and the functional state of the cell population studied. The dynamics of the cyclic nucleotides revealed by these experiments evidently is caused by changes in PDE activity (an early rise and late fall), and this may indicate the possible point of application of the biological activity of thymus factor.

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EXPERIMENTAL STUDY OF THE EFFECT OF PERORAL ADMINISTRATION OF NITROSODIMETHYLAMINE ON FUNCTION AND ENZYMIC ORGANIZATION OF PULMONARY ALVEOLAR MACROPHAGES

R. V. Merkur'eva, N. N. Litvinov, B. V. Aulika, L. Kh. Mukhambetova, L. I. Bushinskaya, S. I. Dolinskaya, E. R. Yakimova, I. N. Konstantinova, and A. V. Votyakov

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The pulmonary alveolar macrophages, as a component of the mononuclear phagocytic system, determines to some degree the resistance of the organism to the unfavorable action of external environmental factors. The important role of the functional state of the alveolar macrophages in the defensive reactions of the body against inhaled chemical pollutants (nitric oxide, hydrogen sulfide, ozone, sulfur dioxide, carbon tetrachloride, etc.) has been demonstrated previously [1, 3, 4]. Meanwhile the possible role of alveolar macrophages in the development of defensive and compensatory mechanisms against the peroral uptake of pollutants, including chemical carcinogens belonging to the nitroso compounds, which are widespread in the environment [5, 6], has not yet been settled. Previous investigations showed that nitrosodimethylamine (NDMA), acting systemically, injures the membranes of the vital intracellular organelles of the liver [8].

It was accordingly decided to undertake a comparative cytological and biochemical study of the function and enzymic organization of alveolar macrophages in the early stages of development of the biological action of NDMA.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats (of which 12 were controls) 12, 24, and 48 h after intragastric administration of NDMA (30 mg/kg body weight). A combination of cytological and biochemical methods of investigation, described previously [3, 7], was used. Together with analysis of the cell composition and counting the total number of macro-

Laboratory of Biochemistry, A. N. Sysin Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow.

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TABLE 1. Activity of Organelle-Specific Enzymes in Alveolar Macrophages of Intact Rats (M ± m)

Conditions	Enzyme activity				
	MDH, μmoles/min/10^6 cells	acetylesterase	β-glucuronidase	β-galactosidase	N-acetyl-β-D-glucosaminidase
		nmoles/min/10^6 cells			
Free enzyme activity	8,80±1,13	0	4,80±1,05	0	0,43±0,23
Total activity	32,2±4,1	12,00±0,56	7,10±1,11	5,33±0,53	5,73±0,73
Limits of variations	24,5—47,6	9,9—13,1	3,5—9,8	3,08—9,92	3,16—7,31

phages, the viability of the cells (in a Fuchs—Rosenthal chamber, using 0.5% trypan blue solution) and the total and free activity (released from the cells into the supernatant) of marker enzymes of various intracellular structures — lysosomes (β-galactosidase, N-acetyl-β-D-glucosaminidase, β-glucuronidase), endoplasmic reticulum (acetylesterase), mitochondria (malate dehydrogenase — MDH), and cytosol (lactate dehydrogenase — LDH), were investigated. Increased permeability of the cytoplasmic membrane and of the above-mentioned intracellular structures of the macrophages was judged by a significant increase in the free activity of the test enzymes and a percentage of their total activity.

Considering the important role of membrane-bound carbohydrate components in maintaining the stability of cell membranes, the concentration of N-acetylneuraminic acid in a suspension of alveolar macrophages was determined by the method described previously [2] in the writers' modification.

EXPERIMENTAL RESULTS

Analysis of the results of investigation of the enzymic organization of the alveolar macrophages of rats of the control group showed that with respect to total activity the various enzymes can be arranged in the following order: strongest activity — mitochondrial MDH, less strong activity — microsomal acetylesterase, and weakest activity (about equal activity of the three lysosomal enzymes: β-glucuronidase, N-acetyl-β-D-glucosaminidase, and β-galactosidase (Table 1).

In the early stages (12 h) after administration of NDMA a decrease was observed in the number (by 25%, $P < 0.001$) and viability (by 30%, $P < 0.05$) of the alveolar macrophages (Table 2).

During the subsequent development of the biological action of NDMA 24 h after its administration the decrease in the number and viability of the macrophages was even greater: on average by 49%; these changes, moreover, persisted 48 h after administration of NDMA. The decrease in the number of alveolar macrophages and in their viability in the earliest stages of the biological action of NDMA (after 12 h) was accompanied by an increase in activity of the microsomal enzyme acetylesterase to 19.9 ± 1.1 units, 65% higher on average than the control ($P < 0.001$).

