GROWTH-PROMOTING FUNCTION OF LEUCOCYTES.

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(Received for publication, April 10, 1922.)

It is well known that the tissues of an adult animal remain capable of resuming the activity characteristic of youth. Even in extreme old age, wounds always heal, although cicatrization may require the formation of a large amount of new tissues. We are as yet ignorant of the mechanisms which cause cells at rest in a growth-inhibiting medium, such as the humors of an old animal, to proliferate again. The activity of a cell at a given instant is function of its activity at the preceding instant and of the concentration of certain substances in its medium.[1] Therefore, it is probable that tissues which have been in a resting condition for several years cannot grow again unless they receive the food material required by the cells for their multiplication. We know that certain substances contained in embryonic juice are endowed with the remarkable property of greatly activating the rate of cell proliferation *in vitro*.[1,2] Possibly such growth-promoting substances must be supplied to adult tissues when they cicatrize or regenerate. One of the sources of these substances may be the leucocytes which, remaining in the embryonic stage of development during the entire life of the organism, probably contain the growth-activating substances characteristic of embryonic tissues. The purpose of the experiments described in this article was to study the value of this hypothesis by ascertaining whether leucocytes contain and secrete growth-promoting substances, and whether tissues and exudates in which they accumulate acquire the power of activating cell proliferation.

[1] Carrel, A., *J. Exp. Med.*, 1913, xviii, 287.

[2] Carrel, A., *J. Exp. Med.*, 1913, xvii, 14. Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 317.

I.

Growth-Promoting Action of Leucocytic Extracts and Secretions.

Leucocytes were obtained from the blood of chickens 1 or 2 years old. After centrifugation, the leucocytes were removed from the surface of the red blood corpuscles, washed several times in Ringer solution, and placed in a small amount of distilled water. After 1 or more days in the ice box, the cell suspension was shaken with once

TABLE I.

Action of an Extract of Leucocytes on the Rate of Multiplication of Fibroblasts.

Experiment No.	Extract No.	Nature of tissue cultivated.	Culture No.	Width of ring of new tissue.		
				Leucocyte extract.	Embryo juice.	Ringer solution.
1	247	Embryonic heart.	247	4.0		2.0
2	247	" "	247	4.0		2.0
3	247	" "	247	5.5	6.0	
4	247	" "	247	5.0	6.0	
5	275	" "	276	5.0		2.5
6	275	" "	276	6.0		2.0
7	275	" "	288	3.5		2.0
8	275	" "	288	3.0		2.5
9	470	" "	490	4.0		2.5
10	470	" "	477	3.0		1.7
11	18488	" "	571	2.5	3.0	
12	18488	1685th passage fibroblasts.	571	4.0	3.0	
13	18488	1685th " "	575	3.0	3.0	
14	18488	1687th " "	587	5.0	6.0	
15	18488	First subculture from Experiment 14.	587	5.0	5.0	

or twice its volume of Ringer solution and centrifuged. The extracts were, therefore, more diluted than the embryonic juice generally used in tissue cultures. Their activity was tested against fragments of chick embryo heart and pure cultures of chicken fibroblasts. The medium was composed of one or two volumes of plasma and one volume of leucocytic extract. In the controls, the leucocytic extract was replaced by Ringer solution or by embryonic juice. The rate of growth was ascertained by measurements of the width of the ring of new tissue which had grown in 48 hours. Fourteen experiments were performed (Table I). Although the extracts were diluted, the

width of the growth was generally 100 per cent greater in the experiments than in the controls containing Ringer solution, and about the same as in embryonic tissue extract. It is evident that dead leucocytes set free substances which promote cell multiplication as do embryonic juices.

An investigation was then made as to whether living leucocytes may secrete growth-promoting substances such as are extracted from them after death. Long ago, growth-activating substances were found to be secreted by embryonic tissues *in vitro*.[1,2] Pulp of embryonic tissue was cultivated in plasma and Ringer solution. The experiments were incubated, while the controls were kept in cold storage. After 1 or 2 days, the serum was extracted from the coagulum and its activity tested against fibroblasts. The activating power of the fluid extracted from the cultures preserved in cold storage was lower than that of the cultures kept in the incubator. It appeared that the tissues growing actively for a short time had modified their medium by secreting in it growth-activating substances. Similar experiments were made with leucocytes. A medium composed of practically equal parts of chicken plasma and hypotonic Tyrode solution was spread on the surface of the cover of Gabritschewski dishes. About thirty fragments of a film of chicken leucocytes were placed in the medium. The control contained no leucocytes. Both dishes were incubated for 48 hours, and large colonies of leucocytes spread into the clot. Then the serum was extracted from the coagulum and tested against a pure strain of fibroblasts. The fluid from the medium which had contained leucocytes was markedly more active. It was evident that, under the conditions of the experiments, leucocytes secreted a substance which activated the rate of growth of fibroblasts.

