

A group of 14 younger rats (52-54 days old) received from one to 3 daily subcutaneous injections of 1 cc of the hormone preparation, and 8 of their litter mates were used as controls. Approximately half of each group of these animals were females. Serum calcium values in the treated animals ranged from 9.7 to 11.4 mg % with a mean value of 10.8, while serum calcium values in the control series ranged from 9 to 12.2 mg % with a mean value of 10.7. In no instance did the serum calcium of a treated animal show a significantly greater value than that found for its litter mate control. Serum inorganic phosphorus determinations were not made.

Summary. A slight increase in serum inorganic phosphorus without a significant increase in serum calcium after multiple injections of anterior pituitary extract in rats is regarded as insufficient proof of the presence of a parathyreotropic factor in the extract examined.

13096 P

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

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Various authors (Borrel,¹ Roskin,² Lipschütz,³ Fischer,⁴ Zweibaum⁵) have indicated that the structure of Rous sarcoma cells shows certain peculiarities, which differentiate them from normal mesenchyme cell's.

These indications are very fragmentary and there is little agreement between the various descriptions. The morphological features of the Rous sarcoma cell are still not sufficiently known.

The structural peculiarities of the Rous sarcoma cells appear with great clarity and they are easily detectable in pure culture of sarcoma cells. Our studies of cell strains, cultured without addition of normal tissue revealed that the sarcoma cells *in vitro* constantly show pro-

¹ Borrel, A., *C. R. Soc. Biol.*, 1926, **94**, 500.

² Roskin, G., *Virchows Arch.*, 1926, **261**, 919.

³ Lipschütz, B., *Z. Kregsforsch.*, 1929, **28**, 491.

⁴ Fischer, A., *Gewebezüchtung*, Munich, 1930.

⁵ Zweibaum, J., *Arch. exp. Zellforsch.*, 1933, **14**, 358.

found morphological alterations of their cytoplasm and nuclei, often associated with marked, indeed extraordinary hypertrophy of the entire cells.

In this note we report on the changes taking place in the nuclei of the sarcoma cells. The studies were made on cultures (preparations *in toto* and serial sections) fixed in *Carnoy* and stained with *Giemsa*.

The changes in the nucleus are very profound and may lead to definite disturbances of the entire nuclear structure. The nuclear ground substances, which is finely reticulated, or finely granular in normal spindle cells, and almost structureless in normal lymphoid cells, in sarcoma cells often appears as if precipitated. In numerous nuclei particulate masses become visible. The individual particles are more or less uniform in size and stand out distinctly on the almost colorless background. At first the particles are equally distributed throughout the nucleus; but later the granular material gathers in the central parts of the nuclei, leaving a wide or narrow zone, a clear halo, between the particulate aggregates and the nuclear membrane (Fig. 1). This zone appears empty, but in some preparations we can see that it is crossed by very fine threads running radially. The granular material appearing in the nucleus is usually

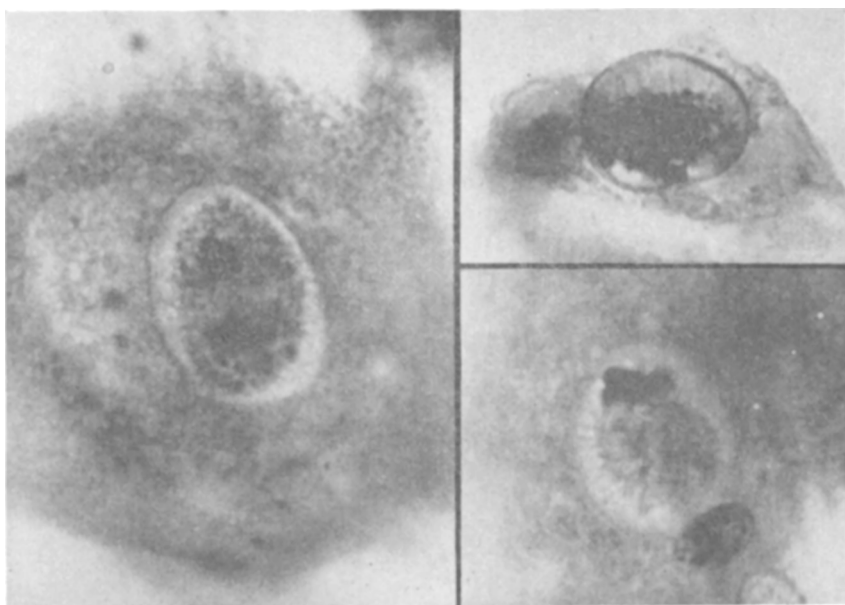


FIG. 1.

