CONTENT OF ADENINE PHOSPHATES IN EXUDATE LEUKOCYTES OF RABBITS WITH DIABETES

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The content of free adenine nucleotides was determined by an enzymic method in perchloric acid extracts from exudate leukocytes of rabbits with alloxan diabetes. The ATP, ADP, and AMP contents were 4.33 ± 0.46, 1.77 ± 0.20, and 1.02 ± 0.18 mg/10^{10} cells, respectively. The total content of adenine nucleotides was 7.03 ± 0.50 mg/10^{10} cells, not significantly different from normal. The ATP and AMP content in diabetes did not differ significantly from their content in the leukocytes of healthy rabbits while the ADP content was 1.5 times higher than the control. The ATP/ADP ratio was reduced almost by half in diabetes. The change in the relative proportions of the adenosine phosphates in diabetes could play an important role in the regulation of the energy metabolism of the cell.

Previous investigations [5] have shown that exudate leukocytes of rabbits with alloxan diabetes have the same respiration as control leukocytes but a more intensive rate of glycolysis.

Since respiration and glycolysis are the main pathways of energy accumulation in leukocytes, it was decided to investigate the content of adenosine phosphates in them. These parameters have frequently been investigated in diabetes, with conflicting results. Some workers observed a decrease in the ATP content in the tissues [4] and whole blood [1] of animals with alloxan diabetes, while others [2, 36] observed no such changes.

EXPERIMENTAL METHOD

Diabetes was induced in rabbits by intravenous injection of alloxan in a dose of 170 mg/kg, and the investigation was carried out not less than two weeks later. Leukocytes were obtained from the exudate by the method described earlier [5]. The content of ATP, ADP, and AMP was determined in the perchloric acid extract from the leukocytes after adjustment of the pH to 7.0 by addition of K_2CO_3. The ATP content was determined by the hexokinase reaction. The composition of the samples (3 ml) was: glucose 2 μmoles, $MgCl_2$ 20 μmoles, tris-HCl buffer (pH 7.4) 150 μmoles, glucose-6-phosphate dehydrogenase 100 μg protein, NADP 0.5 μmole, hexokinase 0.25 mg, extract 1 ml (1×10^8 cells). The ATP concentration in the system was equimolar to the quantity of NADP · H_2 formed and was calculated from the change in absorption at 340 nm.

The ATP content was determined by the pyruvate kinase reaction. Composition of the samples (3 ml): tris buffer (pH 7.4) 150 μmoles, $MgCl_2$ 20 μmoles, phosphoenolpyruvate 2 μmoles, NAD · H_2 0.5 μmoles, lactate dehydrogenase containing phosphoenolpyruvate kinase 150 mg protein, extract 1 ml (1×10^8 cells). The ADP content was calculated by the decrease in optical density at 340 nm.

The AMP content was determined in the same system as ADP by adding myokinase (100 μg protein) to it after stabilization of the readings. It was assumed in the calculation that as a result of the reaction 2 moles ADP were formed from 1 mole ATP (and AMP).

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TABLE 1. Content of ATP, ADP, and AMP (in mg/10^{10} leukocytes) in Extracts from Exudate Leukocytes of Rabbits with Alloxan Diabetes

Group of rabbits	Number of animals	ATP	ADP	AMP	ATP + ADP + AMP	ATP/ADP
Control	11	4,66±0,49	1,17±0,17	1,05±0,21	6,88±0,75	4,3±0,48
Animals diabetes	11	4,33±0,46	1,77±0,20	1,02±0,18	7,03±0,50	2,5±0,28
P		>0,5	<0,05	>0,5	>0,5	<0,05

EXPERIMENTAL RESULTS AND DISCUSSION

The content of adenosine phosphates determined in the extracts from the exudate leukocytes is given in Table 1.

It is clear from Table 1 that the content of ATP and AMP in the exudate leukocytes of rabbits with alloxan diabetes did not differ significantly from that of healthy rabbits, but the ADP content in diabetes was 1.5 times higher than in the control. The total content of free adenine nucleotides in the leukocytes of the experimental rabbits was very close to the control values and the difference between them was not significant.

The value of the ATP/ADP ratio in the leukocytes of rabbits with diabetes was very slightly lower than in the controls, mainly on account of an increase in the ADP content.

These findings, which showed no significant differences in the ATP content in the exudate leukocytes of the experimental and control rabbits, are in agreement with the results of investigations of other tissues of animals with alloxan diabetes. For instance, no difference was found in the total content of adenine nucleotides in the liver of the experimental and control rats [3], and identical quantities of ATP were found in the heart muscle of rabbits with alloxan diabetes and healthy animals [2]. However, a decrease in the ATP content and an increase in the ADP content were found in the skeletal muscle of rats with diabetes [3].

The decrease in the ATP level in the blood of animals with diabetes observed by some investigators is probably connected with a decrease in its content in the red cells [1].

Since the adenine nucleotides participate in the regulation of energy metabolism in the cell, the change in their relative proportions in diabetes could influence the regulation of this metabolism.

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