The pattern of change was similar in the latter stages (after 24 h) with respect to the lysosomal enzyme β-glucuronidase, whose activity was 50% higher than the control, with a mean value of 11.70 ± 1.87 units. An even more marked increase in activity was found not only with acetylesterase, but also with lysosomal N-acetyl-β-D-glucosaminidase, whose activity 48 h after administration of NDMA was almost twice the control level, at 11.60 ± 1.59 units.

Statistical analysis revealed close correlation between the rise in the activity of the enzymes mentioned above and the fall in the number of alveolar macrophages. For acetylesterase, for instance, $r = 0.91$, $P < 0.01$, for N-acetyl-β-D-glucosaminidase $r = 0.80$, $P < 0.05$, and for β-glucuronidase $r = 0.7$, $P < 0.05$.

To assess the biological significance of this correlation between the decrease in the number and viability of the macrophages and activation of enzymes of microsomal and lysosomal origin, it can be tentatively suggested that this phenomenon is based on strengthening of compensatory reactions of the body in the early stages of development of the biological action of NDMA.

TABLE 2. Changes in Number and Viability of Alveolar Macrophages of Rats in the Early Stages of Biological Action of NDMA

Statistical index	Total number of cells, ml suspension				Number of viable cells, ml suspension			
	control	time of investigation after administration of NDMA, h			control	time of investigation after administration of NDMA, h		
		12	24	48		12	24	48
$M \pm m$	$1{,}11 \cdot 10^6 \pm 0{,}07 \cdot 10^6$	$0{,}82 \cdot 10^6 \pm 0{,}05 \cdot 10^6$	$0{,}57 \cdot 10^6 \pm 0{,}09 \cdot 10^6$	$0{,}64 \cdot 10^6 \pm 0{,}09 \cdot 10^6$	$1{,}08 \cdot 10^6 \pm 0{,}08 \cdot 10^6$	$0{,}76 \cdot 10^6 \pm 0{,}06 \cdot 10^6$	$0{,}55 \cdot 10^6 \pm 0{,}08 \cdot 10^6$	$0{,}62 \cdot 10^6 \pm 0{,}09 \cdot 10^6$
P	—	$<0{,}01$	$<0{,}001$	$<0{,}001$	—	$<0{,}05$	$<0{,}001$	$<0{,}01$

A different pattern of change was found in the case of mitochondrial MDH, whose activity fell significantly, proportionally to the decrease in the number of macrophages (after 12 r = 0.81, after 48 h r = 0.71, $P < 0.05$). After 12 h, for instance, the total activity of the enzyme was reduced by 22%, and it fell progressively at later times of the investigation.

The results of investigation of the free activity of the various enzymes showed that administration of NDMA led to labilization of the membranes of the intracellular organelles of the alveolar macrophages; this effect was manifested even in the early stages of observation (12 h after administration) and it was intensified during development of the biological action of NDMA. For instance, free MDH activity was increased on average by 70%, and free N-acetyl-β-D-glucosaminidase activity was increased by 4.8 times (12 h after administration). Evidence that the stability of the cell membrane of the alveolar macrophages was disturbed during the development of the biological effect of NDMA is given by the fact that activity of the cytoplasmic enzyme LDH in the supernatant of the isolation medium of the alveolar macrophages 24 h after administration of NDMA was twice as high as in the control, indicating that the enzyme has escaped through the cytoplasmic cell membrane. Later, 48 h after administration of NDMA, the effect of labilization of the cell membrane of the alveolar macrophages was intensified, as shown by the sharper rise (almost threefold) of LDH activity in the supernatant, whereas total LDH activity was unchanged.

Determination of the content of one of the membrane-bound carbohydrate components (N-acetylneuraminic acid) in the alveolar macrophages revealed a decrease in the early stages (after 12 h) on average by 15% ($P < 0.05$ compared with the control, 2.90 ± 0.04 μg/10^6 cells), and an even greater decrease after 24 h (by 28%, $P < 0.001$); it also remained low 48 h after administration of NDMA.

In the early stages of development of the biological effect of the chemical carcinogen NDMA, when administered by the peroral route, the functional state of the alveolar macrophages is thus disturbed, as shown by a decrease in their number and viability, and by destabilization of the cell membrane and subcellular membranes (those of the lysosomes and mitochondria); these changes are accompanied by disturbance of coordination between enzyme systems in different locations: cytoplasmic, microsomal, lysosomal, and mitochondrial.

The opposite direction of changes in activity of the organelle-specific enzymes evidently reflects both the ability of the alveolar macrophage system to mobilize its defensive and compensatory powers, and also the manifestation of the early stages of the harmful action of the chemical carcinogen.

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