Effect of Na⁺ and K⁺ on Mitochondrial Respiratory Control, Oxygen Uptake, and Adenosine Triphosphatase Activity

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SUMMARY

Isolated rat liver mitochondria incubated with ethylenediaminetetraacetate in the absence of added Mg²⁺ show a loss of respiratory control toward ADP in a medium which contains Na⁺, while in a medium which contains K⁺, this function is preserved; respiratory control in these conditions is restored by including Mg²⁺ into the mixture. On the other hand, mitochondria incubated in the sodium medium with ADP have adequate respiratory responses to added inorganic phosphate.

Mitochondria incubated in the sodium medium in the absence of Mg²⁺ also have a high rate of respiration in the absence of agents which stimulate oxygen uptake. This is accompanied by a loss in the sensitivity to 2,4-dinitrophenol.

Rotenone stimulates a latent ATPase activity in mitochondria incubated with Na⁺ at concentrations which inhibit the oxidation of NADH.

The data obtained seem to indicate that the rotenone site is the energy-coupling site and that K⁺, Mg²⁺, and ADP may protect against the detrimental action of Na⁺ on this system. Evidence is also presented which suggests that ADP may regulate oligomycin-insensitive respiratory activity.

METHODS

Mitochondria from rat liver were prepared according to the method of Schneider and Hogeboom (13) in 0.25 M sucrose and 1 mM EDTA at pH 7.3. The mitochondrial pellet was washed once and suspended in 0.25 M sucrose and EDTA. Oxygen uptake was measured either manometrically or polarographically (Yellow Springs Instrument Company, Yellow Springs, Ohio). The ATPase activity of mitochondria was measured in the conditions indicated under "Results," and the reaction was stopped with trichloroacetic acid at 6% final concentration. Pi was determined in the supernatant according to the method of Summer (14).

RESULTS

During the course of studies on the effect of Na⁺ and K⁺ on oxidative phosphorylation, it was found that mitochondria incubated in the absence of added Mg²⁺ present irregular responses; in some experiments, lower P:O ratios were observed in the potassium medium, but, in some experiments, no difference was found. This difficulty was overcome by including EDTA in the incubation mixture. In the presence of EDTA, a normal response to ADP was observed in mitochondria incubated with Na⁺ in the absence of Mg²⁺.

A latent ATPase activity which becomes apparent in the presence of rotenone is also observed in mitochondria incubated with Na⁺ in the absence of Mg²⁺.

The results of these studies suggest that Na⁺ has a detrimental effect on a component of the oxidative phosphorylation process in intact mitochondria.

Pressman and Lardy (1, 2) reported that K⁺ was required for maximum rates of oxidative phosphorylation and respiration in isolated mitochondria. Although Opit and Clarnock (8) did not confirm the results of Pressman and Lardy (1), a favorable effect of K⁺ has been observed in kidney and brain mitochondria (4, 5). More recent experiments made with the antibiotic valinomycin have also yielded contradictory results. Höfer and Pressman (6) reported that K⁺ had marked effects on oxidative phosphorylation on intact mitochondria, while Smith and Beyer (7), by studies with bovine heart submitochondrial particles, reached the conclusion that K⁺ does not affect ostensibly oxidative phosphorylation. Recently, Papa et al. (8) reported inhibition of oxidative phosphorylation also in submitochondrial particles by higher concentrations of K⁺; however, this effect was also obtained with other cations. There are also reports (9-11) that indicate that the accumulation and oxidation of certain substrates are favorably affected by K⁺, and Harris, Höfer, and Pressman (12) reported that, in certain conditions, a competition exists between K⁺ transport and oxidative phosphorylation.

In this work, data are presented which indicate that in certain experimental conditions a different behavior of the phosphorylative capacity of mitochondria toward added Na⁺ and K⁺ may be shown. An attempt has been made to study the mechanism by which K⁺ and Na⁺ exert these effects.

