Oxidative phosphorylation in an enzyme fraction from mitochondrial extracts

It has been generally observed that oxidative phosphorylation with maximum efficiency occurs only in mitochondria with relatively intact morphology. For this reason the enzymic mechanisms involved in the energy-coupling have been inaccessible to direct study and remain essentially unknown[1]. In this paper we wish to report that oxidative phosphorylation can be demonstrated to occur in a lipoprotein fraction of relatively low particle weight separated from extracts of mitochondria. The P:O ratios observed approach the highest values reported with intact mitochondria.

Washed rat liver mitochondria prepared by the sucrose method are extracted with cold 1% digitonin. The extracts are clarified by centrifugation at 75,000 × *g* for 25 minutes. Centrifugation of the supernatant solution at 100,000 × *g* yields a small sediment which is resuspended in 0.25% digitonin and resedimented at 100,000 × *g*. When an aqueous suspension of this pellet is supplemented with *dl*-β-hydroxybutyrate (BOH), adenosine diphosphate (ADP), inorganic P (P_i), and oxygen, the BOH is oxidized to acetoacetate, which accumulates quantitatively. Accompanying the oxidation is net uptake of P_i, measured by an isotopic method[2]. In the absence of substrate no phosphorylation occurs. Typical data are shown in Table I. The P:O ratios are consistently above 1.5 and many approach the value 3.0. In the absence of added ADP rate of oxidation is greatly reduced, indicating dependence of respiration on P acceptors. The phosphorylation is uncoupled by dinitrophenol (DNP), gramicidin, and dicumarol. Significantly, the phosphorylation does not require addition of Mg^{++} or Mn^{++}, which are in fact inhibitory; Ca^{++} does not uncouple phosphorylation in this isolated enzyme preparation, although it is an extremely effective uncoupling agent in intact mitochondria.

TABLE I

OXIDATIVE PHOSPHORYLATION IN ISOLATED ENZYME SYSTEM

Test system (3.0 ml) contained 0.01 *M* P_i, pH 6.0 ($1.6 \cdot 10^6$ c.p.m. ^{32}P), 0.01 *M* *dl*-BOH, 0.0024 *M* ADP, and enzyme fraction (140 γ N); gas phase air; 15 minutes at 22°. Data in μmoles.

Components	$+\Delta$ *Acetoacetate*	$-\Delta P_i$	*P:O*
Complete system	0.22	0.53	2.4
Complete minus BOH	0.00	0.00	—
Complete plus $5 \cdot 10^{-5}$ *M* DNP	0.22	0.00	0.0
Complete plus 60 γ gramicidin	0.31	0.00	0.0
Complete plus 0.0026 *M* $CaCl_2$	0.22	0.50	2.3

Using these enzyme preparations it is also possible to demonstrate phosphorylation coupled to two well-defined segments of the respiratory chain. The experiments described in Table II involved spectrophotometric measurement of the reduction of ferricytochrome *c* by BOH[3]; reoxidation of ferrocytochrome *c* was blocked with cyanide. The $P/2Fe^{++}$ = P:O ratio was about 1.0 in a large series of experiments. It is seen that the phosphorylation is DNP-sensitive. Omission of either P_i or ADP greatly decreases the rate of reduction. Antimycin A completely blocks both reduction of cytochrome *c* and phosphorylation. Chemically reduced DPNH is oxidized in these preparations, but by an antimycin-insensitive, non-phosphorylating pathway. The experiments in Table III demonstrate phosphorylation coupled to oxidation of ferrocytochrome *c*[4] in this enzyme preparation. The P:O ratios for this segment were as high as 0.9, but most values fell in the range 0.4–0.6.

In the three different types of test, 92–99% of the labeled inorganic phosphate which disappeared during respiration was recovered as newly formed ATP following chromatographic separation of the adenine nucleotides on Dowex-1 columns. No radioactivity was found in ADP or AMP. The isolated enzyme complex specifically requires ADP as phosphate acceptor; the 5′-diphosphates of inosine, guanosine, uridine, thymidine, and cytidine are essentially inactive as P acceptors. The inactivity of inosine diphosphate suggests that the inosine nucleotide-dependent phosphate exchanges observed by Raw[5] in digitonin extracts of mitochondria were not primarily associated with oxidative phosphorylation *per se*.

The enzyme preparations contain slight ATPase activity which is stimulated by DNP and Mg^{++}. The preparations catalyze incorporation of $^{32}P_i$ into ATP in the absence of electron flux[6]. However, the rate and extent of this exchange relative to the rate of oxidative phosphorylation of ADP are very much different from those observed in intact mitochondria.

TABLE II

PHOSPHORYLATION COUPLED TO SPAN: BOH → CYTOCHROME *c*

Test system (1.0 ml) contained 0.01 *M* *dl*-BOH, 0.0024 *M* ADP, 0.005 *M* P_i (pH 6.5; $1 \cdot 10^6$ c.p.m. ^{32}P), 0.005 *M* KCN and enzyme (50 γ N). Ferricytochrome *c* titrated into medium; 5 minutes at 25°, data in mμmoles.

Components	$+ 1/2\ O_2$ / Fe^{++}	$-$ / P_i	*P : O*
1. Complete	48.2	46.5	0.96
Complete minus BOH	0.0	0.0	
Complete plus DNP	106	1.2	0.01
Complete plus 0.1 γ antimycin A	0.0	0.0	
2. Complete	36.2	32.6	0.90
Complete minus ADP	18.0	0.0	0.0
Complete minus Pi	17.5	0.0	0.0

TABLE III

PHOSPHORYLATION COUPLED TO OXIDATION OF FERROCYTOCHROME *c*

Test system (3.0 ml) contained 0.01 *M* P_i (pH 5.5; $1.3 \cdot 10^6$ c.p.m. ^{32}P), 0.01 *M* ascorbate, $4 \cdot 10^{-5}$ *M* cytochrome *c*, 0.0024 *M* ADP, and enzyme (250 γ N); 15 minutes at 23°. Data in μatoms.

Components	O_2 *uptake*	$-$ / P_i	*P : O*
1. Complete system	1.28	0.84	0.66
Complete minus ascorbate	0.00	0.00	
Complete plus DNP	1.42	0.00	0.00

The enzyme preparations have an average particle weight of about 50,000,000 as judged by sedimentation rate (equivalent to less than 1/2,000 of the "particle weight" of the rat liver mitochondrion). Considerable functional and steric organization is still retained in these particles; for instance, it has been found that added DPN and cytochrome *c* do not "mix" freely with the DPN and cytochrome *c* present in the particles.

These preparations have already revealed significant new information on oxidative phosphorylation not heretofore approachable with intact mitochondria; complete experimental details will be presented for publication shortly. This investigation was supported by grants from the U.S. Public Health Service and the Nutrition Foundation, Inc.

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Oxidative degradation of uric acid by cell extracts of a *Pseudomonas*

The aerobic degradation of uric acid by bacteria has been reported by LIEBERT[1], KREBS AND EGGLESTON[2], and DI FONZO[3]. While this work was in progress, FRANKE AND HAHN[4] reported that growing cells and washed cell suspensions of *Pseudomonas aeruginosa* oxidize uric acid to oxalic acid, ammonia and carbon dioxide. Allantoin, allantoic acid, glyoxylic acid and urea were identified as intermediates in the breakdown.