Metabolite Status of the Heart in Acute Insufficiency due to 1-fluoro-2,4-dinitrobenzene[1]

While attempting, with the aid of a differentiated substrate supply, to demonstrate the significance of the various energy yielding metabolic pathways for cardiac contraction, our attention was drawn to the reports of DAVIES et al.[3-7] concerning the effect of 1-fluoro-2,4-dinitrobenzene (FDNB) on the skeletal muscle of frogs. KUBY and MAHOWALD[8] had found that FDNB inactivates ATP:creatine phosphotransferase (creatine kinase). Although FDNB reacts non-specifically with free amino groups and SH groups of proteins, DAVIES et al. proved that it can also be employed to inhibit creatine kinase in the intact tissue. Having produced FDNB poisoning in a normothermic rabbit heart-lung preparation, we hoped to obtain, by analysis of the metabolite concentrations and observation of functional changes, further information on the reactions, associated with breakdown of ATP in the contractile element.

Method. The hearts were perfused by methods described previously[9]. As soon as insufficiency set in, apparent from dilatation of the heart and increased pressure in the right atrium, the volume/min of the sigma pump was reduced. When there was no further output from the left ventricle and the arterial pressure dropped, although the arterial exit tube was constricted, the hearts were frozen by means of metal forceps previously cooled in liquid air. The perfusion solution consisted of 7 parts of a phosphate Ringer solution with the addition of 3% Haemaccel (Behringwerke) and of 3 parts of washed bovine erythrocytes. To the perfusion solution there were added 10 m*M* glucose/l and 5 m*M* pyruvate/l of the extracorpuscular space as substrates. FDNB was dissolved in water by brief boiling. The solution was decanted from non-dissolved FDNB, then 0.9 g% NaCl was added. FDNB solution (5 ml) was pipetted into 100 ml of perfusion solution during perfusion. Every 5 min the addition of 2.5 ml was repeated, usually a total of 15 ml/100 ml perfusion solution, as the hearts completely failed after 25–30 min. Following pulverization of ventricular muscle in a mortar in the presence of dry ice and deproteinization by means of 6 volumes of 0.33 ml of perchloric acid, the concentrations of ATP, ADP, AMP, creatine phosphate, Pi, glucose, glucose-6-P, glucose-1-P, fructose-6-P, fructose-1,6-diphosphate, dihydroxy acetone phosphate, pyruvate and lactate were determined. Except for the inorganic phosphate content, the metabolite concentrations were determined with the aid of optical enzymatic tests[9-12]. The metabolite concentrations are given as μMol/g of tissue (wet weight). The differences of 2 averages were tested for significance with *t*-distribution.

Results. The ATP content, amounting to 3.82 μMol/g in the control hearts, was reduced very significantly to 3.22 μMol/g in the poisoned hearts at the point of total failure ($P < 0.01$). The ADP and AMP contents were similarly lower than the control values, although only the difference in AMP content was statistically significant ($P < 0.05$). The total of free adenine nucleotides was reduced from 4.644 to 3.953 μMol/g ($P < 0.01$). The creatine phosphate concentration was lowered by 1.2 μMol/g. The remaining metabolite concentrations were identical in both test series, with the exception of the G-1-P concentration, which was significantly increased after intoxication. Cardiac failure occurred at glycogen, glucose, pyruvate and lactate concentrations similar to those of the controls.

Discussion. The decrease in high-energy phosphate compounds shows that insufficiency after administration of FDNB is due to energy deficiency, not to inhibition of ATP utilization[13]. This indirectly confirms the findings of INFANTE, KLAUPIKS and DAVIES[5] that ATP induced contractions of glycerine-extracted myofibrils and actomyosin ATPase activity are not inhibited by FDNB. The formation and conduction of excitation were apparently not inhibited since the greatly dilated hearts, without any further volume output, still contracted regularly. The ATP content was significantly decreased by only 15–20% of the control value. From this we conclude that due to

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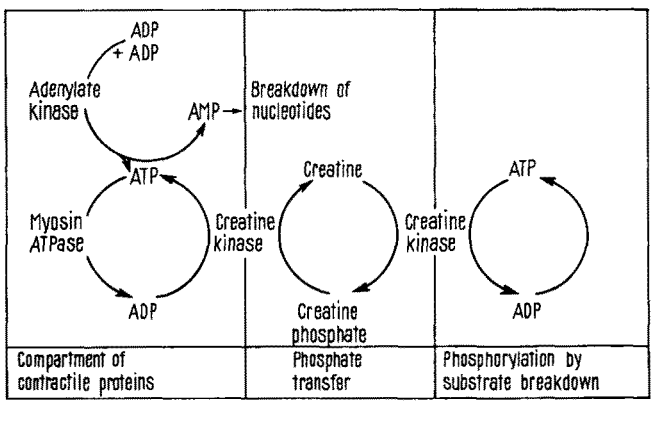

Fig. 1. Compartmentation of adenine nucleotides and phosphate transfer by the creatine/creatine phosphate/creatine kinase system.

