of the primitive fragment of tissue. The ratio between the two relative growths, $\frac{\text{Experiment}}{\text{Control}} = \frac{E}{C}$, is also given. In Experiment 1 the tissues of both experiment and control were divided after the third passage. In Experiment 2 the tissues were divided in the same way after the first passage.

There is a close relation between the relative growths of the experiments and of the controls. This relation, expressed as ratios in the first experiment, was 0.93, 0.93, 0.89, 0.81, 0.83; and in the second,

0.85, 0.88, 0.70, 0.76, 0.82, 0.92. These figures express the fact that in the time elapsed between two passages, the amount of growth is very nearly the same both in the experiment and the control. A curve was plotted for both experiments in which these ratios were expressed in ordinates and the number of passages (48 hour intervals) in abscissæ.



TEXT-FIG. 2. Experiment 2. Ratio between the two relative growths, experiment and control.

DISCUSSION.

A heavy precipitate could be obtained from 10 cc. of diluted plasma by adding 1.2 to 1.6 cc. of a 1 per cent acetic acid solution. But the final product was too acid. 1 cc. of the acetic acid solution produced a precipitate which, after suspension in distilled water, had a hydrogen ion concentration between 6 and 6.3. The hydrogen ion concentration of serum was from 8 to 8.3 and that of embryo juice from 7.3 to 7.5. When the precipitate suspension was dissolved in serum and mixed with embryo juice, the hydrogen ion concentration of the mixture varied from 7.3 to 7.5.

The coagulation was brought about by the addition of embryo juice. The addition of calcium was not essential to promote coagulation. On the contrary, a precipitate formed and liquefaction of

the coagulum occurred after 24 hours. The presence of serum is generally necessary to prevent liquefaction of the coagulum. In the experiments in which fibrinogen suspension was mixed with Ringer's solution or embryo juice alone, without serum, coagulation took place. But progressive liquefaction began soon afterwards. When serum was added in small quantities to fibrinogen suspension and embryo juice, the stability of the clot could be maintained.

The suspension of fibrinogen was used in various concentrations. If the medium contained more fibrinogen than plasma, the tissues did not grow well. When the concentration of fibrinogen was decreased, the coagulum was not dense enough and liquefaction often occurred within 24 hours.

It was found that excellent growth took place in a medium composed of 12.5 per cent fibrinogen suspension, 37.5 per cent serum, and 50 per cent embryo juice. Coagulation occurred within 1 minute and the coagulum was still firm after 48 hours.

The character of the growth of the old strain of connective tissue in the medium was not different from that observed in plasma. The growth was slightly less extensive in the experimental medium than in the control. It was easy to extirpate the fragment of tissue from its medium after 48 hours, and to transplant it into another medium. After every passage the amount of new tissue was about as large in the experiment as in the control. There was no doubt that the culture could have been kept alive for several more generations. This shows the possibility of keeping a strain of connective tissue in a medium composed of serum, fibrinogen, and embryo juice in about the same condition as in plasma and tissue juice.

CONCLUSIONS.

A technique is described by which a medium composed of fibrinogen suspension, serum, and embryo juice may be made.

Fibroblasts grew in this medium about as well as in plasma and embryo juice.

A strain of connective tissue in this medium remained practically as active as the control for several passages.