The Role of Mitochondria in Modifying the Cellular Ionic Environment

CALCIUM-INDUCED RESPIRATORY ACTIVITIES IN MITOCHONDRIA ISOLATED FROM VARIOUS TUMOUR CELLS

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Cyclic stimulation by Ca^{2+} of respiration in mitochondria isolated from Ehrlich ascites-tumour cells occurs only when low phosphate concentrations (approx. 0.5 mM) are also included in the incubation system. Under these circumstances the extra oxygen consumed is related stoichiometrically to the amount of Ca^{2+} taken up by the mitochondria; the values are similar to those obtained with mitochondria from rat liver in the absence of added phosphate. In contrast with liver mitochondria, up to 280 nmol of Ca^{2+} /mg of protein can be added to ascites mitochondria without causing any deleterious effect. Respiration in mitochondria isolated from the Yoshida ascites hepatoma (HA 130) and from the Morris hepatomas 5123C and 9618A is also stimulated by Ca^{2+} in a cyclic manner. However, that in mitochondria from regenerating rat liver responds to Ca^{2+} in the same way as those from normal rat liver. ADP-stimulated respiration in mitochondria from Ehrlich ascites tumour cells, but not from rat liver, is inhibited by low amounts of Ca^{2+} .

Ca^{2+} ions antagonise the activating effects of Mg^{2+} in a large number of cellular enzymic and metabolic processes. By appropriately varying the $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio in defined intracellular environments, the cell is potentially able to use this property of Ca^{2+} to advantage, especially as a means of regulating or modifying cell metabolism (reviewed by Bygrave, 1974; see also Bygrave, 1967; Meli & Bygrave, 1972; Roberts & Bygrave, 1973; Williams, 1970; Rasmussen, 1970; Borle, 1973). Recently much emphasis has been placed on mitochondria as contenders for regulating Ca^{2+} -sensitive cellular metabolic processes (Bygrave, 1974; Meli & Bygrave, 1972; Roberts & Bygrave, 1973; Borle, 1973; Spencer & Bygrave, 1972a, 1973). Indeed, mitochondria isolated from a variety of tissues possess Ca^{2+} -transport properties which endow them well for such a role (Meli & Bygrave, 1972; Bygrave *et al.*, 1971; Spencer & Bygrave, 1972a, 1973).

Recent studies from this laboratory have shown that tightly coupled mitochondria isolated from Ehrlich ascites-tumour cells tolerate unusually high concentrations of Ca^{2+} ; these concentrations do not induce the changes in membrane-associated energy-linked functions normally seen in mitochondria isolated from rat liver (Thorne & Bygrave, 1973a,b, 1974a). This anomalous reaction of the ascites-tumour mitochondria towards Ca^{2+} introduces the possibility that the ionic environment in these cells is not controlled in the same way as it is in cells from rat liver.

In defining further the interaction of Ca^{2+} with energy-linked reactions in tumour mitochondria, we have undertaken a detailed examination of the way in which Ca^{2+} induces respiration in mitochondria isolated from Ehrlich ascites-tumour cells. Where possible, the experiments were designed to enable direct comparisons to be made with those already carried out with the well-documented rat liver system (for reviews on this topic see Lehninger *et al.*, 1967; Rossi & Lehninger, 1964). Some of the principle experiments have been carried out also with mitochondria isolated from several tumours possessing widely different generation times and from regenerating rat liver.

The data obtained provide further evidence that the interaction of Ca^{2+} with mitochondria from some forms of tumours differs in several fundamental respects from that seen with mitochondria from normal and regenerating rat liver. The observed differences are such that they could in part account for, or be associated with, the altered metabolic behaviour seen in these tumour cells.

