Properties of Higher Plant Mitochondria. III. Effects of Respiratory Inhibitors

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Summary. The effects of representative respiratory inhibitors were investigated on the coupled respiration of mung bean mitochondria using succinate and l-malate as substrates. The inhibitors studied were: (I) malonate, (II) amytal and rotenone, (III) antimycin A and 2-nonyl-4-hydroxyquinoline N-oxide (NOQNO), and (IV) cyanide and azide.

Malonate inhibition of succinate oxidation follows a classical type of competitive inhibition with an inhibitor dissociation constant of 0.13 mm. There is no inhibition detectable when malate is used as substrate. In contrast to animal mitochondria, amytal is capable of inhibiting 20 to 40% of succinate oxidation and 90 to 100% of malate oxidation, but inhibition due to rotenone amounts to only 0 to 20% of succinate oxidation and 40 to 50% of malate oxidation. The half-maximal inhibition caused by amytal occurs at 2 to 2.5 mm and that by rotenone at 3 mmoles/mg protein.

The maximal inhibition caused by either antimycin A or NOQNO is 70 to 80% of the state 3 respiration. Very little inhibition was observed on the state 4 respiration, and both inhibitors were capable of titrating stoichiometrically with mitochondrial protein with identical titers, 0.22 mmoles/mg protein for half-maximal inhibition. They differ, however, in that NOQNO does uncouple oxidative phosphorylation in mung bean mitochondria, but antimycin A does not do so. Both cyanide and azide inhibit the state 3 rate 65 to 80%. Inhibition of state 4 respiration can be up to 50% by cyanide, while almost none by azide. Uncoupling action was noted with cyanide, but very little with azide.

It is concluded that the second state 3 rate of succinate oxidation includes 80% succinoxidase, the remaining 20% being contributed by the NADH pathway. Malate oxidation apparently does not involve succinoxidase. Malate oxidation is completely sensitive to amytal, but only 50% inhibited by rotenone. A difference between animal and plant mitochondria appears to be in the flavoproteins associated with NADH oxidation.

From the observations that antimycin A, NOQNO, cyanide, and azide do not cause complete inhibition, it is suggested that a leakage of electrons to oxygen exists before the site of inhibition of antimycin A or NOQNO.

The inhibition of respiratory electron transport in both plant and animal mitochondria has been shown repeatedly to be achieved by the following 4 classes of inhibitors (cf. 9, 12, 15, 25): (I) inhibitors of succinic dehydrogenase, (II) inhibitors of NADH-cytochrome c reductase, (III) inhibitors of electron transport between cytochrome b and cytochrome c, and (IV) inhibitors of cytochrome c oxidase. Class I includes the classical inhibitor, malonate, which acts as a specific, competitive inhibitor of succinic dehydrogenase. From the work on animal mitochondria, the class II inhibitors, amytal and rotenone, are considered to act at the same site in the oxidation of NADH-linked substrates (7, 10, 27, 28). The site of inhibitory action of antimycin A and 2-alkyl-4-hydroxyquinoline N-oxide has been localized, particularly from spectrophotometric observations, between cytochrome b and cytochrome c of the respiratory chain (1, 4, 5, 8, 13, 20, 26). Cyanide and azide are among the classical inhibitors of cytochrome oxidase (16, 21, 22, 31, 34–36, 38).

The literature on plant mitochondrial respiration has so far emphasized qualitative similarities and dis-
similarities with respect to the respiratory properties of animal mitochondria (cf. 2,3,15,25). The dissimilarities are mostly centered around the "cyanide-insensitive" respiration (cf. 15). For example, Hackett et al. (15,16) proposed that an alternate, non-phosphorylating respiratory pathway was functional in sweet potato mitochondria in the presence of inhibitors acting between cytochrome b and oxygen. The main purpose of the present series of publications is to characterize the respiratory properties of plant mitochondria and compare them with those of animal mitochondria. The comparison so far made indicates that there are a number of differences existing between animal and plant mitochondria (18,19). These include: (a) substrate specificity, (b) pattern and rate of substrate oxidation, (c) affinities to substrates and cofactors, and (d) response to the inhibitors of phosphorylation. These differences are mostly quantitative in nature.

