# ANTAGONISTIC GROWTH-ACTIVATING AND GROWTH-INHIBITING PRINCIPLES IN SERUM.

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It is known that the serum of an adult animal restrains the proliferation *in vitro* of homologous fibroblasts and that its inhibiting power increases progressively as the age of the animal advances.[1] It has also been observed that, when serum has been shaken for several hours,[2] or heated for half an hour at 65°C.,[3] its restraining influence on cell activity is still more marked. By contrast, serum which contains leucocytic secretions[4] or aqueous extracts of certain tissues[5] becomes a better culture medium for homologous cells. These facts led to the supposition that the action of serum on homologous fibroblasts was not due to one inhibiting substance only, but rather to the combined action of two substances, one activating and the other inhibiting. In order to test this hypothesis, an attempt was made to separate an activating substance from serum, and to find out whether, after the removal of this substance, the inhibiting effect of serum on homologous cells was enhanced.

## EXPERIMENTAL.

The serum was obtained, as a rule, from plasma of chickens between 1 and 2 years of age, and occasionally from younger or older chickens. 10 cc. of serum were diluted with 90 cc. of distilled water and precipitated by bubbling $CO_2$ through the solution for 10 minutes. The precipitate was thrown down by centrifugation and, after the

[1] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 599.
[2] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 399.
[3] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxv, 647.
[4] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 645.
[5] Carrel, A., *J. Exp. Med.*, 1913, xvii, 14.

supernatant fluid had been pipetted off, dissolved in 10 cc. of Tyrode solution. The pH of the solution was brought to between 7.6 and 8. The supernatant fluid was evaporated *in vacuo* until its volume was reduced to 10 cc., and the pH was adjusted to that of the precipitate solution.

Tyrode solution containing the dissolved precipitate and the treated serum were compared to Tyrode solution and the original serum respectively. The medium was composed of one volume of plasma and three volumes of solution or serum, and contained 1/60 embryonic tissue juices. The tissues were taken from a 10 year old strain of fibroblasts.[6] The rate of growth was ascertained by the increase in the area of the tissues in 48 hours, and the measurements were made according to a technique previously described.[7] As the differences in the rate of growth were expected to be very small, it was necessary to use the greatest care in the application of the technique in order to prevent the errors from being larger than 7 per cent. To express such differences, we have used the ratio between the relative tissue increase in the cultures containing dissolved precipitate or treated serum and that observed in the appropriate controls.

*1. Action of the Substances Precipitated from Serum by $CO_2$ on Homologous Fibroblasts.*—The action on homologous fibroblasts of the substances precipitated by $CO_2$ could not be directly compared with that of the original serum because the rate of cell proliferation increases when the concentration of serum proteins in the culture medium is diminished. As the precipitate consists of only a part of the serum proteins, a better growth of the fibroblasts in the precipitate solution than in the original serum would not necessarily mean that the precipitate contains growth-activating substances. It could occur merely as an effect of a lesser concentration of an inhibiting substance. Therefore, the action of the precipitate had to be compared to that of Tyrode solution, in which the migration of fibroblasts is known to be always more active than in the serum of an adult animal.

In fourteen groups of experiments, a medium containing 75 per cent of Tyrode solution as such was compared to a medium containing the same amount of the solution of the substances precipitated by

[6] Ebeling, A. H., *J. Exp. Med.*, 1922, xxxv, 755.

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

$CO_2$ (Table I). The average rate of migration was 13 per cent greater in the medium containing the $CO_2$ precipitate. As the errors due to the technique are about 7 per cent,[7] it would appear that the precipitated substances have a weak activating effect on homologous fibroblasts. As the proliferation of fibroblasts in Tyrode solution is more active than in the plasma of a chicken 1 or 2 years old,[8] the rate of growth in the precipitate solution is much greater than in the original serum.

TABLE I.