II.

Growth-Promoting Action of Inflamed Connective Tissue Extracts and Peritoneal Exudates.

If leucocytes be capable of setting free growth-activating substances *in vivo* as well as *in vitro*, tissues and exudates where they accumulate must acquire the power of accelerating cell multiplication. Therefore, an attempt was made to ascertain whether inflamed connective tissue contains growth-promoting substances. It is known that extracts of connective tissue stimulate cell proliferation only slightly.[1]

However, control experiments were repeated. Subcutaneous connective tissue taken from a chicken was extracted with Ringer solution. The growth in the cultures containing the extract was about 25 per cent larger than in the controls containing only Ringer solution. In order that leucocytes could accumulate in the connective tissue, a focus of aseptic inflammation was produced by injecting a solution of dilute hydrochloric acid into fragments of sponge placed under the skin of chickens.[3] The connective tissue around the foreign bodies became markedly thickened, although no abscess developed. Fragments of inflamed connective tissue were then removed and cultivated in plasma in order to ascertain whether they contained living leucocytes. They were soon surrounded by a large ring of ameboid cells and after 24 hours the cultures looked almost like those of Rous sarcoma. The inflamed connective tissue was sliced into a pulp, extracted with a small amount of Ringer solution, and the extract tested against fibroblasts and embryonic heart. The medium was composed of one or two volumes of plasma and one volume of extract. The controls contained Ringer solution or embryonic tissue extract. Although the inflamed tissue extracts were dilute, the width of the new tissue was more than doubled by their presence (Table II). There was no doubt that inflamed connective tissue contained substances capable of increasing the rate of multiplication of fibroblasts.

Similar experiments were repeated with peritoneal exudates. Injections of staphylococcus suspension, dog red blood corpuscles, turpentine, or bouillon into the peritoneum of chickens did not determine the production of an exudate. However, pus obtained from a turpentine abscess in a dog was injected into the peritoneum of two chickens, and an opaque fluid, orange-yellow in color, was aspirated from the abdominal cavity 2 days later. This fluid contained a large number of white blood corpuscles. After centrifugation, it was tested against embryonic heart tissue and fibroblasts. In twelve experiments, the width of new tissue was 100 per cent larger than that obtained in Ringer solution and about equal to that in embryonic juice (Table III). It was then certain that a peritoneal exudate containing many leucocytes had acquired the power of stimulating cell multiplication.

[3] All operations were performed under ether anesthesia.

TABLE II.

Action of an Extract of Inflamed Connective Tissue on the Rate of Multiplication of Fibroblasts.

Experiment No.	Extract No.	Nature of tissue cultivated.	Culture No.	Width of ring of new tissue.		
				Inflamed tissue extract.	Embryo juice.	Ringer solution.
1	213	Embryonic heart.	258	1.0		0.5
2	213	" "	258	1.5		0.5
3	213	" "	258	1.5		0.5
4	213	" "	263	4.2		2.7
5	213	1650th passage fibroblasts.	261	7.0	5.0	
6	213	1650th " "	261	5.5	6.0	
7	213	Embryonic heart.	269	3.0		1.0
8	213	" "	269	3.0		1.5
9	213	" "	269	4.0		1.5
10	311	" "	322	3.0		2.0
11	328	" "	332	4.0		2.0
12	328	" "	332	4.0		2.0
13	339	" "	349	1.5		0.8
14	339	" "	350	3.0		0.8

TABLE III.

Action of Peritoneal Exudate on the Rate of Multiplication of Fibroblasts.