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TABLE I

Respiratory control of mitochondria incubated in potassium and sodium media

Oxygen uptake was measured polarographically in a mixture which contained 0.1 M KCl or 0.1 M NaCl, 20 mM Tris- HCl, pH 7.3, 10 mM H2PO4 adjusted to pH 7.3 with Tris, 1.2 mM EDTA, 50 mM sucrose, and 10 mM substrate in a final volume of 5.0 ml. Where indicated, the mixture contained 8 mM MgCl2 or 1.8 mM ADP or both. Temperature was 25°. The experiments with succinate contained 7.4 mg of mitochondrial protein and those with a-ketoglutarate, malate-pyruvate, and glutamate contained 8.4, 8.9, and 6.3 mg of protein, respectively. Respiratory rates before and after the addition of ADP to the mitochondrial mixture are expressed.

<table>
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<th>+ADP</th>
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<th>State 4</th>
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<tr>
<td>Succinate + K+ + Mg++</td>
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<td>115</td>
<td>5.5</td>
<td></td>
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<tr>
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<td>138</td>
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<tr>
<td>a-Ketoglutarate + K+ + Mg++</td>
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<td>68</td>
<td>7.6</td>
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<tr>
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<td>27</td>
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<tr>
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</tr>
<tr>
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<td>Malate-pyruvate + K+ + Mg++</td>
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<td>122</td>
<td>5.1</td>
<td></td>
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<tr>
<td>Malate-pyruvate + Na+</td>
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<td>33</td>
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<td></td>
</tr>
<tr>
<td>Malate-pyruvate + Na+ + Mg++</td>
<td>19</td>
<td>102</td>
<td>5.4</td>
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</table>

The results mentioned above which were obtained polarographically were also observed when oxygen uptake was measured manometrically, and it was found that the stimulation of respiration by dinitrophenol in mitochondria was also absent when the incubation was carried out in the sodium medium without added Mg++ (Fig. 1). The sensitivity to dinitrophenol could be restored by adding Mg++ to the mixture.

A significant finding in these experiments was that mitochondria incubated with Na+ and oxidizing NAD-dependent substrates showed a high rate of respiration in the absence of agents which stimulate respiration (Fig. 1). In the absence of added Mg++, mitochondria in the potassium medium also exhibit a high rate of respiration which would be in agreement with a previous finding reported by Balmcheffsky (15), but the rate in the potassium medium is much less than that observed in the sodium medium. Six experiments were carried out to compare the rate of oxygen uptake of mitochondria with Na+ to the respiratory rate of mitochondria incubated with K+ and dinitrophenol, utilizing glutamate as substrate in the absence of added Mg++. It was found that, in four experiments, the rates in the two conditions were the same, in the other two experiments, the rates of oxygen uptake of mitochondria incubated in the sodium medium were 80 and 70% of the rates observed in mitochondria incubated with K+ and dinitrophenol.

Fig. 2, A and B, shows the results of experiments in which oxygen uptake was measured at different Na+ : K+ ratios. The rate of respiration was linear with time and increased as the Na+ : K+ ratio increased. The same effect of Na+ was observed when respiration was measured at various concentrations of NaCl, maintaining the tonicity of the medium constant by adjusting the concentration of sucrose; each vessel contained 14.0 mg of protein.

1 The dinitrophenol used is 2,4-dinitrophenol.
The effect of dinitrophenol (10-4 M) on the oxygen uptake of mitochondria was clearly inhibitory (Fig. 4). With 0.1 molar Na+ the respiratory rate increased and the subsequent stimulatory effect of the dinitrophenol addition diminished (Fig. 4). At a Na+:K+ ratio of 5 X 10^-4, the effect of dinitrophenol started to become inhibitory, and at higher Na+:K+ ratios, the effect of dinitrophenol was clearly inhibitory (Fig. 4). With 0.1 molar NaCl in the mixture, concentrations of dinitrophenol which ranged from 5 x 10^-4 M to 3 x 10^-4 M inhibited the oxygen uptake of mitochondria.

Oligomycin, an inhibitor of oxidative phosphorylation (16), diminished to some extent the rate of mitochondrial respiration observed in the sodium medium (Table II); however, in the presence of this inhibitor, a marked difference between the effects of the sodium medium and the potassium medium on mitochondrial respiration was still observed. Pi, which increases the oxidation of glutamate and α-ketoglutarate (17, 18), is not required in order to produce a difference in respiratory rates between the all sodium medium and the all potassium medium (Table II).

