CULTIVATION IN VITRO OF MALIGNANT TUMORS.*

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PLATE LXXVII.

The growth of malignant tumors in cultures outside the body has been studied in a large number of experiments. The technique employed has already been described[1] and need not be repeated here. We propose, in this article, to describe the growth characteristics of the different varieties of tumors employed for cultivation.

The tumors employed were as follows: the Rous chicken sarcoma (2), the Ehrlich and the Jensen rat sarcomata, the Flexner-Jobling rat carcinoma, a dog carcinoma, and human sarcomata (3) and carcinomata. By far the largest number of tests was made with the Rous chicken sarcoma, of which nearly four hundred cultures were studied. The next largest number of tests was made in connection with the malignant human tumors, of which nearly two hundred cultures were made. Many cultures were made of the other varieties.

CULTIVATION OF CHICKEN SARCOMA.

The Rous chicken sarcoma grows rapidly in cultures. Moreover, its cells are actively migratory and quickly begin to wander from the tissue fragment into the plasmatic medium. Within two hours the emigration has begun, and within the next few hours proliferation has advanced. The rate of proliferation is shown by the fact that in culture 4 of series M_2, the area covered by the growth in twenty-four hours was forty times that of the original fragment. It is common for the growth area to reach fifteen to thirty times that of the original fragment within twenty-four hours.[2]

Tumor tissue does not necessarily exceed all other tissues in

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[1] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 387.

[2] Carrel and Burrows, *Jour. Am. Med. Assn.*, 1910, lv, 1554.

rapidity of growth in cultures. Indeed, we have found that embryonic tissue, especially splenic, exceeds in this respect the chicken sarcoma.

The factors governing the rate of growth are numerous, as we have already pointed out.[3] In the case of the Rous sarcoma, the state of preservation of the cells is highly important. Many failures could be traced to degenerations of the tumors, particularly myxomatous changes, affecting the fragments cultivated. But the nature of the plasma also affects the result: the most rapid growth was secured with autogenic plasma, and less rapid proliferation with homogenic plasma. Plasma from another chicken bearing sarcoma was less adapted, and sometimes was entirely unsuited to growth; while the addition of minute quantities of sarcomatous extract to normal plasma produced acceleration of growth.[4] Hence, the conditions are complex and will require much patient and ingenious observation for their elucidation.

The appearance of the growing tissue is striking. About the original fragment a continuous and dense layer of elongated and round cells collect, which quickly invade a large part of the medium. The new growth is divisible into two parts. The inner part is composed of radiating, spindle and round cells, the outer part almost wholly of ameboid round cells. The outer layer of cells is surrounded by red corpuscles and debris, doubtless carried mechanically before the advancing cells. The disposition of the new cells may be in an horizontal plane or in the form of a concavity with the greatest depth at the edge of the tissue fragment.

The life of the cultures is short. By the expiration of forty-eight hours, the rate of growth is generally much slower and granules appear in the cells, after which cellular disintegration sets in. A further difference occurs: in cultures showing slow growth, the spindle cells predominate, and in those showing rapid growth, the round cells predominate.

The round cells contain a clear cytoplasm enclosing a few refractile granules and a nucleus often difficult to see in fresh preparations; their ameboid activity is lively. The spindle cells possess

[3] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 416.
[4] Carrel and Burrows, *Jour. Am. Med. Assn.*, 1911, lvi, 32.

clear cytoplasm, enclosing many refractile granules (figure 1) which may cover and obscure the nucleus. They stain red with Sudan III and black with osmic acid, and hence are of the general nature of fatty or lipoidal substances. Similar granules arise in the normal tissue cells of the chicken during cultivation. The ameboid cells are actively phagocytic. Culture 12, series F, consisted of sarcomatous and pigmented cells derived from a fetal eye of a chicken. The sarcoma cells wandered in and out among the pigmented cells, and gradually took up the dark pigment, so that after forty-eight hours they were stuffed with it.

