

GROWTH OF FIBROBLASTS AND HYDROGEN ION CONCENTRATION OF THE MEDIUM.

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The purpose of the experiments described in this paper was to determine the rôle played by the hydrogen ion concentration of the medium with regard to the growth of a strain of fibroblasts cultivated *in vitro* for a long period of time. The fibroblasts used were derived from fresh embryonic chick heart, from 1 or 2 months old strains of connective tissue, and from a strain cultivated for 9 years.¹ The cultures were prepared according to a technique previously described.² The method used for determining the hydrogen ion concentration has been described recently by Felton.³

I.

Method of Obtaining Variations in the Hydrogen Ion Concentration of the Media.

The variations in the hydrogen ion concentration of the media were obtained in the following ways.

1. *Acid Solutions.*—Solutions of acids were made in such concentrations that the addition of a small amount, usually 1 drop, to a certain amount of embryonic tissue juice or plasma would give the hydrogen ion concentration desired. A quantity of juice or plasma sufficient for a whole series of experiments was made in order to secure the same reaction in all. One of the disadvantages of this method was that plasma prepared in quantity underwent changes which resulted in decreasing or entirely preventing coagulation. A second disadvantage was that the embryonic tissue juice sometimes caused subsequent precipitation.

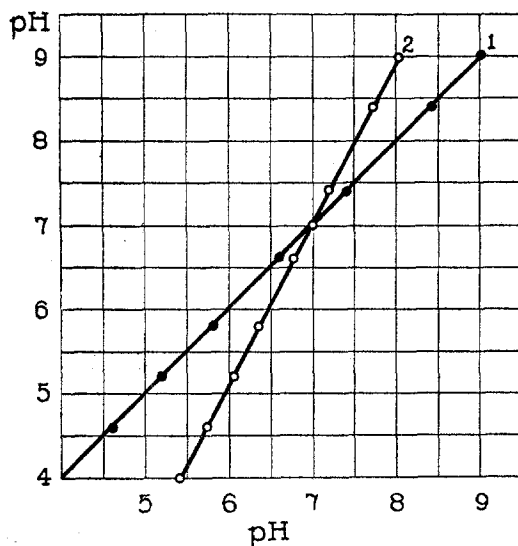
¹Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 231.

²Ebeling, A. H., *J. Exp. Med.*, 1919, xxx, 531-535.

³Felton, L. D., *J. Biol. Chem.*, 1921, xlvi, 299.

2. *Phosphate Solutions.*—Sørensen's standard phosphate solutions were added to embryonic tissue juice in equal parts, to obtain different hydrogen ion concentrations in the media. It was found that if equal parts of fresh embryonic tissue juice and sterile buffer solutions were mixed, for instance with a pH of 7, the growth obtained was nearly as extensive as that in the control in which the juice was diluted with Ringer solution. This showed that phosphate had no appreciable influence on the rate of growth.

A known hydrogen ion concentration was obtained by the following technique. Some preliminary experiments were made in order to find



TEXT-FIG. 1. The abscissæ indicate the pH obtained, and the ordinates the pH of the buffer solution added. Curve 1 represents the pH of Ringer solution, and Curve 2 the pH of embryonic tissue juice, to both of which are added the different standardized buffers as indicated by the ordinates.

out the pH of a mixture of embryonic juice and a given acid. Different numbers of drops of the respective solutions (acid or buffer) were added to tubes holding the same amounts of juice, and the hydrogen ion concentration was tested. These different known reactions of the juice obtained in this way gave a curve in which the amount of acid necessary to obtain a certain hydrogen ion concentration between the

