PRESENT CONDITION OF A STRAIN OF CONNECTIVE TISSUE TWENTY-EIGHT MONTHS OLD.*

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PLATES 1 AND 2.

In previous articles[1] it was shown that connective tissue could be kept outside of the organism in a condition of permanent life. The purpose of the following experiments was to determine the present condition of a strain of connective tissue which, after having undergone 358 passages, has now reached the twenty-ninth month of its life *in vitro*. The strain of connective tissue was derived from a piece of heart extirpated on January 17, 1912, from a chick embryo seven days old. The fragment of heart pulsated for 104 days and gave rise to a very large number of connective tissue cells. These cells multiplied actively during the last two years, and produced a large amount of connective tissue. At present, a great many cultures are obtained from the strain every week.

The dynamic condition of a tissue is manifested by the rate of its growth. The increase in the volume of a fragment of connective tissue can be measured with comparative accuracy. For this the following technique is used. A fragment of tissue is removed from a culture, washed in Ringer solution, and placed in a new medium. It soon becomes surrounded with a ring of new tissue. After forty-eight hours the width of this ring is measured with a micrometer. Under the ordinary conditions of the experiment the thickness of the new tissue is more or less uniform, and its total volume can therefore be estimated fairly accurately by its superficial size. The fragments of tissue usually double in forty-eight hours. But their rapidity of growth is subject to fluctuations dependent upon the character of the medium and upon the condition of the tissue at the time that it is placed in the medium. When these conditions were favorable the ring of new tissue attained, during

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[1] Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516. Ebeling, A., *idem*, 1913, xvii, 273. Carrel, A., *idem*, 1913, xviii, 287.

the last few months, a width of 2 to 2.8 millimeters (figures 1 and 2). A comparison of the amount of tissue produced by a given culture in forty-eight hours this year with that produced in the same time by the same strain of cells a year ago shows that the activity of the strain has increased. Last year the width of the ring of tissue produced in forty-eight hours around the fragments of connective tissue was only 1.5 or 1.8 millimeters.

This increase in the rate of growth is made more apparent by the following experiment. A piece of heart extirpated from a chick embryo eight days old, and a fragment of connective tissue at the beginning of the third year of its life *in vitro* were placed in the same culture medium. After forty-eight hours it was seen that the tissue which had become adapted to the life *in vitro* had increased much more rapidly than the fresh tissue (figure 3). Nevertheless, the tissue adapted to the life *in vitro* was derived indirectly from a fragment of heart extirpated more than two years ago from an embryo seven days old. Thus it is conclusively shown that the proliferating power of the strain has in no wise diminished. Nevertheless, it would be imprudent to conclude from this fact that it has augmented, as the greater rate of increase of the tissues may be due, not to an augmentation of the proliferating power of the cells, but to an improvement in the details of the technique.

Moreover, the fact remains that during the third year of independent life the connective tissue shows greater activity than at the beginning of that period, and is no longer subject to the influence of time. If we exclude accidents, connective tissue cells, like colonies of infusoria, may proliferate indefinitely.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. A fragment of connective tissue extirpated from a culture of the twenty-eight months old strain, one hour after the passage.

FIG. 2. The same tissue, forty-eight hours after the passage.

PLATE 2.

FIG. 3. In the same medium were placed a piece of heart from a chick embryo eight days old (A), and a fragment of connective tissue, No. 8860, which had lived for more than two years outside of the organism (B). The photograph shows the amount of tissue produced in forty-eight hours by both fragments.

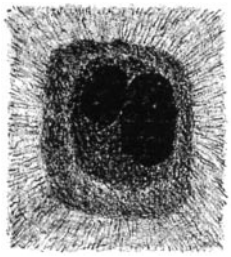

FIG. 1.