In order to gain some insight into the nature of the alteration of mitochondria incubated with Na+ in the absence of added Mg++, the experiments of Fig. 5 were carried out. When Mg++ was added to mitochondria incubated for some time in the sodium medium, the rate of respiration diminished to an extent similar to that observed when Mg++ was included at the beginning of the experiment. However, under the latter conditions, respiratory control and sensitivity to dinitrophenol were maintained, while, in the former case, the sensitivity to dinitrophenol was lost (Fig. 5A).

The behavior of mitochondria toward Na+ is also influenced by added K+. In the experiments of Fig. 5B, mitochondria were previously incubated in an all sodium medium for 12 min. In the subsequent additions of Mg++ and K+ depressed respiration. Five minutes later, the addition of dinitrophenol produced only a slight stimulation of respiration. Fig. 5C illustrates the results of an experiment in which mitochondria were incubated in a combination of Na+ and K+ which supported suboptimal rates of oxygen uptake (cf. Fig. 2). Addition of dinitrophenol under these conditions produced inhibition of respiration. On the other hand, when mitochondria had been exposed to Na+ and K+, the addition of Mg++ produced a diminution in the rate of respiration. The addition of dinitrophenol in this case increased the rate of oxygen uptake (Fig. 5D).

The same pattern of results was obtained when ADP was used to release oxygen uptake. The addition of Mg++ to mitochondria previously incubated with Na+ lowered the respiratory rates, but did not induce respiratory control. However, Mg++ induced respiratory control when mitochondria have been incubated with Na+ and K+. This behavior was observed with glutamate, α-ketoglutarate, or malate-pyruvate as substrates.

These experiments, as well as those of Table I, indicate that a...
Fig. 5. Influence of Na⁺, K⁺, and Mg²⁺ on the effect of dinitrophenol (DNP) on mitochondrial respiration. All incubations were made the same as those in Fig. 1. The first reading was taken after 7 min of equilibrium. MgCl₂ (8 mM) and dinitrophenol (10⁻³ M) were added where indicated. In Experiment A, mitochondria (18 mg of protein) were incubated in 80 mM NaCl from the beginning of the experiment. In Experiment B, mitochondria (17.6 mg of protein) were incubated in 50 mM NaCl from the beginning of the experiment and 50 mM KCl was added together with MgCl₂ at the indicated time. In Experiment C, mitochondria (17.6 mg of protein) were incubated in 50 mM KCl and 50 mM NaCl from the beginning of the experiment. In Experiment D, mitochondria (17.8 mg of protein) were incubated in 50 mM KCl and 50 mM NaCl from the beginning of the experiment.

Table III

Respiratory response of mitochondria incubated with ADP and Na⁺ to P₁

<table>
<thead>
<tr>
<th>Substrate</th>
<th>- P₁</th>
<th>+ P₁</th>
<th>State 3-State 4 transitions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µatoms O₂/min/mg protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>19</td>
<td>100</td>
<td>5.3</td>
</tr>
<tr>
<td>Malate-pyruvate</td>
<td>19</td>
<td>48</td>
<td>2.5</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>11</td>
<td>103</td>
<td>9.4</td>
</tr>
<tr>
<td>Succinate</td>
<td>36</td>
<td>106</td>
<td>2.9</td>
</tr>
</tbody>
</table>

certain order of addition of Na⁺, K⁺, and Mg²⁺ must be maintained in order to preserve the integrity of the system involved in the respiratory response of mitochondria to ADP.

The results obtained with succinate differed from those obtained with other substrates. Although mitochondria incubated in an all sodium medium show a reproducible loss of respiratory control (Table I), in the absence of added Mg²⁺, a higher rate of oxygen uptake in the sodium medium than with the potassium medium is not consistently observed. Furthermore, in those experiments in which a difference was detected, it was of the order of about 20% higher with Na⁺ than with K⁺. These experiments were also conducted in the presence of a rotenone block (19), or in the presence of ATP which activates the aerobic oxidation of succinate (20). No clear-cut difference in the oxygen uptake between the sodium medium and the potassium medium was detected under any of the conditions.

Effect of ADP on Respiratory Pattern of Mitochondria Incubated with Na⁺—The results presented illustrate that the respiration of mitochondria incubated with Na⁺ and P₁ is not stimulated upon the addition of ADP or dinitrophenol. Mitochondria under these conditions also have a high rate of respiration with NAD-dependent substrates which are to a large extent insensitive to oligomycin.

