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Oxydative Phosphorylation of Mitochondria of Ehrlich Ascites Tumor Cells and Normal Rat Liver in the Presence of 2-Deoxy-D-Glucose

by

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The aim of the present experiments was to investigate the influence of 2-deoxy--D-glucose on the concentrations of ATP, ADP and AMP and on the respiratory control during oxydative phosphorylation of the mitochondria of Ehrlich ascites tumor cells and of normal rat liver.

Material and methods

The mitochondrial fraction of the Ehrlich ascites tumor cells and of the normal rat liver were investigated. The inbred strains Dba/212 and C3H of mice were the host of the Ehrlich ascites tumor cells. The tumor was transplanted by intraperitoneal injection of 0.5ml. of ascites fluid diluted 1 : 1 with 0.9 per cent NaCl solution. The period of tumor growth was 7 days. The ascites fluid obtained from 10—15 mice was collected in 50 ml. of 0.9 per cent NaCl solution containing 1 mM EDTA (*pH* 7.0). The cells were washed twice with 0.25 M sucrose solution containing 1 mM EDTA. Centrifugation was performed at 225 g for 5 min. The mitochondrial fraction of the Ehrlich ascites tumor cells was obtained by differential centrifugation of the cellular homogenate after Borst [4]. Mitochondria of rat liver were obtained from a homogenate in 0.25 M sucrose solution containing 10 mM Tris-HCl buffer (*pH* 7.4) and 1 mM EDTA. Differential centrifugation was performed by Schneider's method modified by Meyers and Slater [11]. For homogenization all-glass Potter homogenizers were used. All preparations were performed at 0—4°C. MSE Magnum refrigerated centrifuge was used.

The investigated mitochondrial fractions in the amount of 1—2 mg. of mitochondrial protein were incubated in Warburg vessels at 25°C in the medium used by Borst [4] and composed of: KCl 15 mM, 1 per cent albumin, succinate 10 mM, sucrose 50 mM, EDTA 2 mM, Tris-HCl buffer 50 mM (*pH* 7.5), phosphate buffer 20 mM (*pH* 7.5) and $MgCl_2$ 5 mM, respectively. The total volume of the medium was 2 ml. The medium for rat liver mitochondria did not contain albumin. After 10 min. of initial incubation, ADP in a final concentration of 1.33 mM (according to enzymatic analysis) was added. Mitochondria were incubated in the presence of 20 mM glucose, 20 mM 2-deoxy-D-glucose and in a medium deprived of hexoses. Oxygen uptake was measured by Warburg's method, the measurements being performed every 2 min. over 40 min. Before and after incubation the concentration of adenine nucleotides was measured by enzymatic methods [2]. ATP was determined by means of phosphoglycerate kinase (2.7.2.3) and of glyceraldehydephosphate dehydrogenase (1.2.1.12). For determination od ADP and AMP pyruvate kinase

(2.7.1.40), lactate dehydrogenase (1.1.1.28) and adenylate kinase (2.7.4.3) were used. Extinction was measured with a Hilger spectrophotometer at 340 mμ. Inorganic phosphate was determined by the Fiske and Subbarow method, proteins by the method of Kjeldahl and Lowry method.

Reagents, Phosphoglycerate kinase (2.7.2.3), glyceraldehydephosphate dehydrogenase (1.2.1.12), pyruvate kinase (2.7.1.40), lactate dehydrogenase (1.1.1.28), adenylate kinase (2.7.4.3), ADP and other reagents used for estimation of ATP, ADP, AMP were supplied by Boehringer—Manheim; EDTA and bovine albumin (fr. V) were Light—Colnbrook products; Tris Fluka AG and 2-deoxy-D-glucose Sigma were used. The remaining reagents were obtained from the Laboratory of Chemical Reagents in Gliwice.

