

## MEASUREMENT OF THE INHERENT GROWTH ENERGY OF TISSUES.

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It is well known that the growth energy of the organism decreases progressively with age.<sup>1</sup> The activity of each tissue probably declines at a different rate. Fibroblasts grow more rapidly *in vitro* from the heart of a young chick embryo than from that of an older one, and no cells migrate from a fragment of adult heart.<sup>2</sup> Cardiac muscle fibers lose the property of proliferating *in vitro* very early. Cultures of cutaneous epithelium can be obtained from an embryo, while no epithelial cells grow from the skin of a young chicken. Evidently, there is a definite relation between the growth energy of a given tissue and its age, which could be used for measuring the age of the organism. The rate of healing of an aseptic wound is a function of the age of the patient which is determined when the size of the wound and its index of cicatrization are known.<sup>3</sup> While the growth energy of the tissues slowly decreases in function of time, it may fluctuate under the influence of many other factors, as every physiological and pathological process involves a resumption or a decline of cell activity.

The variations of tissue activity *in vivo* are very probably related to those of the growth-inhibiting and promoting properties of the humors.<sup>4</sup> The decline of the rate of multiplication of fibroblasts cultivated in serum in function of the age of the animal from which the serum is obtained resembles the decrease of the index of cicatrization of a wound in function of the age of the patient.<sup>5</sup> The growth energy

<sup>1</sup> Minot, C. S., *The problem of age, growth, and death*, New York, 1908.

<sup>2</sup> Carrel, A., *J. Exp. Med.*, 1913, xviii, 287.

<sup>3</sup> du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 461.

<sup>4</sup> Carrel, A., *J. Exp. Med.*, 1913, xvii, 14. Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 599; 1922, xxxvi, 365. Carrel, A., *J. Exp. Med.*, 1922, xxxvi, 385. Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 645.

<sup>5</sup> Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 599.

of the tissues decreases at the same time that the blood serum becomes more inhibiting to growth. Some years ago, it was found that fibroblasts in different conditions of activity, when cultivated in the same medium, soon grew at the same rate, and that if two halves of a culture were placed in media of different composition, they multiplied at different speeds after a short time.<sup>2</sup> This experiment demonstrated that the rate of cell proliferation *in vitro* depends on certain properties of the medium. We may, then, assume that the activity of a tissue *in vivo* at a given instant is probably a function of its activity at the preceding instant, and of the concentration of growth-activating and inhibiting substances in the interstitial lymph and the blood plasma.

The knowledge of the relations between the tissues and the humors would certainly be of great importance. We can already detect the presence of the growth-activating and growth-inhibiting substances in the blood and the lymph by determining the growth index of the fluids.<sup>6</sup> But a method for measuring inherent cell activity remains to be developed. This inherent growth energy is not improbably proportional to the residual growth energy, that is, to the energy spent by the tissues in a non-nutritive medium until death occurs. The purpose of the experiments described in this article was to develop a technique for measuring the residual energy of fibroblasts and the relations between inherent and residual energies.

#### EXPERIMENTAL.

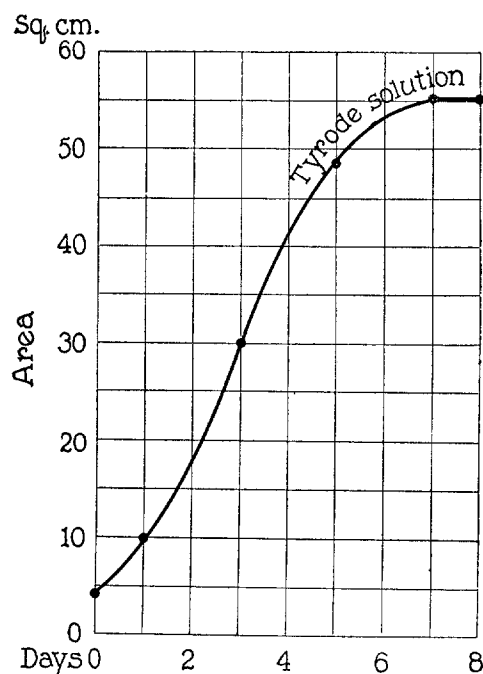
*1. Measurement of the Residual Growth Energy of Fibroblasts.*—A fragment of a pure strain of fibroblasts or of heart tissue was placed in a D-5 flask in 0.5 cc. of plasma and 1.5 cc. of Tyrode solution containing 5 per cent embryonic tissue juice.<sup>7</sup> After coagulation occurred, 1 cc. of Tyrode solution was poured on the surface of the coagulum. Every 48 hours, the fluid was aspirated and replaced by fresh Tyrode solution. Immediately after the preparation of the culture, and every 48 hours, the outline of the tissues was traced in a projectoscope, and the area measured with a planimeter. The extent of the migration of the fibroblasts and the duration of their life represented the residual

<sup>6</sup> Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxv, 17.