Experimental

Tumour line

The Ehrlich ascites-tumour cells were obtained from mice which had been infected for seven to nine days. Their method of propagation is described elsewhere (Bygrave, 1966). The original line of cells

was kindly provided by Dr. Eric L. French, Animal Health Research Laboratories, Parkville, Vic., Australia. The line of Yoshida hepatoma ascites cells (HA 130) was kindly provided by Dr. H. Naora, Research School of Biological Sciences, Australian National University. These cells were propagated in Wistar strain albino rats. Rats bearing the Morris hepatomas (5123C and 9618A) were the generous gift of Dr. John Sabine, Waite Agricultural Institute, University of Adelaide, S. Austral., Australia.

Partial hepatectomy

The operations were carried out by the procedure of Higgins & Anderson (1931). Regenerating livers were used 28 or 48 h after the operation.

Preparation of mitochondria

Mitochondria were prepared from Ehrlich ascites-tumour cells by a slight modification (Thorne & Bygrave, 1973b) of the method of Wu & Sauer (1967). Mitochondria from the remaining tumours and from regenerating and normal rat liver were prepared as described by Spencer & Bygrave (1972a). The isolation medium consisted of 250 mM-sucrose, 2.5 mM-Hepes [2-(N-2-hydroxyethyl)piperazin-N-yl]-ethanesulphonic acid]-KOH (pH 7.4) and 1 mM-EGTA [ethanedioxybis(ethylamine)tetra-acetic acid.] The wash medium consisted of 250 mM-sucrose and 2.5 mM-Hepes (pH 7.4).

O₂ consumption was measured with a Clark-type oxygen electrode (Reed, 1972) coupled to a 25.4 cm (10 in) Rikidenki chart recorder. Ca²⁺ accumulation was measured by using ⁴⁵Ca²⁺ and the millipore filtration technique (Spencer & Bygrave, 1973) with filter papers of pore size 0.45 μm. Protein was determined by a biuret cyanide method (Szarkowska & Klingenberg, 1963).

All chemicals used were of analytical grade. ⁴⁵CaCl₂ was obtained from The Radiochemical Centre, Amersham, Bucks, U.K.

Results

Stimulation by Ca²⁺ of mitochondrial respiration in a phosphate-free medium

Addition of low concentrations of Ca²⁺ to ascites-tumour mitochondria suspended in a phosphate-free medium consisting of sucrose, Hepes and a respiratory substrate, produces a slight but significant increase in the rate of O₂ uptake (Fig. 1). The faster rate of respiration continues for a short time before reverting to the original slower rate. In contrast with experiments with rat liver mitochondria (Rossi & Lehninger, 1964) only one or two additions of Ca²⁺ (200 nmol) may be made to the tumour mitochondria;

Fig. 1. Ca²⁺ stimulation of respiration in ascites mitochondria

The reaction system contained: 250 mM-sucrose, 2.5 mM-Hepes (pH 7.4), 6.5 mM-succinate (a), 10 mM-pyruvate+malate (b) or 10 mM α-oxoglutarate (c) as respiratory substrate and 3.6 mg of mitochondrial protein. The final volume was 2.0 ml and the temperature 25°C. At the times indicated by arrows, 100 μM-CaCl₂ was added. Carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) was added where indicated at a concentration of 5 μM. MT, mitochondria.

after these additions the respiratory system does not respond to further additions of Ca²⁺, but instead either remains at the initial unstimulated rate (with succinate) or shows an even slower rate of respiration (with pyruvate+malate or with α-oxoglutarate).

The pattern of activity described above occurs independently of the particular substrate used. Addition of an uncoupler of oxidative phosphorylation to the medium at the times shown induces a sharp and irreversible increase in the rate of O₂ uptake suggesting that the mitochondria are still fully coupled at this stage of the incubation. The presence of 10 mM-Mg²⁺ produced no beneficial effect on O₂ uptake and so was omitted from the incubation system unless otherwise indicated. The sluggish response of respiration to additions of Ca²⁺ with this medium did not permit adequate measurements to be made on the stoichiometry of the interaction of Ca²⁺ with the respiratory system (but see below).

