# STUDIES ON THE MECHANISM OF OXIDATIVE PHOSPHORYLATION

## I. PREPARATION AND PROPERTIES OF A PHOSPHORYLATING ELECTRON TRANSFER PARTICLE FROM BEEF HEART MITOCHONDRIA

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In our laboratory CRANE, GLENN AND GREEN[1] have described the preparation and properties of a submitochondrial particle which catalyzes the oxidation of DPNH** and succinate by molecular oxygen, and which contains the full complement of cytochromes and other components for these two oxidation processes. This electron transfer particle (ETP) catalyzes other oxidative reactions such as the oxidation of α-ketoglutarate, β-hydroxybutyrate and malate though much more slowly than the oxidation of succinate or DPNH. In the mitochondrion the dehydrogenases of the citric acid cycle are presumably directly linked to the heme chain of the electron transport particle. Since ETP isolated by the methods previously described is deficient with respect to the dehydrogenases of the citric acid cycle other than the succinic and DPNH dehydrogenases, it was hoped that under different conditions a more complex form of ETP with a full complement of dehydrogenases could be isolated from mitochondria and that such a more complete form of the particle would be capable of oxidative phosphorylation. This objective has been achieved but only in part. A particle can indeed be isolated which is more active than ETP in catalyzing the oxidation of metabolites other than DPNH and succinate and which can carry out oxidative phosphorylation coupled to these oxidations. But the specific activity of this particle in catalyzing phosphorylation-coupled oxidations is still lower than that of the original mitochondrion.

We have assumed as a working hypothesis that mitochondria consist of a repeating sub-unit which contains the entire complement of enzymes characteristic of the mitochondrion. In the course of fragmentation of the mitochondrion the sub-units are liberated, and depending upon the extent of damage sustained in the process they either retain all their original properties or become deficient in any of a spectrum of properties.

The present communication deals with the isolation and properties of a phosphorylating electron transfer particle from beef heart mitochondria which we shall refer to as PETP and which must be considered as a more complex form of ETP. Some of these results have been briefly reported[2].

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** The following abbreviations will be used in the text: electron transport particle, ETP; phosphorylating electron transport particle, PETP; diphosphopyridine nucleotide, DPN; reduced diphosphopyridine nucleotide, DPNH; triphosphopyridine nucleotide, TPN.

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## EXPERIMENTAL

### *Methods*

Inorganic phosphate was determined by the method of FISKE AND SUBBAROW[3]; glucose-6-phosphate by reduction of TPN in presence of the specific dehydrogenase[4]; ATPase by liberation of inorganic phosphate[5]; and protein by the biuret method[6]. Oxygen uptake was measured by the standard Warburg technique at 30° in a final volume of 3.0 ml. Oxygen uptake values which are reported in the text have been corrected for uptake in the absence of substrate. The endogenous respiration of PETP preparations was usually essentially zero. In no case was the endogenous respiration more than 10% of the experimental value. Preparations were assayed at the following protein levels: mitochondria, 4 to 6 mg; residue 4 to 6 mg; and PETP 8 to 12 mg. Unless otherwise stated each Warburg flask contained 5 μmoles ATP, 5 μmoles $MgCl_2$, 100 μmoles glucose, 0.5 mg hexokinase, and 20 to 40 μmoles phosphate. Where indicated the following were added: 20 μmoles pyruvate, 5 μmoles L-malate, 20 μmoles α-ketoglutarate, 50 μmoles succinate, 50 μmoles D(—)β-hydroxybutyrate, 10 μmoles *d-iso*citrate, 10 μmoles citrate, 10 μmoles *cis*-aconitate, 1 μmole DPN, 0.5 μmole TPN, 0.1 μmole CoA and 0.5 μmole cocarboxylase.

Oxidation of succinate and DPNH by oxygen was measured under the conditions previously described[1].

### *Reagents and chemicals*

Yeast hexokinase was prepared according to the method of BERGER *et al.*[7], and it was used in about 10 to 100 fold excess in experiments in which oxidative phosphorylation was being measured. The sources of coenzymes were as follows: CoA (Pabst), DPN (Sigma), TPN (Pabst), ATP (Pabst and Sigma), *d-iso*citrate (gift of Dr. Burris) glutathione (Schwarz) and cocarboxylase (Merck). Glucose-6-phosphate dehydrogenase was obtained from Sigma.

