SOME ENERGY LINKED REACTIONS IN THE KEILIN-HARTREE HEART MUSCLE PREPARATION

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Previously we (Kettman and King, 1963) demonstrated an oligomycin-sensitive ATPase in the Keilin-Hartree heart muscle preparation (also cf. Huijing and Slater, 1961). The ATPase was also solubilized and the soluble enzyme was insensitive to the antibiotic. This note reports some energy linked reactions in the Keilin-Hartree preparation. A soluble extract from heart mitochondria stimulates not only the transhydrogenase reaction but also phosphorylation when the heart muscle preparation is used as the electron transport system for succinate oxidation.

Methods and Materials -- The Keilin-Hartree preparation was prepared from beef heart at room temperature according to their method adopted in this laboratory (King, 1961); differential centrifugation was used instead of the precipitation at pH 5.5. The manipulation of some batches was carried out in a cold room (4-9°) and the final preparation was suspended in 0.25 M sucrose. These batches made at low temperatures were labeled as the "cold heart muscle preparation." The alkali extractable soluble factor (AESF) was prepared as outlined in Fig. 1.

Results and Discussion -- The activities of the various reactions were somewhat variable from batch to batch. In general, however, some characteristics stood out clearly. For the succinate supported transhydrogenase (Table I), dependence on aerobosis and electron transport was observed. Oligomycin generally stimulated the reaction. These facts are very similar to the results reported by Danielson and Ernster (1963) using a submitochondrial particle. Apparently this reaction did

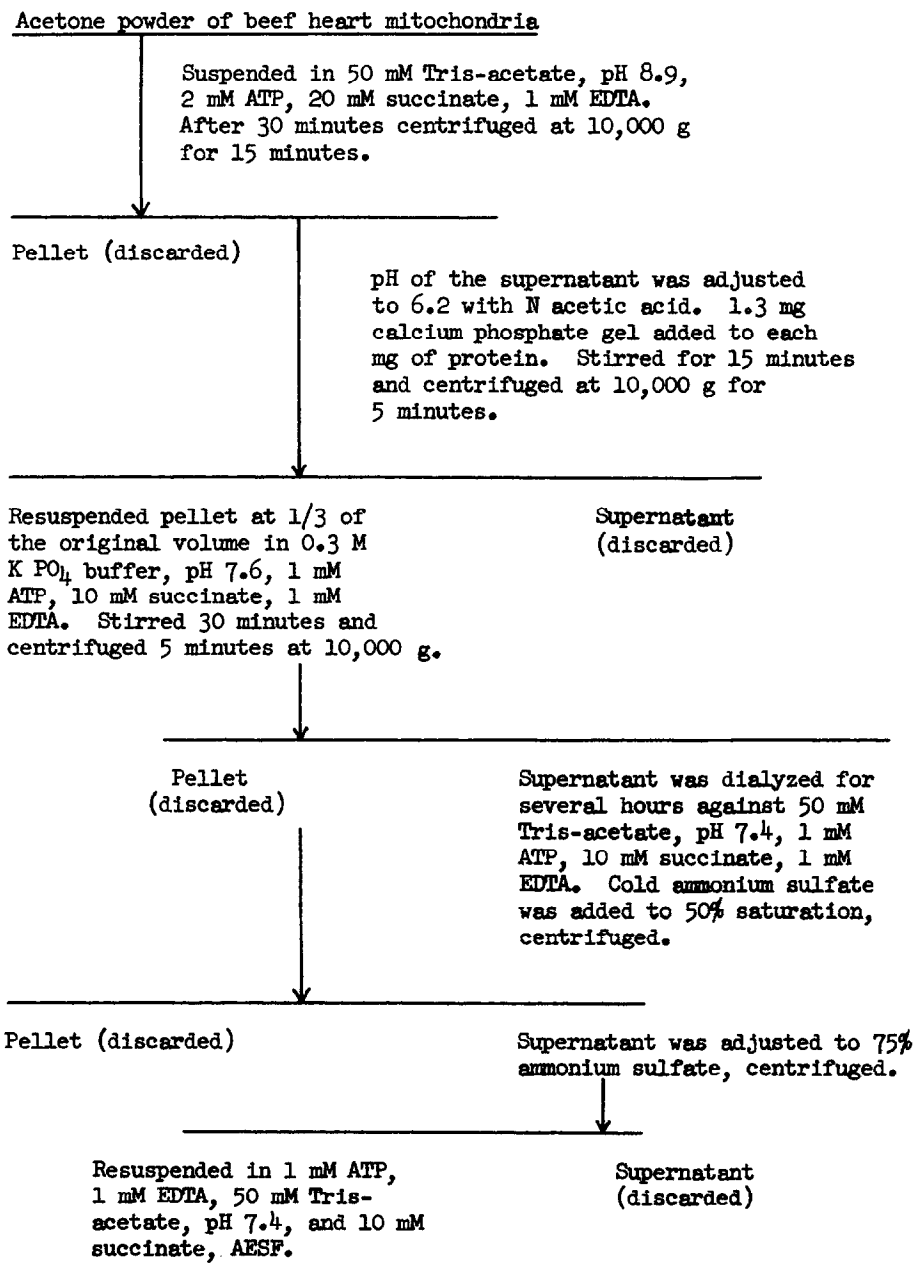

Fig. 1

not require inorganic phosphate. The stimulation by AESF was maximally manifested only in the presence of Mg^{++}. Titration of the heart muscle