Influence of phosphate on stimulation of respiration by Ca²⁺

The experiments shown in Fig. 2 were carried out in an identical way with those shown in Fig. 1 except that 0.5 mM-P_i was included in the incubation system. The effects observed are notable for two reasons. First, it is clear that after the addition of 200 nmol of Ca²⁺ to the incubation system, there is an immediate stimulation of respiration which continues for a short time; this occurs with all substrates tested. After the stimulation there is a return to the original resting rate of respiration.

Fig. 2. Ca²⁺ stimulation of respiration in ascites mitochondria in the presence of phosphate

Reaction conditions were as described in Fig. 1 except that 0.5 mM-P_i was added to the reaction mixture. Mitochondrial protein was 3.6 mg. (a) Succinate; (b) pyruvate + malate; (c) α -oxoglutarate. MT, mitochondria.

As will be shown below, the extent of extra O₂ uptake and the accumulation of Ca²⁺ take place stoichiometrically.

The second major feature of the data is that many successive additions of Ca²⁺ can be made, in each case producing a transient stimulation of O₂ uptake followed by a return to the initial rate. In each situation there is no indication that the mitochondria are becoming uncoupled as occurs in the case of rat liver mitochondria (cf. Rossi & Lehninger, 1964; Thorne & Bygrave, 1973a). With succinate as substrate, for example, it is clear that the mitochondria are still responding efficiently to Ca²⁺ addition, even when the concentration of this ion already present is approaching 600 μ M.

The experiments presented in Figs. 1 and 2 thus show that with mitochondria from Ehrlich ascites-tumour cells a cyclic response of respiration to Ca²⁺ is obtained only when added phosphate is present in the incubation system. Other experiments (data not shown) were carried out in which repeated additions of Ca²⁺ were made to mitochondria in a succinate-supported system, but with different concentrations of P_i also present from the beginning of the incubation. With 0 and 0.25 mM-phosphate present, the resting rate of respiration diminished as the number of additions of the fixed Ca²⁺ concentration increased. However, in the presence of 0.5 mM-phosphate, up to 1 μ mol of Ca²⁺ (280 nmol/mg of protein) could be added *in toto* without producing any obvious deleterious effect on mitochondrial respiration. With 1 mM-phosphate present, 700 nmol of Ca²⁺ could be added, but higher concentrations of Ca²⁺ induced a prolonged high rate of resting respiration. A similar effect occurred with 2 mM-

Fig. 3. Comparison between ascites and liver mitochondria of effect of phosphate concentration on resting respiration after a single addition of Ca²⁺

The reaction system was as described in Fig. 1 with the concentration of P_i indicated present from the beginning of the incubation. The substrate was succinate (6.5 mM), ascites or liver mitochondria (3.6 mg of protein) were present as indicated. The resting rate of respiration was measured after the transient stimulated rate which occurred on the addition of 100 μ M-Ca²⁺. Final volume was 2 ml and the temperature was 25°C. ○, Liver mitochondria; ●, ascites mitochondria.

phosphate, but after the addition of slightly lower concentrations of Ca²⁺. Essentially similar findings were obtained when the experiment was repeated in a medium consisting of buffered KCl.

Data in Fig. 3 show the effect of P_i concentration on the ability of mitochondria from rat liver and the tumour cells to respond to a single addition of 100 μ M-Ca²⁺. The uncoupling action of Ca²⁺ on rat liver mitochondria induced by the permeant anion occurs to the greatest extent between concentrations of 0 and 0.5 mM, where a doubling of the resting rate of respiration occurs (see also Rossi & Lehninger, 1964). In the presence of 2 mM-phosphate the rate is more than trebled. However, with ascites-tumour mitochondria, resting respiration is increased only approx. 16% at 0.5 mM-phosphate and remains virtually unaffected by any further increase in phosphate concentration.

