ACTION ON FIBROBLASTS OF EXTRACTS OF HOMOLOGOUS AND HETEROLOGOUS TISSUES.

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Fibroblasts do not multiply in blood serum[1] after the food material stored in the original tissue is exhausted.[2] Neither can they obtain the nitrogen necessary for their proliferation from chicken bouillon, egg white, egg yolk, nor pure egg abumin.[3] But they live and multiply indefinitely when the medium contains a small amount of embryonic tissue juice.[4] It is, therefore, probable that they require for the building up of protoplasm certain nitrogenous compounds which are synthetized within the organism by other cells. It is known that leucocytes contain such growth-promoting substances.[5] Extracts of muscle and gland tissues of adult animals also possess the power of stimulating the rate of growth of homologous fibroblasts *in vitro*.[6] The juice of heterologous tissues, such as guinea pig tissue, may determine the proliferation of chicken fibroblasts, although the life of these cells in such a medium is not permanent.[7] It seemed that heterologous as well as adult tissues contain certain

[1] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 317; 1923, xxxvii, 759.

[2] Lewis, M. R., and Lewis, W. H., *Anat. Rec.*, 1911, v, 277. Ingebrigtsen, R., *J. Exp. Med.*, 1912, xvi, 421. Burrows, M. T., *Anat. Rec.*, 1916–17, xi, 335. Burrows, M. T., and Neymann, C. A., *J. Exp. Med.*, 1917, xxv, 93. Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 317.

[3] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1923, xxxviii, 487.

[4] Carrel, A., *J. Exp. Med.*, 1912, xv, 516. Ebeling, A. H., *J. Exp. Med.*, 1922, xxxv, 755.

[5] Carrel, A., *J. Exp. Med.*, 1922, xxxvi, 385. Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 645.

[6] Carrel, A., *J. Exp. Med.*, 1913, xvii, 14.

[7] Carrel, A., unpublished experiments.

substances which permit a pure culture of fibroblasts to increase in mass, at least for a time. The aim of the experiments described in this article was to determine as accurately as possible, with the techniques at our disposal, the action of homologous adult tissue extracts and of heterologous embryonic juices and adult tissue extracts on the rate of growth of chicken fibroblasts and on the duration of their life *in vitro*.

EXPERIMENTAL.

Young embryos of chickens, mice, guinea pigs, and rabbits were finely pulped and centrifuged. The supernatant juice was diluted with approximately its volume of Tyrode solution. Muscle and organs of adult chickens, mice, guinea pigs, and rabbits were sliced in a Latapie apparatus, and to the pulp was added its volume of Tyrode solution. After a few hours, the mixture was centrifuged and the supernatant fluid removed.

The juices and extracts were kept in a refrigerator and their H ion concentration was determined daily by the colorimetric method. The pH was about 7.8, and after a few days it became lower, especially in extracts of adult tissues. It was kept constant by the addition of small amounts of sodium hydrate. The juices and extracts were always used within a week after they had been prepared.

The action of the juices and extracts was tested on an 11 year old strain of fibroblasts. The stock cultures were divided into two equal parts. One of the fragments was placed in a medium composed of 1 volume of plasma and 2 volumes of Tyrode solution containing 0.02 of chick embryo juice, and used as a control, while the other fragment was placed in 1 volume of plasma and 2 volumes of tissue extract or tissue juice. The fragments were traced in a projectoscope after 1 and 48 hours and the relative increase was measured by the usual technique.[8] If, after 48 hours, the tissue fragments had doubled in size, they were divided into two equal parts, one half being discarded, and the remaining half cultivated in the same medium. When no marked increase had occurred, the fragments were merely washed and transferred to a fresh medium. The figures on relative increase of the fragments, their absolute dimensions at the beginning of each passage, and the number of passages during which they lived, made it possible to ascertain the action of the various juices and extracts on cell proliferation. The data for each experiment were tabulated, but to save space the tables have been omitted. For each group of experiments, a chart shows the variations in the relative increase of the tissues in function of the time, which is expressed by the number of passages.

