LIVER AND BLOOD ENZYME SPECTRA OF RATS WITH EXPERIMENTAL ANTHRACOSIS ON A DIET SUPPLEMENTED WITH METHIONINE AND PYRIDOXINE

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It is stated in the literature that additional quantities of methionine [3] and pyridoxine [7] may have a beneficial effect in pneomoconiosis, but the biochemical mechanisms of the action of these substances in this condition have not been studied. However, the available information is mainly concerned with silicosis, and virtually no investigations have been undertaken in this connection on anthracosis.

The aim of this investigation was to study the liver and blood enzyme spectra of rats with experimental anthracosis whose diet was supplemented by methionine and pyridoxine.

EXPERIMENTAL METHOD

Experiments were carried out on four groups of male albino rats weighing 130-150 g, receiving balanced, isocaloric, semisynthetic diets. The animals of groups 1 (control) and 2 (experimental) received a diet in which the fat component included lard and sunflower oil in the ratio of 1:1 by weight, whereas rats of experimental groups 3 and 4 received a mixture of butter, lard, sunflower oil, and margarine in the ratio of 1:1.5:1:0.5. Rats of group 3 also received additional methionine (at the rate of 125 mg/100 g diet), and rats of group 4 received extra methionine and pyridoxine (125 and 0.4 mg/100 g diet respectively).

Rats of all the experimental groups inhaled coal dust (for 4 h daily, five times a week, in a chamber in which the dust concentration was 100-120 mg/m^3), and at the same time did physical exercise (running on a treadmill moving at a speed of 6 m/min of running). The need to introduce additional physical exercise and to specify the precise fat composition of the diet received by the experimental animals were dictated by the results of previous investigations [6].

The animals were killed by decapitation 3 and 6 months after the beginning of the experiment.

TABLE 1. Blood Enzyme Activity (in μmoles substrate/min/ml serum) in Rats with Experimental Anthracosis on a Diet Supplemented with Methionine and Pyridoxine (M ± m)

Enzyme	Time after beginning of expt., months	Group of animals			
		1 (control)	2	3	4
Cholinesterase	3	0,91±0,03	0,78±0,03**	0,86±0,04	0,87±0.05
	6	0,87±0,03	0,63±0,02***	0,72±0,02***	0,84±0.06
Aspartate aminotransferase	3	0,0142±0,0004	0,0228±0,0005***	0,0232±0,0007***	0,0224±0.0011***
	6	0,0139±0,0005	0,0226±0,0006***	0,0220±0,0005***	0,0243±0.0007***
Alanine aminotransferase	3	0,0103±0,0004	0,0138±0,0007***	0,0138±0,0005***	0,0122±0.0012
	6	0,0105±0,0005	0,0145±0,0008***	0,0155±0,0007***	0,0114±0.0008
Fructose-1-monophosphate aldolase	3	0,0046±0,0003	0,0085±0,0005***	0,0076±0,0005***	0,0068±0.0009*
	6	0,0051±0,0005	0,0108±0,0005***	0,086 ±0,0006***	0,0075±0.0007*
Alkaline phosphatase	3	0,1343±0,0047	0,1685±0,0046***	0,1632±0,0052***	0,1624±0.0084**
	6	0,1330±0,0041	0,1753±0,0053***	0,1658±0,0065***	0,1674±0.0087**
Acid phosphatase	3	0,0140±0,0009	0,0308±0,0015***	0,0288±0,0020***	0,0230±0.0029**
	6	0,0137±0,0013	0,0396±0,0013***	0,0310±0,0016***	0,0218±0.0027*

Legend. Here and in Table 2: *P < 0.05, **P < 0.01, ***P < 0.001.

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TABLE 2. Liver Enzyme Activity (in μmoles substrate/min/g wet weight of tissue) in Rats with Experimental Anthracosis on a Diet Supplemented with Methionine and Pyridoxine (M ± m)

Enzyme	Time after beginning of expt., months	Group of animals			
		1 (control)	2	3	4
Cholinesterase	3	2,02±0,07	1,74±0,06**	1,83±0,08	1,86±0,06
	6	2,09±0,08	1,48±0,06***	1,81±0,07*	1,92±0,15
Aspartate aminotransferase	3	10,18±0,36	9,80±0,30	10,38±0,71	10,63±0,82
	6	10,46±0,48	9,22±0,43	10,28±0,52	10,81±0,87
Alanine aminotransferase	3	12,49±0,41	10,68±0,34**	11,54±0,38	11,81±0,79
	6	12,69±0,43	9,23±0,51***	10,38±0,48**	11,52±0,61
Fructose-1-monophosphateAldolase	3	7,01±0,13	5,41±0,21***	5,83±0,32**	5,97±0,46*
	6	6,89±0,18	4,57±0,32***	5,36±0,21***	5,64±0,40*
Alkaline phosphatase	3	0,353±0,032	0,433±0,016*	0,453±0,019*	0,440±0,036
	6	0,376±0,032	0,455±0,021	0,475±0,019*	0,483±0,041
Acid phosphatase	3	4,42±0,19	6,62±0,12***	5,80±0,22***	5,62±0,31**
	6	4,32±0,24	7,92±0,18***	6,85±0,27***	6,20±0,35***
Acid RNAase	3	0,781±0,020	1,052±0,032***	0,962±0,023***	0,892±0,029**
	6	0,765±0,021	1,295±0,029***	1,175±0,024***	1,025±0,029***
Acid DNAase	3	0,427±0,006	0,525±0,018***	0,496±0,020**	0,475±0,027
	6	0,477±0,008	0,623±0,020***	0,566±0,018***	0,530±0,032
Cathepsins (per gram $\times 10^{-2}$)	3	0,338±0,011	0,570±0,021***	0,544±0,025***	0,507±0,025***
	6	0,380±0,015	0,757±0,030***	0,692±0,019***	0,620±0,032***

