hypothesis that, besides high secretion of pituitary LH, a decrease in the secretion of pituitary FSH also plays an important role in the regression of mammary gland carcinoma during lactation.

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MIGRATION OF NUCLEOLI AND KARYOPLASM INTO THE CYTOPLASM OF RETICULAR CELLS IN LYMPH NODE CULTURES

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The successive stages of a process characterized by migration of the nucleoli and part of the contents of the nucleus into the cytoplasm of reticular cells were recorded in primary cultures of lymph nodes from albino mice with transplantable leukemia NK/LY. Comparison of the results with data of autoradiography, indicating high proliferative and metabolic activity of the reticular cells, leads the author to suggest that the phenomenon reflects, at the morphological level, the secretory function of the stromal cells of the hematopoietic organs.

KEY WORDS: *experimental leukemia; lymph nodes; reticular cells; tissue culture.*

Among the many problems which face the investigator studying proliferation and differentiation of cells one of the most central is that of the intimate relations between the nucleus and cytoplasm in the living cell.

The object of this investigation was to study the morphological and functional state of the stromal cells of lymph nodes from albino mice with transplantable leukemia NK/LY, in which the successive stages of a process characterized by migration of the nucleoli and of part of the karyoplasm from the nucleus into the cytoplasm of the cells were recorded.

EXPERIMENTAL METHODS

Cultures of lymph nodes were prepared by A. A. Maximov's method. The nutrient medium for culture consisted of two phases: solid and liquid. The solid phase of the nutrient me-

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dium included synthetic medium 199, goose blood plasma, and chick embryonic extract in the ratio of 1:1:0.5. The liquid phase consisted of equal volumes of synthetic medium 199 and human blood serum. Total preparations of lymph node cultures for cytomorphological analysis were fixed in 10% formalin or in methanol and stained by the Jenner–Giemsa method (in Pappenheim's modification for cultures). The character of nucleic acid and protein synthesis was studied by autoradiography with radioactive precursors of DNA [^{3}H]thymidine, RNA [^{3}H]uridine, and protein [^{35}S]methionine. Observations on living cells were made with the MBI-13 microscope in phase contrast. Time-lapse microfilming was carried out with the Konvas motion picture camera at a speed of one frame every 4 sec (with a 40× objective) or one frame every 10 sec (90× objective).

EXPERIMENTAL RESULTS

The zone of growth in primary cultures of lymph nodes from albino mice with transplanted leukemia NK/LY consisted of numerous reticular cells, macrophages, and lymphocytes. According to the indices of proliferation and of assimilation of radioactive precursors of protein and nucleic acids, and to the pattern of interaction between them in culture, all the above-mentioned cells appeared viable [6]. Meanwhile, in some reticular cells parts of the contents of the nucleus with the nucleolus (or nucleoli) were observed to be migrating into the cytoplasm. The initial stage of this phenomenon was shown by changes in the nuclear membrane, which were always accompanied by the appearance of a very small vesicle in the corresponding locus of the cytoplasm. The nuclear membrane, the outline of which in the light microscope is determined by the chromatin adjacent to its inner surface, appeared to be divided in one part of the nucleus by a free space. A vacuole formed in the cytoplasm of the cell in these places, and part of the karyoplasm and nucleolus moved quickly into it (Fig. 1a). Later the vacuole increased in size and the quantity of migrating contents of the nucleus in it increased also (Fig. 1b). The consecutive stages of development of this process could sometimes be observed in cells dispersed over a field of vision. Side by side with them cells with characteristic mitotic figures could be seen (Fig. 2).