In a preliminary series of experiments with respiratory inhibitors, it was found that the inhibitors of class II acted on mung bean mitochondria quite differently from what had been expected from the work on animal mitochondria. The study was thus extended and this paper reports the effects of representative respiratory inhibitors on mung bean mitochondria using succinate and l-malate as substrates.

Materials and Methods

The plant material, the mitochondrial preparation, and the reaction medium were identical with those described in the first paper of this series (18). Respiration was measured polarographically with a Clark oxygen electrode in a 3-nl stirred cuvette. Unless otherwise mentioned, the sequence of additions to the cuvette was as follows: reaction medium, mitochondrial suspension, substrate, and repeated additions of ADP until anaerobiosis. A respiratory inhibitor was introduced either during state 1 (Method 1, 2 mins before addition of substrate) or during the second state 4 (Method 2, 2 mins before the second addition of ADP) (see illustrations in fig 1 of ref. 19). The respiratory rates, respiratory control ratios, and ADP:O ratios were calculated according to Chance and Williams (9). The effects of inhibitors were recorded for all the respiratory states and state 3 to 4 transitions after the introduction of inhibitor, but the state 3 rate, the state 4 rate, and state 3 to 4 transitions immediately after the application of inhibitor are presented in this paper. In all cases, the state 1 application of inhibitor did not cause any appreciable effect on the respiratory rate.

Succinate and malate were chosen as the primary substrates to examine succinoxidase and NADP-linked oxidase system (19). The inhibitors used in this paper were malonic acid disodium salt (Eastman Organic Chemicals), amytal sodium (El Lilly and Co.), rotenone (K and K, Inc.), antimycin A (California Corporation for Biochemical Research), NOQNO (Sigma Chemical Co.), KCN and NaN₃.

Antimycin A, NOQNO, and rotenone were dissolved in ethyl alcohol, and all other chemicals in double distilled water. The pH of chemicals dissolved in distilled water was adjusted to the vicinity of 7. The chemicals to be titrated were prepared immediately before use. The concentration of ADP was determined optically at 260 mµ on the basis of millimolar extinction coefficient of 13.4. The concentration of other chemicals was measured gravimetrically: the molecular weights used for amytal, antimycin A, NOQNO, and rotenone were 250, 549, 287, and 394, respectively. As previously described, a semi-micro Kjeldahl acid digestion method was used to determine the total nitrogen content of the mitochondrial preparations (18,19).

Results

Effects of Malonate. Figure 1 illustrates the effect of malonate on the oxidation of succinate and malate. The oxidation of succinate is clearly inhibited by malonate and the inhibition is observed in all the respiratory states tested. With increasing concentrations of malonate, the respiratory control ratio decreased steadily, whereas the ADP:O ratio remained constant (1.7 ± 0.2). This was the case for both methods of inhibitor applications. Figure 1 further points out that malonate exhibits little or no effect on malate oxidation, suggesting that the contribution of succinoxidase to malate oxidation is practically nil. It should be noted that the maximally inhibited level of both state 3 and state 4 of succinate oxidation reaches the second malate state 4 level (fig 1B).

Figure 2 shows the effect of malonate on the first

![Diagram](https://example.com/diagram1.png)

**Fig. 1.** (Left). Effect of malonate on succinate and malate oxidation. A) method 1 application of malonate; B) method 2 application of malonate. Succinate, 7.5 mM; malate, 30 mM; ADP, 165 µM. Roman numerals (III and IV) in this and subsequent figures denote respiratory state 3 and 4; subscripts (1, 2 and 3) indicate the first, second, and third.