Fig. 4. Accumulation of Ca^{2+} by ascites mitochondria during respiratory stimulation in absence or presence of phosphate

The reaction system was as described in Fig. 2, but with 0.5 mM- P_i present as indicated. Ca^{2+} (containing $0.2 \mu\text{Ci}$ of $^{45}\text{CaCl}_2$) was added at the indicated concentration and, after the transient stimulated rate of respiration, a portion of the mitochondria was sampled for radioactivity by using millipore filtration. Some 3.6 mg of mitochondria protein was present in a final volume of 2.0 ml at a temperature of 25°C . (a) No phosphate; (b) 0.5 mM-phosphate. $\circ$, Succinate; $\bullet$, pyruvate+malate; $\blacktriangledown$, α -oxoglutarate.

Movement of Ca^{2+} during respiratory stimulation

Accumulation of Ca^{2+} by mitochondria concomitant with the stimulation of respiration was determined from experiments carried out in an identical manner with those described in Fig. 1 except that movement of Ca^{2+} into mitochondria was monitored by using $^{45}\text{Ca}^{2+}$ and sampling a small volume of the incubation medium once the transient rapid rate of respiration was complete. The data in Fig. 4 show that in the absence of phosphate only about 80% of the Ca^{2+} added is actually accumulated above about 50 nmol/mg of protein. As the concentration of added Ca^{2+} approaches 250 nmol/mg of protein little further Ca^{2+} is accumulated, although there is the suggestion from the data that in the presence of pyruvate the capacity for Ca^{2+} accumulation is greater than that with succinate or with α -oxoglutarate. Thus, in the absence of added phosphate, maximal accumu-

lation of Ca^{2+} by the ascites mitochondria approaches some 150–200 nmol/mg of protein.

When 0.5 mM-phosphate is present, accumulation of Ca^{2+} is complete up to added Ca^{2+} concentrations of about 200 nmol/mg of protein for succinate and pyruvate+malate, and is even higher for α -oxoglutarate (up to 275 nmol/mg of protein). These data show that the high concentrations of Ca^{2+} added to ascites mitochondria in the presence of P_i and which induce repeated respiratory jumps (Fig. 2), are actually accumulated by the mitochondria. It is important to note that Mg^{2+} and ATP are not present in these incubation media. Indeed Mg^{2+} itself has somewhat deleterious effects when added to the system in the presence of phosphate. The incubation conditions therefore do not represent those necessary for 'massive' loading, yet unusually large amounts of Ca^{2+} are still able to be accumulated and retained by these mitochondria (cf. Rossi & Lehninger, 1964).

Table 1. *Stoicheiometry between O₂ uptake, Ca²⁺ added and Ca²⁺ accumulated by ascites-tumour mitochondria*

Conditions were as described in Fig. 2 with the different substrates present as indicated, with 0.5 mM-P_i and 3.6 mg of mitochondrial protein. The final volume was 2.0 ml. O₂ uptake and Ca²⁺ accumulation were measured 60 s after Ca²⁺ addition as indicated in the Experimental section. The temperature was 25°C.

Substrate	Oxygen uptake (ng-atoms)	Ca ²⁺ added (nmol)	Ca ²⁺ accumulated (nmol)	Activation ratio*	Accumulation ratio†
Succinate	46	200	186	4.3 (2.15)	4.04 (2.02)
Pyruvate+ malate	34	200	197	5.9 (1.96)	5.8 (1.93)
α-Oxoglutarate	26	200	200	7.7 (1.92)	7.7 (1.92)

* ng-ions of Ca²⁺ added/ng-atoms of extra oxygen taken up.

† ng-ions of Ca²⁺ accumulated/ng-atom of extra oxygen taken up. Numbers in parentheses refer to the values obtained per site.

