Biochemical properties of mitochondria from normal and neoplastic tissues

Mitochondria prepared from liver and a variety of other normal tissues show a latent adenosine-triphosphatase (ATPase) activity[1], while in mitochondria from pigeon-breast-muscle[2] and experimentally induced fatty livers[3] the ATPase is active.

The high ATPase activities displayed by cell-free preparations of several tumors adversely affect the Krebs-cycle oxidations and glycolytic reactions in these systems[4,5].

Evidence of the high ATPase activity of tumor mitochondria *in vitro* was gathered from experiments in which normal liver and tumor mitochondria were incubated together in the presence of octanoate. The mitochondria were prepared from mouse liver in isotonic (0.25 *M*) sucrose essentially by the procedure of SCHNEIDER AND HOGEBOOM[6] and washed once with sucrose and once with (0.13 *M*) KCl-(0.013 *M*) phosphate buffer. Tumor mitochondria were prepared in the same way, except that 0.25 *M* sucrose was used for the second washing. The final suspension was made in the KCl-phosphate buffer and kept for one hour at 0° C prior to incubation.

In some experiments not reported here, the mitochondria were used immediately, without changing the results reported below (Table I). When supplemented with the proper substrates, the liver mitochondria showed a vivid β-oxidation, whereas the tumor mitochondria showed no oxygen consumption. In these experiments α-oxy caproate served as a "sparker" of fatty acid oxidation[7,8]. The oxygen consumption resulting from fatty acid oxidation by the liver mitochondria was reduced to zero, however, when tumor mitochondria were added. Mitochondria from many mouse tumors showed this inhibitory effect, *e.g.* transplanted sarcomas (induced by ultra-violet irradiation), ovarium tumors of the sarcomatoid type, hepatomas, lymphosarcomas, myxomas, adrenal carcinomas, spontaneous hepatomas (CBA mice) and spontaneous mamma tumors (C_3H mice). All tumor transplants were grown on mice of the susceptible inbred strain. The rate of inhibition was dependent upon the amount of tumor mitochondria added to the liver mitochondria. Heating the tumor mitochondria (5 min at 100° C) prior to incubation, abolished their inhibitory effect. The inhibitory effect on fatty acid oxidation could be partially counteracted by adding sodium fluoride, which is known as a potent ATPase inhibitor. The addition of F^- to the tumor mitochondria only, never produced any oxygen consumption under these conditions. It follows from this that the addition of F^- to the liver- and tumor mitochondria together restored the β-oxidation by the liver mitochondria. The ATPase originating from the tumor mitochondria is apparently capable of destroying the ATP necessary for the formation of the acyl-CoA bond in the fatty acid activation step.

TABLE I

INHIBITION AND REVERSION OF OCTANOATE OXIDATION BY NORMAL LIVER MITOCHONDRIA IN THE PRESENCE OF TUMOR MITOCHONDRIA AND TUMOR MITOCHONDRIA PLUS FLUORIDE RESPECTIVELY

Additions: Octanoate (2 μM), α-oxy caproate (5 μM), Mg^{2+} (0.005 *M*), ATP (0.0007 *M*), cytochrome *c* (0.00001 *M*) in a total volume of 1.6 ml KCl (0.06 *M*)-phosphate (0.013 *M*) buffer (pH 7.4) including 0.3 ml mitochondrial suspension from mouse liver in KCl-phosphate buffer (corresponding with 250 mg wet weight of tissue) and 0.3 ml mitochondrial suspension from tumor (corresponding with 600 mg wet weight). NaF was added in a final concentration of 0.01 *M*. Oxygen consumption was calculated as the difference between oxygen uptake in the presence of octanoate + α-oxy caproate and that of α-oxy caproate. Protein nitrogen was determined by a micro-Kjehldahl procedure.

Flasks containing mitochondria from;	*mg N per flask*	*µl O_2 consumed after 60 minutes*
I (normal liver)	1.20	187 — 74 = 113
II (*sarcoma* UV 256)	0.55	31 — 29 = 2
I + II		28 — 32 = —4
I + II + F^-		98 — 45 = 53
I (normal liver)	1.35	84 — 33 = 51
II (*ovarium tumor* 5441)	0.82	14 — 15 = —1
I + II		23 — 22 = 1
I + II + F^-		111 — 81 = 30
I (normal liver)	1.54	160 — 76 = 84
II (*spont. mamma tumor*)	0.78	20 — 19 = 1
I + II		28 — 27 = 1
I + II + F^-		107 — 85 = 22

Experiments with octanoate-1-^{14}C confirmed the manometric data. The amount of oxygen consumed during ocatanoate oxidation in the combined system fortified with F^- usually did not reach the initial value which was recorded in the absence of the tumor mitochondria and F^-. Besides the inhibitory effect of F^- on fatty acid oxidation[9], it was shown by the ^{14}C recoveries from $^{14}CO_2$ and ^{14}C-acetoacetate, after incubation with octanoate-1-^{14}C, that a greater amount of octanoate is oxidized by the liver mitochondria in the presence of tumor mitochondria and F^- than is actually indicated by the oxygen consumption calculated according to Table I. This discrepancy appears to be caused by the fact that relatively less α-oxy acid is oxidized in the combined system with F^- in the presence of fatty acid than in its absence, as was demonstrated by experiments on the oxidation of α-oxy caproate-1-^{14}C. Earlier it was shown that α-oxy caproate undergoes oxidative decarboxylation after its preliminary conversion to α-keto caproate[8,10].

No inhibitory effect on the fatty acid oxidation of the normal liver mitochondria could be detected with the supernatant prepared from the tumor mitochondrial suspensions after standing for 1 to 2 hours in the KCl-phosphate buffer (centrifugation for 10 min at 8000 $\times$ g). The inhibitory effect stayed with the sedimented fraction. These and other experiments seem to indicate that the ATPase, originating from the tumor mitochondria remains particulate-bound and does not come into true solution. As a consequence, it is probable that the tumor ATPase affects the ATP outside the liver mitochondria. Whether this should be visualized as a simple diffusion of ATP through the mitochondrial membrane to the suspending medium or as the consequence of a possible localisation of ATP synthesis on the membrane[11,12] is as yet undecided.

It should be emphasized that the ATPase is not tumor specific. Mitochondria prepared from brain and liver mitochondria pre-incubated for half an hour at 37° C in the absence of substrate, show the same properties as described for the tumor mitochondria, although to a smaller extent. At the same time it was found that not all tumors display the same high ATP-splitting activity. Mitochondria from two transplanted interstitial cell tumors of mouse testis were without activity. Mitochondria prepared from granulosa cell tumors of the mouse ovary, suspended in KCl-phosphate buffer as described, exhibited a moderate or complete inhibitory effect on the fatty acid oxidation by normal liver mitochondria. On the other hand, when the suspension was made in isotonic sucrose no effect was noted. These observations together with the data on the osmotic behaviour of the mitochondria (swelling) to be reported later, have led us to the opinion that the mitochondria of most tumors, in contrast with many normal tissues, are liable to damage during preparation, by which the latent ATPase becomes active.

A full account of our work will be published.

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