EFFECT OF DEAFFERENTATION ON THE MORPHOLOGICAL AND HISTOCHEMICAL CHARACTERISTICS OF THE LUNG CELLS OF THE CAT

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With the development of the nervous system in phylogenesis, an increase in the complexity of organization of the cells took place in the tissues of animal organisms, with intensification of their specialization and differentiation. One of the many functions assumed by the nervous system was the maintenance of differentiation of cell forms at its correct height [5].

If the nervous pathways are disturbed, neurotrophic changes develop in the tissues. The principal role in the disturbance of the neurotrophic processes is played by a lesion of the sensory component of the mixed nerve trunk [1,3,6,7]. As T. A. Grigor'eva [1] points out, the complex of tissue changes in the deafferented area is identical to inflammation in the destructive-exudative phase.

During the study of the changes developing in a deafferented area, besides manifestations characteristic of the organ concerned, investigators have also discovered certain general principles. These include the appearance of zones of infiltration of leukocytes and round cells, and of de-differentiation of the cells of the investigated organ. The state of advanced de-differentiation of the tissues of deafferented organs is demonstrated by a disturbance of the formation of a connective-tissue capsule around a foreign body [1,11].

Investigations of the changes arising in the tissues following interruption of the sensory link of the reflex arc have been conducted on various organs. These changes have received least study in the lungs. The few investigations so far carried out [2,4,9] show that following sensory denervation, chronic aseptic inflammation with signs of neutrophil infiltration of the tissues and of de-differentiation of the cells develop in the lungs, just as in other organs. The study of this process is of great importance also because analogous changes develop in the lung tissues in the condition known as vagus pneumonia, which develops after division of the vagus nerve, a large part of which consists of sensory fibers [4].

The object of the present investigation was to study the dynamics of development of the pathological processes taking place in the lungs of cats after sensory denervation, using the methods of histological and histochemical analysis.

EXPERIMENTAL METHOD

The lungs are known to receive their afferent innervation from the vagus nerves and the spinal sensory ganglia.

In the present experiments, the spinal sensory ganglia were removed from 35 adult cats at the level D1-D5 bilaterally. The controls were animals not undergoing the operation and animals on which laminectomy was performed but the spinal ganglia were not removed.

The animals were sacrificed at different times after the operation. Pieces of tissue were taken from all the lobes of the lung and fixed in a 12% solution of neutral formalin or in Carnoy's fluid. The preparations were stained with hematoxylin-eosin, toluidine blue, and azure-II-eosin. The morphological analysis included quantitative analysis of the cell composition of the alveolar epithelium.

Two enzymes of the group of the carboxylic esterases were detected histochemically: nonspecific esterase and lipase. The enzymes were determined by the method of Nachlas and Seligman, as modified by Pearse [17], in conjunction with their differentiation by means of activators and inhibitors [10,12]. No attempt was made to differentiate

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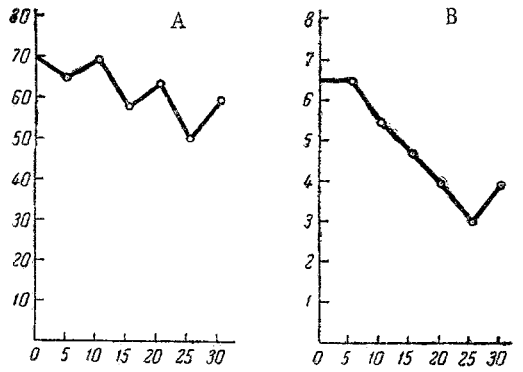

Fig. 1. Changes in the number of large alveolar cells (A) and in nonspecific esterase activity (B) in their cytoplasm after deafferentation of the lung. Here and in Fig. 2: along the axis of abscissas — days after deafferentation, along the axis of ordinates — number of cells in percent (A), activity of enzyme in units of optical density (B).

between nonspecific esterase and esterolytic activity associated with cathespin C. The activity of the enzymes in the cytoplasm of the cells was determined by comparative visual photometry of the stain formed as a result of the enzyme reaction, and expressed as units of optical density. One unit of enzyme activity is equal to 0.1 unit of optical density. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

During the first day after deafferentation, the blood vessels were dilated and congested with blood, and contained an excess of neutrophilic leukocytes. This neutrophilic infiltration started near the vessels and subsequently spread throughout the parenchyma of the lung. The alveolar septa were edematous, and in the lumen of most alveoli a serous exudate was present, containing polymorphs and erythrocytes. The intensity of the exudation and the neutrophilic infiltration increased until the 20th-25th day after the operation, after which their intensity fell slightly, although they persisted throughout the period of observation.

By the 5th-7th day after the operation, the first signs of a disturbance of the normal lung structure appeared. The epithelial cells of the bronchi lost their cilia, and goblet cells increased sharply in number and were distended with secretion. In some cases proliferation of the bronchial epithelium was observed, sometimes leading to complete occlusion of the lumen of the bronchi. The lumen of the other bronchi was filled with exudate, containing neutrophilic leukocytes, macrophages, and desquamated epithelial cells. The bronchial glands were converted into groups of epithelial cells with no signs of organization in the terminal glandular portions.

Starting with the 8th-10th day after the operation, hyperplasia of the lymphoid tissue was observed, especially of its reticular stroma. The reticular cells were round in shape, slightly enlarged, and they contained large nuclei and a delicate chromatin reticulum.

Mitotic figures of various types were repeatedly observed in the cells of the alveolar and bronchial epithelium. Mitotic division of the reticular cells was especially common. The large numbers of macrophages and plasma cells were conspicuous. They appeared on the first days after the operation and were observed until the end of the investigation, both in the lymphoid structures and in other parts of the lung.

