The Apparent Absolute Requirement of Adenosine Diphosphate for the Inorganic Phosphate $\rightarrow$ Water Exchange of Oxidative Phosphorylation*

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SUMMARY

With suitable submitochondrial particles from the heart, the rate of the inorganic phosphate $\rightarrow$ HOH exchange during substrate oxidation in the absence of ADP is less than 1/1000 of the rate in the presence of ADP. The concentration dependence of the exchange on ADP shows simple saturation behavior with an apparent $K_m$ of 0.32 mm. For net oxidative phosphorylation under similar conditions, but with a hexokinase-glucose trap present, the apparent $K_m$ for ADP is 0.58 mm. The ADP analogue, adenosine methylene diphosphonate, is not detectably phosphorylated, does not stimulate the $P_i \leftrightarrow$ HOH exchange in the absence of ADP, and does not inhibit the exchange in the presence of ADP. The submitochondrial particles show the presence of an oligomycin and a 2,4-dinitrophenol-sensitive ADP $\rightarrow$ ATP exchange. These results are consistent with the interpretation that the $P_i \rightarrow$ HOH exchange results from dynamic reversal of ATP formation at the catalytic site, and that in the phosphorylation reaction the first covalent compound formed from $P_i$ or ADP is ATP.

The absence of detectable intermediates promotes consideration of suggestions that the first covalent compound formed by $P_i$ or ADP is ATP itself (1, 6). Additional means of assessing such a possibility thus become of increasing importance. Isotopic exchange reactions provide powerful tools for probing enzyme mechanisms. The $P_i \rightarrow$ ATP, $P_i \rightarrow$ HOH, ADP $\rightarrow$ ATP, and ATP $\rightarrow$ HOH exchanges have been shown to accompany both oxidative and photophosphorylation capacity (cf. References 4 and 6 through 13). The hypothesis has been advanced, based on exchange studies with soluble ATP-linked synthetases, that the occurrence, rates, requirements, and inhibitions of the various exchange reactions can be best explained by a dynamic reversal of ATP formation at a catalytic site, accompanied by different rates of release and binding of substrates (4). More tests of this hypothesis are desirable.

One important aspect concerns the relationship of ADP to the $P_i \rightarrow$ HOH exchange; the hypothesis would predict an absolute requirement. Although earlier studies with digitonin-prepared submitochondrial particles suggested that the $P_i \rightarrow$ HOH exchange might be activated by respiration in absence of adenine nucleotides (12), a later study by Cooper (6) gave convincing evidence of a marked ADP stimulation of the exchange. More recently, Mitchell, Hill, and Boyer (13) demonstrated that exchange in absence of adenine nucleotides was at most a few per cent of that when ADP was present, and Hinkle, Penefsky, and Racker (9) showed 50 to 80% inhibition of the $P_i \rightarrow$ HOH exchange in submitochondrial particles by the presence of an ATP-regenerating system to remove ADP. A principal purpose of the present paper is to examine more critically the possibility that the ADP requirement might be absolute and whether the ADP response is that expected for participation as a substrate or a positive effector. In addition, studies are reported on the effect of an ADP analogue on the $P_i \rightarrow$ HOH exchange, and on a further assessment of the occurrence of ADP $\rightarrow$ ATP exchange in submitochondrial particles. Studies of the ADP $\rightarrow$ ATP exchange were desirable in view of results suggesting that such exchange activity might not be present (10, 14, 15).

EXPERIMENTAL PROCEDURE

Materials—$^{32}P_i$ from various commercial sources was purified before use as described elsewhere (16). Water of approximately 1.5 or 11.8 atom % excess $^{18}O$ was obtained from Yeda Research and Development Company, Rehovoth, Israel.

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mycin was purchased from the Wisconsin Alumni Research Foundation, Madison, Wisconsin. Adenosine 5'-methylene diphosphonate was purchased from Miles Laboratories, Inc. Hexokinase (crystalline, type C-300 from yeast) was purchased from Sigma.

**Preparation and Assay of Submitochondrial Particles**—Submitochondrial particles were prepared by sonic disruption of heavy bovine heart mitochondria essentially by the method of Hansen and Smith (17) as described by Beyer (18). The bovine heart mitochondria were prepared by the method of Sanadi and Fuharty (19) and then frozen overnight prior to sonic disruption. After sonic disruption and centrifugation, the particles were suspended in 0.25 M sucrose, 0.01 M Tris-Cl (pH 7.5) instead of the described suspension mixture.

