Changes in ultrastructure of cells of the SA and AV nodes as well as differences in the degree of disturbance of permeability of the sarcolemma and intracellular membranes for colloidal lanthanum enable the point of application of the ischemic factor to be identified in these formations. Heterogeneity of the ultrastructural changes, the reduction in the number of cristae, and the decrease in energy efficiency of the mitochondria in nodes of the conducting system of the heart lead to a disturbance of their function, on the basis of which rhythm disturbances may arise under the conditions of acute myocardial infarction.

LITERATURE CITED

1. L. A. Alekminskaya, Yu. B. Lishmanov, V. D. Slepushkin, and M. I. Titov, Byull. Éksp. Biol. Med., No. 5, 269 (1985).
2. V. S. Paukov and V. A. Frolov, Elements of a Theory of Pathology of the Heart [in Russian], Moscow (1982).
3. I. A. Chervova and E. R. Pavlovich, Byull. Éksp. Biol. Med., No. 9, 378 (1978).
4. I. A. Chervova and E. R. Pavlovich, Arkh. Anat. No. 8, 67 (1979).
5. V. G. Sharov, R. B. Jennings, and H. K. Hawkins, Arkh. Patol., No. 10, 35 (1980).
6. V. G. Sharov, Byull. Éksp. Biol. Med., No. 12, 757 (1981).
7. V. G. Sharov, Arkh. Patol., No. 3, 3 (1985).
8. M. Mochet, J. Moravac, H. Guillemot, and P. Y. Hatt, J. Mol. Cell. Cardiol., 7, 879 (1975).
9. J. P. Revel and M. J. Karnovsky, J. Cell Biol., 33, 7 (1967).

BRONCHOALVEOLAR CELL COUNT DURING COMPENSATORY HYPERTROPHY OF THE LUNG

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UDC 616.24-008.66-06:616.24-007.64-076.5

KEY WORDS: bronchoalveolar lavage; bronchoalveolar cell count; compensatory hypertrophy of the lungs

Identification of the structural basis for disturbed functions of the damaged lung has not yet been finally achieved. We know that after resection of various kinds the residual lung volume increases rapidly [5, 6, 14]. After leftsided pneumonectomy in rats the relative volume of the right lung only 5 days after the operation amounts to more than 80% of the combined volume of both lungs of control animals [6]. The question arises whether there is an increase in the number of cells settling in the respiratory part of the lung takes place parallel with compensatory growth of the lung. The morphological and functional features of cell populations existing inside the lung are nowadays studied by cytological analysis of material obtained by bronchoalveolar lavage (BAL) [2, 4].

The aim of this investigation was to discover the characteristic features of the bronchoalveolar cell count (BACC) in the normal and postoperative lung and to determine the degree of increase in the number of cells colonizing the internal medium of the lung, in the course of its compensatory hypertrophy.

By bronchopulmonary cell count is meant the ratio between the number of cells obtained by BAL (cells of the monocytic-macrophagal series, lymphocytes, neutrophils, eosinophils, and basophils). Cells of the bronchial epithelium and erythrocytes are not included. The alternative term "endopulmonary cytogram" was first introduced by Avtsyn et al. in 1982 [2].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 215-345 g, kept under animal house conditions. Only animals with no outward signs of disease of any kind were used in the experiments. Under deep pentobarbital anesthesia the left lung (about 37%

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 3, pp. 363-366, March, 1987. Original article submitted May 16, 1986.

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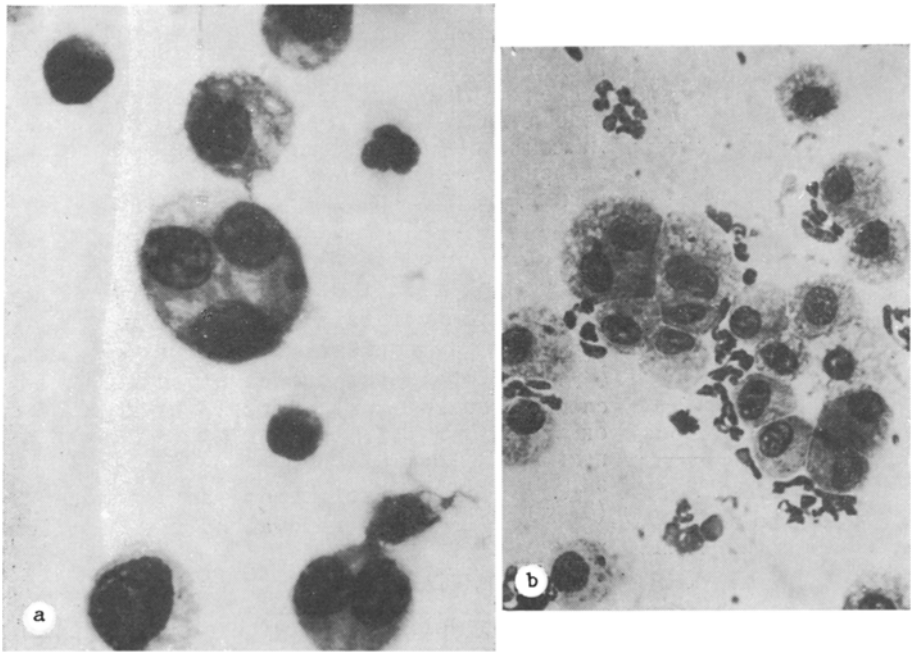