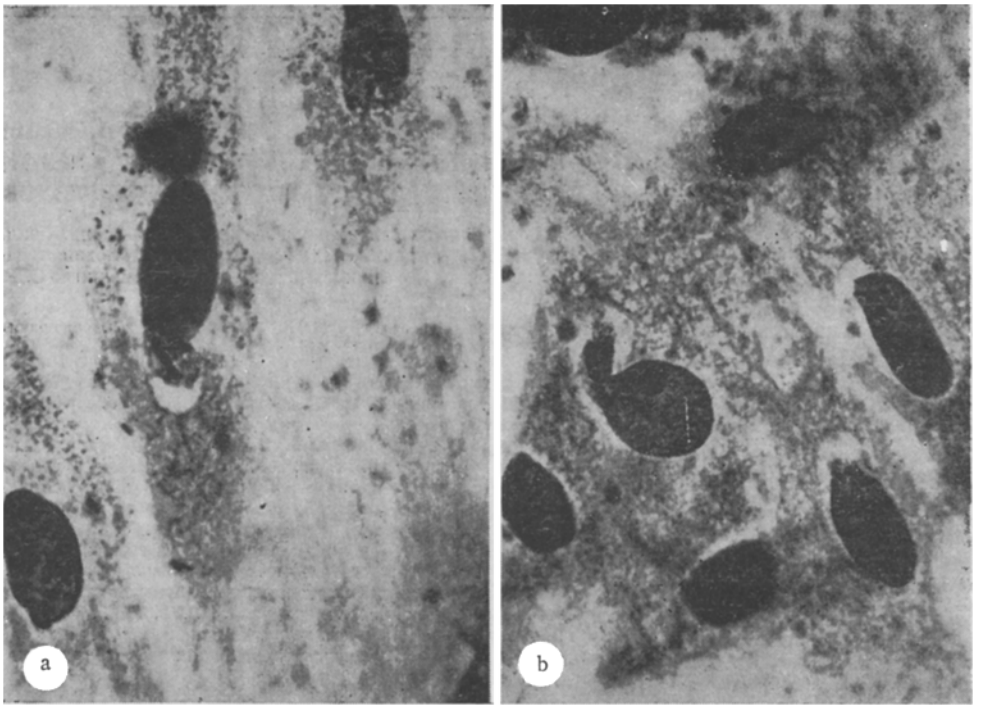

Fig. 1. Different stages of migration of nucleoli and part of karyoplasm into cytoplasm of reticular cells. Total preparations of lymph node cultures. Age of culture 4 days. Fixation with methanol. Staining by Jenner–Giemsa method, 900×. a) Vacuole formation; b) migration of contents of nucleus.

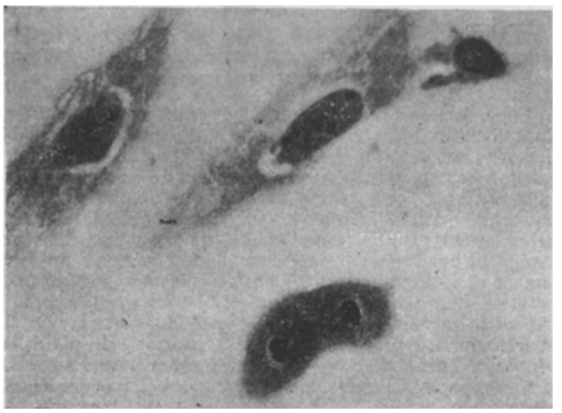

Fig. 2. Mitosis of reticular cell in zone of growth of lymph node culture. Total preparation. Age of culture 4 days. Fixation with methanol. Staining by Jenner–Giemsa method, 900×.

Considering that this phenomenon (and the cells in tissue culture may appear to be nonviable) can be regarded as a manifestation of subnormality or degeneration of the cells, further experiments were carried out using radioactive indicators, in the form of precursors of DNA, RNA, and protein. The results of these experiments showed that among all the cells of the zone of growth the reticular cells were distinguished by the most active assimilation of these precursors (Fig. 3a, b, c). The viability of the reticular cells also was confirmed by observations on living cells by time-lapse microfilming in phase contrast.

There are reports in the literature of the permeability of the nuclear membrane and of migration of nucleoli into the cytoplasm of cells, but this phenomenon has not hitherto been described in lymphocytes. Numerous experiments *in vivo* and *in vitro*, in which labeled amino acids were used, have demonstrated the ease with which small protein molecules can pass through pores in the nuclear membrane. Ribonuclease (mol. wt. 13,000), for instance, can penetrate into the nucleus of many cells. It is assumed that its penetration in this case is connected with hydrolysis of a definite component of the nuclear membrane containing RNA, followed by restoration of the integrity of the membrane [5]. The possibility of transport of macromolecules from nucleus into cytoplasm has been noted by other workers [2, 12]. On the basis of the available information it has been suggested that a region is created close to the nuclear membrane in cells in which nuclear and cytoplasmic materials are concentrated and subsequently organized into specific structures [5]. This assumes the possibility of migration of these structures from the nucleus into the cytoplasm [15]. Migration of the nucleoli and of nuclear material into the cytoplasm was first observed in 1883 under the light microscope in pancreatic cells [14]. In 1960 these observations were confirmed by electron microscopy [16]. Migration of nucleoli from nucleus into cytoplasm has also been observed in nerve cells [4], secretory cells [10], cells in culture [13], and cancer cells [1]. Migration of whole nuclei with a small quantity of karyoplasm has also been frequently observed in the cells of experimental rhabdomyoblastomas [3]. These phenomena have been described in the literature as extrustion or emission (i.e., expulsion) of nucleoli. The mechanism of this process is unknown, although there is information to suggest that when the nucleolus migrates from the nucleus into the cytoplasm a tear develops in the nuclear membrane, through which the extrusion takes place [7, 10]. The view has been expressed also that this process is reversible and that migration of nuclear materials into the cytoplasm takes place by the "lock mechanism" principle [11]. According to A. A. Klishov, migration of the nucleoli and nuclear material from nuclei into cytoplasm is one way of regulation of nucleolo-nuclear and nucleo-cytoplasmic relations.