<sup>7</sup> Carrel, A., *J. Exp. Med.*, 1923, xxxviii, 407.

activity of the tissue, which finds expression ordinarily in an S-shaped curve.

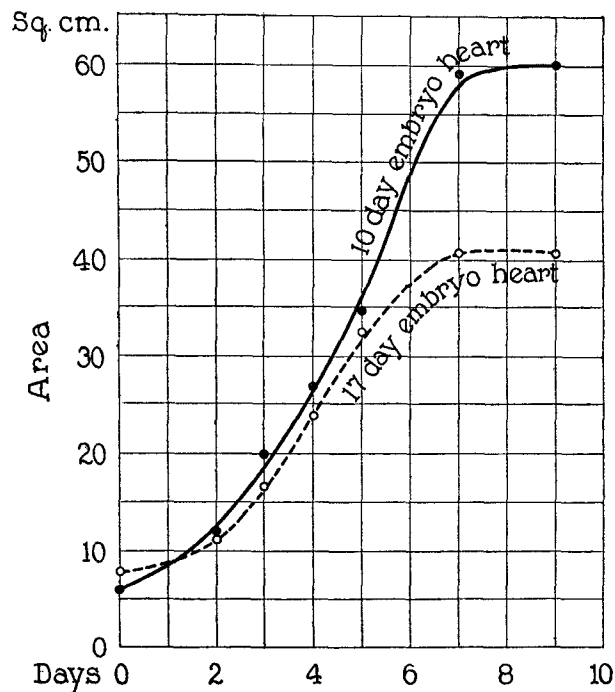
The fragments of fresh tissue usually died before the central part of the original portion disintegrated, that is, before the nutritive substances which they contained had been entirely used. The residual life of a fragment of heart of an 8 day embryo varied from 7 to 12 days. When the original fragment disintegrated early, the rate of multiplication and duration of the life of the fibroblasts were increased.



TEXT-FIG. 1. Residual growth energy of fibroblasts.

Fragments of a pure strain of fibroblasts used all the nutritive substances which they contained before they died. The residual life of a fragment of an 11 year old strain of fibroblasts, in normal condition, generally lasted 7 or 8 days (Text-fig. 1). Under the conditions of the experiments, the period of growth never extended beyond 9 or 10 days. During the first 2 days, there was very little difference in the rate of growth of fibroblasts cultivated in nutrient and non-nutrient

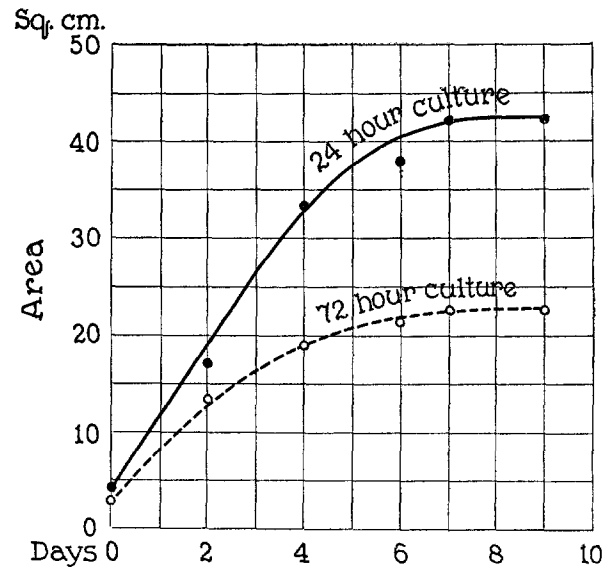
media. As the solid medium was composed of plasma, and the fluid medium of Tyrode solution renewed every 2 days, the tissues were bathed in a fluid which became progressively less concentrated in serum, that is, less inhibiting. The first inflection of the curve might be due to the dilution of the medium under the influence of the Tyrode solution. From the 2nd to the 4th or 5th day, the growth was very active. The effect of starvation became apparent after 4 days. The



TEXT-FIG. 2. Residual growth energy of fragments of heart of 10 and 17 day chick embryos.

growth slackened and the curve began the second inflection. As the solid medium was constantly in contact with Tyrode solution, the decrease in the rate of growth cannot be referred to an accumulation of catabolic substances, but to the lack of nutrient materials. It is evident that the activity of the cells in a medium composed chiefly of Tyrode solution must depend in large measure upon the preceding condition of activity.