Activity of cholinesterase, alanine and aspartate aminotransferases, fructose-1-monophosphate aldolase, and acid and alkaline phosphatases was determined in blood serum and liver homogenate of the rats [1]. Total activity of acid RNAase, acid DNAase, and total cathepsins [4] also was studied in rat liver homogenates. As integral parameters of fibrogenesis, the content of collagen proteins [5] and of total lipids [2] in the lungs was determined.

EXPERIMENTAL RESULTS

It will be clear from Tables 1 and 2 that 6 months after the experiment began cholinesterase activity was significantly lower in the liver and blood of rats of groups 2 and 3, whereas changes in this parameter in rats of group 4 were minimal. The degree of change of cholinesterase activity corresponded to development of fibrosis in the lungs of the experimental animals. For instance, in animals of group 2 the content of collagen and total lipids in the lungs toward the end of the investigation was higher than the control level by 100.5 and 100.0%, whereas in the rats of group 4 by only 26.5 and 28.4% respectively. Similar correlation with the degree of fibrogenesis was found when changes in alanine aminotransferase and aldolase activity were analyzed, for their levels fell in the liver but rose in the blood serum. Meanwhile no such dependence of changes in aspartate aminotransferase and alkaline phosphatase activity were observed. Acid phosphatase activity in the liver and blood, and also the level of other lysosomal enzymes (acid RNAase, DNAase, and total cathepsins) in the liver were significantly higher in all the experimental groups, although these changes were much less marked in the rats of group 4, in which fibrous changes in the lungs also were minimal.

The addition of extra methionine and pyridoxine to the animals' diet optimal as regards fat composition thus diminished the negative manifestations characteristic of experimental anthracosis (disturbances of the liver and blood enzyme systems), and thereby contributed to delay of fibrosis in the lungs. The ineffectiveness of addition of methionine alone evidently confirms the view that an increase in the methionine content in the diet demands corresponding increase in the pyrodoxine content [9], possibly on account of intensified utilization of the latter [8].

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CONTENT OF NICOTINAMIDE COENZYMES IN NORMAL AND REGENERATING RAT LIVER

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The liver regenerating after partial hepatectomy can be regarded as a model for use in studying the metabolic processes and regulatory mechanisms that lie at the basis of cell differentiation, growth, and division. Radical changes in metabolism take place in the regenerating liver within a few hours after the operation [11]. A characteristic feature of this period of regeneration is activation of enzymes related to cell proliferation, whereas activity of enzymes maintaining the specific functions of the liver may be sharply depressed [6].

A reduction of 30% in the total content of nicotinamide coenzymes (NAD^+ + NADH and $NADP^+$ + NADPH) has been observed 24 h after partial hepatectomy [8], but these four dinucleotides have not been determined separately. Yet it may be expected that during regeneration both the total content and the ratio between oxidized and reduced nicotinamide coenzymes, reflecting the functional state of the tissue, will differ from the corresponding values in the intact liver.

The aim of this investigation was to study the time course of changes in the content of nicotinamide coenzymes in rat liver after partial hepatectomy in the initial period of regeneration.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-200 g were used. Partial hepatectomy was performed under ether anesthesia by the method of Higgins and Anderson, and the middle and left lateral lobes were removed. The content of nicotinamide coenzymes in the regenerating liver was studied 4, 18, 24, and 32 h after the operation. Lobes of the liver removed during partial hepatectomy served as the control. The content of reduced and oxidized nicotinamide coenzymes (NAD^+, NADH, $NADP^+$, and NADPH) was determined after preliminary freezing of the liver tissue with liquid nitrogen, followed by extraction by the method described previously [3], with certain modifications as follows. First, to extract reduced nicotinamide coenzymes an aqueous, and not an alcoholic, solution of 0.1 M Na_2CO_3 was used. Second, reduced nucleotides, extracted under the conditions specified previously [3], ensuring destruction of oxidized forms of the coenzymes, were determined after their spontaneous oxidation, which took place while alkaline extracts were kept in a refrigerator at 4°C. The criterion of completeness of oxidation of NADH and NADPH was absence of changes in optical density at 340 nm after addition of 0.1 ml of a solution of phenazine methosulfate (1 mg/ml) to a cuvette containing Tris-HCl buffer and 0.5 ml of the alkaline extract. Because of the low NADH content in the liver, its level was determined after spontaneous oxidation, by the method in [12]. Activity of NAD-kinase, catalyzing NADP synthesis from NAD and ATP, was determined in extracts obtained after homogenization of a weighed sample of liver with five volumes of 0.02 M solution of $KHCO_3$, pH 7.4, containing 1 mM EDTA, followed by centrifugation by the method described previously [1]. The numerical results were subjected to statistical analysis.

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