Results

As indicated by the course of the curve illustrating the respiratory control, the mitochondrial fractions obtained showed symptoms of the coupled oxydative phosphorylation. Figs. 1 and 2 show the results of representative experiments in

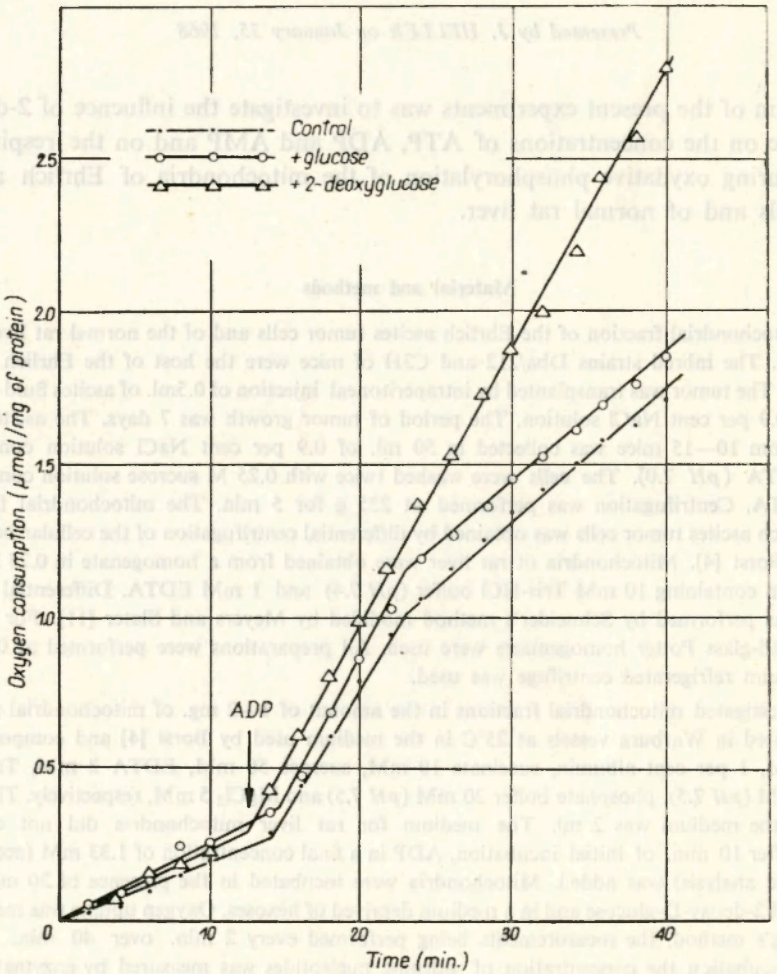

Fig. 1. Influence of 2-deoxyglucose on the oxygen uptake by the mitochondrial fraction of Ehrlich ascites tumor cells

which oxygen uptake by the mitochondrial fraction of the Ehrlich ascites tumor cells and the same fraction of normal rat liver and the influence of 2-deoxy-D-glucose were investigated. It was observed in the control systems (K), that the relatively low oxygen uptake by mitochondria incubated in the absence of hexoses increases after addition of ADP. The increase in oxygen uptake was observed in the experimental conditions within 10 min. Later the oxygen uptake decreased markedly. It was confirmed in the control experiments on the mitochondria of the Ehrlich tumor that in the presence of succinate the P/O ratio was 1.7. Parallelly performed estimations of ATP, ADP and AMP in the control conditions showed that the accumulation of ATP and decrease of ADP and AMP concentrations (Tables I and II) occur during incubation of the investigated mitochondrial fractions. The presented results show that the ability of oxydative phosphorylation is preserved in the investigated mitochondrial fractions.