	ATP	ADP	AMP	ΣATP ADP AMP	ATP/ADP	CP	Pi
A	3.82 ± 0.42	0.574 ± 0.120	0.247 ± 0.027	4.644 ± 0.538	6.8 ± 1.1	5.90 ± 1.05	4.06 ± 0.83
B	3.22 ± 0.38	0.537 ± 0.087	0.192 ± 0.057	3.953 ± 0.463	6.1 ± 0.7	4.73 ± 0.62	4.00 ± 1.03

	Glycogen	Glucose	G-6-P	G-1-P	F-6-P	FDP
A	25.0 ± 6.3	5.15 ± 0.86	0.197 ± 0.061	0.0023 ± 0.0023	0.0405 ± 0.0151	0.0304 ± 0.0223
B	19.7 ± 4.5	5.20 ± 0.72	0.192 ± 0.074	0.0114 ± 0.0055	0.0306 ± 0.0174	0.0208 ± 0.0117

	DAP	Pyruvate	Lactate
A	0.0123 ± 0.0081	0.183 ± 0.096	1.62 ± 0.17
B	0.0142 ± 0.0044	0.180 ± 0.144	1.63 ± 0.30

Metabolite concentrations in the ventricle muscle of the rabbit. Average values with standard deviations of the individual values: (A) sufficient hearts after 30 min of perfusion ($n = 7$); (B) insufficient hearts after FDNB intoxication ($n = 11$).

compartmentation this ATP fraction does not reach the contractile proteins and therefore cannot be exploited for the contraction. After a single movement of the M. rectus abd. of a frog, at 0 °C, the ratio between the quantity of split ATP and the increase of ADP and AMP was balanced, taking into account a functioning adenylate kinase reaction[6]. In continuous cardiac stress by rhythmic contractions, the split ATP was found, on the other hand, neither in ADP nor in AMP, but was degraded beyond the AMP step. This has also been shown by MARÉCHAL and BECKERS-BLEUKX[14] in the sartorius of frogs after tetanus.

The creatine phosphate content was decreased to a much lesser extent than in the other forms of insufficiency due to energy deficiency[9,13,15,16]. The relatively high content of creatine phosphate indicates inhibition of creatine kinase. However, the LOHMANN reaction was not completely blocked (as in the experiments of DAVIES et al. with previous incubation of the muscle tissue), because the creatine phosphate value of the poisoned hearts was below that of the controls. There was no corresponding accumulation of Pi or hexose phosphates[17].

In OLSON's scheme[18] of cardiac metabolism, the creatine phosphate/creatine system is subordinate to ATP formation by oxidative phosphorylation and to splitting at actomyosin. If it were possible to utilize the ATP produced by oxidative or glycolytic substrate breakdown directly for contraction, then an inhibition of creatine kinase should not result in insufficiency. The fact that creatine kinase inactivation leads to failure means that the adenine nucleotides do not diffuse between the sites of phosphorylation and those of breakdown, and that creatine phosphate has the significance of a transport metabolite for a high-energy phosphate. Figure 1 illustrates the course of ATP regeneration in the myofibrils via creatine kinase and adenylate kinase.

From the intermediate products of the Embden-Meyerhof pathway it was not possible to determine any particular changes in concentration which would indicate a direct effect on glycogenolytic metabolism. The glucose-1-P content was increased only by comparison with the control value greatly reduced by the perfusion; it was not increased compared with the content of hearts frozen in vivo. In the case of a disturbance in oxygen consumption as found by INFANTE and DAVIES in the skeletal muscle

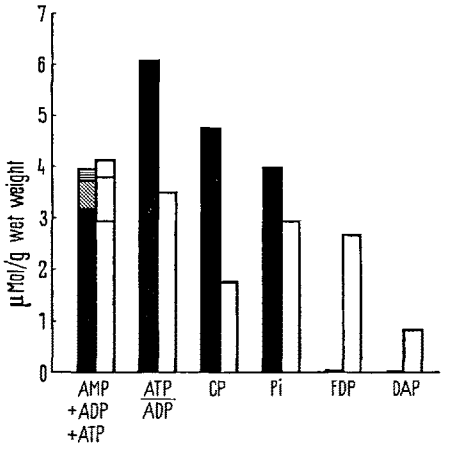

Fig. 2. Comparison between metabolite concentrations in insufficient rabbit hearts after intoxication with FDNB (black columns) and iodoacetate (white columns). CP, creatine phosphate; DAP, dihydroxy acetone phosphate; FDP, fructose 1,6-diphosphate; Pi, inorganic orthophosphate.

of the frog after intoxication[4], the lactate content should increase, as FDNB did not inhibit the glycolytic reactions. Inhibition of oxidative metabolism leads to insufficiency characterized by glycogen breakdown and increase of lactate. It is thus noticeable that hearts treated with FDNB failed with normal glycogen and glucose content.

