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Enzymic activities of tumour mitochondria as evidenced by their inhibitory effect on the oxidation of DL-, D- and L- β-hydroxybutyrate by liver mitochondria

It was demonstrated in the preceding communication[1] that the oxidation of DL-β-hydroxybutyrate (BHB) by mitochondria from mouse livers prepared in isotonic sucrose, was completely inhibited in the presence of mitochondria from various tumours isolated with sucrose containing ethylenediaminetetraacetate (versene). The oxidation of octanoate was also fully suppressed under these conditions[2]. Furthermore it was found that addition of diphosphopyridine nucleotide[1] (DPN) or nicotinamide[3] (an inhibitor of DPNase) partly restored the oxidation when DL-BHB served as the substrate. No such an effect of DPN was seen, however, with octanoate.

The conclusions drawn from these and other experiments were that the tumour mitochondria possessed active splitting enzymes which destroyed DPN and adenosine triphosphate (ATP) and, consequently, abolished the fatty acid oxidative processes of the liver mitochondria. The existence of the DPNase and ATPase activities has been confirmed in direct enzymic assays[3].

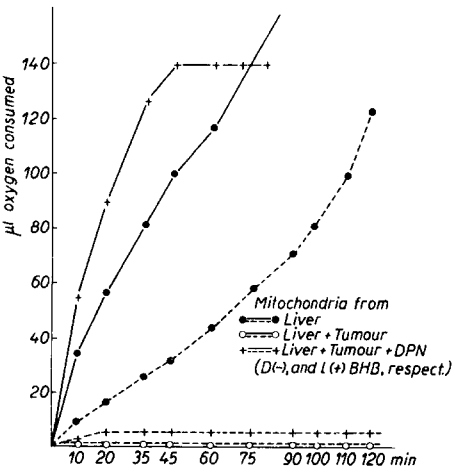

Fig. 1. The oxidation of D- and L-β-hydroxybutyrate by liver mitochondria and liver plus tumour mitochondria with and without DPN addition. Tumour: primary hepatic carcinoma rat (244 days feeding of butter yellow). Isolation of the mitochondria and incubation procedure as described[1], 12 μmoles of each stereoisomer were used. DPN was added in a final concentration of 0.001 *M*. The liver mitochondrial suspensions contained 1.75 mg nitrogen, those from the tumour 1.06 mg nitrogen.

Now, it is known from the studies of LEHNINGER AND GREVILLE[4] that the oxidation of the D(—) isomer of BHB is independent of ATP in that the free acid is oxidized, in contrast wiht the L(+) isomer which can only be oxidized as its coenzyme A (CoA) derivative and thus needs the supply of ATP for the formation of the L-BHByl-CoA bond prior to oxidation.

The oxygen consumption and the acetoacetate production which was recorded by us in the combined system of liver and tumour mitochondria oxidizing DL-BHB in the presence of DPN was usually *one half* of that shown by the liver mitochondria alone. Hence the most probable explanation[2] for this was that DPN addition resulted in the oxidation of the D-isomer by the liver mitochondria, whereas the oxidation of the L-isomer still remained blocked; this was considered as being due to the action of the tumour mitochondrial ATPase.

We have now been able to test this conclusion directly and found it to be correct, by using the pure stereoisomers of BHB. Our sincere thanks are due to Drs. GREVILLE AND LEHNINGER for their courtesy in supplying us with these samples, which were from the same stock as used by them in their own experiments[4].

Fig. 1 illustrates a typical example of the effect of DPN on the oxidation of D- and L-BHB by the liver mitochondria in the presence of mitochondria from a primary hepatic carcinoma of the rat (inbred strain R Amsterdam) induced by butter yellow feeding. In case of the D-isomer oxygen consumption is reduced to zero in the combined system of liver and tumour mitochondria. The DPNase of the latter[1,3] evidently blocks the oxidation by destroying the coenzyme of the liver D-BHB dehydrogenase, since addition of DPN restores the oxidative process. In view of the very high DPNase activity of the mitochondria from the hepatic carcinoma (complete disappearance of added DPN after 30 min contact, measured enzymically[3]) nicotinamide (0.005 *M*) was also added.