Experiment No.	Exudate No.	Nature of tissue cultivated.	Culture No.	Width of ring of new tissue.		
				Exudate.	Embryo juice.	Ringer solution.
1	844	1719th passage fibroblasts.	846	5.0	5.0	
2	844	1719th " "	846	3.0	4.0	
3	844	Embryonic heart.	852	2.0		1.0
4	844	" "	852	2.0		1.0
5	850	" "	858	2.0		0.8
6	850	" "	858	1.5		0.5
7	850	" "	858	3.5		0.5
8	850	" "	858	4.5		0.5
9	850	" "	864	2.5	2.0	
10	850	" "	864	1.75	2.0	
11	866	1727th passage fibroblasts.	852	2.0		1.0
12	866	1727th " "	852	2.0		1.0

III.

DISCUSSION AND SUMMARY.

Two main facts were brought to light by the preceding experiments: first, the presence of growth-activating substances in the leucocytes; second, the setting free of these substances in tissues and fluids where leucocytes accumulate. The existence of growth-promoting substances within the body of the leucocytes was to be expected. Leucocytes are embryonic cells and it is well known that embryonic tissues contain substances which stimulate cell proliferation. But the experiments gave a direct experimental proof of this fact. Then, during the whole life and even in extreme old age, there is a supply of growth-promoting substances within the organism which is potentially capable of restoring the activity of the resting cells. Embryonic tissue juice, as is known, can rejuvenate cells which have ceased to multiply *in vitro* and show evidences of degeneration.[1] If the growth-activating substances of leucocytes can be transferred *in vivo* to tissue cells, they may play a similar rôle.

Therefore, it was important to find out whether the growth-activating substances were set free either by the secretions of the living leucocytes or by diffusion from the body of the cells after they were injured or dead, and whether this phenomenon occurred actually *in vivo*. Indeed, the idea that leucocytes secrete substances necessary to normal physiological processes is by no means new. Long ago, Ranvier described the lymph cells as mobile unicellular glands,[4] and Renaut thought that their function was to bring to fixed cells the necessary food material.[5] This rôle of the leucocytes was considered by him as absolutely essential. According to his theory, differentiated cells could not live in the absence of the lymph cells, which supply them with the substances required for their activity. The presence of such substances in the leucocytes was shown by our experiments and the growth-activating power acquired by inflamed connective tissue demonstrated that the leucocytes could actually bring these substances to the fixed cells.

[4] Ranvier, cited by Renaut, J., Traité d'histologie pratique, Paris, 1889, i, pt. 1, 79.

[5] Renaut, J., Traité d'histologie pratique, Paris, 1889, i, pt. 1, 79, 94.

Under certain conditions, this property of the white cells of the blood may cause the resumption of the activity of tissues which are in a resting state. In the adult organism, the tissues have ceased to grow and the blood plasma has acquired marked inhibiting properties. But growth-promoting substances are still stored in leucocytes, glands, and muscle tissue. The leucocytes could supply fibroblasts or epithelial cells with the necessary food material if they were present where cell proliferation is needed. The existence of mechanisms causing leucocytes to invade tissues in need of repair is certain. The initiation of healing seems to depend on the coming of the leucocytes to the wounded tissue. When they are missing, as happens when the wound is protected from all external irritation, cicatrization is greatly delayed. On the contrary, when staphylococci, turpentine, and other irritants are applied at the surface of the wound, granulations appear after less than 48 hours.[6] These irritants, although different in nature, have the common characteristic of determining an inflammation of the tissues and the migration of leucocytes from the vessels to the surface of the wound. Possibly the white cells bring the substances which adult tissues require in order to cicatrize or regenerate. They would have the function of storing away the growth-promoting substances characteristic of embryonic tissues, and bringing them to the regions of the organism where they are needed.

IV.

CONCLUSIONS.

1. Leucocyte extracts, like embryonic tissue juice, possess the power of increasing the rate of multiplication of fibroblasts *in vitro*.

2. Leucocytes secrete substances *in vitro* which also promote cell multiplication.

3. Peritoneal exudate or connective tissue invaded by leucocytes acquires the power of increasing the rate of multiplication of fibroblasts.

4. Leucocytes are capable of bringing growth-activating substances to tissue cells. They may have the important function of promoting cell multiplication in the parts of the organism where they accumulate under certain conditions.

[6] Carrel, A., *J. Exp. Med.*, 1921, xxxiv, 425.