Of significance is the comparison between the metabolite content in acute failure by FDNB and by iodoacetate (Figure 2). The damage by the latter substance occurs in

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the sequence of energy transfer prior to the damage by FDNB, viz. in the inhibition of the substrate chain phosphorylation. A major decrease in creatine phosphate content was associated with this type of intoxication[9]. Although the ATP content was similar in both types of poisoning, the ATP/ADP ratios differed greatly. After administration of iodoacetate, the ADP content was increased; FDNB produced a marked tendency towards decrease. This, too, suggests that the reduction of the ATP concentration by FDNB is limited to the fraction at the contractile proteins, as the adenylate kinase activity occurs here. The point of attack of iodoacetate in the breakdown of substrate was also marked by the accumulation of fructose-1,6-diphosphate and dihydroxy acetone phosphate before the glyceraldehyde phosphate dehydrogenase reaction. The quantity of phosphate bonded in these 2 metabolites explains the difference in the inorganic phosphate concentrations.

Zusammenfassung. Nach 1-Fluor-2,4-dinitrobenzol-Vergiftung von perfundierten Kaninchenherzen waren bei vollständiger Insuffizienz der ATP-Gehalt sehr signifikant und der Kreatinphosphatgehalt weniger stark vermindert. Glykogen-, Glucose- und Lactatgehalt unterschieden sich von den Kontrollwerten nicht.

G. Gercken und U. Schlette

Chemisches Staatsinstitut der Freien und Hansestadt Hamburg, Abteilung für Biochemie, 2000 Hamburg (Germany), 2 August 1967.

Reduction of Cytochrome *c* by the Mitochondrial Respiratory Chain across a Semi-Permeable Membrane

The succinate-cytochrome *c* reductase system has been defined as a fragment of the respiratory chain capable of catalysing the oxidation of succinate by external cytochrome *c*[1].

The components of such a system and their interrelations have been extensively studied[2], but it is not yet clear how the reduction of the acceptor takes place.

The purpose of this communication is to describe an experimental device that can be used to study the mode of reduction of the terminal acceptor in the succinate-cytochrome *c* reductase system.

We have found that cytochrome *c* reduction can be achieved even when the oxidized cytochrome *c* is separated from the other catalytic components of the system by a semipermeable membrane. The apparatus employed for the study of this reduction is shown in Figure 1.

Membrane was prepared from rolls of commercial brand 'Dexstar' (Visking) 0.064–0.025 mm thick. The reaction was followed by reading the increase of absorbancy at 550 nm on a Beckman DU spectrophotometer. Cyanide was used to inhibit cytochrome *c* oxidase activity. The experimental conditions are described in the caption to Figure 2. The reaction was started by adding cytochrome c in chamber B (Figure 1) after 10 min of preincubation. The submitochondrial particles used as enzyme source were prepared from beef heart mitochondria according to Crane et al.[3].

In Figure 2 cytochrome *c* reduction under normal conditions (no semipermeable membrane) is compared with the reduction that takes place in the presence of a semipermeable membrane.

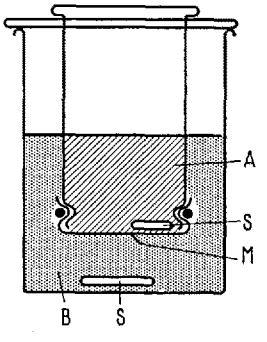

Fig. 1. Experimental device used for reduction through membrane. A, internal chamber (submitochondrial particles and substrate); B, external chamber (cytochrome *c* and substrate); S, magnetic stirrer; M, membrane (diameter 3.2 cm).

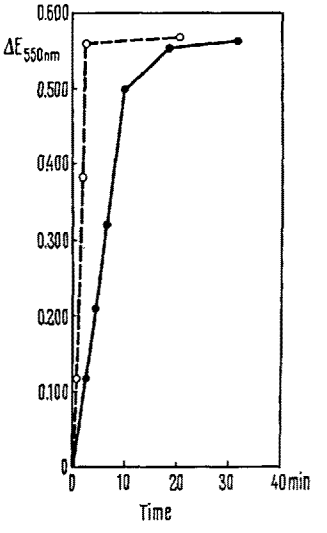

Fig. 2. Comparison of the rate of reduction of cytochrome *c* by submitochondrial particles. Incubation conditions for the reaction. Control: 0.1 *M* phosphate buffer pH 7.3; 2 m*M* succinate; 0.4 m*M* MaCN; 57 m*M* cytochrome *c*; mitochondrial protein 300 µg. Final volume 3.5 ml. In the presence of a membrane the reagent concentration was the same as for the control. Total volume in compartment A was 5.5 ml and in compartment B 12.5 ml. o----o control reaction; ●——● reaction across a semipermeable membrane.

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