A frequent sign of the changes arising in the tissues of the deafferented lung was the appearance of giant cells. Outwardly they resembled megakaryocytes. Their nuclei were large, polymorphic, and sometimes most curiously shaped. In some of the giant cells, the cytoplasm was present in extremely small quantity, and they resembled "bare nuclei." A similar picture has been described by M. I. Pekarskii [8].

Because of the polymorphism of the cells observed in the lungs, it was impossible to determine not only the degree of maturity of the individual cells, but also the type to which they belonged. Besides changes in cell composition, a disturbance of the normal structure of the collagen framework of the lungs was also observed. The collagen fibers, most frequently around the vessels and bronchi, showed changes consisting of loss of the typical fibrous structure, homogenization, and sometimes granular degeneration.

Despite the actively developing process of disturbance of the lung structure, massive death and disappearance of the cells was not observed. The cells of the alveolar, bronchial and glandular epithelium and of the connective tissue, although they lost their specific features, did not die but proliferated actively; many mitotic figures were seen in them; the phagocytic activity of the reticular cells was increased.

The histochemical analysis showed that the cytoplasm of some of the large alveolar cells contained one active enzyme, nonspecific esterase, or two enzymes, nonspecific esterase and lipase. These enzymes were not discovered in some cells.

In the control animals, the number of large alveolar cells containing these active enzymes was relatively stable, amounting on the average to 70% of all the cells (individual variations from 64-79%). The mean nonspecific esterase activity per cell was 6.5 units (individual variations from 6.1-7.8 units).

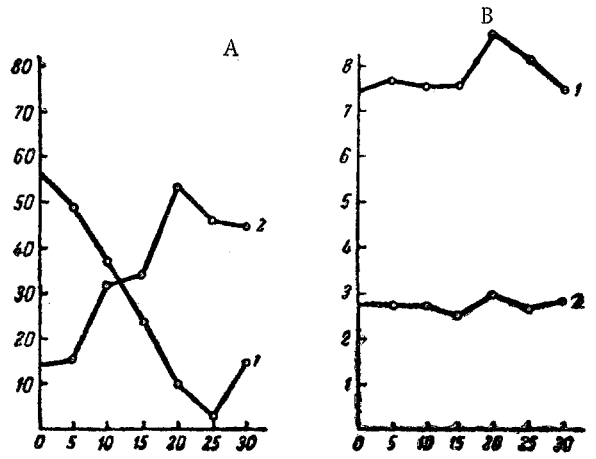

Fig. 2. Changes in the number of large alveolar cells (A) of group 1 (1) and 2 (2) and in nonspecific esterase activity (B) in their cytoplasm after deafferentation of the lung.

After deafferentation, the number of large alveolar cells containing active enzyme remained almost unchanged. Meanwhile, the mean enzyme activity in the cells fell progressively from the 5th until the 25th day after the operation (Fig. 1). After the 30th day, the esterase activity in the cytoplasm of the large alveolar cells recovered slightly. Recovery continued until the 50th-70th day after the operation, but since the number of experiments in the late stages was small, the changes after the 30th day were not statistically significant.

In some of the large alveolar cells, active lipase was discovered besides nonspecific esterase. Combined analysis of the two enzymes in the large alveolar cells showed that the nonspecific esterase activity in the cells which also contained lipase never exceeded 4 units. In the control animals, its mean content in these cells was 2.8 units (individual variations from 2.4 to 3.2 units). The mean number of these cells in the normal lung is 14% (individual variations from 5 to 21%). Meanwhile, in the cells not containing lipase, the mean nonspecific esterase activity was 7.4 units (individual variations from 6.1 to 7.8 units) and their number in the control animals lay between 53 and 61%.

Hence, the large alveolar cells containing carboxylic esterases were represented as cells containing nonspecific esterase (high activity) alone, and also as cells containing esterase (low activity) and lipase.

Considerable changes were observed in the number of cells of each group. The number of large alveolar cells of group 1 fell appreciably (Fig. 2). The fall reached its lowest level (3%) 25 days after deafferentation. The content of cells of group 1 rose slightly after the 30th day. The number of cells of group 2, on the other hand, increased after the 5th day and reached its maximum after 20 days (Fig. 2). Because of the redistribution of the number of cells of groups 1 and 2, the mean esterase activity fell in all the large alveolar cells (Fig. 1).

Only one of these enzymes, nonspecific esterase, was found in the small alveolar cells. Normally the number of these cells varies from 14 to 28%. The mean nonspecific esterase activity in their cytoplasm is 7.7 units (individual variations from 6.7-8.6 units).

After deafferentation, the decrease in the number of cells was not statistically significant, but the fall in activity of this enzyme after the 5th day to 4.0-5.0 units was statistically significant.

The nonspecific esterase activity in the cytoplasm of cells, as Argiris [15,16] points out, corresponds to the degree of their differentiation. The author has previously confirmed this conclusion for connective-tissue cells during their differentiation in the process of regeneration [12,13] and of de-differentiation in tissue culture [14].

The changes in nonspecific esterase in the alveolar epithelial cells after sensory denervation of the lung demonstrate an increase in the number of undifferentiated forms characterized by lowered activity of this enzyme.

Analysis of the results obtained confirms the conclusions reported in the literature concerning the de-differentiation of the cells of organs and tissues after their sensory denervation. Evidence of this is given by the proliferation of epithelium, the polymorphism of cells, the increase in the number of mitoses, the appearance of giant cells, and the hyperplasia of lymphoid tissue.

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