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SITES OF ENERGY CONSERVATION IN OXIDATIVE PHOSPHORYLATION

Sir:

Direct spectroscopic and kinetic studies of respiratory carriers in mitochondria have led to the conclusion that the *reduced* forms of the carriers, especially DPNH, are involved in the "high energy" complexes which are intermediates in the phosphorylation of ADP.[1] Wadkins and Lehninger reached the opposite conclusion on the basis of an experiment on the effect of aerobiosis and anaerobiosis on the ATP-P_i^{32} exchange reaction.[2] We show here that their interpretation of their experimental result is not unique and actually affords support for the conclusion they seek to refute.

The reaction mechanism for oxidative phosphorylation on which Wadkins and Lehninger based their conclusions ignores spectroscopic studies of the rate with which ADP and uncoupling agents interact with the respiratory carriers.[1,3] Such studies indicate that two intermediates intervene between ADP and the carriers. More recently, Myers and Slater, in a study of ATP-ase activity of mitochondria, have concluded that their results support the existence of such intermediates.[4] Cohn and Drysdale have also proposed multiple intermediates in the phosphorus and oxygen exchange reactions.[5] One formulation for the function of two such intermediates in the oxidative phosphorylation mechanism for a particular pair of respiratory catalysts has been represented[1,6]

$$b''' + I \longleftrightarrow b''' \cdot I \qquad (1)$$

$$b''' \cdot I + c'' \longrightarrow c''' + b'' \sim I \qquad (2)$$

$$b'' \sim I + X \longrightarrow b'' + X \sim I \qquad (3)$$

$$X \sim I + P \longleftrightarrow X \sim P + I \qquad (4)$$

$$X \sim P + ADP \longleftrightarrow ATP + X \qquad (5)$$

The ATP-P^{32} exchange reaction is presumed to involve the reversible reactions of X and I in Eq. 4 and 5, and not the respiratory carriers directly, as Wadkins and Lehninger propose. The amounts of X and I available depend indirectly upon aerobiosis and anaerobiosis. Under anaerobic conditions X and I can be bound as $X \sim I$ and $b'' \sim I$, and the exchange will be slow. Under aerobic conditions in the absence of substrate, less binding occurs and the concentrations of X and I are higher, and the exchange will proceed at high ATP concentrations, just as has been found in Wadkins and Lehninger's Table I.[2] If, on the other hand, we assume, as Wadkins and Lehninger assume, that the oxidized form of the respiratory enzyme is the high-energy carrier, we find that binding of X and I as $X \sim I$ and $b''' \sim I$ is maximal under aerobic conditions and minimal under anaerobic conditions. This leads to the conclusion that the exchange reaction should have gone more rapidly under anaerobic conditions than under aerobic conditions which it did not do.

It now may be concluded that Wadkins and Lehninger's data on the 10-fold acceleration of the ATP-P_i^{32} exchange reaction under aerobic conditions support our earlier conclusions: (a) that the reduced forms of the respiratory pigments represent the carriers of the "high-energy" complex and (b) that intermediates exist between the respiratory carriers and ADP.

The ATP-P_i^{32} exchange reaction is at present poorly understood and may not yet provide a substantial basis for proof of any further hypothesis; the reaction is slow in the digitonin preparation, requires high ATP concentrations, and is affected by added ADP at concentrations outside the range of that needed for maximal rate of electron transfer.[7] However, Wadkins and Lehninger's data appear to provide additional evidence that the "high-energy" complex involves the reduced carrier. In their Table I, they find that the incorporation of P_i^{32} into ADP to form ATP^{32} gives over 67 times as much incorporation when the carriers are reduced than when they are oxidized. Since the mitochondria are stated to be anaerobic, this phosphorylation is attributed to the presence of the "high-energy" complexes of the reduced carriers.

Thus the data of Wadkins and Lehninger support in two ways our conclusion[1] that the reduced form of the respiratory carrier serves as the site of the "high-energy" complex. The transition from oxidized to reduced carriers gives first a one-tenth as rapid catalysis of the ATP-P_i^{32} exchange reaction caused by a binding of the reaction intermediates X and I by the reduced carriers and, second, a 67-fold greater anaerobic phosphorylation of ADP caused by "high energy" compounds of the reduced carriers.

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RECEIVED MARCH 20, 1957

THE CONVERSION OF *myo*-INOSITOL TO GLUCURONIC ACID BY RAT KIDNEY EXTRACTS

Sir:

We wish to report the presence of a soluble enzyme system from rat kidney which catalyzes the conversion of inositol to glucuronic acid.