Endogenous nucleotides were removed from the particles as follows, with all treatments at 0-4°C. The particles in the sucrose-Tris solution were placed on a column of Dowex 1, (0.5 X 5.0 cm) (Bio-Rad AG-1 X4, 200 to 400 mesh, Bio-Rad, Richmond, California) previously equilibrated with 0.25 M sucrose, 0.01 M Tris-HCl (pH 7.5). The particles were eluted from the column by washing with the sucrose-Tris solution, care being taken to collect only the portion containing particles to avoid dilution. No loss of phosphorylating capacity was observed with particles subjected to this treatment.

The particles were assayed for total phosphorylation and the P:O ratio essentially as described by Beyer (18). The oxygen uptake was recorded polarographically on a Gilson Medical Electronics Oxigraph with the Clark type of electrode assembly.

The 32Pi incorporated into glucose-6-P was determined by an adaptation of the procedures for extraction of the phosphomolybdate complex with isobutyl alcohol-benzene (20). The protein concentrations were determined by the method of Lowry et al. (21).

**Measurements of 18O Incorporation**—The 18O incorporated into P1 was determined as described by Doyen and Dryan (22).

Separate time course experiments on the exchange reactions showed that they were linear over the measured time periods at the various ADP concentrations. Also incubation of particles in the absence of ADP for up to 10 min was found not to affect the exchange activity upon addition of ADP.

**Determination of ADP and ATP Present after Reaction**—The ADP present in the mixture after reaction was determined by use of pyruvate kinase essentially as described by Reynard et al. (23). The ATP present was determined by absorbing the nucleotides on activated charcoal, collecting the charcoal by filtration, and washing repeatedly to remove the free 32P. The ATP was hydrolyzed by heating the charcoal suspension in 1 N H2SO4 at 100°C for 30 min, and an aliquot of the supernatant used for 32P counting.

**Determination of P -> ATP and ADP -> ATP Exchanges**—The P -> ATP exchange was determined by the use of 32P with separation of P1 from adenine nucleotides by extraction of the phosphomolybdate complex (20). The ADP -> ATP exchange was determined by separating the nucleotides by polyethyleneimine thin layer chromatography, eluting, counting on a scintillation counter, and then calculating the amount of ADP -> ATP exchange as described previously (24).

**RESULTS**

**Relationship between ADP Concentration and P -> HOH Exchange**—Results of measurements of the P -> HOH exchange rate catalyzed by bovine heart submitochondrial particles in the presence of increasing concentrations of ADP up to 1 mM are shown in Fig. 1. Mg2+ was added to give a 0.4 mM excess over the ADP, thus assuring a nearly constant free Mg2+ level at the different ADP concentrations. A DPNH-regenerating system was present to make conditions closely analogous to those used in a comparison experiment in which net ATP formation was measured. Twelve different concentrations of ADP were used, with more measurements at the lower concentrations in an attempt to reveal any tendency for "sigmoidicity" of the curve. The results show that within the range of experimental error the velocity of the P -> HOH exchange shows a hyperbolic dependence on ADP concentration quite analogous to a simple Michaelis-Menten relationship. As noted in Fig. 1, the plot of 1/v against 1/(ADP) gives a straight line, with an apparent K m for ADP of 0.32 mM.

When, under the same conditions as for Fig. 1, the ADP concentration was increased above 2 mM, some inhibition of the exchange was noted; at 10 mM the rate was inhibited by about 55%.

Submitochondrial particles have a considerable ATPase and adenylyl kinase activity; thus ADP concentrations will not

![Graph](http://www.jbc.org/)

**Fig. 1.** Dependence of the P -> HOH exchange on ADP. Reaction mixtures contained in a 1.0-m1 final volume at pH 7.5, 0.25 M sucrose, 0.05 M Tris-sulfate (pH 7.5), 1 mM DPN, 0.05 mg of alcohol dehydrogenase, 10 mM ethanol, 5 mM Pi, MgSO4 (concentration varied so as to maintain 0.4 mM excess over ADP), ADP in the designated concentrations, 0.56 mg of submitochondrial particles (specific activity = 156 mmoles of ATP formed per min per mg of protein at 25°C) and H2O (1.10 atom % 32O excess). All constituents except particles were incubated at 30°C for 3 min to allow temperature equilibrium, followed by a 4 min-incubation with particles. Reactions were stopped with perchloric acid and analyses were made as described under "Experimental Procedure." Points on the curve are averages of duplicates.
remain constant during assays as reported in Fig. 1. The conditions used were chosen to give sufficient H₂O exchange for accurate analyses with minimal decrease in ADP. The actual range of H₂O in the samples reported in Fig. 1 was 0.001 to 0.005 atom % excess. Results of measurements of the net decrease in ADP and increase in ATP are given in Table I. They show that at both 0.1 and 1.0 mM added ADP, about one-fourth of the ADP disappears during the 4-min incubation. There is little net increase in ATP, and the ADP decrease probably represents conversion to ATP and AMP and hydrolysis of the ATP. This disappearance of ADP introduces some uncertainty in the measurements reported in Fig. 1. However, because about the same fraction was lost at low and high substrate concentrations, and the average amount of ADP present was 85 to 90% of that added, the effect would not be expected to change the shape of the curve in Fig. 1, but to decrease the apparent Kₐ slightly.