Stoicheiometry of respiratory stimulation with Ca²⁺ accumulation

Work with mitochondria from rat liver has shown that the stoicheiometric accumulation of Ca²⁺ as well as the stoicheiometric activation of respiration by these mitochondria is affected by the addition of P_i to the incubation system (Rossi & Lehninger, 1964). These same relations can be determined with mitochondria from ascites-tumour cells, but as the above data would suggest this is possible only when small amounts of P_i are also present in the incubation medium. Data in Table 1 were compiled from experiments in which the amount of extra O₂ uptake was measured after the addition of a known quantity of Ca²⁺. At the same time the extent to which the mitochondria had accumulated the added Ca²⁺ was determined by using radioactive Ca²⁺ and employing the sampling techniques described in the Experimental section. The experiments were repeated many times with the substrates indicated. It is clear that the extent to which Ca²⁺ stimulates O₂ uptake is related stoicheiometrically to the number of energy-conserving sites traversed by pairs of electrons moving from substrate to O₂. Both the activation and accumulation ratios give values very close to 2/site; these values are identical with those obtained with rat liver mitochondria in the absence of added P_i.

Ability of Ca²⁺ to stimulate respiration in mitochondria isolated from regenerating rat liver and various lines of tumour cells

The marked difference in the behaviour towards Ca²⁺ between the mitochondria isolated from Ehrlich ascites cells and rat liver seen in the above experiments raised the question as to whether the effects observed with the ascites mitochondria were characteristic of tumour cells in general or only of rapidly dividing cells. To examine this point in more detail mitochondria were isolated from a rapidly growing normal tissue (regenerating rat liver),

from the Morris 5123C and 9618A hepatomas and as well from a Yoshida hepatoma strain of ascites cells (HA 130). The mitochondria obtained in each case exhibited a high degree of acceptor control indicating good coupling of phosphorylation to respiration. An experiment was then carried out with each of the mitochondria species in a manner identical with those described in Fig. 2, but in the presence of 1 mM-P_i and succinate. Repeated additions of Ca²⁺ were made to the incubation system, and the resting-rate of respiration after the transient rapid rate of respiration was plotted as a function of the Ca²⁺ present in the incubation medium (see Fig. 5).

The data show that mitochondria isolated from regenerating rat liver behave in a manner almost identical with that typical of those from normal rat liver. Concentrations of Ca²⁺ as low as 50 nmol/mg of protein slightly uncouples, whereas higher concentrations causes a considerably greater rate of respiration. On the other hand, mitochondria from each of the strains of tumour examined exhibit a pattern of behaviour characteristic of that obtained with the mitochondria from Ehrlich ascites-tumour cells. Even when the concentration of Ca²⁺ in the medium is 200 nmol/mg of protein, the resting rate of respiration with mitochondria from each of the tumour strains remains at values very similar to the rates seen in the absence of added Ca²⁺.

Competition between ADP and Ca²⁺ to induce respiratory stimulation

It was shown by Rossi & Lehninger (1964) with rat liver mitochondria that oxidative phosphorylation of ADP and oxidative accumulation of Ca²⁺ can take place sequentially in a single incubation system. When both Ca²⁺ and ADP are added simultaneously, Ca²⁺ stimulates respiration first; once all of the Ca²⁺ has been accumulated, ADP activates respiration and is phosphorylated.

Fig 6 shows a trace from one of several experiments in which the ability of ascites-tumour mito-

Fig. 5. Ca^{2+} stimulation of respiration in mitochondria isolated from regenerating livers and from various tumour tissues

The reaction system was as described in Fig. 2 except that 6.5 mM-succinate and 1 mM- P_i were added to the reaction mixture. Mitochondria (3.6 mg of protein) were present as indicated in a final volume of 2.0 ml at a temperature of 25°C. The data were recalculated from the original traces and show the rate of resting respiration in the various preparations after successive additions of Ca^{2+} . Mitochondria were obtained from ■, liver; ○, regenerating liver; ●, hepatoma 5123C; □, hepatoma 9618A; ▼, HA 130 ascites, ▼, Ehrlich ascites.

