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Studies on cancer.
II. The significance of the effect of circulation on the growth of cells.

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As early as 1913[1,2] I had already shown that the growth and migration of fixed tissue cells is proportional at all times to the size of the fragment, the cell density of the fragment and indirectly proportional to the thickness of the layer of the medium. All of these proportions hold, however, only to the limits of an active oxygen diffusion to all parts of the culture. Oxygen, as I have pointed out, diffuses readily only 0.5 to 0.7 mm. into clots and later I have found that it diffuses readily no greater than 1 mm. into many dense fragments of tissue. For any of these fixed tissues the maximum activity of the cell is seen about fragments 1 mm. in diameter planted in layers of medium 0.5 mm. in thickness.

Single cells cannot grow in the plasmatic medium of the culture. Connective tissue and mesenchyme cells may stretch out to take their characteristic shapes and migrate short distances. These cells will not grow. Active migration is always proportional to the size of the fragment up to the limits cited above. In the primary plasma cultures an active growth of the mesenchyme cells is seen only about the cellular fragments of the tissues of young embryos and malignant tumors. The cells from these fragments suffer not only this active growth reaction but they later suffer frequently a complete degeneration in the cultures.

This degeneration as it is seen in these active growing cells is peculiar to all of the cultures. It commences early in the centers of all fragments except the least cellular ones cut from scars and adult subcutaneous tissue. It spreads outwards from this center. In the cultures of the more cellular tissues it involves sooner or

1 Burrows, M. T., *Trans. Cong. Am. Phys. and Surgeons*, 1913, ix, 77.

later all the cells except those which migrate directly into the plasmatic medium and out of the line of diffusion of substances from the fragment along the surface of the medium. About the less cellular fragments it never involves the cells which have migrated into the medium. These cells come to rest after a short time unless the medium undergoes liquefaction as it does about the epithelial cells. In this inactive state the mesenchyme and connective tissue cells may remain apparently indefinitely unless new medium is added to the culture.[2]

In the earlier studies of the tissue culture it has also been noted that the rate of activity of any given tissue is inversely proportional to its age. About equal size fragments of the heart muscle of chick-embryos the rate and extent of migration becomes less and less as these fragments are taken from older and older embryos.

Directly associated with this decrease in the rate and extent of migration the degeneration becomes less and less extensive and growth finally ceases. Together with these changes the latent period before any activity is noted in the cultures, increases. About fragments of a 5 day old chick-embryo heart, migration may begin within 1 hour of the time the cultures are prepared. About a simple fragment of a 10 day old chick-embryo heart, no activity is seen before 6 to 12 hours and about a 15 day old chick-embryo heart, before 12 to 18 hours.

At first I thought that these changes were to be related directly to an aging of the cells. It soon became evident, however, that this was not true. It was to be related rather to a gradual development of intercellular substances and blood vessels and a corresponding separation of the cell. The rate and extent of migration, the growth or degeneration of any tissue is proportional to the original cell-density of the fragment whether food and oxygen are maintained or not. The cancer cells of the cultures are not different from the normal cell. The cells from fragment of cellular granulation tissue react like those from equally cellular fragment of the embryo. The cells from cellular fragment of a malignant round cell-sarcoma react like the mesenchyme cells from the young embryonic tissue while those from a fibro-sarcoma react like the cells from older embryos in which

2 Burrows, M. T., *XVII Int. Cong. Med., Gen. Path. and Path. Anat.*, London, 1913, 217

the intercellular substance and blood vessels are the same as that of the fibro-sarcoma.

The only difference that I have been able to find between the cancerous tissue and tissue of young embryos is a richer supply of reserve nutrient substances in the embryonic tissues. The cancerous tissues have little of this reserve. Extracts of cancerous tissues stimulate growth in other cells like extracts of embryonic tissue but they do not prolong it.

While it is true that many authors have assumed that the disappearance of independent growth in embryonic life is the result of an aging of the cell, the best work of this and the last century has shown that this is not true. As Wilson[3] clearly states, growth, differentiation and function are changes not determined by the cell but by more general formative forces or stimuli. Ribbert had fully appreciated this fact in relation to cancer. As he states the remarkable power of tissues to regenerate shows clearly that the cells of the fully developed organism have not lost their property of growth. The growth in cancer Ribbert did not find unique. He thought it could be nothing more than the response that any cell may undergo under the influence of a proper change in the environment.

The difficulty which confronted Ribbert and his school has been the finding of the nature of the environment suitable for this change. The tissue culture as I have already pointed out has aided much in this search. In previous studies[4] I had noted that embryonic heart muscle cells may respond like simple migrating mesenchyme cells, actively growing cells or normally rhythmically contracting cells in the culture. Each of these responses is to be related in each case to a specific environment. The rhythmically contracting cells are stretched through a serum cavity between a surface of the medium in the tissue fragment and the end of a band of fibrin. This same cell removed from these contacts and laid against a fibrin-fibril stretches out to become a simple spindle shaped connective tissue-like cell. When placed at the surface of the medium near a cellular fragment of tissue it flattens to an irregular polygonal or spindle shape, grows actively and divides by mitoses.

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[3] Wilson, E. B., *The Cell in Inheritance and Development*, 1906.

