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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]

$$b^{\cdot\cdot\cdot} + I \longleftrightarrow b^{\cdot\cdot\cdot} \cdot I \qquad (1)$$

$$b^{\cdot\cdot\cdot} \cdot I + c^{\cdot\cdot} \longrightarrow c^{\cdot\cdot\cdot} + b^{\cdot\cdot} \sim I \qquad (2)$$

$$b^{\cdot\cdot} \sim I + X \longrightarrow b^{\cdot\cdot} + X \sim I \qquad (3)$$

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

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

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 $b^{\cdot\cdot} \sim I$, 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 $b^{\cdot\cdot\cdot} \sim I$ 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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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