TABLE I

Influence of 2-deoxy-D-glucose on the concentrations pattern of ATP, ADP and AMP in the suspension of mitochondria of Ehrlich ascites tumor cells during oxydative phosphorylation

No. of exper.	Nucleotides concentration (μmoles/2ml. of incubation medium)											
	before incubation			after incubation (40 min.)								
				without hexoses (K)			+ glucose (20 mM)			+ 2 DG* (20 mM)		
	ATP	ADP	AMP	ATP	ADP	AMP	ATP	ADP	AMP	ATP	ADP	AMP
1	0.31	2.84	1.46	3.80	1.80	0.34	2.40	2.26	1.05	0.48	2.18	1.94
2	0.88	2.19	1.58	3.73	0.75	0.92	2.45	1.56	1.67	0.69	1.22	2.04
3	0.28	2.74	1.50	3.72	0.11	0.30	4.13	0.29	0.33	2.58	1.37	0.74
4	1.95	2.83	1.52	4,80	0.73	0.27	5.57	0.67	0.21	1.38	1.81	1.91
5	1.26	2.69	1.71	6.80	0.33	0.21	6.39	0.21	0.29	2.19	1.45	2.28
6	1.00	2.68	1.71	5.10	0.00	0.26	5.25	0.14	0.36	2.30	1.73	1.27
X**	0.95	2.66	1.58	4.66	0.62	0.38	4.36	0.85	0.65	1.60	1.63	1.70
$\pm w_{tw}$	±0.67	±0.26	±0.10	±1.23	±0.72	±0.28	±1.60	±0.85	±0.58	±0.84	±0.38	± 0.62

* 2DG — 2-deoxy-D-glucose

** $\bar{X} \pm w_{tw}$ mean ± confidence levels calculated by the shortened Dean and Dixon [7] test.

TABLE II

Influence of 2-deoxy-D-glucose on the concentrations pattern of ATP, ADP and AMP in the suspension of mitochondria of normal rat liver during oxydative phosphorylation

No. of exper.	Nucleotides concentration (μmoles/2ml. of incubation medium)											
	before incubation			after incubation (40 min.)								
				without hexoses (K)			+glucose (20 mM)			+ 2 DG* (20 mM)		
	ATP	ADP	AMP	ATP	ADP	AMP	ATP	ADP	AMP	ATP	ADP	AMP
1**	0.26	1.59	1.05	2.42	0.23	0.32	2.52	0.23	0.33	2.26	0.08	0.20
2**	0.17	1.54	1.02	2.26	0.23	0.33	1.90	0.10	0.31	2.36	0.18	0.32
3	0.36	2.85	1.51	4.36	0.19	0.23	4.30	0.18	0.21	4.50	0.30	0.19
4	0.22	2.96	1.47	4.23	0.53	0.21	4.44	0.17	0.19	4.47	0.34	0.34

* 2DG — 2-deoxy-D-glucose

** In experimental Nos. 1 and 2 the original ADP concentration was one half that described in material and methods

The data presented in Fig. 1 indicate, that the presence of glucose does not affect significantly the oxygen uptake by the investigated mitochondria of tumor cells. In this case the relations reflecting the respiratory control are preserved. In most cases in the presence of 2-deoxyglucose the period of increased oxygen uptake after addition of ADP was prolonged. This effect was never observed in mitochondria of normal rat liver (Fig. 2). In this case the presence of glucose and 2-deoxyglucose

