process of concentric indrawing of the wound edges is largely due to growth of granulation tissue, which actively contracts and draws in the wound edges. Under the experimental conditions used, glycine caused a significant increase in the DNA content in the granulations, regularly associated with an increase in the number of cells, including fibroblasts, their earlier maturation, and stimulation of peripheral epithelization. All these effects led to more rapid wound healing. This action of glycine is evidently based on its unique metabolic properties. Glycine is a precursor of many biologically active substances directly related to repair processes: purine bases, glutathione, α-ketoglutaric acid, and many others. Attention is also drawn to the increase in the cyclic AMP content discovered in the present investigation in granulation tissue in the early periods of wound healing and changes in the cyclic AMP/cyclic GMP ratio which, in turn, promote intensification of repair processes.

There is no doubt that administration of glycine in near-physiological doses triggers a system of cascade reactions the final result of which is to accelerate wound healing.

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PROLIFERATIVE ACTIVITY OF CELLS IN DYSHORMONAL FIBROADENOMATOSIS OF THE HUMAN BREAST

K. A. Gudim-Levkovich, L. V. Yakhimovich, S. M. Slinchak, L. P. Kaminskaya, S. A. Kovbasyuk, L. V. Polozun, and Yu. A. Umanskii

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In many cases the development of breast cancer is preceded by so-called dyshormonal dysplasia. These diseases can be regarded clinically and morphologically as precancerous, although not all forms of adenomatosis go on to cancer [2]. One condition that increases the risk of onset of malignant disease is the presence of considerable endocrine disturbances in women [7]. The main criterion for assessment of both dyshormonal dysplasias and malignant diseases is the degree and character of proliferation of the epithelium. Proliferative adenomatosis has been shown to become malignant more often than the nonproliferative form [5, 14]. Nevertheless, clarification of the precise character of proliferative activity of the epithelium from this point of view is beset by great difficulties. This explains the continued search for new methods making the differential diagnosis between dyshormonal fibroadenomatosis and malignant tumors of the human breast easier for both clinicians and pathologists [6].

The object of the present investigation was to study proliferative activity of the epithelium in dyshormonal fibroadenomatosis of the breast.

EXPERIMENTAL METHOD

The method of tissue culture of human breast affected by fibroadenomatosis in diffusion chambers implanted intraperitoneally in animals [3], and using morphological and autoradiographic methods of analysis of the growing cells [10] was used.

Department of Immunology of Carcinogenesis, R. E. Kavetskii Institute for Problems in Oncology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.)

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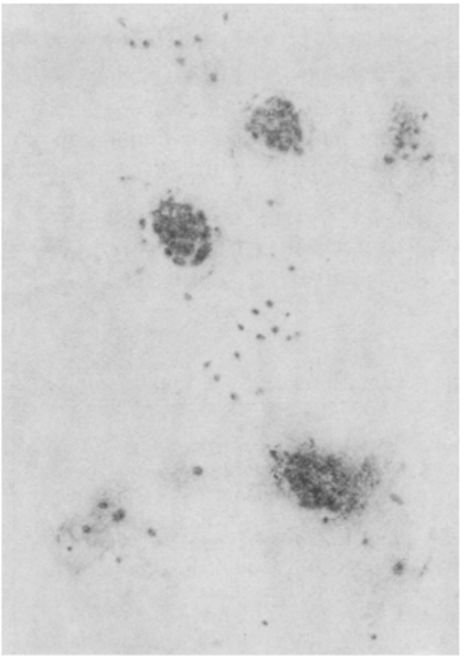

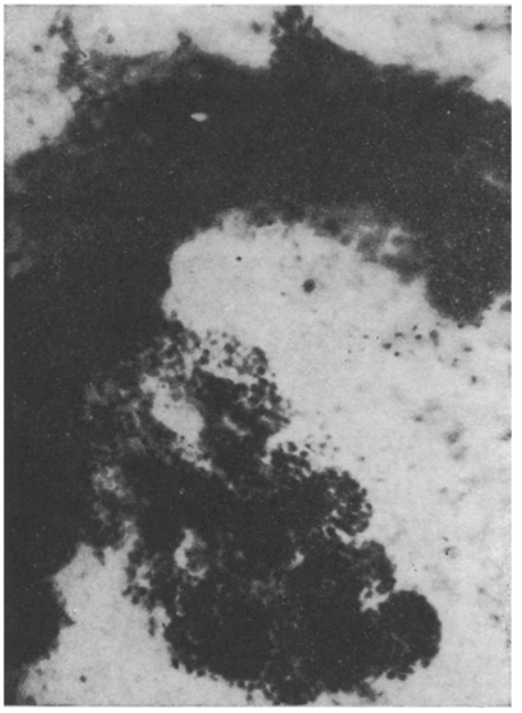

Fig. 2

Fig. 1. Spheroid-like epithelial formations during culture of cells from breast affected by fibroadenomatosis. Carazzi's stain, 140 X.