**Relationship between ADP Concentration and Oxidative Phosphorylation**—If the stimulation of the P₁ → HOH exchange by ADP as noted in Fig. 1 is the result of binding of ADP at the catalytic site for the phosphorylation reaction of oxidative phosphorylation, a similar ADP dependence for net ATP synthesis would be expected. Measurement was made of this dependence under conditions as given with Fig. 1, except that hexokinase (25 μg) and glucose (40 mM) were added to give net P₁ uptake into glucose 6-phosphate. A simple hyperbolic relationship was obtained. The apparent Kₐ found for ADP under our conditions was 0.58 mM, not far from the value of 0.30 mM reported by Brygrave and Lehninger (25) with submitochondrial particles from bovine heart under slightly different conditions.

**Apparent Absolute Dependence of P₁ → HOH Exchange on ADP**—As noted from the results given in Fig. 1 and in the report by Mitchell et al. (13), the submitochondrial particles in the absence of added ADP show a small but detectable apparent P₁ → HOH exchange. Tests of the time course and the oligomycin dependence of this small exchange are given in Fig. 2. The results show that although the ADP-stimulated exchange is obliterated by oligomycin, the small ADP-independent H₂O incorporation into P₁ is not affected by the inhibitor. This result and the marked decline in the rate of the ADP-independent exchange with time suggests that the apparent exchange may be caused by some reaction not connected with oxidative phosphorylation. For example, hydrolysis of a small amount of phosphoprotein or other phosphate compound present could occur. Since such a hydrolysis or other extraneous reaction might not be dependent on added ADP, a short incubation period at 30° in the absence of ADP and H₂O could completely abolish the ADP-independent exchange. This approach was feasible because experiments showed that the particles were still capable of good oxidative phosphorylation rates after incubation for 5 min without ADP.

Results of an experiment in which the submitochondrial particles were incubated for 5 min prior to testing for exchange capacity are given in Table II. A higher concentration of H₂O was used to increase the sensitivity of the assay. As noted in the table, no detectable P₁ → HOH exchange was found in the absence of added ADP. The sensitivity of the experiment was such that a rate as small as about 1/1000 of that in the presence of ADP should have been detected.

**Effect of ADP Analogue on P₁ → HOH Exchange**—If the ADP were stimulating the P₁ → HOH exchange by an action as a positive effector instead of as a substrate, suitable analogues of ADP might stimulate the exchange. Tests were thus made with

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### Table I

<table>
<thead>
<tr>
<th>ADP added</th>
<th>Net decrease in ADP</th>
<th>Net increase in ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.028</td>
<td>0.006</td>
</tr>
<tr>
<td>1.0</td>
<td>0.27</td>
<td>0.021</td>
</tr>
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</table>

### Table II

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed total H₂O</th>
<th>P₁ → HOH exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 1 mM ADP, no particles</td>
<td>0.208, 0.206</td>
<td>0</td>
</tr>
<tr>
<td>Incubated without ADP</td>
<td>0.208, 0.206</td>
<td>0.4</td>
</tr>
<tr>
<td>Incubated with 1 mM ADP</td>
<td>0.755, 0.728</td>
<td>570</td>
</tr>
</tbody>
</table>

*Particles were added to the sample after perchloric acid addition.*
ADP and Oxidative Phosphorylation

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TABLE III
Lack of phosphorylation of adenosine 5'-methylene diphosphonate by submitochondrial particles

Reaction mixtures were as described in Fig. 1, but with nucleotide additions as indicated in this table, and with particles of specific activity = 234 mmoles of ATP formed per min per mg of protein at 25°. The incubation was for 4 min at 30°.