chondria to respond to sequential additions of ADP and Ca^{2+} was examined. Addition first of ADP (200 nmol) in the presence of 1 mM- P_i produces an extra oxygen uptake of 102 ng-atoms followed by a return to the original resting rate of respiration. The addition next of Ca^{2+} (300 nmol) stimulates respiration to a rate faster than that of ADP and produces an extra oxygen uptake of 69 ng-atoms. This also is followed by a return to the original resting rate of respiration. However, a second addition of ADP (200 nmol) fails further to stimulate O_2 uptake. An experiment was then carried out in which various amounts of Ca^{2+} were added to the mitochondria and, after the return to the resting rate of respiration, a fixed amount of ADP (250 nmol) was added. The rates of respiration activated by the Ca^{2+} and by the ADP were then measured for both ascites tumour and the liver mitochondria and expressed as a function of Ca^{2+} added to the incubation system (Fig. 7).

Fig. 6. Effect of sequential additions of ADP and Ca^{2+} on respiratory behaviour in ascites mitochondria

The reaction system was as described in Fig. 1 but with 1 mM- P_i , 7 mM-succinate and 1.9 mg of mitochondrial protein present. At the indicated times 100 μM -ADP or 150 μM - Ca^{2+} was added. Final volume was 2.0 ml at a temperature of 25°C.

As expected, the Ca^{2+} -stimulated respiratory response increases with both species of mitochondria as the concentration of Ca^{2+} is increased. Maximal rates are reached at about 300–400 nmol of added Ca^{2+} with half-maximal rates being attained at about 100–150 nmol. On the other hand, the ADP-stimulated respiratory response differs markedly between the liver and tumour mitochondria. With the former, prior addition of Ca^{2+} (up to 300 nmol) has no influence on the ability of ADP to stimulate respiration. As the concentration of Ca^{2+} is increased with the tumour mitochondria, there is a steady decline in the rate of ADP-stimulated respiration, which reaches almost zero at about 400 nmol of added Ca^{2+} .

Discussion

The observations made in this study amplify previous findings (Thorne & Bygrave, 1973a,b; 1974a; Reynafarje & Lehninger, 1973) on the unusual reactivity of ascites-tumour mitochondria towards

Fig. 7. Effect of Ca²⁺ on ADP-induced O₂ uptake in ascites and liver mitochondria

The reaction was as described in Fig. 6 except that 0.5 mM-P_i was present and the concentration of Ca²⁺ added was varied as indicated. ADP (120 μM) was added after the Ca²⁺-stimulated rate of respiration had returned to the resting rate. Liver or ascites mitochondria (3.3 mg of protein) were present as indicated. ●, ■, Ascites mitochondria; ○, □, rat liver mitochondria. Circles, Ca²⁺-stimulated respiration; squares, ADP-stimulated respiration.

added Ca²⁺. Among the most prominent features of this behaviour already revealed is the resistance exhibited by these mitochondria to the well-known uncoupling action of Ca²⁺ (Thorne & Bygrave, 1974a). As a consequence a number of energy-linked reactions intimately linked to the process of oxidative phosphorylation as well as the oxidative phosphorylation of ADP itself, are only slightly affected by extremely high amounts of this ion. Moreover, the ascites tumour mitochondria are able to retain high amounts of accumulated Ca²⁺ for considerably long periods of time without suffering any apparent damage (H. McIntyre & F. L. Bygrave, unpublished work). The findings of Reynafarje & Lehninger (1973) suggest that Ca²⁺ is also transported more efficiently in the L1210 tumour mitochondria than in normal liver mitochondria. These mitochondria were shown to exhibit unusual anomalies in the stoichiometric relations between respiration, Ca²⁺ binding and H⁺ ejection.

The data presented in this communication reveal two further major features of unusual behaviour by ascites-tumour mitochondria. The first relates to the influence of P_i on Ca²⁺-induced respiration and the second, to the inhibition by added Ca²⁺ of ADP-induced respiration.