The increase in cells is associated with karyokinetic division of the nuclei. During the first twenty-four hours, this nuclear division is observed chiefly in the inner area, close to the original fragment; but later it occurs throughout the growing area. At about the period of cessation of growth and of life of the culture, it occurs only at the outer edge. This demonstration constitutes the final proof that in the cultivation of tissues outside the body actual multiplication of cells and growth of tissue occur.

Finally, it may be stated that we found no characteristic for sarcoma cells, as such, that was not also to be found in some normal tissue cells of the chick.

CULTIVATION OF RAT SARCOMATA.

Sarcomata of the rat, so far as they are represented by Jensen's and Ehrlich's strains, behave in cultures very much as the Rous chicken sarcoma does. There are, however, some important differences of detail. The growth begins usually after a latent period of from four to twelve hours, and continues from four to six days. The new cells are round and spindle-shaped, and the fragments become surrounded by a dense network of real tissue. Thus it comes about that the line of demarcation between the original fragment and the new growth is almost invisible. In process of growth the round cells occupy the most advanced position in the medium, and are followed by the spindle cells. The morphology of the tumor cells is distinct from that of the connective tissue cells. Karyokinesis has been present in specimens which have grown for as great a time as six to eight days.

CULTIVATION OF HUMAN SARCOMATA.

Three human sarcomata were subjected to cultivation. They consisted of two giant-celled tumors and a fibrosarcoma. We failed to cultivate the giant-celled tumors, probably for the reason that they caused rapid liquefaction of the medium. The medium about the fibrosarcoma underwent partial liquefaction, but as a thin and firm clot adhered to the cover-glass, growth was still possible, and after a latent period varying from six to twenty-four hours, it took place. Polygonal and spindle-shaped cells wandered into the medium and remained isolated and did not produce a dense new tissue, as did the sarcoma of the rat.[5]

On account of the extreme tenuity of the plasmatic medium it was possible to follow all the details connected with the proliferating cells. The fusiform cells developed sharp processes, and their clear protoplasm was seen to become filled with refractile granules that surrounded the large central nuclei. The cytoplasm also came to contain small round or irregular bodies, of which some showed side knobs like buds (figure 2), the nature of which is still undetermined. The bodies were opaque and occupied vacuoles in the living cells; in stained preparations, they took on a pale tone from the nuclear dye. Within these pale bodies, a far darker and minute spot sometimes appeared. Similar cell inclusions were observed in the cells of the original, uncultivated tumor.

CULTIVATION OF CARCINOMATA.

Cultivation of carcinoma was attempted with (1) an adenocarcinoma of the breast of an old dog (bitch), (2) the Flexner-Jobling rat carcinoma, and (3) several carcinomata of human beings; one from the lip, and the others from the male and female breasts.

Success was achieved with the dog tumor, in which growth of the adenomatous portion was noted to begin on the third day. The growth consisted of blunt tubules or columns of cells presenting an epithelial-like appearance. The tubules resembled those which we have observed in cultures of the thyroid gland[6] and the kidney. The cells composing them were round or polygonal; the nuclei were

[5] Carrel and Burrows, *Jour. Am. Med. Assn.*, 1910, lv, 1732.
[6] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 416.