Table III illustrates the results of experiments with mitochondria which have been previously incubated in a medium containing Na⁺ and excess ADP; the addition of P₁ to this reaction mixture produces an increase in the respiratory rate; under these conditions, mitochondria remain coupled despite the presence of Na⁺.

The experiments of Fig. 6 illustrate the fact that the detrimental effects of Na⁺ toward mitochondria are protected against by ADP provided that it is added together, with, or before the Na⁺. In Experiment A, mitochondria were added to a mixture containing K⁺ and a limiting amount of ADP; the addition of excess P₁ produced an increase in respiration until the ADP was exhausted; a second and third addition of ADP produced typical State 4-State 3 transitions. On the other hand, in the case of mitochondria incubated together with Na⁺ and a limiting amount of ADP (Fig. 6B), the addition of excess P₁ increases the rate of respiration, but, upon the consumption of the ADP, the rate of respiration does not return to that of the basal state. Instead a high rate of respiration is sustained which is increased only slightly by a second addition of ADP.

Fig. 6. Respiratory control of mitochondria incubated with Na⁺ and K⁺. Oxygen uptake was measured polarographically as in Table I. The substrate was glutamate. Mitochondria were added to a mixture which contained the indicated amount of ADP and 0.1 mM NaCl or 0.1 mM KCl and the rest of the reagents as in Table III. At the indicated time, P₁ or ADP were added at the indicated final concentration. The numbers on the left of the traces indicate the actual rates of respiration.
and in the potassium medium with and without added Mg++. Rothenone beyond those which completely suppress oxygen uptake, phosphorylation of ADP by mitochondria was measured in the sodium a slight stimulation of 4TPase is observed at concentrations of Mg++. close parallelism between the inhibition of respiration and the studies were conducted in media containing Ii+. However, as I. With respect to the rotenone-stimulated ATPase not affect the ATPase activity of mitochondria, however, their results of these experiments are in agreement with those of Table medium by rotenone could only be observed in the presence of taneously by including ATP in the medium, was carried out. A suppression of ADP was measured where indicated. Temperature was 25°C.

rate and upon the exhaustion of the Pi the respiratory rate diminished almost to the basal rate. A second addition of Pi, produced a second increment in the respiratory activity in both experiments.

Stimulation of ATPase Activity by Rotenone—The ATPase activity of mitochondria incubated with Na+ and K+ was studied in order to find out whether the high rate of oxygen uptake observed in the presence of Na+ is accompanied by a high ATPase activity. The ATPase activity of mitochondria was found to be slightly higher in the sodium medium than in the potassium medium, but the difference was not consistently observed. The apparent slightly higher ATPase activity in the sodium medium was thought to be due to a lower rate of phosphorylation due to the oxidation of endogenous substrates, and, thus, the ATPase activity of mitochondria was studied in the presence of rotenone which inhibits the oxidation of NADH (19).

Ernster, Dallner, and Azzone (21) reported that rotenone did not affect the ATPase activity of mitochondria, however, their studies were conducted in media containing K+. However, as shown in Fig. 7, rotenone induced an ATPase activity in mitochondria incubated with Na+. Four experiments (not shown) were carried out to compare the rotenone-stimulated ATPase activity to the dinitrophenol-stimulated ATPase activity in mitochondria incubated with Na+. It was found that the rotenone-stimulated ATPase activity was between 18 and 20% of the dinitrophenol-stimulated ATPase activity.

Evidence against the possibility that the rotenone-stimulated ATPase activity is only apparent is presented in Tables IV and V. In the absence of Mg++, rotenone induced a much stronger stimulation of ATPase activity in the sodium medium, while in the presence of Mg++, rotenone induced mitochondrial ATPase activity to the same extent in both the sodium and the potassium media. The increase in ATPase activity in the potassium medium by rotenone could only be observed in the presence of Mg++. Table V shows the results of experiments in which the phosphorylation of ADP by mitochondria was measured in the sodium and in the potassium medium with and without added Mg++.