*Action of the $CO_2$ Precipitate on the Rate of Growth of Homologous Fibroblasts.*

| Group No.* | Culture No. | Rate of growth in. | | Ratio, $\frac{E}{C}$. | Tyrode solution. | | Precipitate solution. | |
|---|---|---|---|---|---|---|---|---|
| | | Tyrode solution (C). | Precipitate solution (E). | | pH | Refractive index. | pH | Refractive index. |
| 1 | 29987 | 2.86 | 3.54 | 1.23 | 8.0 | 1.3280 | 7.4 | 1.3314 |
| 2 | 30050 | 3.47 | 3.70 | 1.06 | 8.0 | 1.3280 | 7.6 | 1.3286 |
| 3 | 30098 | 4.20 | 4.43 | 1.05 | 8.0 | 1.3280 | 7.8 | 1.3304 |
| 4 | 30154 | 4.22 | 4.50 | 1.07 | 8.0 | 1.3280 | 7.8 | 1.3304 |
| 5 | 30184 | 4.17 | 4.20 | 1.01 | 8.0 | 1.3290 | 7.6 | 1.3320 |
| 6 | 30229 | 3.24 | 3.72 | 1.15 | 8.0 | 1.3290 | 7.8 | 1.3320 |
| 7 | 30282 | 3.65 | 4.20 | 1.15 | 8.0 | 1.3290 | 7.6 | 1.3316 |
| 8 | 30312 | 3.15 | 3.42 | 1.09 | 8.0 | 1.3290 | 7.6 | 1.3316 |
| 9 | 30650 | 3.27 | 3.60 | 1.10 | 8.0 | 1.3290 | 7.8 | 1.3298 |
| 10 | 30709 | 2.92 | 3.28 | 1.12 | 8.0 | 1.3290 | 7.6 | 1.3305 |
| 11 | 31217 | 3.80 | 3.86 | 1.02 | 8.0 | 1.3280 | 7.8 | 1.3301 |
| 12 | 31239 | 3.63 | 3.81 | 1.05 | 8.0 | 1.3280 | 7.7 | 1.3291 |
| 13 | 1928 | 4.26 | 5.33 | 1.25 | | | | |
| 14 | 1959 | 2.75 | 5.14 | 1.87 | | | | |
| Average..... | | | | 1.15 | | | | |

* Each group averaged from four to five experiments.

*2. Action of the Treated Serum on Homologous Fibroblasts.*—The treated serum was reduced to its original bulk of 10 cc. by evaporation *in vacuo* and compared to the original serum as regards its action on homologous fibroblasts. Since serum is rendered less concentrated by the removal of the globulins, a lessening in its inhibiting action on fibroblasts might be expected. Should it prove instead to be more growth-restraining than the original serum, in spite of its lower

[8] Carrel and Ebeling,[1] Text-fig. 12.

concentration in proteins, there would be no doubt that the removal of the globulins had increased its inhibiting power. In fourteen groups of experiments the rate of proliferation of fibroblasts was found to be 26 per cent slower in the serum deprived of globulinthan in the original serum (Table II).

TABLE II.

*Action of the Treated Serum on the Rate of Growth of Homologous Fibroblasts.*

| Group No.* | Culture No. | Rate of growth in. | | Ratio, $\frac{E}{C}$. | Original serum. | | Treated serum. | |
|---|---|---|---|---|---|---|---|---|
| | | Original serum (*C*). | Treated serum (*E*). | | pH | Refractive index. | pH | Refractive index. |
| 1 | 31187 | 2.38 | 1.69 | 0.71 | 7.8 | 1.3356 | 7.6 | 1.3310 |
| 2 | 31191 | 3.68 | 2.98 | 0.81 | 7.8 | 1.3356 | 7.6 | 1.3310 |
| 3 | 31192 | 5.36 | 3.46 | 0.64 | 7.5 | 1.3360 | 7.6 | 1.3310 |
| 4 | 31210 | 2.91 | 2.17 | 0.75 | 7.5 | 1.3360 | 7.6 | 1.3310 |
| 5 | 31209 | 2.64 | 2.06 | 0.78 | 7.4 | 1.3320 | 7.4 | 1.3310 |
| 6 | 31201 | 3.99 | 3.21 | 0.80 | 7.4 | 1.3320 | 7.4 | 1.3310 |
| 7 | 31218 | 3.26 | 2.87 | 0.88 | 8.0 | 1.3385 | 8.0 | 1.3345 |
| 8 | 31225 | 4.45 | 3.45 | 0.78 | 8.0 | 1.3385 | 8.0 | 1.3345 |
| 9 | 31228 | 3.25 | 2.84 | 0.87 | 8.0 | 1.3385 | 8.0 | 1.3345 |
| 10 | 31234 | 2.87 | 2.19 | 0.77 | 7.8 | 1.3370 | 8.0 | 1.3320 |
| 11 | 31240 | 2.94 | 2.36 | 0.80 | 7.8 | 1.3370 | 8.0 | 1.3320 |
| 12 | 1774 | 5.40 | 3.82 | 0.70 | | | | |
| 13 | 1784 | 6.10 | 3.02 | 0.50 | | | | |
| 14 | 1786 | 5.30 | 2.36 | 0.45 | | | | |
| Average..... | | | | 0.74 | | | | |

* Each group averaged from four to five experiments.