**Fig. 2.** (Right). Nature of malonate inhibition of succinate oxidation. A) double reciprocal plots; concentration on each line indicates malonate concentration. B) slope of lines of figure A plotted against malonate concentration.
state 3 respiration of succinate oxidation. The mitochondria, ADP, and malonate were successively added to the reaction cuvette; then various concentrations of succinate were introduced. The malonate concentrations were changed from one series to another. There was no noticeable uptake of oxygen detected before addition of succinate. A series of double reciprocal plots (fig 2A) indicates that the malonate inhibition follows a classical type of competitive inhibition.

From the slopes of this figure, $\frac{K_m}{V_m} \left( 1 + \frac{I}{K_i} \right)$, plotted against malonate concentrations (fig 2B), the $K_i$ value is calculated as 0.13 mM. The apparent $K_m$ for succinate in this experiment is found to be 0.6 mM. This value compares well with previous results (18).

Effects of Amytal and Rotenone. Experiments were carried out to investigate the effect of amytal on succinate oxidation (fig 3). The maximal inhibition of the state 3 rate was 20% (first state 3) and 40% (second state 3) of the untreated controls. This maximal inhibition was caused by amytal concentrations higher than 5 mM. The half-maximal inhibition was 2 to 3 mM. All succinate state 4 rates were practically uninhibited with the amytal concentrations used in this study (fig 3). A slight decrease of ADP:O ratio was noted; from 1.5 to 1.2 for the first state 3 to 4 transition and from 1.8 to 1.5 for the second transition. A slight uncoupling effect of amytal at high concentrations is also reported in the literature with animal mitochondria (7, 11).

It has been observed with animal mitochondria that amytal inhibits the oxidation of NAD+-linked substrates (11, 30). In agreement with this concept, figure 3 shows strong amytal inhibition of malate oxidation. When amytal was applied during state 1, both the first state 4 and the first state 3 rates were inhibited (fig 3A), whereas amytal introduced during the second state 4 inhibited primarily the second state 3 (fig 3B). The half-maximal inhibition is about 2 mM for the first state 4 and the first and the second state 3's. This value is in agreement with the titer with pigeon heart mitochondria (7), but is 1 order of magnitude higher than those reported for other animal mitochondria (7, 11, 30). The oxidation of malate was slightly uncoupled by this chemical with the method 1 application; the ADP:O ratio decreased from 2.3 for control to 1.8 for 3 mM amytal.

![Fig. 3. (Left). Effects of amytal on succinate and malate oxidation. A) method 1 application of amytal; B) method 2 application of amytal. Succinate, 7.5 mM; malate, 30 mM; ADP, 165 μM.](image1)

![Fig. 4. (Right). Effect of rotenone on succinate and malate oxidation. A) method 1 application of rotenone; B) method 2 application of rotenone. Succinate, 5 mM; malate, 33 mM; ADP, 185 μM; mitochondria, 166 μg.](image2)
There was practically no uncoupling when the inhibitor was applied during the second state 4.

Figure 4 illustrates the effect of rotenone on the oxidations of succinate and malate by mung bean mitochondria. When this inhibitor was added during state 1 (fig 4A), no inhibition was found for succinate oxidation, but with malate 15% of the first state 4 and 40% for the first state 3 were inhibited at high concentrations with half-maximal inhibition at 2.5 \(\mu M\). The introduction of rotenone during the second state 4 produces a different picture (fig 4B); while state 4 rates for both succinate and malate oxidation are not inhibited by this chemical, 20% of succinate state 3 rate and 50% of malate state 3 rate were inhibited at high concentrations. The half-maximal inhibition is attained at 0.5 \(\mu M\) and 1 \(\mu M\) for the oxidation of succinate and malate respectively. During this titration, the ADP:O ratio in succinate oxidation did not change, but it decreased in malate oxidation from 2.1 to 1.3 for the first state 3 to 4 transition and from 2.5 to 1.3 for the second transition. It thus appears that the effect of rotenone on the oxidation of succinate is very little, but malate oxidation is inhibited incompletely and with some uncoupling.