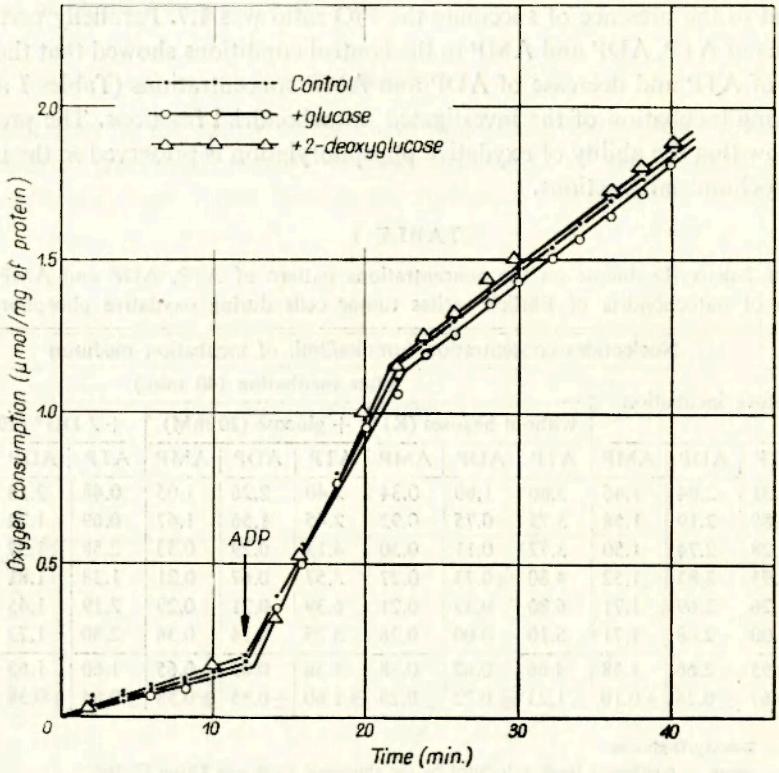

Fig. 2. Influence of 2-deoxyglucose on the oxygen uptake of the mitochondrial fraction of rat liver

did not change the course of the oxygen uptake curves for the investigated phosphorylating system. Table I shows the results concerning the influence of 2-deoxyglucose on the concentrations of adenine nucleotides in the suspension of Ehrlich ascites tumor cells mitochondria during oxydative phosphorylation. It was demonstrated that an incresae in ATP and decrease in ADP and AMP concentrations occur during incubation of mitochondria in a medium deprived of hexoses (control system). Similar quantitative changes in the investigated nucleotides were observed also in the presence of glucose. However, the pronounced decrease of ATP accumulation, a smaller decrease of ADP concentration and no decrease of AMP concentration, as compared with the control group, were found during the incubation of mitochondria isolated from tumor cells in the presence of 2-deoxyglucose. The mean results of the discussed experiments are shown in Fig. 3. On the contrary, 2-deoxyglucose