### *Preparation of mitochondrial suspensions*

Suspensions of particles prepared from beef heart muscle by the method described previously by CRANE *et al.*[1] and which we have referred to as mitochondrial suspensions are mixtures of intact and fragmented mitochondria. Such a mixture can be resolved by differential centrifugation into two fractions which may be referred to as the heavy and light mitochondrial fraction respectively. These are by no means homogeneous fractions. The heavy mitochondrial fraction contains a considerably higher proportion of intact mitochondria than the light fraction while the light mitochondrial fraction contains a considerably higher proportion of mitochondrial shells than the heavy fraction. There are other differences which will be considered at a later point. The details for separating the crude mitochondrial suspension into the heavy and light fractions are as follows. Either the frozen or fresh suspension was diluted with 0.25 *M* sucrose to bring the concentration of protein down to 20 to 24 mg per ml, and the pH was then adjusted to 7.1 with dilute alkali. The suspension was thoroughly homogenized at low speed in a motor-driven Potter-Elvehjem type homogenizer (glass-teflon), and then centrifuged in the No. 30 rotor of the Spinco preparative ultracentrifuge at 12,000 r.p.m. for 10 min. The sediment usually consisted of two well defined layers: an upper, light-colored, gelatinous layer (light mitochondrial fraction) and a well-packed lower layer (heavy mitochondrial fraction) which was dark-colored. The separation of the two layers was accomplished by adding 0.25 *M* sucrose to cover the sediment completely and then sloughing off the upper layer by agitation with a metal pestle rotated at high speeds. The lower layer was suspended in 0.25 *M* sucrose. The sedimentation and separation procedure was then repeated to remove any residual gelatinous material. The thick suspension of heavy particles was stable with respect to oxidative phosphorylation for several months when stored at —10°.

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*Preparation of PETP*

The heavy mitochondrial suspension was diluted with 0.25 *M* sucrose to bring the protein concentration down to 50 mg per ml and was then mixed at −5° with 0.6 vols. of 0.5 *M* potassium phosphate buffer of pH 7.8, 0.313 vols. of 95% ethyl alcohol and 0.23 vols. of a solution containing per ml 9 μmoles ATP, 9 μmoles Versene, 7.5 μmoles pyruvate, 1.5 μmoles malate, 9 μmoles magnesium chloride, 0.15 μmole CoA (SH form), 1.5 μmoles DPN, 7.3 μmoles glutathione and 0.73 μmole cocarboxylase. After homogenization the suspension was centrifuged for 5 min at 15,000 r.p.m. in the No. 30 head of the Spinco. The sediment contained the fraction hereinafter referred to as the residue. The supernatant fluid which contained PETP was then centrifuged at 30,000 r.p.m. for 30 min. Both the first and second sediments (separately) were washed twice with about 20 volumes of 0.25 *M* sucrose. Residue and PETP were finally suspended in 0.25 *M* sucrose fortified with 1 μmole phosphate buffer of pH 7.2 and 1 μmole ATP per ml. All manipulations were carried out at 0° unless otherwise stated.

Table I contains a summary of the recovery of protein during the isolation procedure and also the distribution of succinic and DPNH activity between residue and PETP. The yield of PETP varied from about 6 to 12% of the protein content of the starting mitochondrial suspension. Reextraction of the residue did not increase the yield significantly. Less than 2% of the protein in the original heavy mitochondrial suspension was found in solution after sedimentation of the residue and PETP fractions. Even this small amount can be reduced further by prolonged centrifugation of the supernatant at 40,000 r.p.m. Thus it would appear that relatively little soluble protein was released during the isolation procedure.

TABLE I

DISTRIBUTION OF ENZYMIC ACTIVITIES IN FRACTIONS OBTAINED DURING PREPARATION OF PETP

| *Fraction* | *Protein mg* | *Per cent of original protein* | *DPNH oxidation μmoles/min/mg protein* | *Succinate oxidation μmoles/min/mg protein* |
|---|---|---|---|---|
| Mitochondria | 2856 | 100 | 0.82 | 0.28 |
| Residue | 2499 | 84 | 0.73 | 0.16 |
| PETP | 230 | 8 | 3.35 | 0.72 |
| Soluble | 84 | 3 | — | — |

PETP could be prepared conveniently by yet another method. The alcohol-phosphate procedure was applied directly to the mitochondrial suspension prior to resolution into the heavy and light fractions. The supernatant fraction (after removal of the particles sedimenting at 15,000 r.p.m.) was now found to consist of light and heavy particles (non-mitochondrial) which are readily separable by the same tactics described above for separating the light and heavy fractions of the original mitochondrial suspension. The heavy well packed particles have the properties of PETP while the light fluffy particles have the properties of ETP. Table II summarizes the data on the recovery of protein and units in the PETP and ETP fractions obtained by this method.