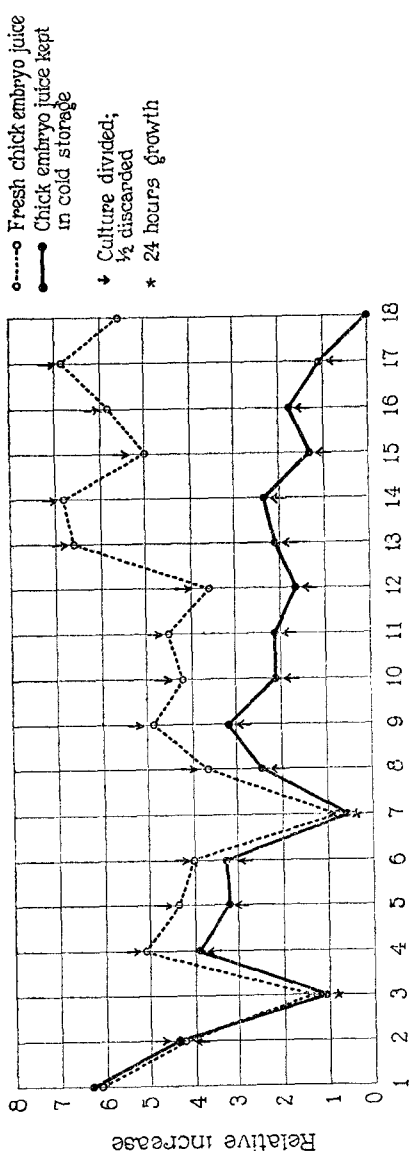
1. Action of Fresh Chick Embryo Juice and of the Same Juice Preserved in Cold Storage on the Duration of Life of Homologous

[8] Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 231.

Fibroblasts.—Before studying the action of the various homologous and heterologous juices on the growth of chicken fibroblasts, it was necessary to ascertain in more precise manner than has been done heretofore the influence of the spontaneous modifications undergone by the juices in function of time. It is well known that the increase in the H ion concentration prevents the stimulating action of the juice on cell proliferation,[9] and that this increase occurs spontaneously. But as it is also possible that some other deterioration takes place, even when the pH does not vary, the action of fresh juices was compared with that of juices kept in cold storage at a constant pH. In the following experiments, fresh juice and juice kept in cold storage were compared. When the juice had been kept in the refrigerator for more than 1 week, its action became weaker than that of the fresh extract. The tissues which had been cultivated in the juice kept for 34 days in the refrigerator died after seventeen passages, while those cultivated in fresh extract continued doubling in size every 48 hours (Text-fig. 1). It was not possible to determine whether the cause of death was intoxication or starvation. This experiment showed that, when the H ion concentration was kept constant by the addition of sodium hydrate, some changes occurred in the embryonic tissue juice which rendered it unsuitable for maintaining fibroblasts in a condition of indefinite growth. But this effect could hardly have been detected if the tissues had not been in contact for several passages with the juice. Tissue juices and extracts should be used, therefore, when freshly prepared.

2. Action of Extracts of Adult Homologous Tissues on the Growth and Duration of Life of Fibroblasts.—The extracts made from muscle brought about an increase in the mass of the tissues during the first passage. In one group of three experiments, the tissues could be divided into two parts at the third passage (Text-fig. 2), and apparently the muscle tissue extract had an action similar to that of embryonic tissue juice. However, it was observed that the cells contained more fat granules than do normal cultures. After either four or five passages, the tissues died. The controls in Tyrode

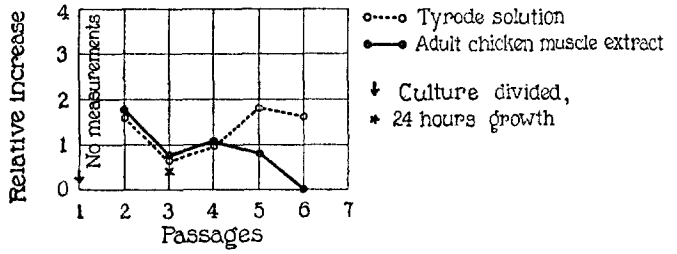
[9] Fischer, A., *J. Exp. Med.*, 1921, xxxiv, 447.



