CYTOBIOCHEMICAL CHARACTERISTICS OF PULMONARY ALVEOLAR MACROPHAGAL FUNCTION DURING THE DEVELOPMENT OF PROTECTIVE REACTIONS AGAINST ATMOSPHERIC CHEMICAL POLLUTANTS

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The alveolar macrophages are one of the most important cellular mechanisms protecting the lungs against the unfavorable effects of chemical atmospheric pollutants [5, 9, 12-14]. This is due to the important role of the system of mononuclear phagocytes in determining resistance of the organism to various factors [2, 10, 15, 16]. In previous experiments, the writers used original techniques to estimate the functional state of alveolar macrophages [3] and showed that if widespread pollutants of the atmospheric air such as sulfur dioxide and carbon tetrachloride enter the body by inhalation, enzymic disorganization is observed in the surface layer of the pulmonary macrophages [4, 6]. However, the problem of the biological importance of the cytobiochemical characteristics, based on changes in relations between the activity of the various factors and the number of alveolar macrophages during development of protective reactions in the early stages of manifestation of the biological action of chemical pollutants, remains unsolved.

In the investigation described below cytobiochemical parameters of the state of function of alveolar macrophages in rat lungs were determined at different stages of protective mechanisms and of their transition into an unfavorable (membrane-injuring) effect by analysis of correlation [1, 11] between statistically significant changes in the number of cells and enzyme activity in various situations.

EXPERIMENTAL METHOD

A detailed description of the experimental arrangements (continuous inhalation of sulfur dioxide in different concentrations), isolation of alveolar macrophages from rat lungs, cytologic analysis and biochemical methods of determination of enzyme activity linked with subcellular structures — lysosomes (β-glucosidase, β-galactosidase, acid phosphatase) and of cytosol-soluble lactate dehydrogenase, were published previously [4, 5]. Altogether 76 animals (60 experimental and 16 control) were used.

EXPERIMENTAL RESULTS

By comparative analysis of the results showing significant ($P < 0.05$) correlation between changes in the biochemical and cytologic parameters studied, it was possible to assess the functional role of cytobiochemical disturbances in the pulmonary alveolar macrophages and, on that basis, to obtain characteristics of the different stages of manifestation of protective reactions and of their possible transition into an unfavorable biological effect during exposure to atmospheric pollutants.

The initial stage of manifestation of protective reactions of the body (at the pulmonary macrophagal level) corresponded to direct correlation between the significant increase in lysosomal enzyme activity and in the number of cells. This phenomenon, which was observed in the case of β-glucosidase (activation on average by 2.4 times) and the number of alveolar ma-

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phages (an increase of 2.7 times; $r = 0.99$; $P < 0.001$), in our opinion is evidence of a compensatory increase both in the number of macrophages and in activity of the lysosomal glycosidases, which are responsible for catalytic breakdown of conjugated sugars that accumulate abnormally in cells under the influence of xenobiotics [8].

The next phase of the compensatory reactions corresponded to a combined reduction in the number of cells and increase in activity of certain enzymes, which was found most frequently in observations on lysosomal glucosidases, namely β-glucosidase, acid phosphatase, β-galactosidase, and lactate dehydrogenase of the alveolar macrophages during prolonged exposure (4 and 6 weeks) to sulfur dioxide. Similar levels of correlation (r varied from 0.82 to 0.93; $P < 0.001$), expressed as a significant increase (on average, twofold) in enzyme activity against the background of an appreciable fall in the number of cells (by 50%), can be assessed as manifestation of the stage of strain on compensatory processes in the system of pulmonary alveolar macrophages. The strain on compensation takes place mainly because of increased activity of several lysosomal and cytoplasmic enzymes, evidently due to the need to increase the intensity of breakdown of metabolic products accumulating in these cells and activation of glycolysis as a source of energy production.

The next stage of development of the protective reactions was established by determining correlation between the simultaneous significant increase in the number of alveolar macrophages and a decrease in activity of certain enzymes. Correlations of this sort was found during inactivation of the lysosomal template enzyme β-galactosidase by 67.7% and of lactate dehydrogenase by 8.62%, accompanied by a simultaneous increase in the number of macrophages by 2.2 times ($r = -0.78$ and $r = -0.72$, respectively; $P < 0.01$). In that case, evidence of the presence of decompensation was given by a decrease in activity of enzyme systems located in lysosomes and cytosol of the macrophages, against the background of an increased number of the cells themselves.

Evidence of a marked unfavorable biological effect was given by significant correlation between the simultaneous decrease in enzyme activity and in the number of alveolar macrophages (by 31.1%; $r = 0.80$; $P < 0.01$). This phenomenon appeared in the early stages (after 2 weeks) of exposure to sulfur dioxide (0.05 mg/m^3) in the case of the firmly structured lysosomal β-glucosidase. This character of correlation between the decrease in the number of cells and in the activity of enzymes located in them points to inhibition of pulmonary alveolar macrophagal function.