<table>
<thead>
<tr>
<th>ADP</th>
<th>Adenosine 5'-methylene diphosphonate</th>
<th>*32P1 uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>mM</td>
<td>mmoles/ml/min</td>
</tr>
<tr>
<td>0</td>
<td>0.20</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>0</td>
<td>0.60</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>0</td>
<td>4.0</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>0</td>
<td>10.0</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>0.20</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>0.60</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>1.00</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>1.00</td>
<td>0.20</td>
<td>70</td>
</tr>
<tr>
<td>1.00</td>
<td>0.60</td>
<td>80</td>
</tr>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>78</td>
</tr>
</tbody>
</table>

TABLE IV
Inability of adenosine 5'-methylene diphosphonate to stimulate Pi ⇔ HOH exchange

Reaction mixtures were as described in Fig. 1, but with nucleotides added as indicated in this table. Values given are averages from duplicate incubations.

<table>
<thead>
<tr>
<th>Nucleotide added</th>
<th>Pi ⇔ HOH exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>mmoles/ml/min</td>
</tr>
<tr>
<td>adenosine 5'-methylene diphosphonate, 0.1</td>
<td>0</td>
</tr>
<tr>
<td>adenosine 5'-methylene diphosphonate, 1.0</td>
<td>0</td>
</tr>
<tr>
<td>ADP, 1.0</td>
<td>210</td>
</tr>
</tbody>
</table>

TABLE V
Effect of oligomycin and 2,4-dinitrophenol on Pi ⇔ ATP and ADP ⇔ ATP exchanges

Reaction mixtures contained, in 1.0-ml final volume, 0.25 mM sucrose, 0.50 mM Tris-sulfate (pH 7.5), 10 mM mgPi (12,380 cpm per pmole), 6 mM ATP, 2 mM ^32C-ADP (56,900 cpm per pmole), MgSO4 as indicated, and 0.54 mg of submitochondrial particles (specific activity, 143 mmoles of ATP formed per min per mg of protein at 25°). In the samples indicated, 1 mM 2,4-dinitrophenol or 1 μg of oligomycin were added. Duplicate incubations, each containing either ^32C-ADP or *32P1, were performed. Mixtures without particles were incubated at 30° for 3 min, then for 4 min with particles. Assays were made as described under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Mg++ added</th>
<th>Pi ⇔ ATP</th>
<th>ADP ⇔ ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control + Oligomycin</td>
<td>+ 2,4-Dinitrophenol</td>
</tr>
<tr>
<td>mM</td>
<td>mgmoles/ml/min</td>
<td>mgmoles/ml/min</td>
</tr>
<tr>
<td>5.0</td>
<td>13</td>
<td>0.5</td>
</tr>
<tr>
<td>8.5</td>
<td>18</td>
<td>0.6</td>
</tr>
<tr>
<td>10.0</td>
<td>18</td>
<td>0.2</td>
</tr>
</tbody>
</table>

ADP and 5'-methylene diphosphonate in which the oxygen of ADP is replaced by a CH2 group. This analogue has been reported to function very poorly as a phosphate acceptor, but to give either no or slight inhibition of net synthesis in presence of ADP (26) when tested with submitochondrial particles prepared by digitonin treatment. We found no detectable phosphorylation by particles under our conditions, as noted in Table III. Also, as shown by data in the table, the analogue does not inhibit phosphorylation of ADP. Binding of the analogue at an "effector" site might occur, however. For example, the corresponding methylene phosphate analogue of ATP functions well as an effector of adenylate deaminase (27). Results of tests to find if the ADP analogue would activate the Pi ⇔ HOH exchange are given in Table IV. They show no detectable activation.

Sensitivity of ADP ⇔ ATP Exchange to Phosphorylation Inhibitors—If the function of ADP in promoting the Pi ⇔ HOH exchange of oxidative phosphorylation is to serve as the initial phosphate acceptor, and if the exchange is due to the dynamic reversal of ATP formation, an ADP ⇔ ATP exchange should accompany the other exchanges. Assessment of this possibility in submitochondrial particles is complicated by an active ADP ⇔ ATP exchange not associated with oxidative phosphorylation but probably catalyzed by adenylate kinase or nucleoside diphosphokinase. Indeed, Zalkin, Pullman, and Racker (14) did not observe a measurable inhibition by 2,4-dinitrophenol of the ADP ⇔ ATP exchange catalyzed by submitochondrial particles. They suggested that the ADP ⇔ ATP exchange might be small if ADP was much more tightly bound at the catalytic site than ATP or Pi. However, the Km for ADP is not unduly low, and added ADP is readily removed from particles by washing. A further assessment of the possible sensitivity of the ADP ⇔ ATP exchange to oligomycin and to 2,4-dinitrophenol thus seemed desirable, using well washed particles and relatively short time periods.