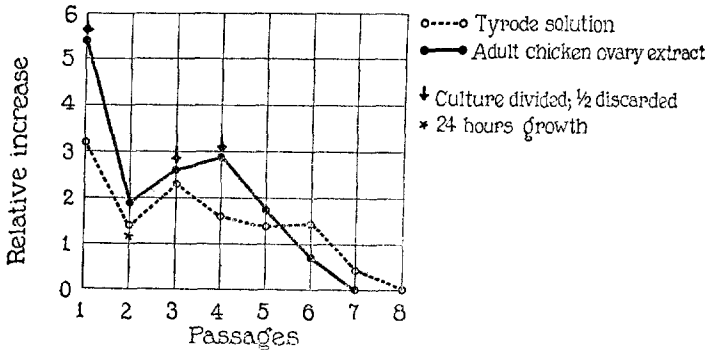
TEXT-FIG. 1. Action of fresh chick embryo juice and of chick embryo juice kept in cold storage on the rate of growth of homologous fibroblasts.

solution did not increase in size to such an extent that they could be divided. They were still living after six passages (Text-fig. 2).

In one group of three experiments, extracts of ovary also had a stimulating action. The mass of the tissues increased, but not permanently. The cultures were large enough to be divided after the first, third, and fourth passages. Death occurred at the seventh



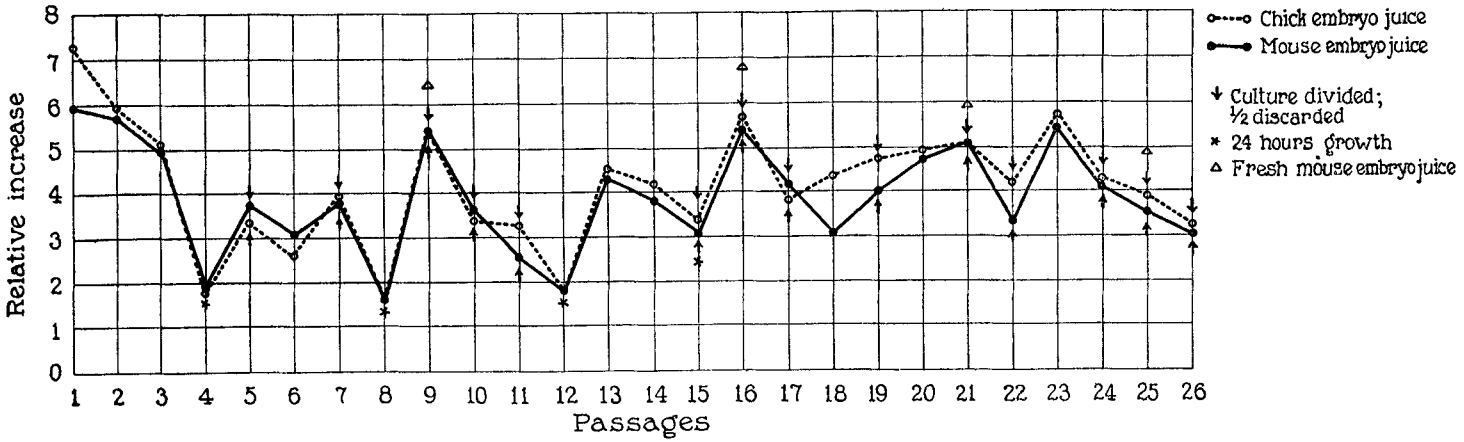
TEXT-FIG. 2. Action of adult chicken muscle extract on the duration of life of homologous fibroblasts.



TEXT-FIG. 3. Action of adult chicken ovary extract on the duration of life of homologous fibroblasts.