The oxidation of DPNH by molecular oxygen catalyzed by the original unfractionated mitochondrial suspension showed only a partial requirement for added

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TABLE II

ACTIVITIES OF FRACTIONS PREPARED FROM UNFRACTIONATED MITOCHONDRIAL SUSPENSIONS

| *Fraction* | *Total protein mg* | *DPNH oxidation μmoles/min/mg protein* | *Pyruvate + malate oxidation μatoms $O_2$/min/mg protein* | *Phosphorylation μmoles P/min/mg protein* |
|---|---|---|---|---|
| Mitochondria | 9640 | 2.0 | | |
| Residue | 5130 | 1.42 | | |
| PETP (packed) | 738 | 3.4 | 0.024 | 0.067 |
| ETP (fluffy) | 1090 | 8.1 | 0.002 | 0.002 |

cytochrome *c*. Dr. F. L. CRANE in our laboratory has established that the particles in the suspension may be classified into two types—one of which shows no requirement for added cytochrome *c* ("closed" type electron transfer chain[8])—while the other shows a partial or complete requirement for added cytochrome *c* ("open" type electron transfer chain). All of the particles with closed chains end up in the PETP or ETP fractions while all of the particles with open chains end up in the residue fraction. There is thus a close correlation between the proportion of particles in the original unfractionated mitochondrial suspension which show no requirement for added cytochrome *c* and the yield of PETP.

Table III shows the recovery of phosphorylation units (μmoles inorganic phosphate esterified per min per mg protein) in residue and PETP as compared to the units present in the original mitochondrial suspension. There was some loss in the capacity for oxidative phosphorylation during the isolation procedure but qualitatively (as measured by P/O ratios) there was in many though not all preparations no diminution in the efficiency of the process. The loss in phosphorylation capacity is of course directly referable to a decline in the specific rate of oxidation of substrates of the citric acid cycle.

TABLE III

DISTRIBUTION OF OXIDATIVE PHOSPHORYLATION ACTIVITY IN FRACTIONS OBTAINED DURING PREPARATION OF PETP

| *Fraction* | *Oxidation units** | | *Phosphorylation units** | |
|---|---|---|---|---|
| | *— cofactors* | *+ cofactors* | *— cofactors* | *+ cofactors* |
| Mitochondria | 116 | 179 | 300 | 405 |
| Residue | 88 | 113 | 221 | 254 |
| PETP | 3.3 | 6.2 | 9.2 | 9.0 |

* μatoms oxygen or μmoles phosphate per minute.
Cofactor mixture: 1 μmole DPN, 0.1 μmole CoA, 0.5 μmole cocarboxylase.
Substrate: pyruvate + malate.

*Stability*

Some data on the stability of suspensions of PETP and crude mitochondria are shown in Table IV. There was no demonstrable deterioration of these suspensions in the course of storage at —10° for extended periods of time. A gradual loss in activity was observed when a preparation was subjected to continued freezing and thawing or storage at 0°.

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TABLE IV

STABILITY OF MITOCHONDRIA AND PETP TO FREEZING

| *Preparation* | *Frozen in sucrose for* | *ΔP μmoles* | *ΔO μatoms* | *P/O* |
|---|---|---|---|---|
| Mitochondria | 1 day | 19.3 | 6.7 | 2.88 |
| Mitochondria | 7 days | 16.0 | 6.4 | 2.50 |
| PETP | 0 days | 10.2 | 5.6 | 1.82 |
| PETP | 7 days | 9.2 | 5.6 | 1.64 |

Substrate: pyruvate + malate.
ΔP = disappearance of inorganic phosphate corrected for blank.
ΔO = oxygen uptake corrected for blank.
Usual Warburg experiments and conditions.

*Composition*

The composition and spectral properties of PETP will be considered in detail elsewhere. PETP preparations showed higher ATPase activity than either the residue or the original mitochondrial suspension but as yet no correlation has been found between the level of ATPase activity in PETP and the P/O ratio. It would appear from such data that ATPase does not function under phosphorylating conditions. Presumably ATP generated in the course of oxidative phosphorylation might not be available as substrate for ATPase.