Results of measurements of the effect of 2,4-dinitrophenol and of oligomycin on the ADP ⇔ ATP exchange are given in Table V. In these experiments the initial ATP, ADP, and Pi concentrations were as used by Zalkin et al. (14), but the ATPase inhibitor that was included in their reaction mixture was not present. The reaction was tested at three different Mg++ levels because of the probable differential sensitivity of the various ADP ⇔ ATP exchange reactions to Mg++. For example, Bygrave and Lehninger (15) showed that the oligomycin-sensitive component of the ADP ⇔ ATP exchange activity of intact mitochondria is saturated at lower Mg++ concentrations than is the oligomycin-insensitive component. As noted in Table V, both oligomycin and 2,4-dinitrophenol nearly completely abolished the Pi ⇔ ATP exchange at all Mg++ levels. In addition, a definitive inhibition of the ADP ⇔ ATP exchange was noted; at the 10 mM Mg++ concentration, oligomycin inhibited by 25% and 2,4-dinitrophenol by 55%.

DISCUSSION

The results in this paper point strongly to an absolute requirement of ADP for the Pi ⇔ HOH exchange reaction of oxidative phosphorylation. The three explanations that appear most probable to us for consideration of the ADP requirement are as follows.

1. ADP is required as a positive effector (allosteric activator) for catalysis of transient metaphosphate formation from Pi, or for
water formation accompanying formation of a phosphorylated intermediate, for example as follows.

\[ \text{HOPO}_3^- + \text{H}^+ \xrightarrow{\text{(ADP)}} [\text{PO}_4^3^-] + \text{HOH} \quad (a) \]

or

\[ \text{HOPO}_3^- + \text{YH} \xrightarrow{\text{(ADP)}} \text{Y-P}^\sim + \text{HOH} \quad (b) \]

where \( \sim \) is used to denote input of energy from oxidation-reduction by an unknown mechanism.

2. Reaction of ADP with a phosphorylated intermediate allows subsequent exchange with water of a group that acquired an oxygen from \( P_1 \), for example.\(^1\)

\[ \text{HOPO}_3^- + \text{X} \xrightarrow{\text{Y}} \text{Y-O-PO}_4^2^- + \text{XH} \]

\[ \text{Y-PO}_4^2^- + \text{ADP} \xrightarrow{\text{ATP}} \text{YOH} \]

\[ \text{YOH} + \text{XH} \xrightarrow{\text{ADP}} \text{X} \xrightarrow{\text{Y}} \text{Y} + \text{HOH} \]

3. Water oxygen is formed from \( P_1 \) only when ATP itself is formed as follows.

\[ \text{HOPO}_3^- + \text{ADP} \xrightarrow{\text{ADP}} \text{ATP} + \text{HOH} \]

4. ADP allows formation of a pentacovalent phosphorus derivative that exchanges oxygen with water.

None of these explanations can be rigorously excluded or proved by present data. Examination of information pertinent to each explanation is instructive, however.

The first explanation, a positive effector role for ADP, was aptly suggested by Slater (28) and Ter Welle and Slater (29) to explain observations such as the ADP stimulation of respiration in presence of arsenate, and by Hinkle et al. (9) to explain ADP stimulation of the \( P_1 \Rightarrow \text{HOH} \) exchange. The apparent absolute requirement of ADP for the \( P_1 \Rightarrow \text{HOH} \) exchange argues against an effector role because some activation would be anticipated in the absence of the effector. Requirement of an effector can be sufficiently low, however, such that rates in its absence are undetectable. Such apparent absolute requirement has been demonstrated for acetyl-CoA activation of the pyruvate carboxylase reaction (30) and for acetyl glutamate activation of the carbamyl phosphate synthetase reaction (31). We are unaware of any case, however, in which an absolute requirement of a substrate as a positive effector has been demonstrated. If ADP served as its own activator for oxidative phosphorylation, a sigmoid response of net phosphorylation as well as the \( P_1 \Rightarrow \text{HOH} \) exchange and properties of the \( P_1 \Rightarrow \text{HOH} \), \( P_1 \Rightarrow \text{ATP} \), ATP \( \Rightarrow \text{HOH} \), and ADP \( \Rightarrow \text{ATP} \) exchanges associated with oxidative phosphorylation. Not only does the mechanism explain an ADP requirement for the \( P_1 \Rightarrow \text{HOH} \) exchange, but it also gives explanation for ADP stimulation of the \( P_1 \Rightarrow \text{ATP} \) exchange (9, 13) and ATP \( \Rightarrow \text{HOH} \) exchange (9, 13) for \( P_1 \) stimulation of the oligomycin-sensitive ADP \( \Rightarrow \text{ATP} \) exchange (15, 32), and for \( P_1 \) stimulation of the ATP \( \Rightarrow \text{HOH} \) exchange (9). The explanation is given additional credence by the more rapid exchange of substrate oxygens than of substrate carbon atoms with purified synthetases and an adequate theoretical basis for the understanding of these differences in exchange rates (4).