A stoichiometric relationship exists between rotenone concentration and mitochondrial protein (10). This stoichiometry with mung bean mitochondria (fig 4) is 17.5 mmol/mg protein for maximal inhibition and 3 mmol/mg protein for half-maximal inhibition.

**Effects of Antimycin A and NOQNO.** Figure 5 illustrates the sensitivity of succinate oxidation to antimycin A. The first and second state 4 rates are inhibited by about 30%, whereas the first and second state 3 rates are markedly inhibited with the half-maximal inhibition at 18 \(\mu M\). It is of interest to note that antimycin A did not bring about the total inhibition of respiration, but at higher concentrations did decrease the state 3 rate to the state 4 level. The remaining respiration corresponds to about 20% of uninhibited second state 3 level. As expected, the respiratory control ratio decreased with increasing concentrations of antimycin A. The change in the ADP:O ratio, on the other hand, was very little; from 1.4 to 1.2 for figure 5A and from 1.7 to 1.3 for figure 5B.

Inhibition of malate oxidation by antimycin A is shown in figure 6. Similar to succinate oxidation, the first state 4 rate was slightly inhibited and no inhibition was observed for the second state 4 at the antimycin A concentrations used. State 3 rates were, however, markedly inhibited; a half-maximal inhibition is attained with an antimycin A concentration of 18 \(\mu M\). Here again, the complete inhibition of state 3 rate could not be observed. Higher concentrations of antimycin A decreased the state 3 rate of malate oxidation close to the state 4 level, the remaining respiration being 25 to 30% that of the uninhibited control. It should be added that the ADP:O ratio was hardly affected by antimycin A application; 2.3 ± 0.1 for the first state 3 to 4 transition and 2.3 ± 0.1 for the second transition.

![Fig. 5. (Left). Effect of antimycin A on succinate oxidation. A) method 1 application of antimycin A; B) method 2 application of antimycin A. Succinate, 7.5 mm; ADP, 167 \(\mu M\); mitochondria, 148 \(\mu g\) N.](image)

![Fig. 6. (Right). Effect of antimycin A on malate oxidation. A) method 1 application of antimycin A; B) method 2 application of antimycin A. Malate, 30 mm; ADP, 167 \(\mu M\); mitochondria, 148 \(\mu g\) N.](image)

![Fig. 7. Titration of antimycin A effect of malate second state 3 rate with various concentrations of mitochondria. Mitochondrial concentration given on each curve as \(\mu g\) N/e ml reaction mixture. The maximum inhibition (dotted line) is the same as the second state 4 after antimycin A addition. Malate, 33 mm; ADP, 150 \(\mu M\). A) rate of oxygen uptake vs. antimycin A concentration; B) antimycin A concentration for half-maximal effect plotted against mitochondrial concentration.](image)
The above observation that the same value of half-maximal inhibition was obtained for both succinate and malate oxidation indicates that the same antimycin A sensitive electron transport chain is utilized for the oxidation of both succinate and NADH, in the manner identical to the electron transport system in animal mitochondria (cf. 9). It has been reported with phosphorylating and non-phosphorylating animal mitochondria that the inhibiting concentration of antimycin A changes with the protein concentration of preparations used (14, 29, 33). In the following experiments, therefore, the inhibition by antimycin A was titrated against various concentrations of mitochondria. For this purpose, the procedure used in figure 6B was applied and the second state 3 rate was recorded. The summary of a series of experiments is shown in figure 7A. The relationship between mitochondrial concentration and the concentration of antimycin A required to cause a half-maximal inhibition is plotted in figure 7B. A calculation determining the ratio of antimycin A concentration to mitochondrial concentration reveals a value of 1.4 μmole antimycin A/mg N or 0.22 μmole/mg protein. Our value is about 100 times lower than the value reported earlier with sweet potato mitochondria (37).

