SUBSTRATE DEPENDENT COMPARTMENTATION OF ATP IN RAT LIVER MITOCHONDRIA.

F.A.Hommes and J.A.C.M.van der Beek

Department of Biochemistry, University of Nijmegen,
School of Medicine, Nijmegen, The Netherlands.

Received September 24, 1963

It has been reported earlier (Hommes, 1962) that addition of increasing amounts of hexokinase to rat liver mitochondria in the absence of externally added adenine nucleotides, results in an increased respiration, until a plateau level is reached at which a further addition of hexokinase does not stimulate the rate of oxygen uptake. This phenomenon has been interpreted as an indication for the existence of a permeability barrier for ATP, as this maximal rate of oxygen utilisation was far below the rate obtainable with added ADP. The availability of ATP for the hexokinase reaction was apparently rate limiting. This effect was observed with succinate or glutamate, malate, malonate as substrate. Rather peculiar kinetics of oxygen uptake were observed when β-hydroxy butyrate was used as substrate. After an initial stimulation of respiration, an inhibition was observed, which could not be released by ADP or uncouplers of oxidative phosphorylation.

Klingenberg, in his recently proposed theory of dynamic organization of hydrogen pathways in mitochondria (Klingenberg, 1963), has indicated that a medium degree of reduction of mitochondrial pyridine nucleotides is essential for maximal conditions of the dehydrogenases. It was therefore of interest to investigate the inhibition of the above described system with β-hydroxy butyrate as substrate in more detail.

Methods: Rat liver mitochondria were prepared essentially as described by Schneider (1948). The extent of pyridine nucleotide reduction was monitored spectrophotometrically, using a Chance-Aminco double beam spectrophotometer (Chance, 1954) at the wave length pair 340-374 mμ (Chance and Williams, 1956). Oxygen consumptions were measured polarographically with a Clark oxygen electrode (Chance and Williams, 1955). Hexokinase was purchased from Boehringer and Soehne.

Results: The kinetics of oxygen utilisation are shown in fig.1. Addition of hexokinase to mitochondria in the presence of glucose and β-hydroxy butyrate

results in a slightly increased respiration. After about 4 min. a burst of respiration is observed, followed by an inhibited state.

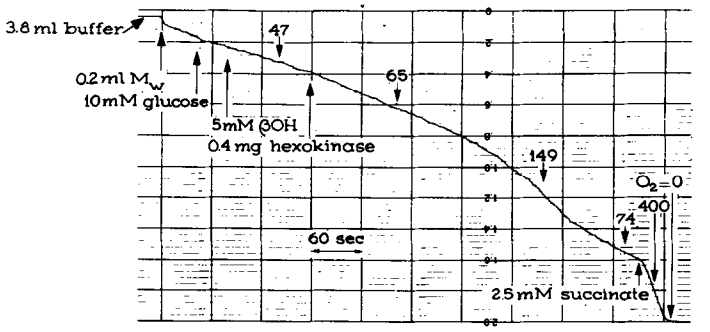

Fig. 1. Kinetics of oxygen utilisation by rat liver mitochondria in the presence of glucose, β-hydroxy butyrate (β-OH) and hexokinase. Protein concentration: 4.1 mg per ml. The numbers are the rate of oxygen consumption in mμ moles O_2 per min. The reaction was carried out in a medium containing 0.23 M sucrose, 10 mM K_2HPO_4, 10 mM KCl, 5 mM $MgCl_2$ and 10 mM triethanol amine hydrochloride, pH 7.4 .

Fig. 2 shows the corresponding experiment in which the redox-state of pyridine nucleotides was measured spectrophotometrically. The inhibited phase is accompanied by a complete oxidation of mitochondrial pyridine nucleotides, in agreement with the observation of Klingenberg, namely that a completely oxidized state of the mitochondrial pyridine nucleotides is inhibitory for DPN linked dehydrogenases.

However, such inhibition phenomena were not observed with glutamate, malate, malonate as substrate. This system is also pyridine nucleotide linked and therefore the inhibition phenomena observed with β-hydroxy butyrate as substrate seem to be rather specific. Succinate oxidation is also unimpaired as can be seen in fig. 1. The rate of oxygen utilisation is the same as in the control experiment under these conditions.

Oxidation of pyridine nucleotide in the presence of β-hydroxy butyrate indicates that the process of oxidative phosphorylation by which DPNH is oxidized is a faster process than the reduction via the dehydrogenase. This oxidation is only possible in these tightly coupled mitochondria in the presence of phosphate acceptor, provided by the glucose-hexokinase system. This means that ATP generated by oxidative phosphorylation must be freely available to the glucose-hexokinase system in order to provide the phosphate acceptor ADP. This in turn indicates that ATP generated in β-hydroxy butyrate oxidation is not compartmentalized , at least not to such an extend that it

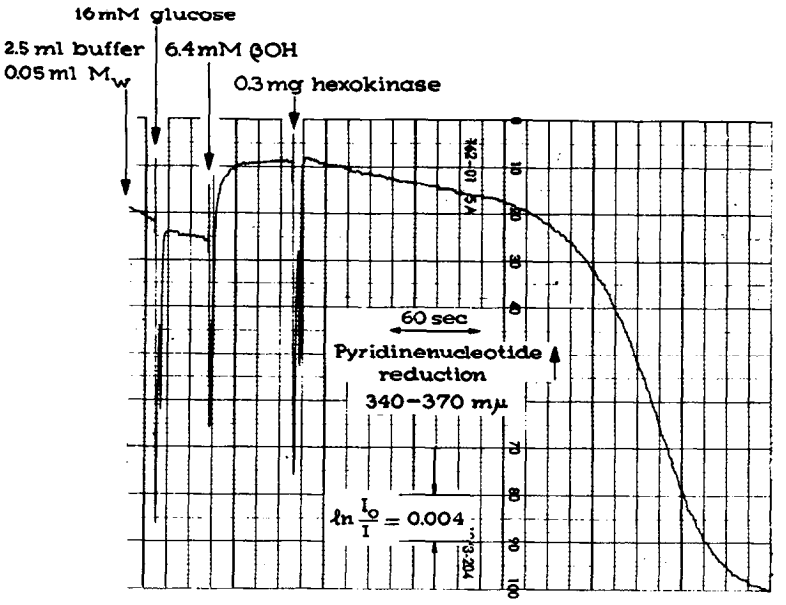

Fig. 2. Kinetics of oxidation and reduction of pyridine nucleotides in rat liver mitochondria in the presence of glucose, β-hydroxy butyrate (β-OH) and hexokinase. Experimental conditions as in fig. 1. Protein concentration: 1.6 mg per ml.

controls β-hydroxy butyrate oxidation, in contrast to the ATP generated by oxidation of succinate or glutamate, malate, malonate.

These differences may reveal some unique characteristics as to the organization of the dehydrogenases in the mitochondrial cristae.

References:

Chance, B., Science 120, 767 (1954).
Chance, B. and G.R.Williams, J.Biol. Chem. 217, 383 (1955).
Chance, B. and G.R.Williams, Adv. Enzym. 17, 65 (1956).
Hommes, F.A., Fed. Proc. 21, 142 (1962).
Klingenberg, M., in B.Chance: Energy linked functions of mitochondria, Acad.Press, New York, 1963, pg. 121.
Schneider, W.C., J.Biol.Chem. 176, 259,(1948).