We showed that for Ca²⁺ to stimulate respiration in a cyclic manner approx. 0.5 mM-phosphate needs to be present in the incubation system. Only under

these conditions could a stoichiometric relation between extra O₂ uptake and Ca²⁺ accumulation be observed. Concentrations of phosphate less than 0.5 mM led to an eventual permanent decline in the rate of respiration once high concentrations of Ca²⁺ were present in the system. On the other hand, concentrations of phosphate in excess of this amount eventually induced failure of the rate of O₂ uptake to return to the initial resting rate. The latter situation occurred, however, only when the Ca²⁺ concentration approached exceptionally high values.

The effects of P_i on Ca²⁺-stimulated respiration thus differ between ascites tumour and rat liver mitochondria in that with the latter, low concentrations of the anion abolish the stoichiometric relationship between extra O₂ uptake and Ca²⁺ accumulation by inducing respiration to continue at the stimulated rate. With the tumour mitochondria on the other hand, it is necessary to have the anion present before the stoichiometric cyclic respiratory response is obtained. Moreover oligomycin, which prevents the deleterious effect of phosphate in rat liver mitochondria, has no influence on the beneficial effect of phosphate in the ascites mitochondria.

The second major observation made in the present study was that Ca²⁺ inhibited respiration stimulated by ADP in the ascites-tumour mitochondria. This inhibition was not evident in an identical experiment carried out with rat liver mitochondria. Elsewhere we have shown that oxidative phosphorylation of ADP is inhibited by added Ca²⁺ in these mitochondria (Thorne & Bygrave, 1974a). We have also established that Ca²⁺ inhibits the atractyloside-sensitive translocation of ADP into the ascites tumour mitochondria (Thorne & Bygrave, 1974b). Thus the possibility exists that the inhibition of ADP-stimulated respiration in mitochondria from Ehrlich ascites-tumour cells arises because Ca²⁺ prevents the entry of ADP into the mitochondria. It is noteworthy that ADP translocation by rat liver mitochondria is not inhibited but stimulated by added Ca²⁺ (Spencer & Bygrave, 1972a,b, 1974).

It is likely that the differing effects of Ca²⁺ on the respiratory activities of ascites tumour and liver mitochondria in the absence and presence of phosphate described above, are of potential biological significance to the metabolism of tumour cells, especially since mitochondria obtained from rapidly dividing normal cells, i.e. regenerating rat liver, respond to Ca²⁺ in a similar manner to those from adult rat liver. In contrast, mitochondria isolated from both of the solid hepatomas and from the Yoshida ascites hepatoma respond to Ca²⁺ in a similar manner to the mitochondria isolated from Ehrlich ascites-tumour cells.

For some years it has been considered that the control mechanisms integrating respiration and

glycolysis in normal cells might be deranged in tumour cells (reviewed by Wenner, 1967). Forms of derangement which have been suggested include a breakdown in the movement of cofactors common to both respiration and glycolysis (i.e. ADP, ATP, P_i) between the mitochondria and the cytoplasm (Lynen & Koenigsberger, 1951; Kvamme, 1955; Racker, 1956; Chance & Hess, 1959); adenine nucleotides and P_i are able to directly influence the activity of many glycolytic enzymes (Rose & Rose, 1970), whereas ADP and P_i are clearly obligatory for coupled respiration and oxidative phosphorylation. We have established elsewhere that Ca^{2+} movements between the cytoplasm and mitochondria might also be an important system involved in the control and integration of glycolysis and respiration (Meli & Bygrave, 1972; Spencer & Bygrave, 1972a). The nature of the interaction of Ca^{2+} and phosphate with the membrane-associated energy-linked reactions seen here in some forms of tumour mitochondria, together with the ability of Ca^{2+} to inhibit the oxidative phosphorylation of ADP in the Ehrlich ascites-tumour mitochondria are observations that are directly relevant to the question of deranged control of energy metabolism in tumour cells. Such observations also shed further light on the potential importance of bivalent ions as a controlling element in metabolism in normal cells (Bygrave, 1974; Meli & Bygrave, 1972).

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