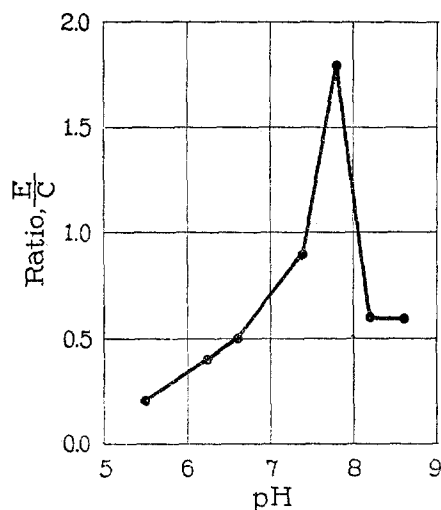
points found empirically could be calculated by interpolation (Text-fig. 1). Equal volumes of the standardized buffer solutions and of tissue juice were used. Upon ascertaining what pH resulted in the tissue juice when it was mixed with its own volume of buffer solution, the reactions obtained gave a straight line when a curve was plotted and it was possible to calculate readily which buffer should be used to produce the hydrogen ion concentration desired in the tissue juice (Text-fig. 1). Another test made in the same way was then necessary; namely, with the plasma-juice mixture. This had to be done rapidly in order to mix the indicator and juice-plasma before coagulation took place. In other words, when fibroblasts are to be cultivated in a medium of, let us say, pH 6, preliminary experiments for those particular media will show what buffer solutions should be added to the juice which will give pH 6, when combined with plasma. The test should be made in two steps for each experiment, one for the juice-buffer, and the other for the juice-buffer-plasma mixture, because of the slight differences in hydrogen ion concentration of the different juices and plasmas. The tissue juice may vary from pH 6.8 to 7.2, and the plasma from pH 7.4 to 8.

II.

Cultivation.

After the embryonic juice had been tested to determine its hydrogen ion concentration, it was mixed with equal parts of a buffer solution, calibrated pipettes being used to obtain the same sized drops. A selected culture was divided in two equal parts and washed in Ringer solution for about 30 seconds. One fragment was cultivated in a medium composed of 1 drop of plasma and 1 drop of a mixture of embryonic tissue juice and buffer solution. The other fragment, or the control, was cultivated in 1 drop of plasma and 1 drop of embryonic tissue juice to which had been added the same volume of Ringer solution instead of buffer solution. Mica cover-slips were used. 1 hour after the preparation of the culture, a drawing was made under the projectoscope. After 48 hours incubation, the outline of the new growth was drawn and calculations were made, by the method described by Ebeling.² When the purpose of the experiments was the study of the influence of the same hydrogen ion concentration for several genera-

tions, the culture was divided in two parts at each passage. One fragment was kept as control and the other placed in the experimental medium, care being taken to keep the other factors constant.



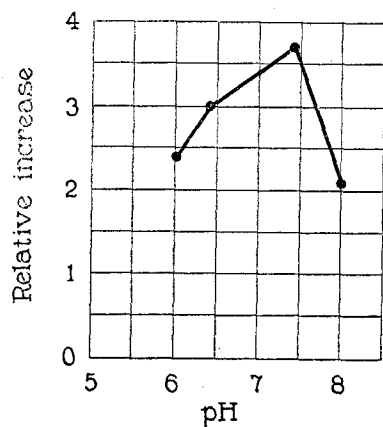
TEXT-FIG. 2. The different hydrogen ion concentrations of the media in which the fourth passage of the old strain of fibroblasts is cultivated are indicated by the abscissæ. The ordinates indicate the relative increase of growth, $\frac{E}{C}$. The different hydrogen ion concentrations in the medium were obtained by adding phosphate buffer solutions to the extract.

III.

Rate of Growth.

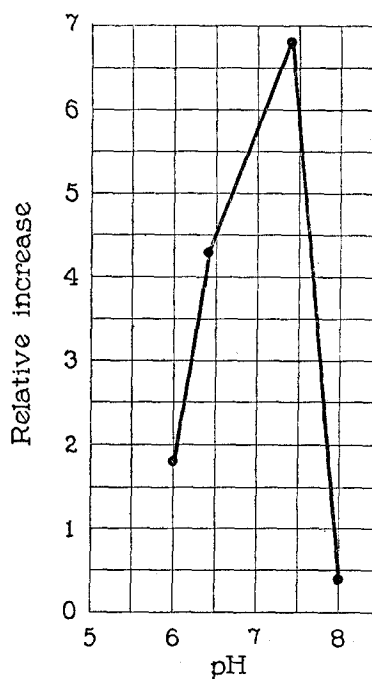
When fibroblasts were cultivated in media containing different hydrogen ion concentrations varying from pH 5.5 to 8.5, a curve was obtained for the rate of growth with a distinct maximum between pH 7.4 and 7.8 (Text-figs. 2 to 4). The rate of growth decreased very rapidly with increasing hydrogen and hydroxyl ion concentrations, and the plotted curve was nearly symmetrical. The results obtained by using different acids, such as acetic, phosphoric, sulfuric, and hydrochloric, were much the same. It seemed that the anions did not have any remarkable influence on the growth. The curves reached a maximum at