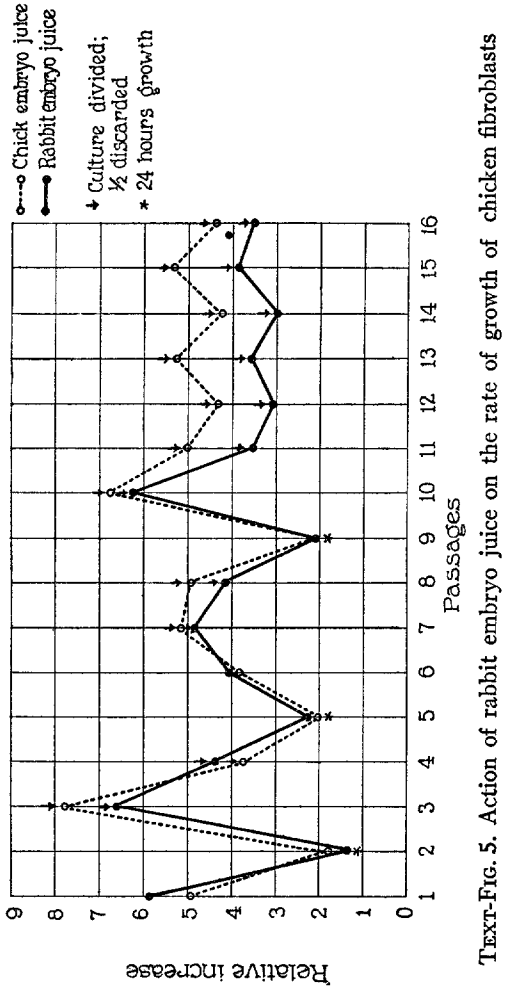
passage. The controls did not increase in mass and could not be divided, but they survived seven passages (Text-fig. 3). Thus, it appeared that the extracts of adult tissues were not capable of maintaining indefinitely the life of the fibroblasts *in vitro*, as fresh embryonic juice does.

3. Action of Heterologous Embryonic Tissue on the Growth and Duration of Life of Fibroblasts.—In one group of three experiments,



TEXT-FIG. 4. Action of mouse embryo juice on the rate of growth of chicken fibroblasts.

the action of the juice of mouse embryos was tested on chicken fibroblasts, the controls being cultivated in chick embryo juice. The supply of mouse embryo juice was renewed after 12, 26, 36,

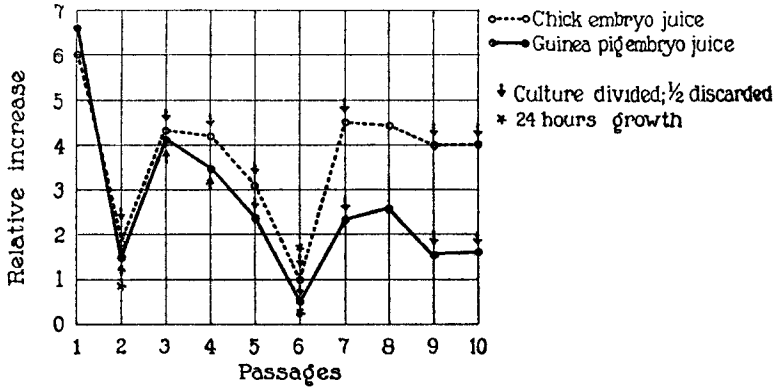


TEXT-FIG. 5. Action of rabbit embryo juice on the rate of growth of chicken fibroblasts

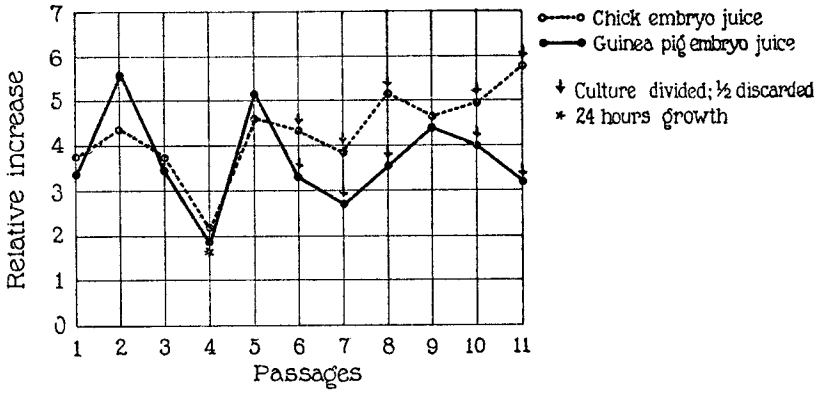
and 44 days. Text-fig. 4 shows that the connective tissue fragments doubled in mass in 48 hours in mouse embryo juice, as well as in chick embryo juice. After twenty-six passages, the rates of growth were still practically the same in both substances and the

