THE GROWTH OF TISSUE IN ACID MEDIA.*

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PLATE 7.

It has often been shown that the higher animals are extremely sensitive to changes in the reaction of their circulating fluids, and that if the blood be made acid death results. There seems to be a general belief that the cells themselves are unable to withstand an acid reaction of the medium about them. The truth of this belief can now be tested by the use of tissues growing *in vitro*.

In the course of some work with the cells of a chicken sarcoma proliferating *in vitro* I was led to add blue litmus to the plasma medium, and observed that a marked acid change accompanied cell growth. All the tissue fragments were at first stained blue and those which remained inert did not lose this color; but the others which were destined to proliferate became violet or pink within a few hours, often indeed before any sprouting out of cells was observable; and any considerable degree of growth always resulted in a focal pink change in the medium. The mere elaboration of acid by growing tissues is in itself no cause for remark. But the reaction with litmus means that a considerable amount of acid is produced, since this indicator when in plasma is relatively insensitive, owing to the high combining power for acid of the proteins. And that the cells should be capable of further active growth in the medium which they have rendered acid deserves attention and calls for further observations.

Chicken plasma, chicken sarcoma, and the heart muscle and large blood vessels of embryo chicks have been used for the present experiments. The ordinary, hanging-drop plasma preparation was found

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to be unsuitable since it presents too thin a layer for the proper observation of color changes. Instead small Petrie dishes have been used and the tissue bits distributed in a layer of plasma one to two millimeters thick. With pure azolitmin the results are not good, and with pure litmus (Merck's reagent) they are no better than with the watery solution obtained from Kahlbaum's cube litmus and used for tinting litmus milk. This solution as prepared by adding 250 cubic centimeters of water to 500 grams of litmus, heating to 80° C. for one hour, and filtering, is distinctly though only slightly alkaline. It contains numerous impurities including carbonates. When added to chicken plasma, as yet unclotted, in the proportion of one part to ten it colors the medium a deep blue. Such a medium is better than a violet one because the color changes are sharper.

The findings with the chicken sarcoma and with non-neoplastic connective tissue differ only in intensity. The production of acid by the chicken sarcoma in quantities sufficient to change the reaction of the medium is prompt and marked, as is the sarcoma's growth. In one instance where eight or ten small fragments of the tumor grew in a dish with 0.8 of a cubic centimeter of plasma mixture, the diffuse pink change in the medium at the end of two days' incubation was found by test to be equivalent to that brought about in a similar bulk of the unclotted medium by 0.12 of a cubic centimeter of N/10 hydrochloric acid. Fragments of tumor or of late embryos (twenty days) placed in the tinted plasma are all at first colored blue, and only those which start to grow show the change to violet and pink. The color differences between active and inert tissue bits are very striking in dishes where some fragments grow and others perish (figure 1). They constitute a good macroscopic index to proliferation. The growing tissue itself appears as a whitish, unstained zone, or halo, in a pink medium. Material from very young embryos, which survives practically *in toto* the transfer to the plasma medium, likewise remains unstained, and some of the visceral tissues from such a source appear to affect the reaction of the plasma medium very little in their growth.

Tissues grow excellently in a medium tinted with methyl orange, but the acids they produce do not affect this indicator,—presumably because they are organic. With congo red a slight violet change is

sometimes observed in the zone of proliferation, but the reaction is only definite in the case of cells into which the stain has penetrated. These are practically always cells distended with fat droplets and far gone in degeneration. Their cytoplasm may be colored a bright violet. Dead tissue fragments stain red usually, but growing tissue is unaffected by the stain and is seen as a pale halo in the midst of the red medium. The occasional violet tinting of isolated cells cannot be due to free carbon dioxide, for though congo red reacts to it in water this does not happen when the gas is bubbled through unclotted plasma tinted with the indicator.