*Phosphorylation properties*

Table V summarizes some representative data on the P/O ratios obtained for the oxidation of various metabolites with PETP, residue, and mitochondria respectively. With the exception of β-hydroxybutyrate all the metabolites were oxidized in more than one step. Thus the oxidation of α-ketoglutarate involved largely the two step conversion of α-ketoglutarate to malate, and only in small part the further oxidation of malate. Since the succinic oxidation step did not involve phosphorylation[2], the corrected P/O ratio for oxidation of α-ketoglutarate should be roughly twice the observed ratio, *i.e.*, 4. Malate and pyruvate were used in conjunction since neither substrate alone was oxidized at an appreciable rate. Oxalacetate could quantitatively substitute for malate. The product of interaction of these two substrates is citrate as shown by citrate accumulation in the presence of fluorocitrate. In absence of added TPN and DPN, citrate, *cis*-aconitate, or *iso*citrate did not undergo oxidation at a

TABLE V

OXIDATION AND PHOSPHORYLATION RATES IN VARIOUS FRACTIONS WITH DIFFERENT SUBSTRATES

| *Substrate* | *Mitochondria* | | *PETP* | | *Residue* | |
|---|---|---|---|---|---|---|
| | *O** | *P*** | *O* | *P* | *O* | *P* |
| Pyruvate + malate | 0.137 | 0.360 | 0.032 | 0.080 | 0.122 | 0.312 |
| α-Ketoglutarate | 0.033 | 0.062 | 0.018 | 0.040 | | |
| β-Hydroxybutyrate | 0.047 | 0.105 | 0.015 | 0.023 | | |
| L-Glutamate | 0.047 | 0.112 | 0.007 | 0.018 | | |

* μatoms oxygen/min/mg.
** μmoles phosphate esterified/min/mg.

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significant rate. However oxidation proceeded satisfactorily in the presence of these cofactors. The addition of TPN was not required for maximal rate of oxidation with pyruvate + malate as substrates*. Glutamate oxidation probably involves a three step conversion to malate. The calculated P/O value for the oxidation of glutamate to malate is 2.3 assuming 3 for the glutamate to α-ketoglutarate step, 4 for the α-ketoglutarate to succinate step and 0 for the succinate to fumarate step. The P/O ratio for the oxidation of β-hydroxybutyrate is usually closer to 2 than 3 although there is some variation in this regard from preparation to preparation of PETP.

All these phosphorylation reactions are abolished in whole or in part by 2,4-dinitrophenol at relatively low concentrations (*cf.* Table VI). Since one out of each four phosphorylations catalyzed in the α-ketoglutarate to succinate step is DNP-insensitive[9,10] there is always some residual phosphorylation in presence of dinitrophenol when α-ketoglutarate or glutamate is used as substrate.

TABLE VI

EFFECT OF 2,4-DINITROPHENOL ON PHOSPHORYLATION AND OXIDATION CATALYZED BY PETP

| *Conditions* | *ΔP μmoles* | *ΔO μatoms* |
|---|---|---|
| Pyruvate + malate | 10.5 | 9.3 |
| + DNP $10^{-4}M$ | 0.9 | 6.0 |
| α-Ketoglutarate | 9.6 | 5.9 |
| + DNP $10^{-4}M$ | 2.3 | 6.2 |

Assayed in presence of cofactors under conditions of the Warburg experiments.

The presence of inorganic phosphate is mandatory to attain the maximal rate of oxidation of pyruvate and malate catalyzed by PETP (Table VII). However neither ATP nor the hexokinase acceptor system influenced the rate of oxidation significantly. All the activity values shown in Table VII are low in consequence of the extensive washing of PETP in the absence of ATP and phosphate. There is present even in well washed suspensions of PETP a significant amount of bound inorganic phosphate and of adenine nucleotide[11].

