for a period of 36 h, 7.8 ± 2.1‰ of unlabeled mitoses were collected after transplantation, If, however, the label was injected for 48 h, hardly any unlabeled mitoses were found (only 0.25 ± 0.25‰; five animals were used in each experiment). The facts are evidence that in the terminal stage of development a few cells may be in the G_2 period and then in the reversible restingR_2 state for over 36 h, but this time cannot be increased to 48 h. With an increase in the length of stay in the reversible resting R_2 state the cells evidently proceed into the irreversible resting state.

During development of AH22A the size of the G_2 subpopulation thus undergoes relatively little change, probably as a result of interaction between the factors determining its size, Between the 5th day of development and the terminal stage, a subpopulation of cells in AH22A can be found in the reversible resting R_2 state, and which return to the mitotic cycle in the early period after stimulation of division. The size of this subpopulation increases somewhat as the tumor ages. However, in the terminal stage its size is small (47‰), and on that account there can be no question of any significant accumulation of cells in the reversible resting R_2 state. This state of affairs and the fact that the size of the G_2 subpopulation + the subpopulation of cells in the reversible resting R_2 state is reduced in a delayed tumor are evidence that the R_2 state is a relatively short-term transient state during emergence of the cells from the G_2 period of the mitotic cycle.

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CORRELATION ANALYSIS OF FREE MAMMARY GLAND STROMAL CELLS DURING AGE CHANGES AND DEVELOPMENT OF SPONTANEOUS TUMORS

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The fact that communication between cells taking part in immunologic reactions takes place is not nowadays in dispute. However, the nature of this communication and the extent to which changes in it determine the character of the pathological process has not yet been investigated. Many workers [1, 4] consider that the development of the immunologic response is determined by cooperative relations between cells belonging to the systems of specific and nonspecific immunity.

We know that the development of a pathological process is based on insufficiency or disturbance of certain components of coordinating systems. However, despite much research in im-

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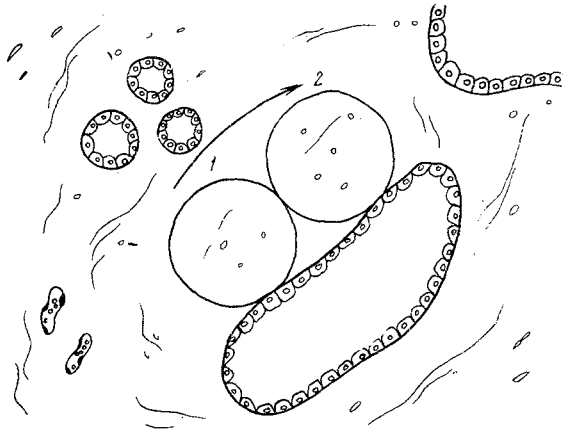

Fig. 1

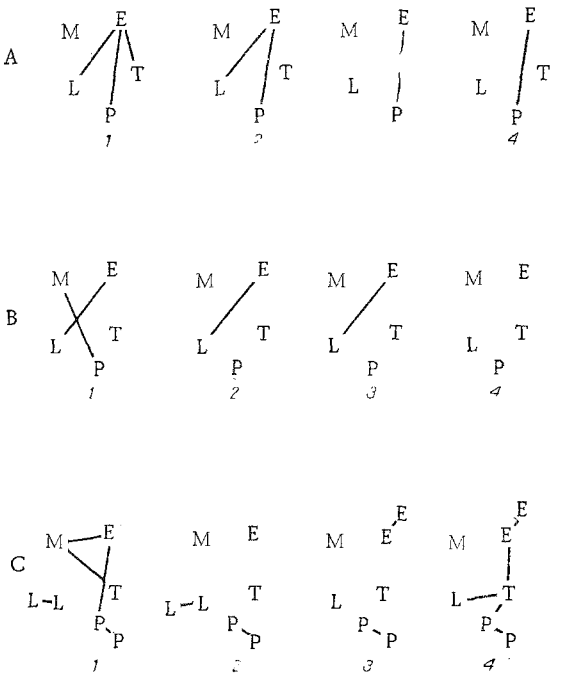

Fig. 2

Fig. 1. Method of counting cells in area of infiltration in consecutive fields of vision (example shows counting around epithelial structures). 1, 2) fields of vision. Arrow indicates direction of scanning.

Fig. 2. Correlation between cells in zone of epithelial component (A), between cells infiltrating zone of blood vessels (B), and in different (vascular and epithelial) zones (C). L) Lymphocyte, P) plasma cell, M) macrophage, E) eosinophil, T) mast cell. 1-4) Groups of animals.

munology and, in particular, the immunology of tumor growth [2, 6, 7], no information is available on cooperative relations between free-lying cells infiltrating the stroma.

The object of this investigation was to study, by morphological methods, changes in the character of communication between cells depending on the degree of proliferation of the epithelium during age changes in the epithelium of the mammary gland and the development of spontaneous tumors.

EXPERIMENTAL METHOD

Experiments were carried out on 49 noninbred rats aged 20 months, divided into four groups depending on the intensity of the morphological changes in the mammary gland. When division into groups was undertaken attention was paid to proliferative changes in the mammary gland epithelium and the complex character of the involutional changes. Group 1 (12 animals) was conventionally described as "physiological." Age atrophy of the epithelium with no signs of hypersecretion or pathological proliferation predominated in the mammary gland. Group 2 (12 animals) was described as the group with "pathology of the involutional period." Atrophic involutional changes in the epithelium of the mammary gland were intermingled with foci of secretory activity and moderate proliferation with no signs of atypical growth. Group 3 included animals (16) with signs of active secretion, atrophy, and pathological proliferation of the epithelium in the mammary gland to give a picture of fibrocystic mastopathy. Group 4 consisted of nine animals with tumors of the fibroadenoma type and with epithelial tumors with signs of infiltrative growth.