Fig. 1. Cells of BAW from rat lung. a) BAW from control animal, consisting predominantly of alveolar macrophages with different numbers of nuclei; stained by Pappenheim–Kryukov method; b) high neutrophilia of BAW 24 h after left-sided pneumonectomy: increased adhesion of alveolar macrophages. Stained by Pappenheim–Kryukov method, 1000 ×.

TABLE 1. Volume of Physiological Saline Injected into Lungs and of BAW Withdrawn and BACC of Control and Postoperative Rats

Experimental conditions	Number of rats	Volume of physiological saline injected, ml	Volume of BAW withdrawn, ml	Number of cells in 1 ml of BAW ($\times 10^6$)
Control	8	11,7±0,3	8,5±0,5	0,26±0,03*
Mock operation:				
1st Day	4	11,3±0,3	8,3±0,5	0,41±0,1*
3rd Day	5	11,4±0,2	8,8±0,3	0,21±0,03
5th Day	4	11,7±0,6	9,7±0,4	0,20±0,02
Removal of left lung:				
1st Day	5	9,7±0,7	6,6±0,6	0,30±0,06*
3rd Day	5	9,1±0,7	7,4±0,4	0,30±0,04*
5th Day	5	10,1±0,1	8,3±0,2	0,33±0,07*

Legend. Asterisk indicates that differences between control and experiment are not significant ($P > 0.05$).

of the weight of both lungs) was removed from the experimental rats. Intact rats and rats undergoing mock operation, involving thoractomy in the region of the 5th intercostal space, followed by interrupted suture of the wound in layers, served as the control. Under deep pentobarbital anesthesia phyiological saline, heated to 37°C, was injected intratracheally into the control and experimental animals 1, 3, and 5 days after the operation until the lung was completely filled, when it was reaspirated by means of a syringe, in order to obtain bronchoalveolar washings (BAW). The lungs were fixed in 10% formalin solution and histological sections stained with hematoxylin and eosin were examined. After BAL, all the organs were inspected. Rats with signs of pathology were rejected. The volume of physiological saline injected into and withdrawn from the lungs was determined in the animals used in the experiment. The number of cells in 1 ml of BAW was counted in a Goryaev's chamber and the ratio between dead and viable cells was determined after staining with a 1% solution of trypan blue. In preparations obtained from a cellular suspension of BAW, stained by the Pappenheim–Kryukov method (Fig. 1) 1000 cells in each case were examined under the light microscope. The results were subjected to statistical analysis by the Fisher–Student test. Differences between control and experiment were regarded as signifi-

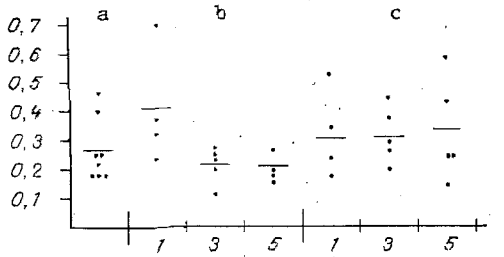

Fig. 2. Number of cells in 1 ml BAW from lungs of control and experimental rats at different times after operation. Abscissa, time after operation (in days); ordinate, number of cells in 1 ml of washings ($\times 10^6$); a) control, b) mock operation, c) removal of left lung.

ficant at the $P \leqslant 0.05$ level. The cytological investigation was conducted on BAW from 36 rats (8 control, 13 after a mock operation, 15 after removal of the left lung).

EXPERIMENTAL RESULTS

The lungs of animals of all groups were well filled with physiological saline. It will be clear from Table 1, which gives the lung volumes according to the volume of physiological saline injected into them, that by the 5th day after left-sided pneumonectomy the residual right lung contained on average 10.1 ml, compared with 11.7 ml for both lungs in the control. The internal volume of the right lung was thus 84% of the volume of both lungs in the control. If it is recalled that the right lung in rats accounts for about 63% of the volume of both lungs [6], according to calculation, in the control it ought to contain on average 7.4 ml. In fact, the degree of filling of the single residual lung on the 5th day after left-sided pneumonectomy was increased by 36.2% ($P < 0.01$). The volume of BAW obtained in the control averaged 73%, after the mock operation 73-78%, and after removal of the left lung 68-83%. These data are evidence of the improved elasticity of the hypertrophied lung.