Nuclei of Rous sarcoma cells; culture No. 12708; Giemsa's stain. $\times 1600$.

distinctly acidophilic. Occasionally the particulate masses are gathered not in the center but in certain peripheral areas of the nucleus. The remaining parts then appear empty.

The basophilic chromatin disappears progressively from the nucleus; the chromatin particles become smaller and less distinguishable. Margination of the basi-chromatin on the nuclear membrane is only rarely found.

The nucleoli usually become round or rod shaped, and appear as very large, compact, deeply stained, robust bodies. Often their shape is very irregular; not infrequently the nucleoli break up into several fragments; these either remain clumped together or may be found scattered throughout the entire nucleoplasm. Occasionally vacuoles are seen in altered nucleoli.

The changes described above do not take place simultaneously in all cells of a particular culture; in some the nuclei are profoundly changed; in others little or not at all. Apparently, the cells are involved successively.

In some cells, usually hypertrophied basophilic round cells, nuclear changes can be observed, different from those already described. Nuclei of such cells contain sharply defined inclusions, amorphous and hyaline in appearance, lying between the nuclear membrane and a nucleolus (Fig. 2). These inclusions are stained a bluish tint with Giemsa. The ground substance in which these inclusions are embedded is as a rule also altered.

In addition to the changes described the nuclei of sarcoma cells show a great tendency to fragmentation. The form of nucleus often

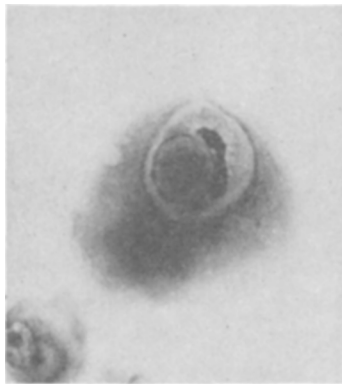


FIG. 2.

Intranuclear inclusion in Rous sarcoma cell; culture No. 12708; Giemsa's stain.
× 660.

becomes irregular, parts are pinched off, and thus one can frequently find the nucleus broken up into 10-15 fragments; some are attached to each other by fine filaments. At times such fragments show a fully normal nuclear structure, others lack nucleoli, still others have no chromatin substance whatever, and some again appear completely emptied. Often the picture observed suggests that some nuclear fragments dissolve in the surrounding cytoplasm.

A detailed description of the changes in the nuclei of Rous sarcoma cells and a discussion of their significance will appear elsewhere.

13097 P

Cellular Composition of Pure Rous Sarcoma Cultures *in vitro*.

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We wish to report on experiments undertaken in order to investigate the cellular composition of pure Rous sarcoma cell colonies *in vitro*. They were carried out on cultures, 20-50 passages old, of our Rous sarcoma strains, which were cultivated without the addition of normal tissue. They include observations on living cultures and on preparations *in toto* and serially sectioned ones, fixed in Carnoy or Zenker Formol, and stained with Giemsa.

The results of these investigations can be summarized as follows:

The pure Rous sarcoma culture consists of two cell types: spindle cells and round cells. The structure of the spindle cell corresponds exactly to that of the normal fibrocyte. The round cell is characterized by a strongly basophilic, completely homogeneous or finely granular cytoplasm and a relatively large, round, somewhat excentric nucleus. The nucleus has a thick, unfolded membrane, 1-2 heavy nucleoli, and more or less finely divided chromatin. The size of the round cells varies between 5 and 15 μ . They are almost round, with regular or somewhat angular contours. Their pseudopodia are slightly developed, their movements are sluggish and they phagocytize but little.

The fibroblast-like cells and the basophilic round cells are not distinct cell types but different aspects of the same cell. In every culture numerous transitional stages enable us to follow, step by step,