*3. Action on Homologous Fibroblasts of Normal Serum, and of Serum Diluted and Reduced by Evaporation to Its Original Volume.*—The question arose as to whether the increase in the inhibiting action of serum might not be due merely to some deterioration of the proteins occurring during evaporation. To determine the point, 10 cc. of serum were diluted with 90 cc. of distilled water and reduced by evaporation to the normal volume. The actions of the normal and of the evaporated sera on homologous fibroblasts were compared (Table III). No marked differences were found in the rate of growth.

*4. Simultaneous Action of Treated Serum and Globulin Precipitate on Homologous Fibroblasts.*—The precipitate was dissolved in the

serum from which it came and the action of the solution on fibroblasts was compared to that of the original serum (Table IV). The differences in the rate of growth of fibroblasts cultivated in normal

TABLE III.

*Action of Diluted and Reconcentrated Serum on the Rate of Growth of Homologous Fibroblasts.*

| Experiment No. | Culture No. | Rate of growth in. | | Ratio, $\frac{E}{C}$. | Original serum. | | Diluted and reconcentrated serum. | |
|---|---|---|---|---|---|---|---|---|
| | | Original serum (*C*). | Diluted and reconcentrated serum (*E*). | | pH | Refractive index. | pH | Refractive index. |
| 1 | 31249 | 2.39 | 2.39 | 1.00 | 8.2 | 1.3373 | 8.2 | 1.3369 |
| 2 | | 2.93 | 2.70 | 0.92 | | | | |
| 3 | | 3.25 | 3.25 | 1.00 | | | | |
| 4 | | 2.91 | 2.85 | 0.98 | | | | |
| 5 | | 3.64 | 3.42 | 0.94 | | | | |
| Average..... | | | | 0.97 | | | | |

TABLE IV.

*Action of the $CO_2$ Precipitate Dissolved in the Treated Serum on the Rate of Growth of Homologous Fibroblasts.*

| Experiment No. | Culture No. | Rate of growth in. | | Ratio, $\frac{E}{C}$. | Original serum. | | $CO_2$ precipitate dissolved in treated serum. | |
|---|---|---|---|---|---|---|---|---|
| | | Original serum (*C*). | $CO_2$ precipitate dissolved in treated serum (*E*). | | pH | Refractive index. | pH | Refractive index. |
| 1 | 31271 | 3.60 | 3.20 | 0.89 | 8.1 | 1.3372 | 8.0 | 1.3365 |
| 2 | | 3.50 | 3.30 | 0.94 | | | | |
| 3 | | 2.84 | 2.72 | 0.96 | | | | |
| 4 | | 3.17 | 2.90 | 0.91 | | | | |
| Average..... | | | | 0.93 | | | | |

serum, and in the solution of the precipitate in the supernatant serum were only 7 per cent; that is to say, they came within the limits of error of the method.

## CONCLUSIONS.

The substances precipitated by $CO_2$ from the serum of chickens 1 or 2 years old slightly increased the activity of pure cultures of a 10 year old strain of fibroblasts, an indication that a growth-promoting substance had been obtained from the serum. The rate of growth of fibroblasts was slower in serum deprived of its globulins and the increase of the inhibiting effect of the treated serum is thus manifested. It would appear that the inhibiting action of serum is referable to one or several growth-activating substances which precipitate by $CO_2$, and to one or several growth-retarding substances which remain in the supernatant serum after the precipitate has been thrown down by centrifugation.

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

1. A substance enhancing the proliferative activity of homologous fibroblasts is precipitated from serum by $CO_2$.

2. The serum from which the precipitate has been removed inhibits the proliferation of homologous fibroblasts more markedly than does the original serum. The solution of the precipitate in the treated serum has about the same effect on fibroblasts as the original serum.

3. The restraining effect of serum on the activity of homologous fibroblasts is due in part to the antagonistic action of growth-activating and inhibiting substances.

We wish to express our thanks to Dr. Albert Fischer for the preparation of the globulins used in a few of the experiments.