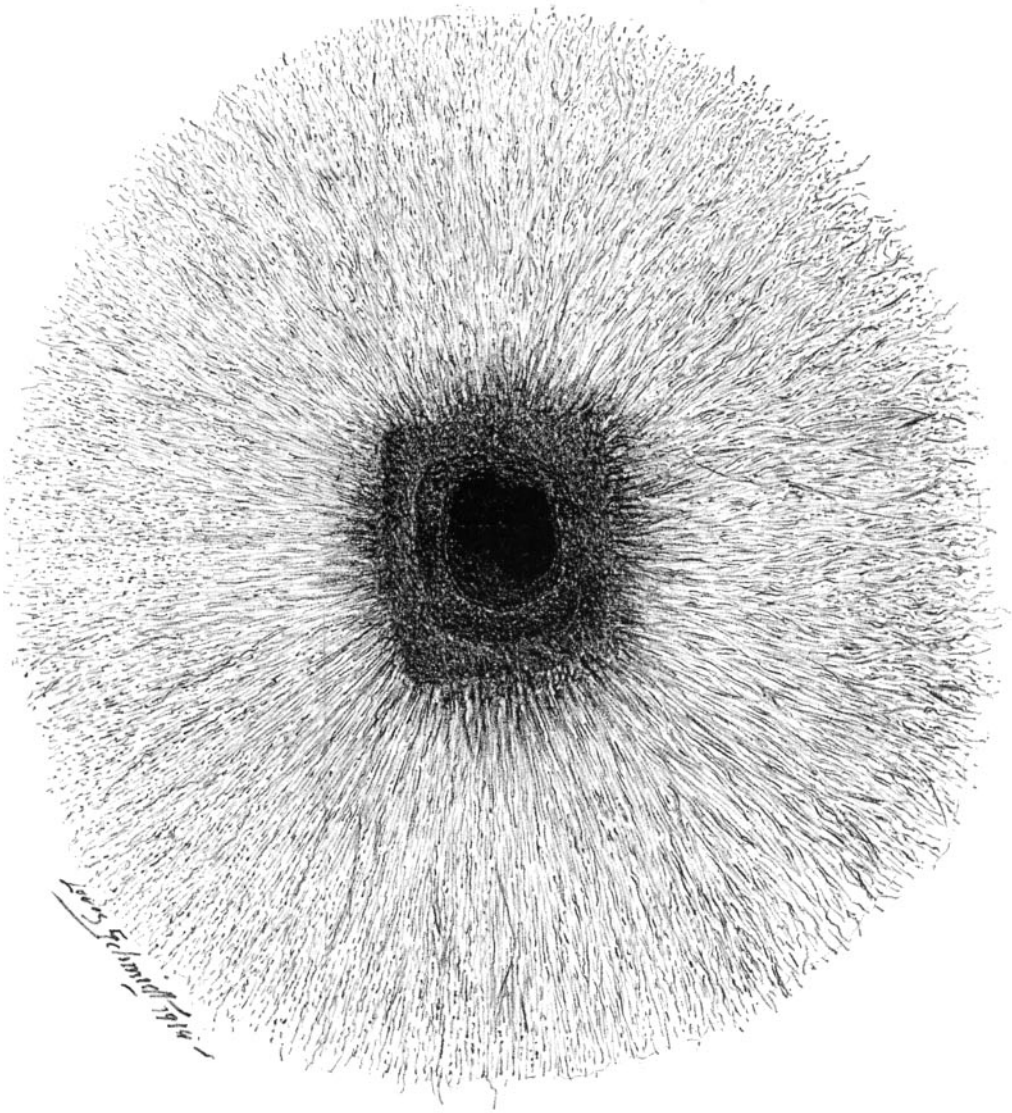

FIG. 2.

(Carrel: Condition of Strain of Connective Tissue.)

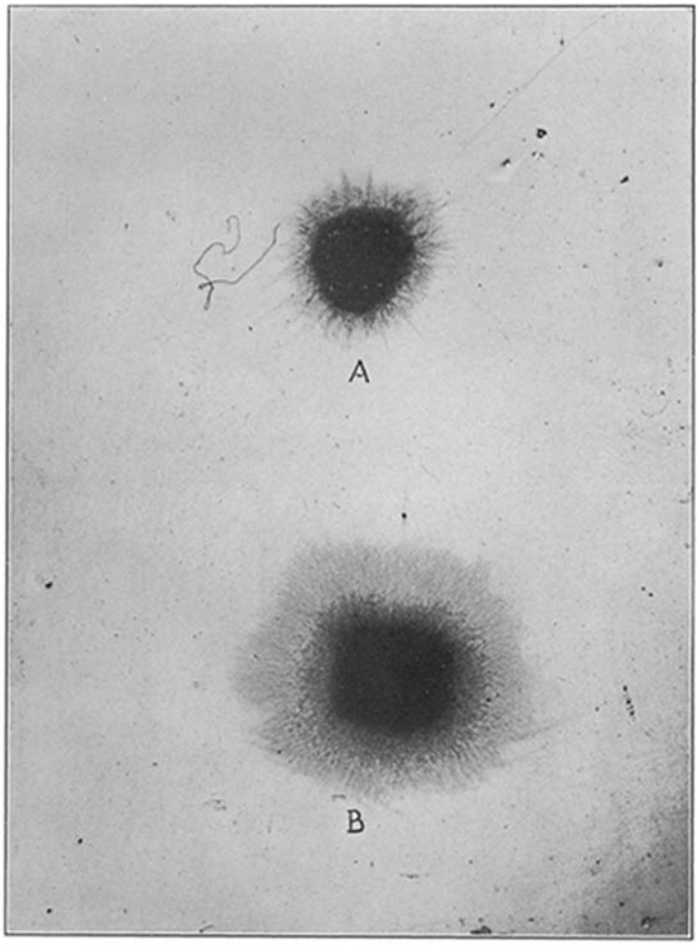

FIG. 3.

(Carrel: Condition of Strain of Connective Tissue.)