experiment was discontinued. No evidence had been obtained that the tissues would die sooner in mouse embryo juice than in chick embryo juice.

In one group of three experiments, rabbit embryo juice acted on chicken fibroblasts in the same manner as chick embryo juice. The



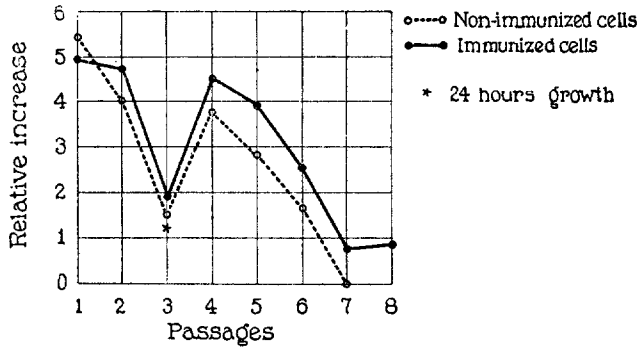
TEXT-FIG. 6. Action of guinea pig embryo juice on the rate of growth of chicken fibroblasts.



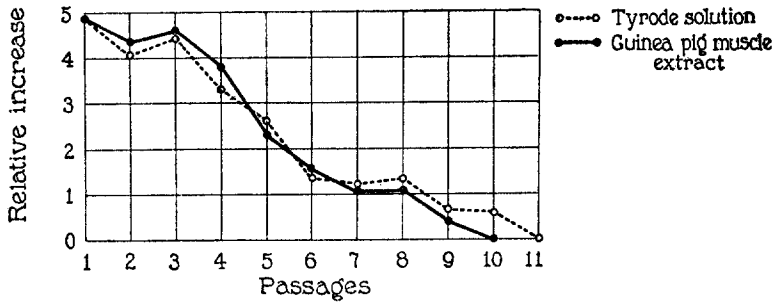
TEXT-FIG. 7. Action of guinea pig embryo juice on the rate of growth of chicken fibroblasts.

tissues increased markedly in size and no difference could be observed between fibroblasts nourished on chick embryo juice and rabbit embryo juice. Every 48 hours, the tissues doubled in size. This experiment was continued for 29 days. The curve shows that the

rates of growth of the fibroblasts in both juices were in general similar (Text-fig. 5). The differences observed after the 19th day were due probably to the fact that the supply of rabbit embryo juice had not been renewed. A similar slackening in the growth was observed when homologous tissue juice was used under the same conditions.



TEXT-FIG. 8. Action of rabbit serum on the rate of growth of chicken fibroblasts cultivated previously in chicken and rabbit embryo juices.

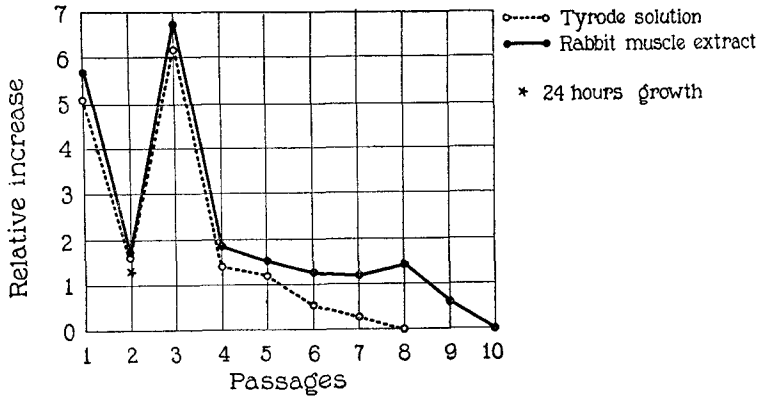


TEXT-FIG. 9. Action of adult guinea pig muscle extract on the duration of life of chicken fibroblasts.