pH 7.4 and fell very rapidly on both sides of this point, appearing always to be steeper on the alkaline side in spite of the fact that the resistance against the alkalinity was more marked. The same type of curve was found in all experiments. When the media were more alkaline or more acid, precipitation occurred, coagulation was interfered with, and it was impossible to draw any conclusions from the results. If the series of experiments were repeated by carrying the fibroblasts to a new medium containing the same hydrogen ion concentration as was used in the preliminary culture, it was observed that the absolute



TEXT-FIG. 3.

TEXT-FIG. 3. The different hydrogen ion concentrations of the media in which the first passage of the old strain of fibroblasts is cultivated are indicated by the abscissæ. The ordinates indicate the relative increase of growth. Phosphate buffer solutions were used.



TEXT-FIG. 4.

TEXT-FIG. 4. The different hydrogen ion concentrations of the media in which the fifth passage of the old strain of fibroblasts is cultivated are indicated by the abscissæ. The ordinates indicate the relative increase of growth. Phosphate buffer solutions were used.

TABLE I.

Experiment No.	Culture No.	Buffer solution added.*		Juice-buffer mixture.			First passage.			Second passage.			Third passage.			Fourth passage.		
		pH	pH	Control.	Experiment.	Ratio, $\frac{E}{C}$.	Control.	Experiment.	Ratio, $\frac{E}{C}$.	Control.	Experiment.	Ratio, $\frac{E}{C}$.	Control.	Experiment.	Ratio, $\frac{E}{C}$.			
1	264	4.0	5.5	7.0	0.4	0.6	6.2	3.1	0.5	3.0	2.7	0.9	6.1	1.7	0.2			
2	265	4.6	6.0	5.4	0.1	0.1	6.3	2.4	0.3	6.6	3.0	0.4	6.5	0.9	0.1			
3	266	5.2	6.2	3.1	7.3	0.4	16.5	4.7	0.2	3.0	1.7	0.5	6.6	3.0	0.4			
4	267	5.8	6.6	1.8	1.5	0.7	8.7	4.1	0.4	4.6	3.3	0.7	3.0	1.7	0.5			
5	268	6.6	7.2	4.7	3.9	0.8	12.4	1.8	0.1	4.2	5.1	1.2	5.8	3.4	0.5			
6	269	7.0	7.4	10.8	10.8	1.0	12.2	11.9	0.9	4.8	6.8	1.4	7.3	6.6	0.9			
7	270	7.4	7.8	10.4	6.6	0.6	4.3	5.1	1.1	9.9	12.1	1.2	2.1	3.9	1.8			
8	271	8.4	8.2	11.8	4.5	0.3	12.1	7.9	0.6	10.3	8.1	0.7	3.7	2.3	0.6			
9	272	9.0	8.6	7.2	6.3	0.8	11.1	7.7	0.7	12.7	8.4	0.6	7.6	4.9	0.6			

* 1 cc. of buffer solution was added.

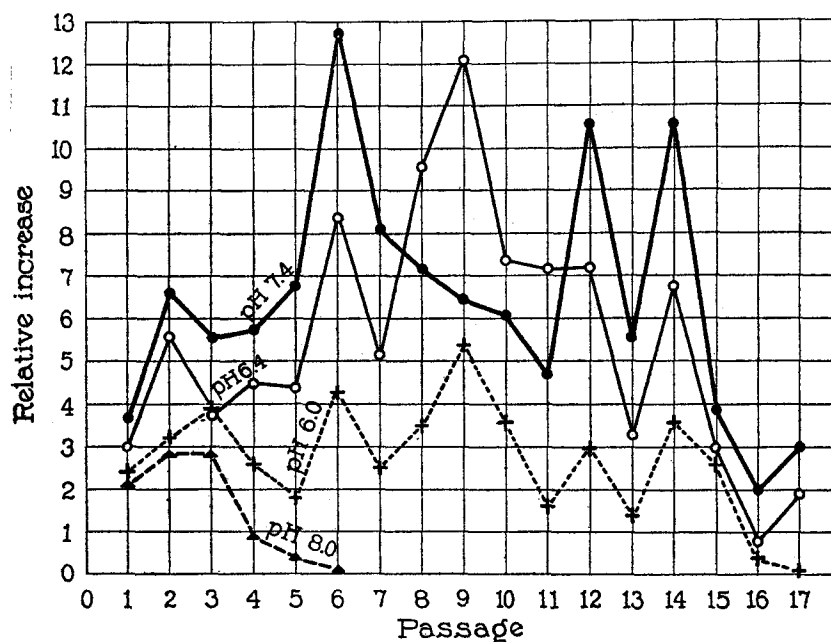
TABLE II.