The enzyme was prepared by homogenizing rat kidneys in a Potter–Elvehjem homogenizer in a

medium made of equal volumes of 0.2 *M* phosphate buffer *p*H 7.2 and 1.15% KCl. The homogenate was centrifuged in the Spinco ultracentrifuge at 90,000 × *g* for 30 minutes. The enzyme system contained in the supernatant fluid was salted out by the careful addition of solid ammonium sulfate at 0° (56 g. per 100 ml. initial volume) and was redissolved in 0.05 *M* phosphate buffer of *p*H 7.2. The assay for enzymatic activity was carried out in test tubes by incubating an aliquot of the enzyme solution with inositol. At the end of the incubation the tubes were heated for 2 minutes at 100° and the protein-free filtrate was assayed for glucuronic acid using the orcinol method of Mejbaum.[1] Table I summarizes a typical experiment. When inositol was omitted from the incubation mixture or when the enzyme solution was heat-

TABLE I

RATE OF FORMATION OF GLUCURONIC FROM INOSITOL

The reaction mixture contained 0.2 ml. of 1 *M* phosphate buffer *p*H 7.2, 0.2 ml. of 0.5 *M* inositol, 0.15 ml. of enzyme solution and distilled water to a final volume of 2 ml.; gas phase oxygen, incubation at 34°.

Time of incubation (minutes)	5	10	15	20	30	40
Glucuronic acid (γ)	50	75	101	118	147	160

inactivated prior to its use the amount of orcinol-reacting substances was 3–5% of that formed in the complete system.

In a large scale experiment in which 15 ml. of enzyme solution was used, 78.3 mg. of glucuronic acid was formed representing a 10% conversion of the added inositol. The glucuronic acid was isolated by column chromatography on Dowex-1 X10 (acetate form) using 0.4 *N* acetic acid as the eluting solvent and was identified by paper chromatography using the solvent systems: pyridine–ethyl acetate–acetic acid–water,[2] ethanol–water (88:12), acetone–water (80:20), *n*-butanol–acetic acid–water (100:21:50), and ethanol–acetic acid–water (80:10:10). The spots were revealed by spraying the paper with aniline oxalate and heating at 100° for 5 minutes. In all cases the isolated uronic acid migrated in a manner indistinguishable from authentic glucuronic acid and was resolved from guluronic, galacturonic, mannuronic, iduronic, 2-ketogluconic, 5-ketogluconic, and ascorbic acid, as well as from the corresponding lactones of the above uronic acids.

The sodium salt of the isolated uronic acid was crystallized from ethanol (*Anal.* Calcd. for $C_6H_9O_7Na \cdot H_2O$: C, 30.77: H, 4.70. Found: C, 30.86; H, 4.74). The free acid has a m.p. of 162–163° while the lactone had a m.p. of 176–178°. The brucine salt of the isolated uronic acid had a m.p. of 162–165°.

The isolated glucuronic acid as well as its lactone were optically inactive, indicating that it is a racemic mixture. *Escherichia coli* (Strain B) adapted on D-glucuronic acid utilized approximately half of a known amount of the isolated uronic acid. The remaining half was recovered from the culture medium and its specific rotation at equilibrium was found to be $[\alpha]^{24}_D$ −33.3° (*c* 0.48 in H_2O, *1 l* dm.) compared to −36.0° expected for L-glucuronic acid. The enzymatic formation of glucuronic acid from inositol occurs with the simultaneous uptake of oxygen (1–1.2 μmoles of oxygen per μmole of glucuronic acid formed) and the disappearance of an equivalent amount (± 10%) of inositol (determined by bioassay[3]).

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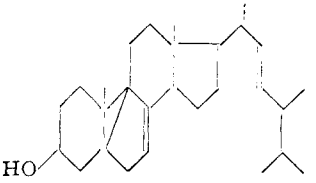
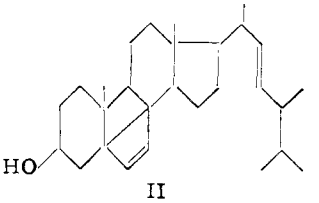
THE STRUCTURE OF PHOTOISOPYROCALCIFEROL[1]

Sir:

Recent studies by Velluz, Havinga and their associates[2] have established that the first step in the ultraviolet irradiation of ergosterol (9α-H, 10β-CH_3, 9,10-*anti*) is a bond cleavage reaction with the formation of a 9,10-*seco*-sterol type of intermediate. On the other hand, Dimroth and Windaus[3] have shown that a similar irradiation of the 9,10-*syn* epimer, isopyrocalciferol (9β-H, 10β-CH_3), is a bond forming reaction. The product, photoisopyrocalciferol, showed the presence of only two double bonds (on quantitative hydrogenation), showed no maximum in the ultraviolet spectrum and upon oxidation yielded a non-conjugated unsaturated ketone. The photo isomer, upon heating, was reconverted to the starting homoannular diene. On the basis of these data, the following two structures (I and II) were postulated and the present work has established the correctness of structure II.



I



II

(1) This work was supported, in part, by Grant A-709 (C4)-Bio (5) of the U. S. Public Health Service, National Institutes of Health, Department of Health, Education and Welfare.

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