The effect of NOQNO on the oxidation of succinate and malate was examined by applying this inhibitor during the second state 4. As shown in figure 8, the pattern of inhibition resembles that by antimycin A; little or no inhibition was observed on second state 4 and the state 3 rates were markedly inhibited approaching the state 4 rates at high concentrations. The level of full inhibition corresponds to 25 % (succinate) and 25 % (malate) of respective untreated controls. The half-maximal inhibition of the state 3 rate is attained at a concentration of 40 μM with both substrates. During this titration, the respiratory control decreased. The ADP:O ratio also decreased from 1.7 to less than 1.0 with succinate and from 2.5 to less than 1.0 with malate. This uncoupling effect of NOQNO differs from the antimycin A effect.

Since NOQNO exerts its inhibition at the same site in the electron transport chain as does antimycin A (5, 20, 26), and its general mode of action resembles antimycin A, the inhibition by NOQNO was titrated against various concentrations of mitochondria. Malate was used as the substrate. Figure 9A shows the actual measurements with 3 different mitochondrial concentrations, and the concentration of NOQNO required for the half-maximal inhibition is plotted against mitochondrial concentration in figure 9B. The ratio of NOQNO to mitochondrial nitrogen was calculated from the slope of the latter figure as 1.4 μmole/mg N, identical with the antimycin A titer. This chemical is thus as effective an inhibitor as is antimycin A of electron transport, but it differs from antimycin A in its ability to uncouple phosphorylation.

**Fig. 8.** (Left). Effect of NOQNO on second state 3 and state 4 rates of succinate oxidation (A) and malate oxidation (B). Succinate, 7.5 mM; malate, 30 mM; ADP, 170 μM.

**Fig. 9.** (Right). Titration of NOQNO effect on malate second state 3 rate with various concentrations of mitochondria. Number on each curve denotes mitochondrial concentration in μg N/3 ml reaction mixture. The maximum inhibition (dotted line) is taken as the second state 4 rate after NOQNO addition. Malate, 33 mM; ADP, 187 μM. A) rate of oxygen uptake vs. NOQNO concentration; B) NOQNO concentration for half-maximal effect plotted against mitochondrial concentration.

**Fig. 10.** (Left). Effect of KCN on succinate oxidation. A) method 1 application of KCN; B) method 2 applications of KCN. Succinate, 7.5 mM; ADP, 190 μM.

**Fig. 11.** (Right). Effect of KCN on malate oxidation. A) method 1 application of inhibitor; B) method μM.

**Effects of Cyanide and Azide.** Figures 10 and 11 illustrate the effect of KCN on the mitochondrial oxidation of succinate and malate. In general, the inhibition of state 4 rates is small. State 3 rates with both substrates, on the other hand, are markedly inhibited. It is of interest to note that, similar to antimycin A and NOQNO effects, both the first and the second state 3 rates approach the level of the
The effect of azide on the oxidation of succinate and malate was studied only by applying this chemical during the second state 4 (fig 13). No inhibition of state 4 rates was found. State 3 rates, however, were markedly inhibited by this inhibitor. A half-maximal inhibition is obtained at 45 μM with succinate as substrate and 35 μM with malate as substrate. Similar to the cyanide effect, the maximal inhibition approaches the second state 4 rate. The level of maximal inhibition corresponds to about 35% with succinate and 32% with malate of the uninhibited state 3 rate. As the azide concentration increased, the respiratory control ratio decreased. The ADP:O ratios, on the other hand, were much less affected: the ratio remained constant below 30 μM azide and very gradually decreased at higher concentrations. With 100 μM, for example, the ratio was 1.6 in succinate oxidation and 2.1 in malate oxidation.

Discussion

Action of Respiratory Inhibitors. Table I summarizes all the results with respiratory inhibitors that have been presented in this paper. In this table the inhibitors examined are grouped according to their site of inhibition in the electron transport system of animal mitochondria.

