translocase reacts preferentially with ADP towards the outside of the mitochondrion and with ATP towards the inside. On the other hand, exogenous ATP can be utilized for the ATP–ADP and ATP–P_i exchange reactions, for the uncoupler-induced ATPase and for the endergonic reduction of α-oxoglutarate *plus* ammonia[11,12] or of acetoacetate[13] with succinate as hydrogen donor. KLINGENBERG AND PFAFF[1], PFAFF, KLINGENBERG AND HELDT[3] and PFAFF[14,15] have demonstrated by direct measurement that in coupled mitochondria the adenine nucleotide translocase reacts more rapidly with exogenous ADP than with exogenous ATP, and that in the presence of uncoupler this difference vanishes.

In this paper evidence is presented that when the level of high-energy intermediates of oxidative phosphorylation in the mitochondrion is low, exogenous ATP can be used as energy donor for citrulline formation. The results of an illustrative experiment are presented in Table I. Citrulline synthesis was inhibited by oligomycin or rotenone, even though exogenous ATP (3 mM) was present. Presumably then, in the absence of oligomycin or rotenone, the ATP for citrulline synthesis is generated within the mitochondrion during oxidative phosphorylation (with endogenous substrate). 2,4-Dinitrophenol also inhibited. However, when 2,4-dinitrophenol and oligomycin were present together, slightly more citrulline (1.8 μmoles) was formed than in the control (1.4 μmoles). Rotenone (Table I) or arsenite (not shown) had very little effect in the presence of 2,4-dinitrophenol *plus* oligomycin, indicating that the substrate-linked phosphorylation step could not have contributed to the synthesis under these conditions. Furthermore, the synthesis in the presence of 2,4-dinitrophenol *plus* oligomycin *plus* rotenone was abolished by atractyloside*. These results show that exogenous ATP was utilized for citrulline synthesis in the presence of 2,4-dinitrophenol *plus* oligomycin.

TABLE I

EFFECT OF 2,4-DINITROPHENOL ON CITRULLINE SYNTHESIS IN ISOLATED RAT-LIVER MITOCHONDRIA

The reactions were carried out in round-bottomed tubes in a Dubnoff metabolic shaker at 25° for 30 min. The reaction mixture (1 ml) contained 15 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 50 mM Tris–HCl buffer (pH 7.4), 10 mM L-ornithine, 10 mM NH_4Cl, 5 mM potassium phosphate buffer (pH 7.4), 16.6 mM $KHCO_3$, 3 mM ATP, 25 mM sucrose, 2% ethanol, 6.3 mg mitochondrial protein and additions indicated. The gas phase was 95% O_2 and 5% CO_2 and the final pH was 7.4. The reaction was stopped by addition of $HClO_4$ and citrulline was determined as described by CHARLES, TAGER AND SLATER[16].

Additions	*ΔCitrulline (μmoles)*
None	1.4
Oligomycin (10 μg)	0.2
Rotenone (2 μg)	0.2
2,4-Dinitrophenol ($5 \cdot 10^{-5}$ M)	0.3
Oligomycin + rotenone	0.6
2,4-Dinitrophenol + oligomycin	1.8
2,4-Dinitrophenol + rotenone	0.5
2,4-Dinitrophenol + rotenone + oligomycin	1.6
Atractyloside (300 μg)	3.2
2,4-Dinitrophenol + rotenone + oligomycin + atractyloside	0.1

* The stimulation by atractyloside of citrulline synthesis in the absence of inhibitors is discussed elsewhere[17].