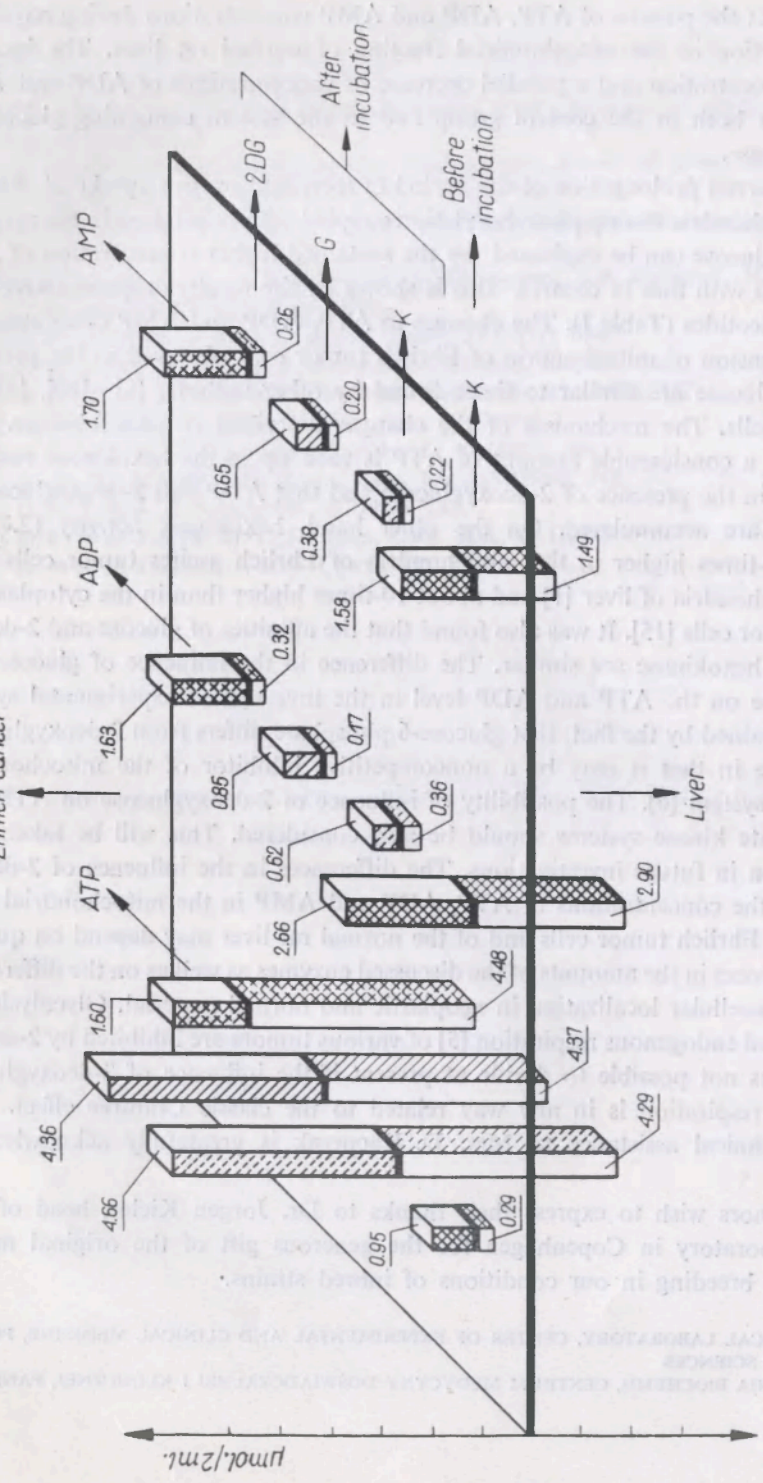

Fig. 3. Influence of 2-deoxyglucose on the pattern of ATP, ADP and AMP concentrations in suspensions of mitochondria of the Ehrlich tumor and of the rat liver

did not affect the pattern of ATP, ADP and AMP concentrations during oxydative phosphorylation in the mitochondrial fraction of normal rat liver. The increase of ATP concentration and a parallel decrease of concentrations of ADP and AMP were similar both in the control group and in the system containing glucose or 2-deoxyglucose.

The observed prolongation of the period of increased oxygen uptake of Ehrlich tumor mitochondria during phosphorylation coupled with respiration in the presence of 2-deoxyglucose can be explained by the sustained higher concentration of ADP as compared with that in control. This is shown by the results of quantitative analysis of nucleotides (Table I). The changes in ATP, ADP and AMP concentrations in the suspension of mitochondria of Ehrlich tumor cells observed in the presence of 2-deoxyglucose are similar to those found by other authors, [8]—[10], [13], in the intact cells. The mechanism of the changes described remains obscure. It is known that a considerable amount of ATP is used up in the hexokinase reaction in the cells in the presence of 2-deoxyglucose and that ADP and 2-deoxyglucose-6--phosphate are accumulated. On the other hand, hexokinase activity (2.7.1.1.) is about 20-times higher in the mitochondria of Ehrlich ascites tumor cells than in the mitochondria of liver [1] and about 10-times higher than in the cytoplasm of Ehrlich tumor cells [15]. It was also found that the affinities of glucose and 2-deoxyglucose for hexokinase are similar. The difference in the influence of glucose and deoxyglucose on the ATP and ADP level in the investigated experimental system may be explained by the fact, that glucose-6-phosphate differs from 2-deoxyglucose--6-phosphate in that it may be a noncompetitive inhibitor of the mitochondrial hexokinase system [6]. The possibility of influence of 2-deoxyglucose on ATP-ases and adenylate kinase systems should be also considered. This will be taken into consideration in future investigations. The differences in the influence of 2-deoxyglucose on the concentrations of ATP, ADP and AMP in the mitochondrial fractions of the Ehrlich tumor cells and of the normal rat liver may depend on quantitative differences in the amounts of the discussed enzymes as well as on the differences in their intracellular localization in neoplastic and normal material. Glycolysis [3], [12], [14] and endogenous respiration [5] of various tumors are inhibited by 2-deoxyglucose. It is not possible to decide at present if the influence of 2-deoxyglucose on cellular respiration is in any way related to the classic Crabtree effect.

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