Succinate and glutamate were the substrates. In the sodium medium, Mg++ increased the phosphorylation of ADP significantly, while in the potassium medium, the inclusion of Mg++ in the incubation mixture did not affect the phosphorylation. The results of these experiments are in agreement with those of Table I. With respect to the rotenone-stimulated ATPase activity, it can be observed that although Mg++ increases the phosphorylation of ADP significantly in the sodium medium, the rotenone-stimulated ATPase activity is not affected by Mg++. In the potassium medium, phosphorylation does not require exogenous Mg++, while the stimulation of ATPase by rotenone is enhanced by Mg++. These disparities indicate that the stimulation of ATPase activity by rotenone is independent of the phosphorylation capacity of mitochondria.

Ernster et al. (21) titrated the rotenone site of mitochondria and indicated that the rotenone-sensitive respiratory catalyst is present at the lowest molar ratio among the known components of the electron transport system. An experiment similar to that made by Ernster et al. (21), in which glutamate-supported respiration and ATPase induction by rotenone were measured simultaneously by including ATP in the medium, was carried out. A close parallelism between the inhibition of respiration and the stimulation of ATPase activity was obtained (Fig. 8). Although a slight stimulation of ATPase is observed at concentrations of rotenone beyond those which completely suppress oxygen uptake,

### Table IV

<table>
<thead>
<tr>
<th>Additions</th>
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<td>Glutamate</td>
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</tr>
<tr>
<td>Glutamate + Mg++</td>
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<td>9.3</td>
</tr>
<tr>
<td>Succinate</td>
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<td>2.5</td>
</tr>
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<td>Succinate + Mg++</td>
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### Table V

<table>
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<td>Succinate + Mg++</td>
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*Fig. 7. Effect of rotenone (Rot) on the ATPase activity of mitochondria incubated in sodium and potassium media. The incubation mixture contained: 0.1 mM KCl or NaCl; 7 mM ATP, 20 mM Tris-HCl, pH 7.3, 1 mM EDTA, 10 mM sucrose, and 8.6 mg of mitochondrial protein in a final volume of 5.0 ml; 2.4 μM rotenone were added where indicated. Temperature was 25°C.*
Fig. 8. Effect of rotenone on the oxygen uptake and the ATPase activity of mitochondria. Mitochondria were incubated under the same conditions as those in Fig. 1 in 0.1 M NaCl and 7.3 mM ATP. Glutamate was the substrate and rotenone as indicated. The reaction was started after 7 min of pre-equilibrium by tipping in the mitochondria from the side arm (19 mg of protein). The reaction was stopped by trichloroacetic acid after 15 min of incubation following reading of the manometer. Pi was determined in the supernatant following centrifugation. O—O, ATPase activity; •—•, respiration.

the curves are strongly indicative that the inhibition of respiration and the stimulation of ATPase activity are due to the interaction of rotenone with a single site. It should be mentioned that the respiration of mitochondria incubated with K+ is completely inhibited by the same concentrations of rotenone which completely suppress the oxygen uptake of mitochondria incubated with Na+, but, in the former case, no stimulation of ATPase activity by rotenone is detected.

DISCUSSION

In this work, a comparison of the effects of Na+ and K+ on the respiratory control and oxidative phosphorylation of mitochondria incubated with EDTA and in the absence of added Mg++ is presented. However, if Mg++ is included in the incubation mixture, lower rates of ADP or dinitrophenol stimulated respiration are consistently observed in the presence of Na+, as compared to K+, and P:O ratios are not affected by the cationic environment. The results in the presence of Mg++ are in agreement with the previous work of Pressman and Lardy (2) in which higher rates of respiration were observed with K+ than with Na+. These higher rates in the potassium medium may be due to the beneficial effect of K+ on substrate transport which has been documented in several reports (9–11).

The most marked difference in the behavior of oxidative phosphorylation reactions of mitochondria to Na+ and K+ may be observed in the presence of EDTA and absence of added Mg-Cl₂. Under these conditions, the behavior of mitochondria incubated with various combinations of Na+ and K+ depends on the concentration of Na+ used in the mixture rather than on the concentration of K+. Variations in the K+ concentration from 10 to 100 mM do not affect the respiratory and phosphorylation patterns of mitochondria. On the contrary, clear-cut effects of Na+, such as a stimulation of respiratory activity and loss of respiratory control to ADP, becomes apparent at 25 mM Na+ and reach a maximum at 75 and 100 mM Na+. The concentration of sucrose (50 to 150 mM) does not ostensibly affect the effect of Na+. The marked differences between Na+ and K+ previously reported seem to be due to the use of EDTA in the preparation and incubation of mitochondria and, also, to the omission of Mg++ in the experimental mixtures, conditions which differ from those of other investigators (1–3).