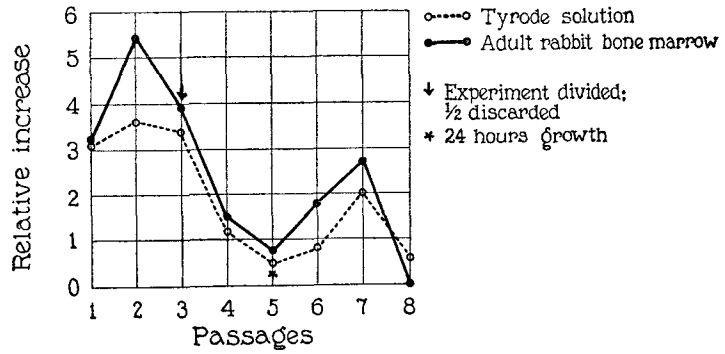
In two groups of three and four experiments respectively, the action of guinea pig embryo juice was studied in the same manner. In a first series of experiments, the tissues doubled in mass every 2 days in both media. After seven passages, growth was more extensive in the chick embryo juice because the supply of guinea pig juice had not been renewed (Text-fig. 6). In a second series of ex-

periments, after 20 days the rate of growth of chicken tissue fed on guinea pig juice was the same as that of the control (Text-fig. 7).

It is evident that protoplasm can be synthetized by chicken fibroblasts nourished on guinea pig, mouse, and rabbit embryonic tissues.



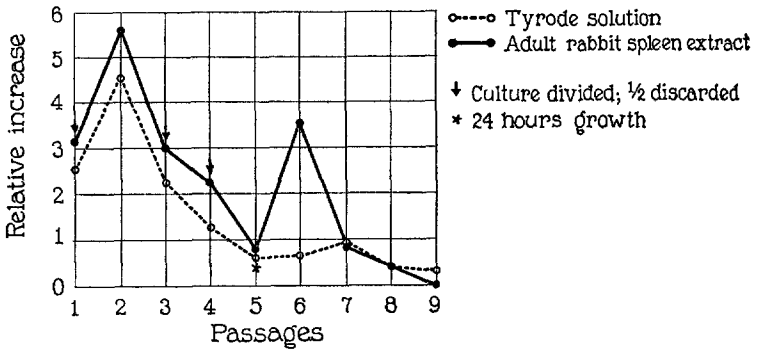
TEXT-FIG. 10. Action of adult rabbit muscle extract on the duration of life of chicken fibroblasts.



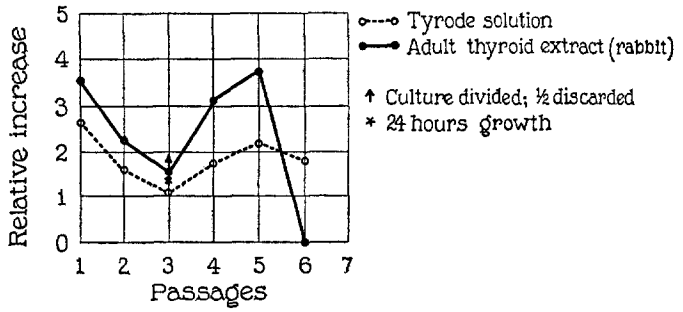
TEXT-FIG. 11. Action of adult rabbit bone marrow extract on the duration of life of chicken fibroblasts.

No differences traceable to variations in the media could be observed either in the rate of migration or the amount of tissue produced. Nevertheless, in one group of three experiments, an attempt was made to investigate whether chicken fibroblasts fed on rabbit juices were modified in their specificity. Strains of chicken fibroblasts

derived respectively from cultures maintained on chicken and rabbit embryo substances were cultivated in rabbit serum. The retarding action of rabbit serum was less marked on the fibroblasts grown in rabbit tissue juice than on the normal fibroblasts (Text-fig. 8). The fibroblasts previously nourished on rabbit tissue juice under-



TEXT-FIG. 12. Action of adult rabbit spleen extract on the duration of life of chicken fibroblasts.