The occurrence of an ADP \( \Rightarrow \text{ATP} \) exchange associated with oxidative phosphorylation could be interpreted as suggesting a phosphorylated intermediate, but is also obviously explainable by reversal of ATP formation. If the third mechanism given above is correct, then any system capable of showing a 2,4-dinitrophenol- and oligomycin-sensitive ADP \( \Rightarrow \text{ATP} \) exchange should also have capacity for net oxidative phosphorylation, for a \( P_1 \Rightarrow \text{HOH} \) exchange, for a \( P_1 \Rightarrow \text{ATP} \) exchange, and for an ATP \( \Rightarrow \text{HOH} \) exchange. Conversely, any system with these properties should show an oligomycin- and 2,4-dinitrophenol-sensitive ADP \( \Rightarrow \text{ATP} \) exchange. Both conditions appear to be met, but further probing of these aspects may be desirable because literature reports are not sufficiently definitive. The detection of oligomycin- and 2,4-dinitrophenol-sensitive ADP \( \Rightarrow \text{ATP} \) exchange may be difficult in some systems because of the relatively large exchange activity caused by enzymes not directly involved in oxidative phosphorylation. The additional assessment reported herein (Table V) clearly demonstrates the presence of oligomycin- and dinitrophenol-sensitive ADP \( \Rightarrow \text{ATP} \).

\(^1\) This type of sequence is a variant of what has been called the "chemical hypothesis" of oxidative phosphorylation (26). Because a nonchemical mechanism for ATP formation is impossible, a preferable designation might be a "covalent precursor" hypothesis.
exchange capacity in submitochondrial particles. We do not have an adequate explanation for the apparent discrepancy between these results and those reported by Zalkin et al. (14).

A point of interest is that the amount of oligomycin-sensitive ADP \( \rightleftharpoons \) ATP exchange noted in Table V is about equal to the amount of P\(_1 \rightleftharpoons \) ATP exchange. This would be expected if the exchange occurred by a dynamic reversal of ATP formation and if the rates of dissociation of ADP and P\(_1\) from the catalytic site were approximately equal. A similarity of the sensitivity of the P\(_1 \rightleftharpoons \) ATP and ADP \( \rightleftharpoons \) ATP exchanges to oligomycin and to other inhibitors has been noted by Cooper and Kulka (32) in studies with digitonin particles of rat liver mitochondria.

Bygrave and Lehninger (15) noted the absence of detectable oligomycin-sensitive ADP \( \rightleftharpoons \) ATP exchange with damaged mitochondria not showing respiratory control. This could reflect increase in ADP \( \rightleftharpoons \) ATP exchange activity not associated with oxidative phosphorylation and masking of the small but important oligomycin-sensitive component. Response of some systems to Mg\(^{++}\) appears complex. Schnaitman and Pedersen (10) observed that the oligomycin-sensitive ADP \( \rightleftharpoons \) ATP exchange and respiratory control of particles from digitonin-fractionated mitochondria was lost when 5 mM Mg\(^{++}\) was added to the reaction mixture with 10 mM ATP and 5 mM ADP already present. Some bound Mg\(^{++}\) must be initially present in the particles. Conceivably, the extra Mg\(^{++}\) addition allows catalysis of the ADP \( \rightleftharpoons \) ATP exchange by latent nucleoside diphosphokinase of the particles and decreases oxidative phosphorylation capacity.

In all experiments on ADP \( \rightleftharpoons \) ATP exchange, a complication which arises is that addition of oligomycin and dinitrophenol to any system capable of oxidative phosphorylation may result in different concentrations of substrates, both free and bound, at active sites and thus make direct comparisons between ADP \( \rightleftharpoons \) ATP exchange rates under the different conditions difficult. A more crucial test can be achieved with preparations low in extraneous exchange activity.

REFERENCES

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