A striking fact with regard to the elaboration of acid in the plasma medium, and one which must be of much importance for tissue cultures is the small extent to which it diffuses. It is relatively unaffected, as regards neutralization, by the amount of plasma beyond the zone of growth, and this even when the plasma web has been thinned by dilution. When growth is stopped by placing the preparation in the ice-chest, and alkaline plasma is present beyond the zones of growth, neutralization gradually occurs in the latter, but so slowly that it may be incomplete at the end of forty-eight hours. Under conditions *in vitro* which do not permit of an artificial circulation the growing tissue finds itself almost at once in an acid medium. When growth is prolonged by washing the tissue in Ringer solution and reimplanting it in fresh plasma, its alkaline reaction is temporarily restored, only to disappear as growth begins anew. The more rapid the growth of a tissue *in vitro* the less are the opportunities for its products of metabolism to be diluted by diffusion, and so the more unfavorable the conditions under which its growth must continue. This circumstance probably accounts to a considerable degree for the difficulties which Lambert[1] has experienced in keeping rat tumor alive *in vitro*. Similar difficulties are experienced with the chicken sarcoma.[2] When first implanted in plasma it grows almost explosively and produces markedly acid foci; but reimplanted it usually fails to grow.

No attempt has been made to determine the nature of the acids

[1] Lambert, R. A., *Jour. Exper. Med.*, 1913, xvii, 499.
[2] Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516.

elaborated during growth *in vitro*. Carbonic and lactic acids are presumably present in greatest amount.

SUMMARY.

Connective tissue cells of chick embryos and cells of a chicken sarcoma, proliferating *in vitro,* soon render acid the plasma about them, but they nevertheless continue to grow well. Evidently the tissue cell will withstand a considerably greater change in the reaction of the fluids about it than has usually been supposed.

Under conditions of *in vitro* life in plasma, which do not provide for an artificial circulation, the acid produced by growing tissues diffuses only slowly and is subject to little dilution from this source. About tissues which grow very rapidly *in vitro,* as, for example, tumor tissues, there must be a marked concentration of metabolic products, and this may largely account for the poor results of attempts at the continuous propagation of such tissues *in vitro*.

EXPLANATION OF PLATE 7.

FIG. 1. Chicken sarcoma in litmus plasma; twenty-four hours' incubation. A pink change indicates tissue growth. The tumor fragments remaining inert are stained blue. Two color photograph; preparation one third larger than natural size.

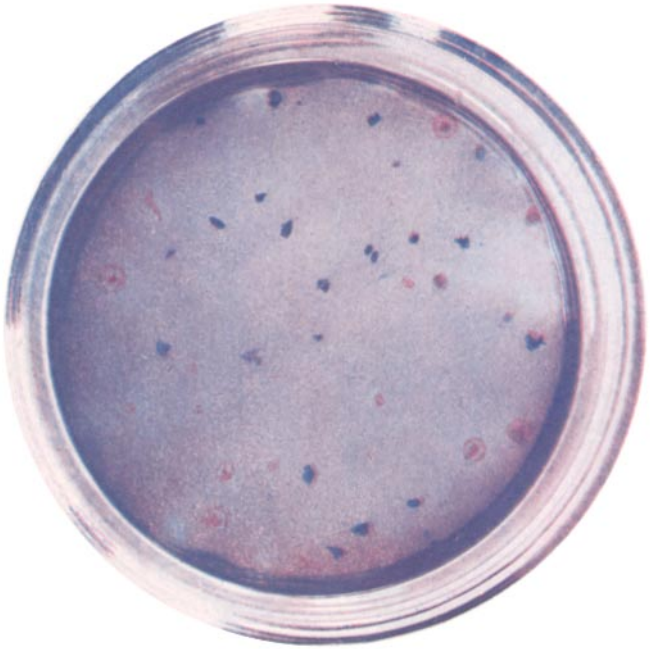

FIG. 1.

(Rous: Growth of Tissue in Acid Media.)