The number of cells in 1 ml of BAW from most animals of all three groups was between $0.20 \cdot 10^6$ and $0.41 \cdot 10^6$ (Fig. 2). The mean cell count in the control and experimental animals did not differ significantly. Meanwhile an increased cell count was observed in the BAW of 5 rats: $0.46 \cdot 10^6$-$0.70 \cdot 10^6$.

The BACC of both control and experimental rats was represented mainly by cells of the monocytic-macrophagal series: 88.9 ± 3.2% in the control, 83.4 ± 5.1% after the mock operation, and 81.7 ± 3.5% after removal of the left lung. Lymphocytes accounted on average for 4.2 ± 1.4, 3.2 ± 0.5, and 2.5 ± 0.6% (Fig. 3). Only in the BACC of one control rat was a relative increase found in the lymphocyte count (11.3%). The number of neutrophils in BACC averaged 6.9 ± 3.5% in the control rats, 12.9 ± 5.3% in rats after the mock operation, and 15.6 ± 3.7% for rats after pneumonectomy ($P > 0.05$). Among the alveolar macrophages in the control and experimental series on average 1.0-3.8% of the cells were nonviable. A distinguishing feature of the BACC of some animals with one residual lung was a relative increase in the number of eosinophils (to 0.6%). However, differences in the mean values of the eosinophil counts in the control and experiment at different times after the operation were not significant. The causes of the eosinophilia are not sufficiently clear. Increased adhesion of alveolar macrophages was observed in the BAW of the pneumonectomized rats (Fig. 1b).

The high BACC in 4 of the 5 rats correlated with high (over 20%) neutrophilia (Fig. 1b). Among the control rats high neutrophilia (30.8%) was found in one of the eight animals, after the mock operation in two of the 13 rats (36.5 and 67.7%), and after left-sided pneumonectomy in four of the 15 rats (26.4, 31.0, 41.4, and 46.4%). In 4 rats high neutrophilia in BAW was observed against the background of a normal total cell count. Low- or middle-lobar focal bronchopneumonia, resembling hypostatic pneumonia in character [3], was found in all the animals with high neutrophilia. These results are in agreement with those of a previous investigation [1], when a high percentage of spontaneous diseases of the upper respiratory tract and lungs was found in inbred and noninbred rats.

The question arises, what could facilitate this inflow of cells from the blood stream into the respiratory portion of the lung during its compensatory hypertrophy. We know that

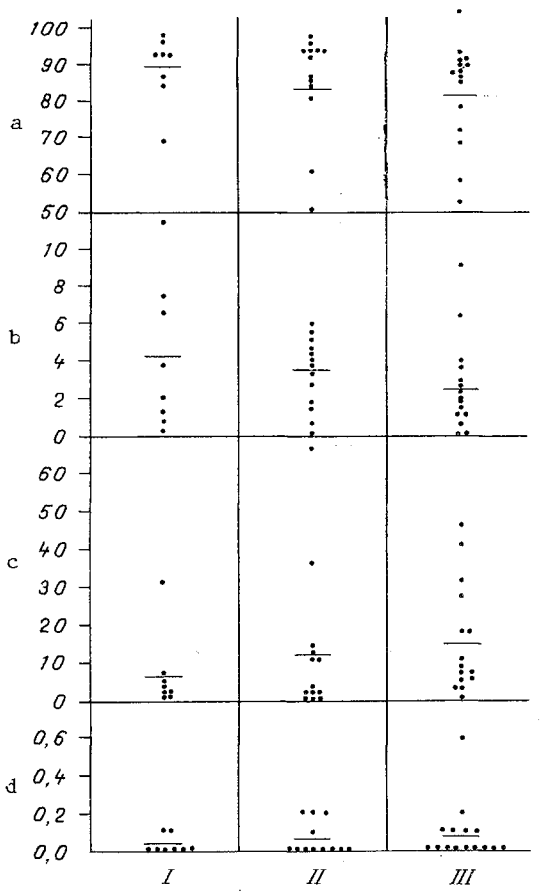

Fig. 3. BACC of control and experimental animals. Vertical axis: a) monocytes-macrophages (in percent); b) lymphocytes (in percent); c) neutrophils (in percent); d) eosinophils (in percent). I) Control; II) mock operation; III) removal of left lung.

monocytes and neutrophils can migrate through the air-blood barrier into the alveolar cavity, and also through the mucous membrane of the air passages into their lumen [12]. Bronchoscopy and the procedure of BAL themselves stimulate migration from the blood stream into the alveoli [9, 10]. It can accordingly be postulated that the mechanical factors (stretching of the alveoli and capillaries) and an increase in permeability of the components of the air-blood barrier, observed at different times after operations on the lungs [7], facilitate the release of cells into the alveolar cavity of the hypertrophied lung. The possibility likewise cannot be ruled out that stimulation of uniform migration of cells from the blood stream into the alveoli is due to an increase in concentration of various modulators of cell behavior in the liquid phase of the supracellular alveolar layer. Their role may be performed by chemical attractants, secreted by alveolar macrophages [11, 13], and also by surface-active substances secreted by type II alveolocytes [8].