TEXT-FIG. 13. Action of adult rabbit thyroid extract on the duration of life of chicken fibroblasts.

went but a slight change. This effect may have been due to the fact that the specificity of the chicken fibroblasts so nourished had decreased, or that they had become immunized against rabbit specific substances.

4. Action of Heterologous Adult Tissue Extract on the Growth and Duration of Life of Fibroblasts.—In one group of four experiments,

chicken fibroblasts cultivated in extract of adult guinea pig muscle did not increase in mass, and died sooner than the controls in Tyrode solution (Text-fig. 9).

In one group of three experiments, the extract of adult rabbit muscle had a similar action. The fibroblasts died 4 days sooner in muscle extract than in Tyrode solution (Text-fig. 10).

In one group of three experiments, rabbit bone marrow caused the fibroblasts to double in mass at the third passage. But both the experiment and control tissues died at the eighth passage (Text-fig. 11).

In one group of four experiments, rabbit spleen extract increased the mass of the tissues to the same extent, but the duration of life was no longer than in the controls (Text-fig. 12).

In one group of three experiments, extract of thyroid gland produced some increase in the mass of the tissues. Death occurred suddenly at the sixth passage (Text-fig. 13).

SUMMARY.

Extracts of homologous adult tissues determine an increase in the mass of pure cultures of chicken fibroblasts nourished thereon comparable to that resulting from embryonic tissue juice. But the effect of these extracts differs markedly from that of the latter, since cell multiplication does not continue indefinitely. After a few passages, the fibroblasts cultivated in adult tissue extracts grew more slowly than in Tyrode solution. The cytoplasm became dark and filled with fat granules, and death followed. It is possible that the tissues of adult animals contain, as does the serum, substances which are toxic for the homologous cells, and which progressively overcome the effect of the growth-activating substances. The effect of heterologous adult tissue extracts did not differ markedly from that of homologous tissues. The chicken connective tissue increased slightly in mass, but died sooner than the controls in Tyrode solution.

By contrast, tissue juices derived from the embryos of mice, guinea pigs, and rabbits acted on chicken fibroblasts in the same manner as chick embryo juices. The increase in mass of the cultures was regular and rapid. They doubled in size every 48 or 72 hours, and the

rate of growth did not decrease after 30 days. It appears that embryonic tissue juices are not necessarily toxic for heterologous fibroblasts, and that they can be used in the building up of protoplasm in the tissues of a different species. In experiments made long ago,[6] the action of tissue juice was described as being specific. The premature death of the fibroblasts cultivated in heterologous juices at that time would now appear to have been due to spontaneous changes in the pH and the deterioration that even normal chick embryo juice at a pH of 7.8 undergoes spontaneously. In the recent experiments, when freshly prepared homologous and heterologous juices were used, their action on chicken fibroblasts in pure culture was identical. However, the fibroblasts produced in cultures nourished by rabbit juice grew better when transferred to rabbit serum than did ordinary chicken fibroblasts. It has not been determined as yet whether this effect is due to an immunization of the fibroblasts against rabbit humors, or to some decrease in the specificity eventuating in cells intermediate between rabbit and chicken fibroblasts.

It may be concluded that, under the conditions of the experiments:

1. Pure cultures of chicken fibroblasts increase in mass under the influence of extracts of adult homologous tissues. But they ultimately die while the fibroblasts cultivated in embryonic tissue juices live indefinitely.

2. The increase in mass of chicken fibroblasts cultivated in the juices of mouse, guinea pig, rabbit, and chick embryos is about identical.

3. Chicken fibroblasts produced in cultures nourished by rabbit embryonic tissue juice are less sensitive to the inhibiting action of rabbit serum than ordinary chicken fibroblasts.

4. Cultures of chicken fibroblasts in extracts of adult tissues of mice, guinea pigs, and rabbits increase slightly in mass, but the increase is temporary and death occurs after a few passages.