CONTENT OF ADENYL MONONUCLEOTIDES AND RESPIRATORY PHOSPHORYLATION IN MITOCHONDRIA OF RATS WITH TRANSPLANTABLE TUMORS

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The content of adenyl mononucleotides, the process of oxidative phosphorylation, and the ATPase activity of the liver mitochondria of rats with transplantable sarcoma 45 and Walker's carcinosarcoma were investigated at different stages of tumor growth. The fall in the ATP level observed in the liver mitochondria of the rats with tumors was due, first, to inhibition of its formation as a result of the partial uncoupling of oxidative phosphorylation and, second, to an increased rate of its breakdown as a result of increased ATPase activity.

KEY WORDS: tumor growth; oxidative phosphorylation; ATPase; adenyl mononucleotides; liver.

Contrary to the widely held view, not more than half of cancer patients die from metastases and the rest die from what are termed complications: secondary infection, dysfunction of organs, and degenerative changes in the tissues. An important cause of these phenomena could be the low efficiency of energy metabolism in the tissues. There is only indirect evidence in the literature that ATP synthesis in remote tissues is disturbed during the development of malignant tumors. Evidence that this is so is given by the observed partial uncoupling of oxidative phosphorylation in the liver mitochondria of animals with transplantable tumors [3, 5-7]. The content of ATP and ADP in the blood is known to be reduced during induced carcinogenesis [4]; information on changes in the levels of these compounds in the platelets [9] and erythrocytes [8] of cancer patients, however, is contradictory.

Experiments were carried out to study changes in the ATP, ADP, and AMP content in the liver mitochondria of animals with transplantable tumors and the possible causes of those changes.

EXPERIMENTAL METHOD

Mitochondria were isolated by differential centrifugation in the cold simultaneously from the liver of one control and two experimental rats. The respiratory activity of the mitochondria and the phosphorylation of added ADP, and the response of the mitochondria to DNP and rotenone were investigated polarographically (the PA-3 polarograph). The medium for isolation of the mitochondria contained 0.25 M sucrose and 0.01 M Tris buffer, pH 7.4. The incubation medium consisted of: sucrose 0.125 M, Tris buffer 0.01 M, KH_2PO_4 0.02 M, EDTA 0.001 M, KCl 0.07 M, and $MgCl_2$ 0.005 M, pH 7.4. The final concentrations were: ADP 250 μM, DNP $1.25 \cdot 10^{-4}$ M, rotenone $2 \cdot 10^{-6}$ M, and succinate and glutamate 10 mM. The following were determined: the respiration rate of the mitochondria in medium with oxidation substrate (V_2), the respiration rate after addition of ADP (V_3), the respiration rate after phosphorylation of the added ADP (V_4), and the respiratory controls after Chance and Lardy. The above values were expressed in nanoatoms oxygen/min/mg protein. Free tissue mononucleotides were determined by high-voltage electrophoresis [1]. The results were subjected to statistical analysis by means of the Iskra-122 computer.

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TABLE 1. Concentration of Adenyl Mononucleotides (in μmoles/g tissue) in Liver Mitochondria of Rats with Transplantable Tumors

Group	Number of rats	AMP	ADP	ATP	ADP/ATP
Control	23	0,59± 0,017	0,85± 0,01	2,39± 0,045	0,36± 0,006
Walker' carcinosarcoma					
I stage	7	0,80± 0,09	1,30± 0,085	1,88± 0,125	0,74± 0,02
P		<0,05	<0,001		<0,001
II »	6	0,61± 0,058	0,94± 0,059	1,80± 0,078	0,53± 0,06
P				<0,001	<0,01
III »	8	0,75± 0,024	0,67± 0,026	1,19± 0,056	0,57± 0,02
P		<0,001	<0,001	<0,001	<0,001
Sarcoma 45:					
I stage	8	0,72± 0,069	1,01± 0,180	2,10± 0,075	0,48± 0,015
P				<0,01	<0,001
II »	7	0,77± 0,039	1,20± 0,089	2,02± 0,058	0,60± 0,062
P		<0,001	<0,001	<0,001	<0,001
III »	9	0,56± 0,295	1,05± 0,131	1,35± 0,069	0,78± 0,057
P				<0,001	<0,001

Legend. Only statistically significant differences from control are shown.

