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 cytoplasma.

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*.

L. DOLJANSKI AND E. TENENBAUM. (Introduced by L. Halberstaedter.)

From the Department of Experimental Pathology, Cancer Laboratories. The Hebrew University, Jerusalem.

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,

the change of one cell form into the other. The transformation of the spindle cell into the basophilic round cell takes place by means of contraction of the former. The fibroblast-like cell draws in its processes; its body becomes round, the cytoplasm more and more basophilic, the nucleus becomes more compact, its membrane easily visible and the nucleoli very prominent. The basophilic round cell, arising from the contraction of the spindle-cells, recalls at times lymphocytes and even more haemocytoblasts as seen in cultures of blood and hemopoietic tissues. They differ distinctly from large, amoeboid polyblasts (macrophages) as well in form, nuclear structure, and cytoplasm as in their behavior. The process of transformation of spindle-cell into basophilic round cell is reversible; observations and experiments show that the basophilic cell can stretch itself out and recover the form of the spindle cell. Sometimes, the basophilic round cell may also take on an appearance closely resembling that of amoeboid histiocytes; it becomes progressively larger, its cytoplasm more and more vacuolated and pale; it acquires an amoeboid appearance and its pseudopodia become much better developed.

Our observations indicate a connection between the transformation of fibroblast-like cells into basophilic round cells and the peculiar fundamental ability of sarcoma cells to attack proteolytically and to liquefy their plasmatic growth medium. The change in the physical state of the medium surrounding the cell produces a change in its form. Whereas the cell is stretched out in a semi-solid medium, it becomes round when the plasma is liquefied. The sarcoma cell with its marked proteolytic activity itself creates the conditions that determine the diversity of its form. By adding or withholding embryonic extract, the proteolytic power of the sarcoma cell can be diminished or increased. In this manner the appearance of the sarcoma cells *in vitro* can, to a large extent, be experimentally influenced.

The fact that normal fibroblasts may undergo the same changes enables us to understand more fully the mechanism of transformation which the sarcomatous fibroblasts in culture undergo. Such changes in normal fibroblasts were first observed by Rous and Jones[1] when the plasmatic medium, in which the cells were grown, was digested with trypsin; and they can also be seen when an untreated culture of normal fibroblasts occasionally liquefied the medium spontaneously. Under these conditions the normal fibroblast too is transformed into

[1] Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, **23**, 549.

a round cell, often practically indistinguishable from the basophilic round cell, resulting from transformation of the sarcomatous fibroblast.

In view of these findings, the discussion, which cell type in the sarcoma culture, the fibroblasts or the "macrophages" carries the malignant attributes, appears to be futile. The pure Rous sarcoma consisting of one cell type; a mesenchyme cell, appearing at times as a spindle cell, at others as a basophilic round cell is the actual carrier of the Rous sarcoma agent. The changes induced by the sarcoma agent in the spindle cells and basophilic round cells will be described in another communication.

13098

Age Variations in Resistance of Albino Rat to Diphtheria Bacilli and to Diphtheria Toxin.

ERICH SELIGMANN. (Introduced by C. W. Jungeblut.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.

As reviewed in a previous paper on experimental diphtheria in the albino rat[1] this animal possesses a high but not complete resistance to diphtheria toxin and to living diphtheria bacilli. Amounts of toxin exceeding by 2000 times the fatal dose for guinea pigs (calculated on body-weight) are required to kill albino rats by subcutaneous injection. Again, when large numbers of living bacilli are injected into these animals by the same route only slight transitory lesions are observed. Attempts to explain the mechanism of this natural resistance have been made by several authors. Thus Goodman,[2] Pettit,[3] Coca, Russel and Baughman,[4] Sbarsky[5] have thought that the rat's body-cells are incapable of fixing diphtheria toxin; Ledingham,[6] on the other hand, has expressed his belief that the reticulo-endothelial system of the rat is particularly capable of aiding in the absorption of localized abscesses in the subcutis

1 Seligmann, E., and Jungeblut, C. W., *J. Immunol.*, 1941, **40**, 119.

2 Goodman, H. M., *J. Infect. Dis.*, 1907, **4**, 509.

3 Pettit, A., *Ann. Inst. Pasteur*, 1914, **28**, 663.

4 Coca, A. F., Russel, E. F., and Baughman, W. H., *J. Immunol.*, 1921, **6**, 387.

5 Sbarsky, *Biochem. Z.*, 1926, **169**, 113.

6 Ledingham, J. G. C., *J. State Med.*, 1926, **34**, 2.