The observation of this phenomenon in cells of different tissues and organs emphasizes its wide distribution. When this phenomenon is examined, special attention must be paid to investigations by the electron autoradiography method [8]. The workers who used this method argue that the nucleoli of mouse liver cells, which synthesize and transport RNA to the nuclear membrane, participate in the release of portions of RNA into the cytoplasm. The nucleolus is one of the cell components which responds most actively to changes in the functional

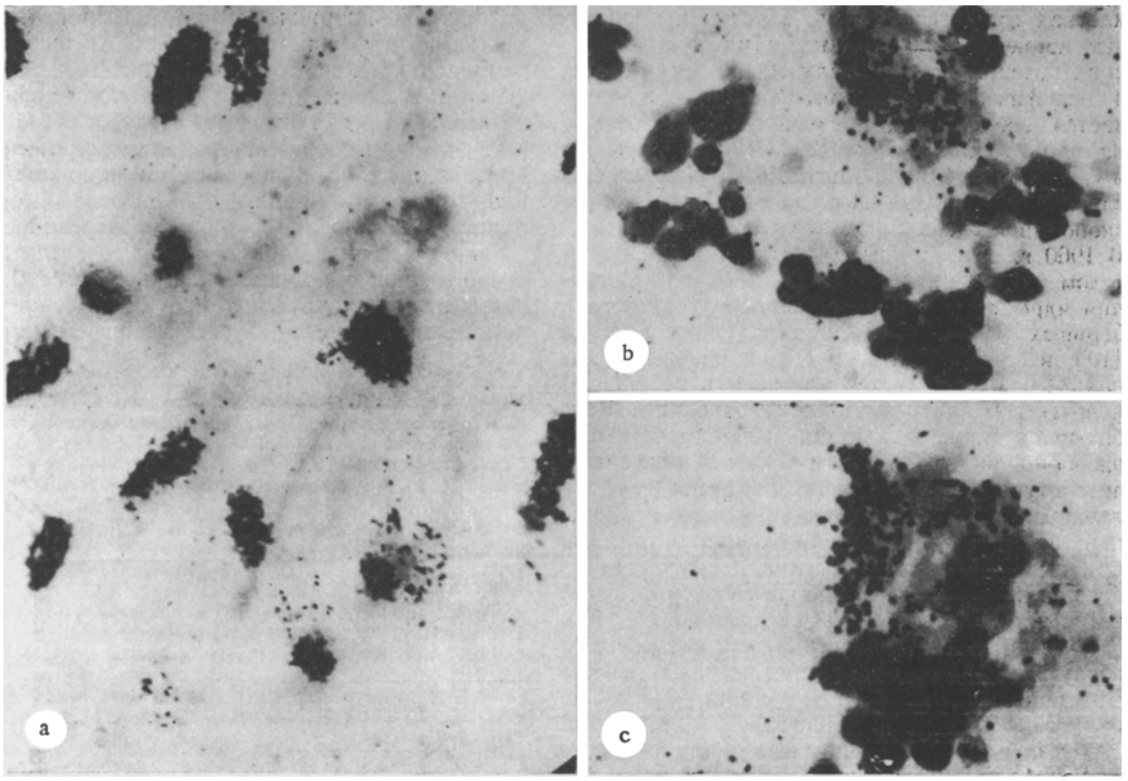

Fig. 3. Metabolic status of reticular cells in lymph node cultures. Total preparations. Age of culture 4 days. Fixation with 10% formalin. Staining by Jenner–Giemsa method, 900×. a) [^{3}H]Thymidine, b) [^{3}H]uridine, c) [^{35}S]-methionine.

state of the cell. The response of the nucleolar apparatus of cells is particularly marked during pathological processes and the development of leukemia, the morphological substrate of which consists of cells of the hematopoietic organs, whereas the response of the nucleoli of the stromal cells is perfectly regular. Considering that the phenomenon found in reticular cells is analogous to changes in the nuclei of secreting cells of many different tissues of the body, it can be postulated that this state reflects the secretory function of the reticular cells at the morphological level. This hypothesis can be put forward to explain the mechanisms of cellular interaction between cells of the lymphoid complex. Reticular cells actively synthesize nucleic acids, proteins, and carbohydrates, and secrete their metabolic products into the surrounding medium; the possibility cannot be ruled out that the stromal cells of the hematopoietic organs thereby create the microenvironment which, according to some workers, induces processes of proliferation and differentiation of the hematopoetic cells [9]. During the development of leukemia it is the stromal cells which can induce neoplastic changes in the precursor cells of all branches of hematopoiesis.

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