It can be seen from Fig. 1 that the oxidation of D-BHB in the combined system of liver

and tumour mitochondria fortified with DPN, took place at a more rapid rate than that of the liver mitochondria alone. Since the tumour mitochondria *per se* did not oxidize DL-BHB in the presence of DPN, the oxidation of D-BHB by the liver mitochondria, as compared with that in the combined system with DPN, might be limited either by the amount of endogenous DPN present, or by the dependence of phosphorylation, which accompanies the passage of hydrogen through the respiratory chain, on phosphate acceptors. DPN addition to the liver mitochondria alone did not enhance the rate of oxidation but rather slowed it down; this was also noted earlier in experiments with DL-BHB. Neither was it possible to demonstrate that ATP breakdown, after adding the "high energy" phosphate trapping system glucose-hexokinase, enhanced the liver mitochondrial oxidation of D-BHB. This may suggest a mechanism controlling oxidation other than by the level of phosphate-bond acceptor system (*cf.*[5,6]). It is further seen that, whereas the liver mitochondria do not stop taking up oxygen after the theoretical amount for acetoacetate formation is reached, the combined system does. The latter was also found when the glucose-hexokinase system was added to the liver mitochondria oxidizing D-BHB. For oxidation to occur beyond the stage of free acetoacetate, whatsoever the precise mechanism of action, an activating reaction, needing ATP, is bound to happen. When ATP is split, however, before it can serve this purpose, either by the hexokinase or by the tumour mitochondrial ATPase, further oxidation is rendered impossible. It should be remembered, since the D-isomer is oxidized as the free acid and thus needs no ATP supply such as the L-isomer, that the oxidation of the former to acetoacetate can not be inhibited by the tumour ATPase.

The ATP-dependent oxidation of the L-isomer of BHB by the liver mitochondria occurs at a slower rate than the oxidation of D-BHB (usually half as rapid). In the combined system of liver and tumour mitochondria, oxidation of L-BHB remained suppressed despite the presence of DPN. This was anticipated in view of the active tumour mitochondrial ATPase which might drain off the ATP necessary for the initial activation reaction. Also when the sucrose-prepared liver mitochondria were supplied with extra ATP, generated by the oxidation of added α-oxy caproate[7], the same inhibition was observed.

Since octanoate is known to be oxidized also as its CoA derivative, it follows that DPN addition to the combination liver and tumour mitochondria *plus* octanoate and α-oxycaproate would not have any promoting effect on the oxidative response either.

Similar results were obtained with the mitochondria from the transplanted mouse sarcoma UV 256 and the adrenalcortex carcinoma T 17572. In these cases only DPN (no extra nicotinamide) was added because the DPNases of these mitochondria were less active (50 and 63% of added DPN disappeared after 30 min contact resp.[3]) than the former but still high enough to give an appreciable resp. complete inhibition of the liver mitochondrial D-BHB oxidation.

Addition of fluoride (0.01 *M*), an inhibitor of the ATPase, to the combined system of liver and sarcoma mitochondria in the presence of DPN and L-BHB resulted in an oxygen uptake of 25 μl, whereas in the absence of F^- no O_2 was consumed.

Mitochondria prepared with sucrose-versene from tumours, which did not inhibit the octanoate or DL-BHB oxidation of the liver mitochondria[1,2] and whose ATPase and DPNase activities were found[3] to be smaller than the afore-mentioned, did not suppress the oxygen consumption of the liver mitochondria in the present experiments. It should be noted that the mitochondria from these tumours when prepared in 0.25 *M* sucrose without versene did inhibit the octanoate oxidation of the liver mitochondria (*cf.* Table X and XI, reference [2]); this inhibition has now been found to be relieved by DPN. After one washing of the mitochondria with KCl-phosphate buffer of pH 7.4, DPN no longer had this effect (ATPase determining factor).

The sucrose-versene mitochondria from three transplanted mouse hepatomas (T 26473, CBA 71 and T 28202) and one testis tumour (T 5358) oxidized only the D- and not the L-isomer of BHB in the presence of DPN and α-oxy caproate.

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