The relative percentages of the different free-lying cells around the terminal portions of the ducts and also between the small blood vessels in a zone remote from the epithelial component were determined in sections stained with hematoxylin-eosin and azure-eosin. Cells were counted in consecutive fields of vision in the immediate vicinity of the epithelium or vessels, up to a total count of 100 free cells. The following types of cells were distinguished: lymphocytes, plasma cells, macrophages, mast cells, and eosinophilic leukocytes (eosinophils) (Fig. 1).

TABLE 1. Coefficients of Correlation between Number of Different Free-Lying Cells in Subepithelial Cells (A), in Vascular Zone (B), and between Areas of Cellular Infiltration in Different (vascular and epithelial) Zones (cross analysis; C)

Zones and cells	Group 1	Group 2	Group 3	Group 4
A				
Lymphocyte (L)	L–E (–0,62)	L–E (–0,65)	—	—
Macrophage (M)	—	—	—	—
Plasma cell (P)	P–E (0,67)	P–E (0,70)	P–E (0,94)	P–E (0,78)
Eosinophilic leukocyte (E)	E–P (0,67)	E–P (0,70)	E–E (0,94)	E–P (0,78)
	E–L (–0,62)	E–L (–0,65)	—	—
	E–T (–0,69)	—	—	—
Mast cell (T)	T–E (–0,69)	—	—	—
B				
Lymphocyte (L)	L–E (–0,74)	L–E (–0,76)	L–E (–0,98)	—
Macrophage (M)	M–P (0,70)	—	—	—
Plasma cell (P)	P–M (0,70)	—	—	—
Eosinophilic leukocyte (E)	E–L (–0,74)	E–L (–0,76)	E–L (–0,98)	—
Mast cell (T)	—	—	—	—
C				
Lymphocyte (L)	L–L (0,60)	L–L (0,64)	—	—
	—	—	—	L–T (0,89)
Macrophage (M)	M–T (–0,58)	—	—	—
	M–E (0,63)	—	—	—
Plasma cell (P)	P–P (0,66)	P–P (0,78)	P–P (0,97)	P–P (0,89)
	P–E (0,79)	—	—	—
	—	—	—	P–T (–0,76)
Eosinophilic leukocyte (E)	E–M (0,63)	—	—	—
	E–P (0,79)	—	—	—
	—	—	E–E (0,69)	E–E (0,80)
Mast cell (T)	T–M (–0,58)	—	—	—
	—	—	—	T–L (0,89)
	—	—	—	T–E (–0,94)
	—	—	—	T–P (–0,76)

Note: Coefficients of correlation given in parentheses.

The results were analyzed on the ES 10-20 computer with calculation of the coefficient of correlation [3, 5]. Correlation between the different types of cells, allowing for their different tissue localization, was determined. The degree of accumulation of cells in the zone of direct contact with the epithelial components was compared with that in the vascular zone, and cross-correlation analysis was undertaken in obviously different zones (Fig. 2). In the data presented in this paper only mathematically significant coefficients of correlation (over ±0.5) were taken into consideration.

EXPERIMENTAL RESULTS

During determination of correlation between the number of cells around the epithelium, paired correlations were discovered between all cells except macrophages in group 1. However, if proliferation and pathological changes were present in the epithelium, the number of paired correlations was reduced and reached a minimum in group 3 (the "pretumor" group). Correlation between eosinophils and plasma cells was preserved in group 4. It will be noted that the coefficient of correlation for the preserved eosinophil—plasma cell relationship increased sharply in group 3 (Table 1, A).

Analysis of relations between the number of cells around the vessels showed that in group 1 ("physiological") accumulation of the different types of cells also was accompanied by correlation changes but, by contrast with the area of infiltration around the epithelium, the mast cell remained independent. With the appearance of pathological proliferation the number of correlations decreased. In group 4 ("tumor" group) accumulation of all cells took place independently of one another. The coefficient of correlation for lymphocyte—eosinophil in group 3 reached a maximum (Table 1, B).

The clearest correlations were revealed by cross-correlation analysis (Table 1, C). In the animals of group 1 (i.e., those with a normal, physiological involution of the mammary gland) correlations were found between all types of cells (coefficients 0.6-0.7). With the appearance of the initial signs of dyshormonal pathology (group 2), however, disturbance of the correlations took place abruptly: only correlations between cells of the same type, located around the epithelium and vessels, remained. In group 3 ("mastopathy") the same picture was preserved. Group 4, in which at first glance apparent restoration of correlations was observed, is particularly interesting. These correlations were seen to be qualitatively different, for new variants of correlation appeared (mainly with the mast cells) (Table 1, C). The mast cells lose their autonomy and became the predominant cell component in cooperation between cells of the specific and nonspecific systems of immunity.

Correlation analysis of the character of relationships between different free-lying stromal cells thus shows that during physiological involution paired correlations were found between all cells studied. The development of pathological proliferation of the epithelium was preceded by a change in the character of connections between the free-lying stromal cells. Parallel with progression of the pathological process, a decrease in the number of paired correlations was observed. Tumor development was accompanied by the appearance of qualitatively new variants of correlations rather than by a decrease in existing paired correlations. With the development of free tumor changes the most marked decrease was observed in the number of paired correlations between different types of cells.

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