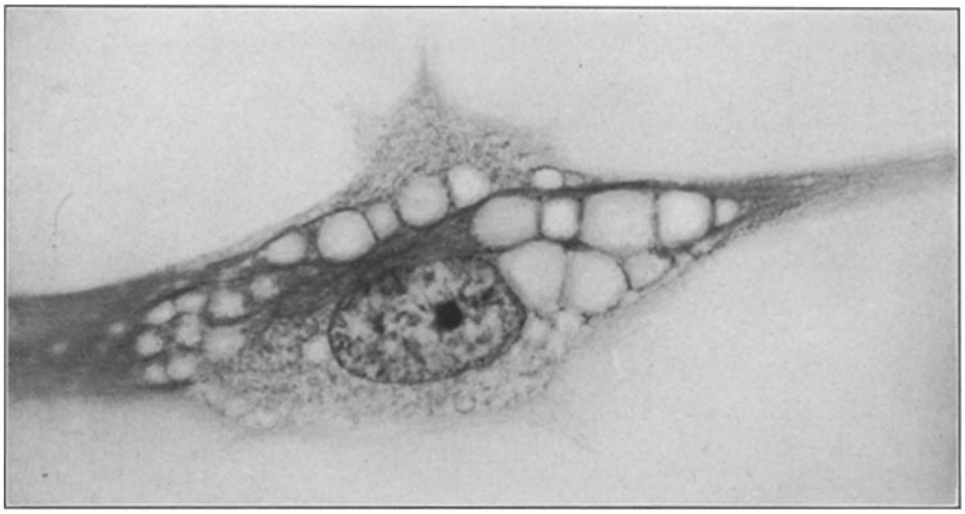

FIG. 1.

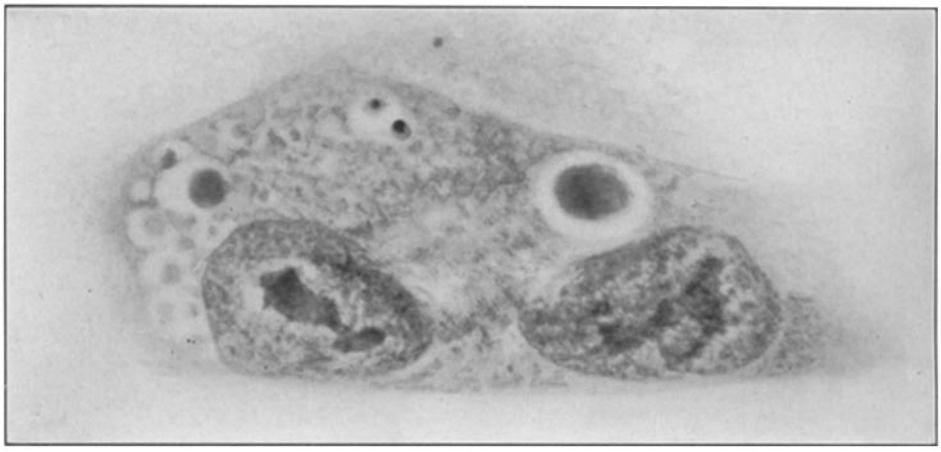

FIG. 2.

central; the cytoplasm was clear and homogeneous, except for small refractile granules. We have observed cells similar to these in cultures of the skin and liver.

In the Flexner-Jobling rat carcinoma the growth was composed of epithelial-like cells resembling those observed in the cultures of the spontaneous carcinoma of the dog.

Lambert and Hanes have succeeded in cultivating a mouse carcinoma.[7]

Thus far we have not had signal success with the cultivation of human carcinoma, for the reason that the six specimens with which we worked rapidly liquefied the plasmatic medium. Some growth was obtained from a small carcinoma of the breast; generally liquefaction occurred both in the cultures prepared from the tumor and from the pectoral muscle and lymphatic glands. In order to succeed with cultures of human carcinoma it will be necessary to master and overcome the process of liquefaction. We have already found that not all human tissues cause liquefaction of the plasmatic medium, so we believe that the conditions can be discovered and prevented.

It can therefore be concluded that experimental malignant tumors grow *in vitro* extensively, and that the cultivation of human tumors is also possible, although much more difficult on account of the liquefaction of the plasma.

EXPLANATION OF PLATE LXXVII.

FIG. 1. An isolated spindle cell from a culture of the Rous chicken sarcoma. The large clear spaces mark the sites of dissolved fat granules. Stained with hematoxylin and eosin.

FIG. 2. An isolated multinuclear cell with inclusions from a culture of human fibrosarcoma of the fibula. Stained with hematoxylin and eosin.

[7] Lambert and Hanes, *Proc. Soc. Exper. Biol. and Med.*, 1911, viii, 59.