[4] Burrows, M. T., 2nd Pan Am. Sci. Cong., Washington, D. C., Sect. VIII, Pt. 2, 494

In a further analysis of muscular contraction I showed that this whole phenomenon can be explained as a differential surface tension phenomenon established by specific contacts of the cell with a heterogeneous environment.[5] In the same manner it can be shown that all functional activity in the organism, like the shape and arrangement of the cell, can be directly related to the immediate environment surrounding the cells.[2]

From these later observations it became evident that growth is not something peculiar to the younger cells. It is a reaction which may be the property of any fixed tissue cell when placed in the proper environment. The environment suitable for such growth is one where the cells are crowded together and the circulation is reduced.

In this regard, as it is evident, body cells are not different from many unicellular organisms. Wildiers,[6] 1902, had shown that single yeast cells will not grow in a large bulk of medium. For growth to take place a certain bulk relation must exist between the medium and the yeast. Webb, Williams, and Barber[7] found that a single tubercle bacillus or a single spore of anthrax will not infect an animal. Several must be inoculated. So in the same manner Robertson[8] has pointed out that the growth rate of paramecium increases with the number of organisms present in the medium.

Wildiers had also noted that this inability for the single yeast cell to grow is due to the lack of some product liberated by these cells. Extracts of yeast added to the medium allow the active growth of a single cell to proceed.

In the early history of a tissue culture Carrel and I[9] had already noted that an active growth stimulant may be readily extracted from fresh embryonic and cancer-tissue. This stimulant is not present in most adult tissues but as the above experiments show this stimulant must develop in these tissues when they are cut away from the body and placed in the culture. Again these experiments indicate that the amount of this stimulant generated is directly proportional to the original cell-con-

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5 Burrows, M. T., *Am. J. Physiol.*, 1917-18, xlv, 556.

6 Wildiers, *E. La Cellule*, 1902, xviii, 313.

7 Webb, G. B., Williams, W. W., and Barber, M. A., *J. Med. Res.*, 1909, xx, 1.

8 Robertson, T. B., *Biochem. J.*, 1921, xv, 596.

9 Carrel, A., and Burrows, M. T., *J. Am. Med. Assn.*, 1910, lvi, 32.

tent of the fragment. In proof for this I have extracted many of these fragments with isotonic sodium chloride solution after they had remained for a time in the culture and recovered the stimulant as indicated by the action of these extracts on other cells.

While these experiments of Carrel's and mine were interesting in showing that the active independent growth of cells in early life and in cancer may be the direct result of the presence of a growth stimulating substance in these tissues, they became of little importance in that they failed to show the source of this substance.

In my earlier experiments with the tissue culture I had already shown that autolysis as it occurs from the absence of oxygen does not liberate a growth stimulating substance. Extracts of such tissues retard or completely inhibit all cell activity. The growth of nerve fibers is strikingly inhibited about the larger fragments of the neural tube while it is most active about smaller ones.[10] In a recent paper before this Society I have again shown that the development of the stimulus for growth is dependent on the presence of oxygen, while the growth reaction itself like muscular contraction (Fletcher) is not dependent on this gas. The cells from fragments of the heart muscle of 5 day old chick-embryos will show a limited growth in the absence of oxygen. The cells in older fragments demand oxygen for the same limited growth. The amount demanded by these older tissues is directly proportional to their age and the latent period for their growth in an ample oxygen supply.[11]

Drew[12] has recently stated that autolyzing tissue liberates a growth stimulating substance. This idea as it is evident from the above observation is wholly unfounded. His own experiments also disprove his contentions. He extracted the stimulus from a kidney incubated for one hour. This is not long enough for autolysis to set in.

From the work cited above on the tissue culture it becomes evident that growth in body cells is directly related to a crowding of the cells or to the establishment of conditions suitable for the accumulation of certain products of their normal metabolism about them. The earlier experiments of Carrel and mine had

[10] Burrows, M. T., *J. Exp. Zool.*, 1911, x, 63.

[11] Burrows, M. T., PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 133.

[12] Drew, A. H., *Brit. J. Exp. Path.*, 1923, iv, 46.

already shown that the stimulating substance for the growth of cells is soluble in salt solution. The studies reported in paper No. 1 of this series, indicate clearly that it may be washed away from the tissue with serum. These observations show that this stimulating substance is a product of the normal metabolism of the cell. These cells can grow only when they are crowded together in an environment where this product of their metabolism is not rapidly removed from them.

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Studies on cancer.

III. Cellular growth and degeneration in the organism.

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As it is well known an active independent growth of cells in the body is peculiar alone under normal conditions to the earlier periods of development. At first this growth is quite generalized. Later it becomes localized, first in one part and then another. In man it ceases entirely with the laying down of the last kidney tubule and glomerulus at about 10 days after birth. Subsequent to this time all growth is merely the enlargement of pre-formed organs and tissues. It is like the hypertrophies and hyperplasias of later life.

In later life it is again well known that hypertrophy and hyperplasias are related directly to the functional activity of the part. Increase the work of the heart, it grows. Decrease its activity, it atrophies. So, in the same manner, the removal of a part of any organ or another organ of the same kind leads to an active enlargement of the remaining parts. This enlargement continues to the re-establishment of a certain size which corresponds to the functional demand made upon the part.

As a moderate increase in the functional demand made upon an organ or a part is associated with the growth of that organ