Fig. 2. Aggregates of cells of proliferating epithelium during culture of tissue from breast affected by fibroadenomatosis. Carazzi's stain, 140 X.

Pieces of tissue and cell suspensions were applied to millipore filters of diffusion chambers by the method described previously [10]. After culture for 6 days the filters were removed and stained by Carazzi's method. Autoradiographs were obtained from some of the preparations. Proliferative activity of the cells was judged by their ability to incorporate ^{3}H-thymidine into their nuclei. The labeling index was determined and expressed as the percentage of ^{3}H-thymidine-labeled cells among a total of 1000 cells examined in each preparation. The epithelial cells and fibroclasts labeled with ^{3}H-thymidine also were acounted separately and their relative labeling index calculated, i.e., the percentage of labeled epithelial cells and fibroblasts among their own population.

EXPERIMENTAL RESULTS

During culture of both a cell suspension and of tissue fragments for 6 days simultaneous growth of epithelial connective-tissue cells was observed. Growth of the epithelial cells reflected their capacity for organotypical differentiation, with the formation of gland-like structures lying on the basement membrane. They formed papillary structures with morphological signs of high proliferative activity of the cells, resembling a breast lobule (Fig. 1).

The results of a study of proliferative activity of the cells showed (Table 1) that the labeling index in the growing cells differed in different cases of fibroadenomatosis and varied from 5.0 to 20.6%. Analysis of the cell composition showed that epithelial cells accounted on average for up to 70% of the total, and fibroblasts up to 29.1%. The percentage of labeled epithelial cells was 8.3 and of fibroblasts 4.6, but the relative labeling index of these cells differed only a little (12.2 and 14.6 respectively).

If the labeling index of the epithelial cells was low (from 5.0 to 7.7%) a monolayer or laminar type of growth of the epithelium was found on the filters, with the formation of discrete structures of organotypical character.

In cases with a high labeling index of the epithelial cells (up to 20.6%), however, growth of the epithelial cells during culture of both fragments and suspensions in diffusion chambers was characterized by the formation of large papillary proliferating structures and, in one case, by growth of spiral formations composed of epithelial cells, or spheroids (Fig. 2), which are specific for cells of malignant tumors cultured under such conditions [8, 9, 11-13, 15].

Comparison of the labeling index with the results of histological investigations showed that these parameters depend on the form of dyshormonal fibroadenomatosis of the breast. The number of DNA-synthesizing cells was low in cell cultures

TABLE 1. Labeling Index of Tissue Cells from Human Breast with Fibroadenomatosis, Cultured in Diffusion Chambers (in %)

Patient's surname	Total labeling index	Labeling index of		Relative labeling index of	
		epithelial cells	fibroblasts	epithelial cells	fibroblasts
Ga-a	10,8	4,2	6,6	7,5	15,0
M-ts	12,3	5,8	6,5	12,7	26,5
R-k	7,7	4,4	3,3	6,1	12,0
Z-K	13,9	12,1	1,8	14,5	9,7
L-K	12,2	7,1	5,1	11,4	13,4
S-o	18,2	13,1	5,1	16,8	19,6
B-a	20,6	15,0	5,6	24,1	16,6
Sh-ya	15,3	9,2	6,1	12,9	21,0
Ch-K	5,0	3,2	1,8	4,2	7,5
Mean	12,8±1,75	8,3±1,32	4,6±0,53	12,2±1,11	14,6±2,12

from forms of fibroadenomatosis in which growth of connective-tissue cells predominated. Cystic forms of breast fibroadenomatosis with hyperplasia of epithelial cells, with their poorly developed stroma, were characterized like all other proliferative forms, by high values of their labeling index. According to Gorevaya and Nekrasov [1], it is these dyshormonal dysplasias which are accompanied by considerable endocrinological disturbances in the patients and which often terminate in the development of malignant breast tumors.

On culture of cells from the human breast affected by fibroadenomatosis in diffusion chambers proliferative activity of the cultured cells was thus shown to be dependent on the type of dyshormonal dysplasia in each concrete case and, in particular, on the character of proliferation of the epithelium. Morphological forms which, by the level of proliferative activity of their cells, closely resembled cells of malignant tumors. Considering that the method of cell culture in diffusion chambers reflects maximal proliferative activity of the cells compared with their culture *in vitro* [4], it is concluded that this method can be used for screening cases of dyshormonal fibroadenomatosis of the breast that have a tendency of become malignant.

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