In the process of compensatory growth of the lung after unilateral pneumonectomy the increase in respiratory surface is accompanied by a rapid rise in the number of cells colonizing the respiratory part of the organ and performing a protective barrier function. It must be pointed out that the cellular composition of the BAW during compensatory hypertrophy of the rat lung, uncomplicated by inflammation, is indistinguishable from the cell composition of the BAW of intact animals and animals undergoing a mock operation. The predominant cell population is formed by cells of the monocytic-macrophagal series. The BACC of postoperative pneumonia is characterized by high (more than 20%) neutrophilia, and the inflow of the various cells from the blood stream into the alveoli takes place proportionally, so that the physiological ratio between monocytes-macrophages, lymphocytes, and leukocytes in the internal medium of the regenerating lung is preserved.

LITERATURE CITED

1. É. Kh. Abdrashitova, Vest. Akad. Med. Nauk SSSR, No. 9, 81 (1983).
2. A. P. Avtsyn, G. I. Lukomskii, L. K. Romanova, et al., Sov. Med., No. 7, 8 (1982).
3. I. K. Esipov (ed.), The Lung in Pathology [in Russian], Novosibirsk (1975).
4. G. I. Nepomyashchikh, V. N. Efremov, V. P. Tumanov, and L. M. Nepomnyashchikh, Byull. Éksp. Biol. Med., No. 1, 105 (1986).
5. L. K. Romanova, Byull. Éksp. Biol. Med., No. 11, 100 (1960).
6. L. K. Romanova, Experimental and Clinical Regeneration of the Lungs [in Russian], Moscow (1971).
7. L. K. Romanova, Regulation of Repair Processes [in Russian], Moscow (1984).
8. L. N. Fillipenko, O. V. Petrov, and L. K. Romanova, Byull. Éksp. Biol. Med., No. 2, 169 (1977).
9. A. B. Cohen and G. K. Batra, Am. Rev. Resp. Dis., 122, 239 (1980).
10. V. V. Damiano, A. Cohen, A.-L. Tsang, et al., Am. J. Pathol., 10, 349 (1980).
11. J. E. Gadek, G. W. Hunninghake, R. L. Zimmerman, and R. G. Crystal, Am. Rev. Resp. Dis., 121, 723 (1980).
12. C. Hulbert, C. Walker, and C. Hogg, Am. Rev. Resp. Dis., 124, 310 (1981).
13. J. W. Hunninghake, J. E. Gadek, H. M. Fales, and R. G. Crystal, J. Clin. Invest., 66, 473 (1980).
14. D. E. Rannels, D. M. White, and C. A. Watkins, J. Appl. Physiol., 46, 326 (1979).

STATE OF THE MAST CELL POPULATION IN RATS WITH EXPERIMENTAL ATHEROSCLEROSIS

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UDC 616.13-004.6-092.9-07:
616.155.36-076.3

KEY WORDS: mast cells; anticlotting system; atherosclerosis

Heparin is the principal humoral agent of the anticlotting system and it enters the blood stream during activation of that system. Heparin is synthesized, stored, and secreted in the mast cells (MC) which, in response to various stimuli, secrete it from granules along with histamine and other biologically active substances [7]. It has been shown [9] that excitation of the function of the anticlotting system by α-thrombin and its analog, DIP-α-thrombin, is characterized both by elevation of the anticoagulant potential of the blood and by a marked fall in the saturation index of MC with heparin. These results are evidence that the MC population is involved in the effector response of the anticlotting system. However, the problem of the status of the MC population in various functional states of the anticlotting system has received little study. We know that if animals are kept for a long time on a diet rich in cholesterol and fat, this leads to the development of atherosclerosis and depression of the function of the anticlotting system [1]. The mast cell population was previously stated to play a role in the development of atherosclerosis [3].

In connection with the facts described above it was decided to investigate changes in the state of the MC population of animals with experimental atherosclerosis and the effect of DIP-α-thrombin, a specific activator of the function of the anticlotting system, on this state. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Male albino rats were kept for 8 months on a diet rich in cholesterol and fat, which contained animal fat with added cholesterol, methylthiouracil, vitamin D_2, and cholic acid

*Deceased.

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 0007-4888/87/0003-0414$12.50