2. *Residual Growth Energy of Tissues from Animals of Different Ages.*—In several experiments, two fragments of the ventricle of the hearts of 10 and 17 day chick embryos were placed in a D-5 flask, according to a technique previously described.<sup>7</sup> The curve expressing the residual activity, as determined by the experiment and recorded in Text-fig. 2, shows that the duration of life of both tissues was about the same, but that the growth of the older tissue was 30 per cent less



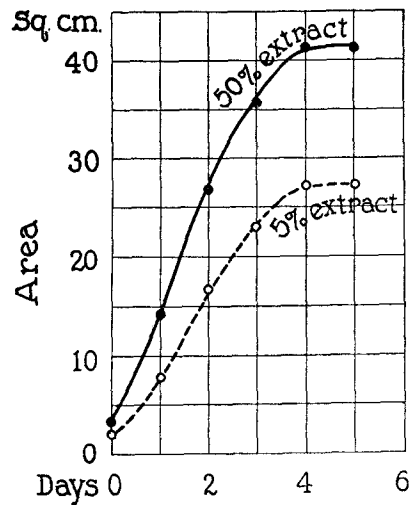
TEXT-FIG. 3. Residual growth energy of fibroblasts kept previously in a hanging drop for 24 and 72 hours respectively.

extensive than that of the younger. There was certainly a marked difference in the inherent activities of the two tissues referable to the difference in age of the embryos from which they were derived.

3. *Residual Growth Energy of a Pure Strain of Fibroblasts after 24 and 72 Hours Cultivation in a Hanging Drop.*—When fibroblasts are cultivated in a hanging drop of plasma and embryonic tissue juice, the S-shaped growth curve shows that great variations occur in the inherent activity of the tissues during 72 hours. The period of optimal growth lasts from about the 12th to the 48th hour. Later, the curve flattens

and becomes almost parallel to the time axis after 72 hours.<sup>8</sup> This second inflection is due to the spontaneous disappearance of the thermolabile growth-promoting substances of the medium and to the accumulation of catabolic products. It is probable that fragments of a pure strain of fibroblasts brought to different states of activity by cultivation for 24 and 72 hours in a hanging drop would show corresponding differences of residual activity.

Several fragments of an 11 year old strain of fibroblasts were cultivated on hollow slides in hanging drops composed of 1 volume of plasma and 1 volume of embryonic tissue juice. After 24 and 72 hours,



TEXT-FIG. 4. Residual growth energy of fibroblasts kept previously for 48 hours in a hanging drop containing 5 and 50 per cent embryonic tissue juice respectively.

respectively, fibroblasts were taken from the hanging drop cultures, imbedded in the medium in a D-5 flask, and their residual growth observed. The duration of life was about the same, but the extent of migration of the fibroblasts cultivated for 72 hours was about 45 per cent less than that of those cultivated for 24 hours (Text-fig. 3).

4. *Residual Growth Energy of a Pure Strain of Fibroblasts Cultivated in Media Containing 5 and 50 Per Cent Embryonic Tissue Extracts.*—Two halves of a fragment from an 11 year old strain of fibroblasts were placed

<sup>8</sup> Ebeling, A. H., unpublished experiments, 1919.

in media containing respectively 5 and 50 per cent embryonic tissue juice, in order to modify the inherent activity. After 48 hours, both fragments were placed in a flask in the ordinary medium and the residual activity was measured. The duration of life of both fragments was about the same, but the area covered by the cells of the fragments previously cultivated in 50 per cent embryonic tissue juice was much larger. In the experiment recorded in Text-fig. 4, the activity of the cells, as expressed by the extent of their migration, was 25 per cent greater for the tissues previously cultivated in 50 per cent embryonic tissue juice than for those cultivated in 5 per cent.

#### CONCLUSIONS.

1. The residual growth energy of fibroblasts is expressed by the extent of their migration and multiplication in a non-nutrient medium.
2. The residual energy of fibroblasts is related to their inherent energy and the variations of the inherent energy can be ascertained by the measurement of the residual energy.