The effect of depletion of high-energy intermediates of oxidative phosphorylation on the utilization of exogenous ATP for citrulline synthesis is shown in Fig. 1. In the experiment of Fig. 1A, mitochondria were preincubated for 5 min with dicoumarol. Oligomycin *plus* rotenone were added after 5 min, followed 5 min later by serum albumin (to bind the dicoumarol). When ATP was added after a further 5 min, an appreciable synthesis of citrulline occurred. However, if succinate was added together with the ATP, very little citrulline formation took place. Succinate did not inhibit citrulline synthesis if antimycin was also present. β-Hydroxybutyrate did not inhibit citrulline synthesis under these conditions, in which rotenone was present (not shown). The experiment of Fig. 1B shows that oligomycin must be present in order to obtain citrulline synthesis from ATP after the preincubation.

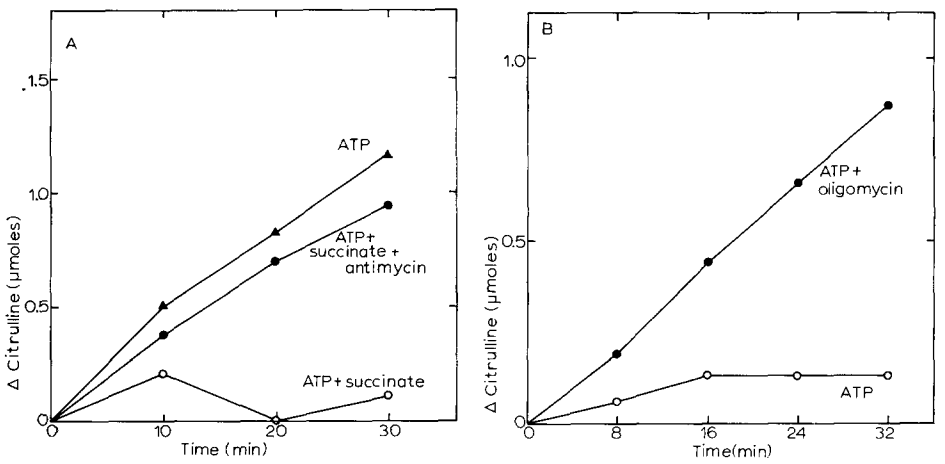

Fig. 1. Effect of depletion of high-energy intermediates of oxidative phosphorylation on the utilization of exogenous ATP for citrulline synthesis in rat-liver mitochondria. Each point represents a separate incubation. A. Experimental conditions as in Table I, except that ATP was initially absent and mitochondria (6.3 mg protein) were preincubated with 20 μM dicoumarol. After 5 min, 10 μg oligomycin and 2 μg rotenone were added, followed 5 min later by 8 mg serum albumin. After another 5 min, citrulline synthesis was initiated by adding 10 mM ATP (▲—▲), or 10 mM ATP *plus* 10 mM succinate (○—○ and ●—●). In curve (●—●) 0.5 μg antimycin was added at the same time as oligomycin *plus* rotenone. B. Preincubation of the mitochondria (6.1 mg protein) as described in A, except that oligomycin was omitted in one set of incubations (○—○). At zero time, 10 mM ATP was added in both sets of incubations.

These results show that exogenous ATP can be used for citrulline synthesis in mitochondria depleted of high-energy intermediates of oxidative phosphorylation. They also show that the subsequent generation of high-energy intermediates, either during succinate oxidation in the presence of oligomycin, or from ATP itself in the absence of oligomycin, leads to a very marked inhibition of this synthesis. Even when mitochondria were not preincubated with uncoupler, a stimulation of citrulline synthesis was observed when oligomycin and rotenone were added together (Table I, line 5) probably because the formation of high-energy intermediates both from substrate oxidation and from ATP was inhibited. The information available at present does not allow a distinction to be made between a direct effect of high-energy intermediates, for instance on the adenine nucleotide translocase (*cf.* ref. 9), or an indirect

one. Experiments with other ATP-utilizing systems should be done in order to establish the generality of this phenomenon.

MEISNER[18] has recently reported that the net efflux of adenine nucleotides from the mitochondria may be an energy-requiring process. However, since this net efflux is atractyloside insensitive, it is probably unrelated to the effect reported in this paper.

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