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Cytoplasmic Changes in Rous Sarcoma Cells Cultivated *in vitro*.

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We have previously reported some nuclear alterations in Rous sarcoma cells grown in pure culture.[1] The following is a brief account of the cytoplasmic changes which are generally developed in Rous sarcoma cells cultivated *in vitro*.

The most outstanding changes take place in the central area of the cell. The cytoplasm of the central cell region at first takes on a cloudy appearance. Indefinite at first, this zone gradually becomes better defined and finally assumes a very sharp outline (Fig. 1). In general, the segregated central area is 3-6 times as large as the nucleus, is spherical in shape and always appears to be denser than the surrounding cytoplasm, but shows no pronounced structural peculiarities. In the first stage of its development the central area is always somewhat eosinophilic; in the more advanced stage of the segregation process its tinctorial characteristics become rather indefinite; with Giemsa it stains at times slightly red and at times slightly blue, and mostly in mixed tones. The nucleus lies almost invariably outside the central area; in

[1] Tenenbaum, E., and Doljanski, L., Proc. Soc. Exp. Biol. and Med., 1941, **47**, 236.

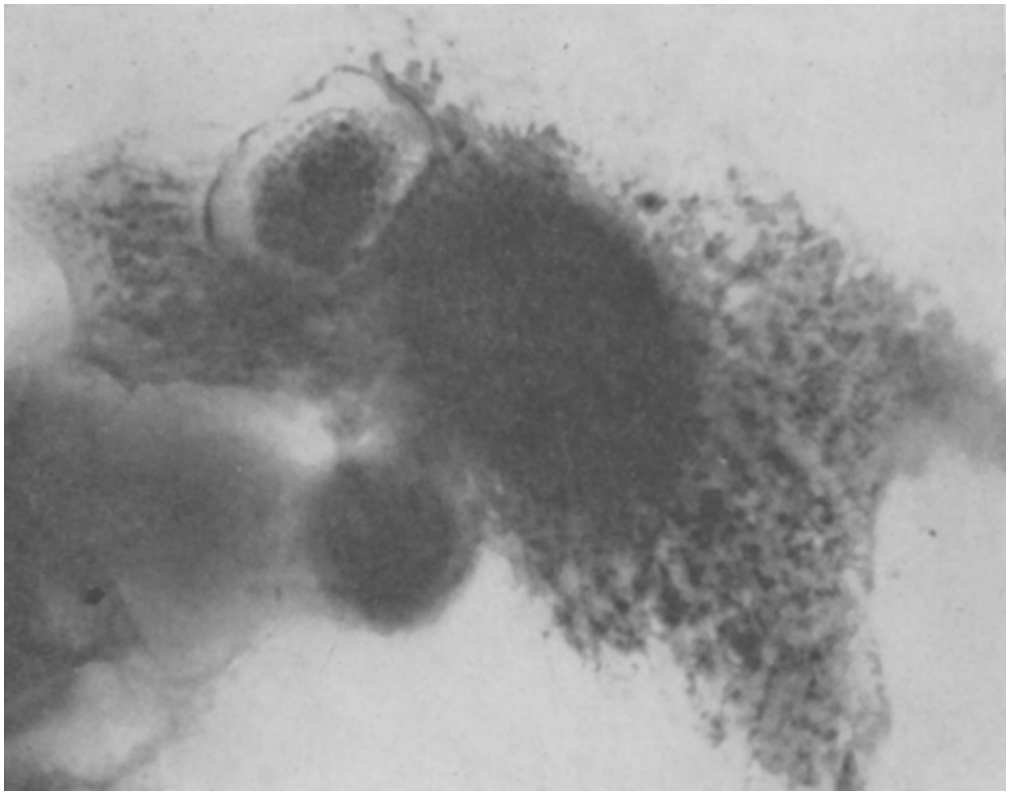

Fig. 1.
Rous sarcoma cells; culture No. 12711; Giemsa's stain. × 1790.