On the basis of the correlation described above between the direction and degree of change in the number of alveolar macrophages and enzymic disorganization of the lysosomes and cytosol in these cells, cytobiochemical criteria have been suggested for assessing the state of function of alveolar macrophages in different stages of protective reactions of the body to inhaled chemical pollutants. Four phases of manifestation of compensatory mechanisms in alveolar macrophages and their transition into an unfavorable biological effect were distinguished, and it seems worthwhile to present them in the following order. The first stage is an active protective reaction, when a combined increase is observed both in the number of cells and in the activity of enzymes located in them. The next stage reflects "strain" on the compensatory mechanisms, as shown by a decrease in the number of macrophages, correlating with a simultaneous increase in intracellular enzyme activity. The stage of transition of the protective reactions into an unfavorable effect was manifested as enzyme inactivation coupled with an increase in the number of cells. Finally, a distinctly unfavorable effect was expressed as a simultaneous fall both in the number of alveolar macrophages and in enzyme activity, which in some cases was combined with labilization of the cell and lysosomal membranes [7]. When the results as a whole are evaluated it must be noted that during development of the biological action of sulfur dioxide the system of alveolar macrophages of the rat lungs was mainly in a stage of strain on compensatory and protective reactions.

The combined cytobiochemical criteria of the functional state of the pulmonary alveolar macrophages described above thus enable the mechanisms of development of compensatory processes in the body to be identified (at the cellular level) and the transition from protective reactions into an unfavorable biological effect to be characterized. The results provide a theoretical basis for the recommendation of a wider experimental use of the comparative cytobiochemical study of the state of function of the pulmonary alveolar macrophages in connection with the control of environmental factors.

LITERATURE CITED

1. F. P. Vorob'ev, N. K. Goloborod'ko, and A. M. Manuilova, Mathematical Experimental Planning in Biochemistry and Medicine [in Russian], Khar'kov (1977).
2. J. Carr, Macrophages. A Survey of Their Ultrastructure and Function [Russian translation], Moscow (1978).
3. D. Coffin, D. Gardner, M. Waters, et al., Byull. Éksp. Biol. Med., No. 11, 632 (1978).
4. R. V. Merkur'eva, B. V. Aulika, N. N. Skvortsova, et al., Gig. Sanit., No. 9, 75 (1978).
5. R. V. Merkur'eva, B. V. Aulika, N. N. Skvortsova (R. W. Merkurjewa, B. W. Aulika, N. N. Skworzowa), et al., Z. Ges. Hyg., 26, 34 (1980).
6. R. V. Merkur'eva, B. V. Aulika, I. S. Shaternikova, et al., Zh. Gig. Epidemiol. (Prague), 113 (1980).
7. R. V. Merkur'eva, Vopr. Med. Khim., No. 2, 35 (1982).
8. G. I. Sidorenko and R. V. Merkur'eva, Gig. Sanit., No. 8, 8 (1981).
9. G. B. Fedoseev, T. R. Lavrova, and S. S. Zhikharev, Cellular and Subcellar Mechanisms of Protection and Injury of the Bronchi and Lungs [in Russian], Leningrad (1980).
10. I. Ya. Uchitel', Macrophages in Immunity [in Russian], Moscow (1978).
11. D. Himmelblar, Analysis of Processes by Statistical Methods [Russian translation], Moscow (1973).
12. D. H. Barry and L. E. Mawdsley-Thomas, Thorax, 25, 612 (1970).
13. W. G. Hocking and D. W. Golde, New Engl. J. Med. 301, 580 and 639 (1979).
14. S. D. Lee, Biochemical Effects of Environmental Pollutants, Ann Arbor (1977).
15. R. Masse, P. Fritsch, D. Nolibe, et al., Pathol. Biol., 23, 464 (1975).
16. S. S. Spicer, P. L. Sanne, M. Eguchi, et al., J. Reticuloend. Soc., 26, 45 (1979).

VERTICAL ORDER AND MOSAIC PATTERN OF NEUROGENESIS IN THE MOUSE NEOCORTEX

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The mammalian neocortex is characterized by a combination of horizontal and vertical orderliness in the organization of its cells. Horizontal orderliness is manifested as the layers of cortical cells and fibers, vertical orderliness by a number of vertically organized functional neuronal assemblages (minicolumns, macrocolumns) and by morphologically detectable vertical groupings of neuron bodies and their apical dendrites and synapses [1, 2]. The most conspicuous morphological features of the columnar organization are to be found in the developing neocortex [9]. Many autoradiographic studies of the order of formation of neurons in the mammalian neocortex have shown how the times of formation of neurons depend on their arrangement in layers of the cortex, i.e., on the horizontal orderliness of the arrangement of the neurons in the neocortex [3, 5]. Meanwhile, no conclusions have been drawn regarding the connection between the times of formation of neurons and the vertical organization of neocortical structures.

Meanwhile, we know that in the tectum of amphibians and mammals, which also possesses horizontal layers and columnar neuronal assemblages, vertical orderliness of neurogenesis is quite clearly defined [4, 6, 8, 10]. Since the tectum and neocortex are based on similar principles of organization of their cells, the present writers postulated that a vertical orderliness of neurogenesis also exists in the neocortex and has remained unnoticed as a result of the superposition of a more marked laminar sequence of neuron formation on it. It was also postulated that the summation of these two tendencies in neurogenesis may lead to a mosaic order of neuron formation in the structure. To test these hypotheses the investigation described below was undertaken.

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