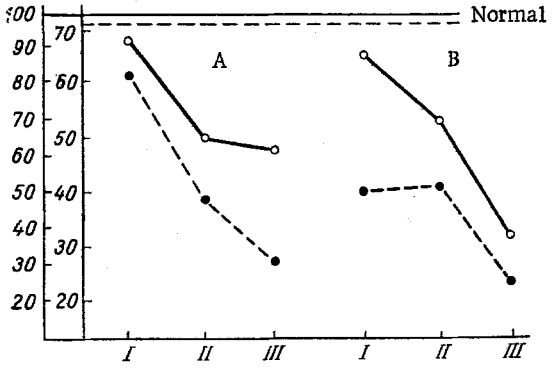

Fig. 1. Velocity of ADP phosphorylation by liver mitochondria of rats with tumors at different stages of their development: A) rats with sarcoma 45; B) rats with Walker's carcinosarcoma. Abscissa, stages of tumor development; ordinate, rate of phosphorylation of ADP (in mmoles/min/mg protein). Solid line) succinate as oxidation substrate; broken line) glutamate.

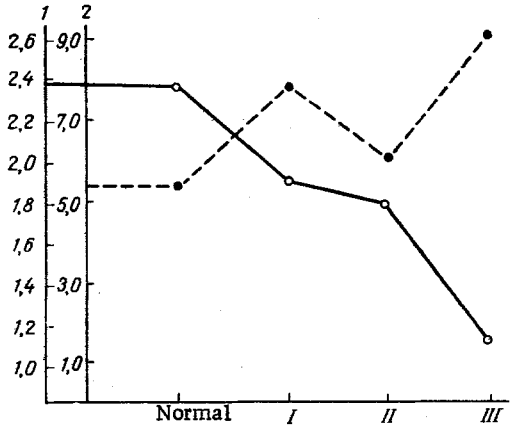

Fig. 2. Activity of Mg-ATPase and ATP level in liver mitochondria of rats with Walker's tumor. Abscissa, stages of tumor development; ordinate, 1) ATP level (in μmoles/g tissue); 2) ATPase activity (in μg inorganic phosphorus/mg protein). Solid line) ATP level; broken line) ATPase activity.

Experiments were carried out on 150 male albino rats weighing 180-200 g. The experimental animals were given a subcutaneous injection of 1 ml of a thick suspension of tumor cells (about 1 million) of a slowly growing sarcoma 45 and rapidly growing Walker's carcinosarcoma. The rats with sarcoma 45 were killed 7-8 days (period of initial growth or, conventionally, stage I), and 15 days (period of rapid growth of the tumor, stage II) and 30 days (terminal period, stage III) after transplantation; the rats with Walker's tumor were killed 3 days (period of initial growth), 6-7 days (period of rapid growth), and 10-12 days (terminal period) after transplantation.

EXPERIMENTAL RESULTS AND DISCUSSION

The ATP level in the liver mitochondria of rats with tumors fell progressively: this phenomenon was seen more clearly in animals with rapidly growing tumors. The ADP level, on the other hand, rose a little except in stage III in the rats with Walker's carcinosarcoma.

The AMP level also was higher than normally. The increase in the ADP—ATP ratio in the liver mitochondria of the animals with tumors indicated a reduction in the degree of coupling of respiration with phosphorylation in them; the relatively low value of this ratio in stage III of development of Walker's carcinosarcoma, on the other hand, was the result not of better coupling than in stage I, but of the simultaneous sharp decrease in both ADP and ATP (Table 1).

In the course of development of the malignant tumor the degree of uncoupling of respiration and phosphorylation increased, as reflected in a sharp decrease in V_3, a decrease in the respiratory control, and the fall in the ADP/O ratio. Meanwhile the velocity of nonphosphorylating oxidation (V_2) was unchanged, and V_4 was increased only in the period of initial growth of the tumor.

The velocity of ADP phosphorylation by the liver mitochondria of animals with tumors is shown in Fig. 1 in relation to the stage of tumor development. As the results show, the velocity of ATP synthesis was substantially reduced with development of the tumor. These results, and also those of the experiments with rotenone and DNP, showed that the NAD-dependent portion of the respiratory chain was the most vulnerable; the succinate stage was less severely affected.

The cause of the observed decrease in the ATP concentration in the liver mitochondria of animals with tumors could be an increase in the rate of ATP breakdown as well as the partial uncoupling of respiration and phosphorylation. In conjunction with V. A. Zaitsev, the writers have shown [2] that activity of Mg-dependent ATPase is increased in the early stages after transplantation of sarcoma 45 and Walker's carcinosarcoma. The degree of lowering of the ATP level was inversely proportional to the increase in enzyme activity, especially in the terminal stage of tumor development (Fig. 2).

The fall in the ATP level in the liver mitochondria of rats with transplantable sarcomas was thus the result of at least two phenomena: inhibition of its formation (uncoupling of oxidative phosphorylation) and stimulation of its breakdown (increased activity of mitochondrial ATPase). A decrease in the concentration of the universal high-energy compound, ATP, can give rise to severe disturbances in the vital activity of the cell.

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