* The conditions for the oxidation of citrate, *iso*citrate and *cis*-aconitate present some puzzling features. When mitochondria are allowed to act upon limited amounts of pyruvate + malate in equimolar concentrations the observed oxygen uptake corresponds closely with the theory for complete oxidation to $CO_2$ and water of both pyruvate and malate. Thus citrate which is formed by the condensation of acetyl CoA and oxalacetate is undergoing oxidation through the cycle by way of *iso*citrate. Nonetheless the addition of TPN is not essential for *iso*citrate oxidation when this is generated from pyruvate + malate. When citrate or its equilibrium forms are added directly to beef heart mitochondria no appreciable oxygen uptake obtains in absence of added TPN. This anomalous result may be explained on the basis that when pyruvate and malate undergo oxidation the proper coenzyme form for the oxidation of *iso*citrate is generated from bound pyridinenucleotide. This generation process does not proceed or is inhibited when citrate is added as such and addition of TPN then becomes mandatory. No significant phosphorylation appears to be coupled with the TPN-catalyzed oxidation of *iso*citrate by mitochondria, residue or PETP.

One further anomaly of beef heart mitochondria is the fact that the pyruvic oxidase cannot be assayed by the reaction with ferricyanide unlike the equivalent enzyme from pigeon breast muscle.

The above results parallel closely those reported by C. M. MONTGOMERY AND J. L. WEBB in the *J. Biol. Chem.*, 221 (1956) 347, for the oxidation characteristics of rat heart mitochondria.

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TABLE VII

REQUIREMENTS FOR COMPONENTS OF ASSAY SYSTEM FOR OXIDATION PHOSPHORYLATION

| | *Rate of phosphorylation* μmoles/min/mg protein | *Rate of oxygen uptake* μatoms/min/mg protein |
|---|---|---|
| Complete system | 0.036 | 0.025 |
| minus phosphate | — | 0.011 |
| minus ATP | 0.027 | 0.022 |
| minus hexokinase, glucose | 0 | 0.027 |
| minus phosphate, hexokinase, ATP, $Mg^{++}$, glucose | — | 0.008 |

Complete system contains:

30 μmoles phosphate, 2 μmoles ATP, 20 μmoles pyruvate, 5 μmoles L-malate, 0.5 mg hexokinase, 100 μmoles glucose, 5 μmoles $MgCl_2$, 100 μmoles Tris pH 7.1, and 10.6 mg PETP protein twice washed.

Glucose-6-phosphate is formed as the product of glucose esterification by ATP in presence of the hexokinase system. This was demonstrated in a boiled extract of the incubation mixture by reduction of TPN in presence of the glucose-6-phosphate dehydrogenase.

## DISCUSSION

It was recognized as early as 1951 in our laboratory[12,13] that intact mitochondria are not essential for oxidative phosphorylation and that sub-mitochondrial units can show at least in part the same phenomenon. More recently LEHNINGER and his colleagues[14–16] have treated rat liver mitochondria with digitonin and obtained in low yield sub-mitochondrial particles which carry out oxidative phosphorylation in presence of β-hydroxybutyrate (P/O ratios from 1 to 3). These digitonin-treated particles have largely lost the capacity to oxidize substrates of the citric acid cycle.

The almost exclusive use of liver mitochondria for studies of oxidative phosphorylation has delayed recognition of the facts (1) that instability is not necessarily an invariant attribute of the phosphorylation system; (2) that heart is a far superior source for a stable phosphorylating system; and (3) that slaughter house material can be used successfully for preparation of actively phosphorylating mitochondria.

We were unable to understand the rationale of the method of preparation of PETP until Dr. HANS RIS of the Department of Zoology, University of Wisconsin, examined the different stages in the preparation by electron microscopy. A full documentary report of this work will be given in a subsequent communication of this series. According to the photographs the starting beef heart particulate suspension is a mixture of principally (1) intact mitochondria with dense cristae; (2) small masses of dense cristae which are not contained within the mitochondrial form; and (3) spherical mitochondrial shells or vesicular forms of varying diameter with few or no cristae. There are in addition relatively small amounts of non-mitochondrial structures such as sarcosomes and myofibrils.

When the crude mitochondrial suspension is fractionated into the heavy and light fractions in 0.25 *M* sucrose, 1 and 2 are concentrated in the former and 3 in the latter. The alcohol-phosphate treatment applied to the heavy mitochondrial

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fraction leads to the concentration of the intact mitochondria into the residue and of the mitochondria-free masses of dense cristae into the PETP fraction. The light mitochondrial fraction which contains principally vesicular forms and relatively little of mitochondria-free cristae is an inferior starting point for the preparation of PETP (*cf.* Table VIII) but is an excellent starting point for the preparation of ETP. Thus when the alcohol-phosphate treatment is applied directly to the unfractionated mitochondrial suspension (*i.e.* with both heavy and light components) three easily separable fractions are obtained *viz.* residue, PETP and ETP.