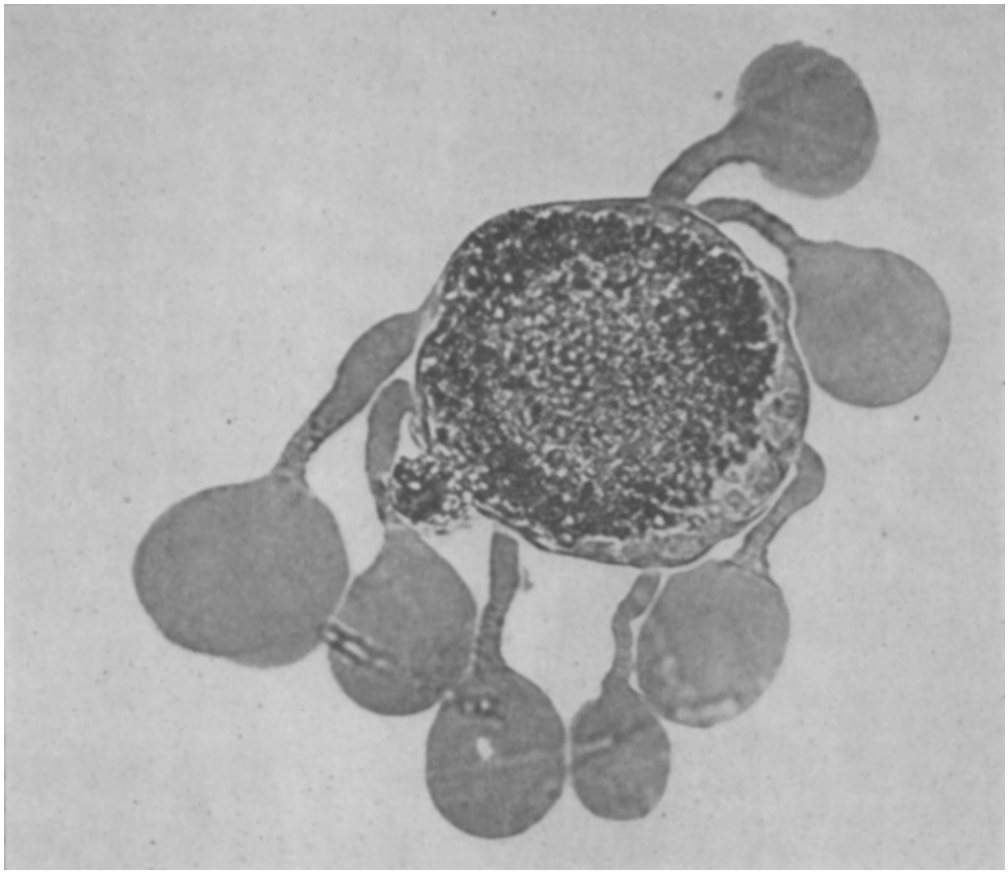

FIG. 2.
Rous sarcoma cell; culture No. 11764; living. × 585.

binuclear cells the central area is situated between the two nuclei.

As the result of the segregation of the central cell region the sarcoma cell often appears to be built of two clearly distinct zones—central area and peripheral cytoplasm. The peripheral cytoplasm of the sarcoma cell involved is not only sharply marked off from the central region, but in itself presents striking structural and behavioral peculiarities. It possesses the character of a flat membrane constantly changing in outline. The vigorous movement of this membrane never leads to significant displacement of the cell. Pseudopodia projected by these cells have a very unusual aspect (Fig. 2). They often appear as coarse rigid rods, which move in all directions on their base, as on a joint. The distal ends of these pseudopodia sometimes swell and become spherical. The protoplasmic balls so formed float on their flexible stems, and often become detached.

In correspondence with the heterogeneity of structure of the cytoplasm of the sarcoma cells, the distribution of the vacuoles is not diffuse but regional, being restricted often to the boundary region between both. The vacuoles are usually numerous; some contain fat while others are devoid of fat. With neutral red, the vacuolar system of the sarcoma cells can be clearly demonstrated.

Often the peripheral cytoplasm of the sarcoma cell contains a close network of thick or thin threads and granules which stain intense blue with Giemsa and display the aspect of hypertrophic mitochondria. Frequently, small eosinophilic granules are found scattered throughout the sarcoma cells. Here and there

this eosinophilic material is also found in the form of large homogeneous clumps, which are occasionally enclosed in a vacuole. Accumulations of eosinophilic material bearing a great resemblance to the intracellular eosinophilic masses may at times also be found extracellularly.

The cytoplasmic changes described above occur simultaneously with the nuclear alterations previously noted by us in sarcoma cells maintained in pure culture. Both are accompanied by great and sometimes extreme hypertrophy of the cells involved.

Our observations indicate that the peculiar nuclear and cytoplasmic alterations which we have described, and which have also been pointed out in part by earlier investigators (Borrel, Fischer, Roskin, Zweibaum), represent different aspects of a slowly progressive and decidedly pathological process. Although the changes in the sarcoma cells are extremely varied and complex their combination forms a definite syndrome of serious cell disease which, in conditions of life *in vitro*, inevitably overtakes cells bearing the causative agent of Rous sarcoma, and leads ultimately to the death of the sarcoma cell colonies.

14116

Presence in Various Grains of Factors Inhibiting Tumor Growth.

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The following report deals with the results of experiments on the influence of various grains on tumor growth. These studies were stimulated by experiments of Lewisohn and coworkers.[1] They observed that the therapeutic results of intravenous therapy of yeast extracts on spontaneous mammary carcinomas in mice could be improved by feeding polished rice instead of a normal diet. The question was raised, whether this improvement was due to a lack of substances in the polished rice diet essential for tumor growth, or whether the polished rice contains an additional tumor inhibitor. To study this problem in detail, we employed a rapid test for tumor growth inhibitors, developed in this laboratory by Laszlo and Leuchtenberger.[2]

Method. In the rapid test, treated groups of transplanted tumor mice (Sa.180) are compared with untreated controls as to the size and weight of their tumors. At the start of the experiment, 7-10 days after transplantation, groups of 7 mice are formed, each bearing a transplanted Sa.180 of the same size from the same transplant. If these matched groups are injected intravenously twice daily with saline solution for 2 consecutive days, the tumor-sizes and tumor-weights of treated animals and controls will agree after 48 hours. If one or more of these groups are injected intravenously twice daily on 2 consecutive days with the standard preparation (yeast extract as previously described) the tumor growth in these groups is inhibited. The extent of this inhibition is such that after 48 hours the differences of mean terminal size and mean terminal weight in treated and untreated groups permit the detection of tumor growth inhibitors even in a small number of animals.

Results. In a series of experiments it was found that at least four intravenous injections of 0.1 cc (corresponding to 5.25 mg solids) of the standard are necessary for an inhibitory effect, which is statistically significant, if mice are fed a normal diet. If mice are fed a polished rice diet, 4 injections of 1.75 mg solids of the same standard are sufficient for a similar inhibition of tumor growth. Control groups

1 Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., Laszlo, D., and Dische, Z., *Cancer Research*, 1942, **2**, 818.

2 Laszlo, D., and Leuchtenberger, C., to be published in *Cancer Research*, 1943.