Experiment No.	Culture No.	Buffer solution added.*		Relative increase.																
		pH	pH	Passage No.																
				1	2	3	4	6	7	8	9	10	11	12	13	14	15	16	17	
1	375-1	4.0	6.0	2.4	3.2	3.9	2.6	1.8	4.3	2.5	3.5	5.4	3.6	1.6	3.0	1.4	3.6	2.6	0.4	0
2	375-2	5.8	6.4	3.0	5.6	3.7	4.5	4.3	8.3	5.2	9.6	12.1	7.3	7.2	7.2	3.3	6.8	3.0	0.8	1.9
3	375-3	7.0	7.4	3.7	6.6	5.6	5.8	6.8	12.7	8.1	7.2	6.5	6.1	4.7	10.6	5.6	10.6	3.9	2.0	3.0
4	375-4	9.0	8.0	2.1	2.8	2.8	0.9	0.4	0	0	0	0	0	0	0	0	0	0	0	0

* 1 cc. of buffer solution was added.

increase of new growth became less and less in the most alkaline and most acid media (see also Tables I and II and Text-fig. 5). The descent of the curve on both sides of the maximum became more abrupt in the succeeding generations because of the retardation of growth in the media with highest alkalinity and acidity. (Compare Text-fig. 3, which represents the first passage, and Text-fig. 4, which represents

the fifth passage.) By following the growth of fibroblasts, generation after generation, in media containing the same hydrogen ion concentration it was observed that in the highest acidity (pH 5.5) cell proliferation ceased after four to six passages. The fibroblasts showed more resistance to higher alkalinity, but at the highest (pH 8.5) they grew for about eight to ten passages. It may be seen from the curve (Text-fig. 2) that the growth of tissue at pH 5.5 was very near the reaction where



TEXT-FIG. 5. The growth of the four cultures of the experiments in Table II, expressed graphically.

no growth will take place, although at pH 8.5 it was further from this point, and growth was observed for more than ten passages. It may be assumed, therefore, that growth can continue for a long time in this hydrogen ion concentration, but with a smaller increase of cell proliferation. The optimum growth occurred between pH 7 and 7.8. This was the normal reaction of a mixture of plasma and embryonic tissue juice. It may be seen from the curves that a slight variation in the hydrogen ion concentration of the culture medium resulted in marked changes in

the rate of growth of fibroblasts. It is interesting to note that the differences were mostly of a quantitative nature. The only morphological change of the cells observed was that, in the acid media, they showed more vacuoles than in the alkaline.

IV.

CONCLUSIONS.

1. The rate of growth of fibroblasts is markedly modified by slight changes in the hydrogen ion concentration of the medium. The curves expressing the rate of growth in function of the hydrogen ion concentration of the medium are nearly symmetrical on both sides of the maximum.

2. The optimum growth of fibroblasts occurs at pH 7.4 to 7.8. A slight change from this reaction has a remarkable influence on the rate of growth.

3. Fibroblasts show more resistance to higher alkalinity than to higher acidity. They grew for only four to six generations in a medium having a pH of 5.5, and for more than ten generations in one of 8.5.

4. The influence of different hydrogen ion concentrations on fibroblasts was only of a quantitative nature.

I wish to acknowledge my appreciation to Dr. Felton for his advice regarding his own method for measuring the hydrogen ion concentration of small amounts of fluid.