In submitochondrial particles, Smith and Beyer (7) reported no effect of up to 30 mM K+ on oxidative phosphorylation; on the other hand, Papa et al. (8) reported inhibition of oxidative phosphorylation of submitochondrial particles by 50 and 100 mM K+. The inhibiting effect of K+ reported by the latter group was also attained with other cations and may be interpreted as a non-specific effect of ionic strength. Nevertheless, no K+ requirement for oxidative phosphorylation has been demonstrated in submitochondrial particles, and, thus, it is possible that the effects presently reported do not represent a primary action of K+ on oxidative phosphorylation, but rather a detrimental effect of Na+ on mitochondria structure which secondarily influences oxidative phosphorylation.

A point that should be taken into serious consideration is that the described lack of respiratory control to ADP in mitochondria incubated with Na+ is due to factors related to the permeability of substrates. However, this possibility seems unlikely since, under the conditions in which loss of respiratory control is observed, a high rate of oxygen uptake is detected with all NAD-dependent substrates assayed. Furthermore, the results of Table I and Table III show that mitochondria incubated in mixtures of identical composition have different respiratory and phosphorylation patterns which depend only on the order of addition of Na+, ADP, and Pi. The most logical explanation for this particular behavior of mitochondria is not on a permeability basis, but on a complex interaction of Na+, ADP, and Pi with a particular mitochondrial structure.

Results are also presented which indicate that rotenone stimulates a latent ATPase activity in mitochondria to an extent of about one-fifth of that induced by dinitrophenol. This stimulation is observed with Na+, and with K+, if Mg++ is added to the mixture. The following results indicate that the activation of ATPase by rotenone is not the result of the inhibition of oxidation of endogenous substrates with a resulting lower phosphorylation of ADP.

1. In the sodium medium, Mg++ increases the phosphorylation of ADP significantly, but does not affect the rotenone-stimulated ATPase.

2. In the potassium medium, the phosphorylation of ADP is not affected by Mg++, but Mg++ is required for the stimulation of ATPase activity by rotenone.

3. The inhibition of oxygen uptake with NAD-dependent substrates is of the same extent with Na+ as with K+ and yet a higher rotenone-stimulated ATPase activity is observed with Na+.

Ernster et al. (21) indicated that the rotenone site is rate-limiting for the oxidation of NADH, and Chance et al. (22) reported that Site I of oxidative phosphorylation may correspond to the rotenone site. Moreover, in mitochondria from Saccharomyces cerevisiae, both Site I and the rotenone site are lacking (23, 24). The stimulation of ATPase activity by rotenone which, according to the above mentioned findings, is independent of the phosphorylation of ADP and the inhibition of oxygen uptake show a strong parallelism to increasing concentrations of rotenone. All these
findings would lend support to the postulation that the energy-coupling site corresponds or is very closely associated with the rotenone site.

In this respect, it should be mentioned that Myers and Slater (25) reported that antimycin A, at concentrations beyond those which inhibit electron transport, increases the ATPase activity of mitochondria. Siekevitz et al. (26) showed that Antimycin stimulates the ATPase activity of mitochondria slightly and also inhibits the dinitrophenol-stimulated ATPase activity. It is possible that these effects of Antimycin and antimycin A resemble in their mechanism the action of rotenone presently described. Piericidin has been reported to act similarly to rotenone in the inhibition of electron transport (27), and, although the binding of piericidin is less specific than that of rotenone (28), it would be interesting to study whether piericidin also stimulates a latent ATPase activity in mitochondria incubated with Na+.

A plausible explanation for the results described in this paper would be to implicate that the energy-transducing mechanism involves a conformational change of a protein entity such as the electron carrier at the energy-coupling site. This would explain the unmasking of ATPase activity in the absence of Pi and the inhibition of electron transport by stoichiometric amounts of rotenone, as well as the finding that ADP protects against the detrimental action of Na+, although the stimulatory effect of Na+ on mitochondrial respiration in the absence of Pi is oligomycin insensitive. It is possible that these effects of Amytal and antimycin A resemble in their mechanism the action of rotenone presently described.

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