TABLE VIII

ACTIVITY OF RESIDUE AND PETP PREPARED FROM HEAVY, LIGHT AND MEDIUM MITOCHONDRIAL SUSPENSIONS

| | *Type of mitochondrial suspension* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Heavy fraction* | | | | *Medium fraction* | | | | *Light fraction* | | | |
| | *— cofactors* | | *+ cofactors* | | *— cofactors* | | *+ cofactors* | | *— cofactors* | | *+ cofactors* | |
| | *P** | *O*** | *P* | *O* | *P* | *O* | *P* | *O* | *P* | *O* | *P* | *O* |
| Mitochondria | 0.360 | 0.137 | 0.461 | 0.176 | 0.237 | 0.092 | 0.320 | 0.141 | 0.173 | 0.059 | 0.205 | 0.097 |
| Residue | 0.312 | 0.122 | 0.271 | 0.137 | 0.242 | 0.096 | 0.278 | 0.124 | 0.105 | 0.042 | 0.161 | 0.094 |
| PETP | 0.080 | 0.032 | 0.104 | 0.074 | 0.045 | 0.016 | 0.044 | 0.030 | 0.021 | 0.010 | 0.023 | 0.030 |

Cofactor mixture contains 1 μmole DPN, 0.1 μmole CoA, and 0.5 μmole cocarboxylase.
* μmoles phosphate esterified/min/mg protein.
** μatoms oxygen uptake/min/mg protein.
Substrate-pyruvate + malate.

The following tentative conclusions may be drawn from these observations. The original crude mitochondrial suspension already contains the various particle types. As has been indicated, some of these particle types are separable by differential centrifugation in 0.25 *M* sucrose while other particle types are more readily prepared in the alcohol-phosphate medium. Although the primary role of the alcohol-phosphate fractionation procedure appears to be that of facilitating the centrifugal separation of already formed units rather than that of fragmenting intact mitochondria, particle modification is not excluded. The non-mitochondrial vesicular forms with few or no cristae are the principal constituents of the ETP fraction which is very active in electron transport but essentially inactive in oxidative phosphorylation. Intact cristae seem to be the essential requirement for oxidative phosphorylation, either free or mitochondria-bound.

The fact that only a limited fixed amount of active PETP can be extracted from the crude mitochondrial suspension can now be explained on the basis that, as prepared, the suspension contains only a limited amount of mitochondria-free cristae. No more is produced during the alcohol-phosphate treatment.

It is significant that the mitochondria-free cristae show a "closed" type of electron transport system whereas the mitochondria-bound cristae show an "open" type. The conversion of an open to a closed system must parallel therefore the process by which cristae are torn loose from the intact mitochondria.

The origin of the active constituent particles of the PETP is thus uncertain. Either they were formed by the fragmentation of intact mitochondria during the homogenization of the beef heart tissue and/or during the subsequent procedures in the preparation of the mitochondrial suspension or they were formed by autolytic

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processes in the period of time which elapses between the collecting of the tissue and the processing. Dr. HANS RIS has reported to us that direct examination of the same heart tissue which was subsequently used for preparation of mitochondria failed to disclose any evidence of fragmented or vesicular mitochondria and it thus appears probable that the active particles of PETP are formed in consequence either of the homogenization of the tissue in the macro blendor or of processes which proceed during the subsequent centrifugation and resuspension phase.

The loss of oxidative capacity which accompanies the liberation of cristae from mitochondria is a process which has to be controlled before a PETP preparation can be obtained which is equal to mitochondria in oxidative capacity.

The conversion or transition of cristae into vesicles is clearly visible in electron microscope photographs of beef heart mitochondrial suspensions which have been exposed to the alcohol-phosphate reagent.

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SUMMARY

Methods for the preparation of actively phosphorylating electron transport particles from beef heart mitochondrial suspensions are described. The stability of the phosphorylation process both in the beef heart mitochondria and the derivative particles is of a different order of magnitude from that of rat liver mitochondria. The maximum P/O ratios attainable with PETP are close to the highest values recorded in the literature. 2,4-Dinitrophenol abolishes oxidative phosphorylation except for substrate level phosphorylation accompanying oxidation of α-ketoglutarate.

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