Table I

Succinate supported transhydrogenase *

Additions	μmole TPNH formed/min./g protein
1. None	38
2. + oligomycin 4 μg	66
3. + AESF (1.2 mg)	60
4. + 2 mM sulfide or in the absence of O_2	0

* The assay mixture contained 20 μg rotenone, 50 mM Tris-acetate, pH 7.4, 15 μg crysalline lactate dehydrogenase (Sigma), 200 mM lactate, 200 μM DPN, 200 μM TPN, 10 mM succinate and 0.5 to 1.0 mg of the heart muscle preparation; total volume, 3 ml. Ten mM $MgCl_2$ was added with AESF. Increase in absorbance at 340 mμ was measured on a Cary spectrophotometer, model 11, at about 25° using a 0-0.1 slide wire. Succinate or TPN was used to start the reaction. The reading was made against the blank in the spectrophotometer which contained all constituents except succinate.

preparation with AESF was made and a maximal activity could be obtained. The stimulations of AESF and oligomycin were non-additive.

The ATP-linked transhydrogenase in the cold heart muscle preparation was also demonstrated (Table II), which is at variance with the reports by Haas (1964). Oligomycin inhibited the reaction. Likewise, AESF caused some depression. The failure to demonstrate the ATP driven reduction of DPN by succinate (the Chance reaction)(cf. Hommes, 1963) in the heart muscle preparation was indicated in Table III. This observation disagrees with the report by Gawron et al. (1964), but agrees with Haas (1964). The lack of ATP interaction in the reduction of DPN by succinate and the presence of ATP driven transhydrogenase cannot be explained satisfactorily. Indeed, Ernster (1964) has explained that the Gawron observation is due to the action of the contaminating malate dehydrogenase rather than direct hydrogen transfer. However, our observation seems to be similar to the ATP supported ion accumulation by a

Table II

ATP supported transhydrogenase *

Additions	μmole TPNH formed/min./g protein
1. None	25
2. + oligomycin (4 μg)	0
3. + AESF (1 mg)	13
4. - ATP	0

* Conditions similar to Table I. Assay mixture contained: 2 mM sodium sulfide, 50 mM Tris-acetate, pH 7.4, 200 μM DPN, 200 μM TPN, 15 μg lactate dehydrogenase, 200 mM lactate, 10 mM $MgCl_2$, 1.5 mM ATP and 0.5 to 1.0 mg of the heart muscle preparation. The reaction was started by TPN when the enzyme had been preincubated with ATP for approximately 5 minutes at room temperature or by ATP when the enzyme had been preincubated with TPN. A lag period was observed when ATP was used to start the reaction.

non-phosphorylating preparation described by Vasington and Greenawalt (1964).

A slight but significant stimulation of oxidative phosphorylation by AESF was constantly observed. Demonstration was best with the cold heart muscle preparation. The heart muscle preparation prepared at room temperature showed no phosphorylation. Because of the apparent presence of an uncoupler(s) in AESF (cf. Table II), a selective adsorption technique was involved similar to that described by Green et al. (1963). Incubation followed by centrifugation, resuspension, and assay gave the results outlined in Table IV. Racker (1964) has also indicated a reconstitution of oxidative phosphorylation in the heart muscle preparation.

From these results, it appears that the comments of King (1963) are correct; the term "non-phosphorylating preparation" now has less meaning. Indeed, the proposal by Slater (1953) concerning the involvement of

Table III

ATP supported reduction of DPN by succinate *

Additions	μmole DPNH formed/min./g protein
1. None	0
2. + AESF	0

* Conditions similar to Table I. Assay mixture contained: 50 mM Tris-acetate, pH 7.4, 2 mM sodium sulfide, 200 μM DPN, 10 mM succinate, 10 mM $MgCl_2$, 1.5 mM ATP and 1 mg of the heart muscle preparation. The reaction was started by succinate when the enzyme had been preincubated with ATP for approximately 5 minutes at room temperature or by ATP when the enzyme had been preincubated with succinate.

Table IV

Stimulation of oxidative phosphorylation by AESF *

	P/O		
Preparation	No AESF	+ AESF	△ P/O
Cold HMP 443	0.29	.45	0.16
Cold HMP 411	0.06	.18	0.12

* The cold heart muscle preparation (HMP) and AESF were incubated together with 10 mM $MgCl_2$, and 20 mM Tris-acetate, pH 7.4. The mixture was centrifuged for 25 minutes at 50,000 r.p.m., the residue was resuspended in 0.25 M sucrose. The assay system was similar to that used by Penefsky et al. (1960) with 20 mM succinate as the substrate. The AESF was purified to the gel eluate stage and dialyzed.

non-phosphorylated high energy intermediates in oxidative phosphorylation has now been gradually substantiated in certain aspects by experiments.

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