The first group includes only malonate, which is a specific competitive inhibitor of succinic dehydrogenase (figs 1 and 2). The Ki calculated from the effect of malonate on the succinate first state 3 respiration is 0.13 mM (fig 2B). The malonate Ki value obtained from the first state 3 respiration of succinate oxidation probably represents the inhibitor constant free of complications. The different Ki values in table I for various respiratory states probably reflect the possible contribution of the NADH oxidase path which was not accounted for in the calculations. It should be mentioned that the Ki values for malonate of heart muscle and plant succinic dehydrogenase are reported as 0.2 mM (17) and 0.24 mM (32), respectively.
Table I. Summary of the Effects of Respiratory Inhibitors on Succinate and Malate Oxidation by Mung Bean Mitochondria

<table>
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<th>Group</th>
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<th>Method 2</th>
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<td>III₁</td>
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<td>A)</td>
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B) Degree of maximum inhibition (% of untreated control)

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C) Uncoupling effect on oxidative phosphorylation**

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* Calculated on the basis of competitive inhibition from figure 1.

** The effects of these inhibitors depend upon mitochondrial concentration (see text). The values obtained for half-maximal effect of malate as substrate in method 2 are: Rotenone, 3 µmole/mg protein; Antimycin A, 0.22 µmole/mg protein; NOQNO, 0.22 µmole/mg protein.

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Rotenone has been claimed to be by far the most potent inhibitor of the electron transport system of animal mitochondria and its titer value changes linearly with mitochondrial concentrations (7, 10, 27, 28). Ernster et al.'s (10) titer for half-maximal inhibition is about 0.003 µmole/mg protein and that for complete inhibition 0.025 to 0.028 µmole/mg protein. These values can be compared with our values; 3 µmole/mg protein and 17.5 µmole/mg protein for half-maximal and maximal inhibitions respectively (fig 4). Clearly this chemical is at least 500 times less effective on mung bean mitochondria than it is on rat liver mitochondria. The half-maximal inhibition by amytal is attained at 0.2 to 0.5 mM (rat liver mitochondria; 7, 11) and 2 mM (pigeon heart mitochondria; 7). Our value of 2 to 4 mM for the value of 'K' for amytal agrees closely with that of pigeon heart mitochondria. The difference in K values between rotenone and amytal with animal mitochondria is as much as 4 orders of magnitude. Both chemicals, at high concentrations, apparently inhibit completely NAD+-linked substrate respiration (7, 10, 27, 28). These results indicate a strong dissimilarity in the action of amytal and rotenone between animal and plant mitochondria.

Amytal inhibition of succinate oxidation is found in animal mitochondria, the half-maximal effect is at 2.5 mM with succinate alone (30) and at 7 mM in the presence of glutamate (7). Rotenone inhibition of succinate oxidation has, however, not been reported with animal mitochondria.

The site of action of amytal and rotenone has been identified to be on the substrate side of cytochrome b (7, 28), probably on the substrate side of flavoprotein (7). Furthermore, rotenone is generally considered from the work on animal tissue and mitochondria to mimic practically all the known features of the amytal effects on the electron transport system: both chemicals specifically inhibit the NAD+-linked respiration of mitochondria (7, 10, 27, 28). Our re-
sults (figs 3 and 4) in general, agree with this concept. However, the observations that amytal is capable of bringing about 90% inhibition and rotenone only 40 to 50% inhibition of malate state 3 point out a difference in the mode of the 2 inhibitors. In succinate oxidation the maximal inhibition observed was 40% with amytal, whereas it was 20% with rotenone. Furthermore, with the method 1 application, amytal inhibits the first state 3 of succinate oxidation by about 20%, while rotenone does not inhibit this state at all. All these observations point out that rotenone is a more specific inhibitor of the oxidation of NADH-linked substrates, whereas amytal causes some inhibition of succinoxidase system as well. If this is the case, the difference between plant and animal mitochondria is associated with the flavoproteins in the intra-mitochondrial NADH oxidase path.

The pattern of inhibition by the third group of inhibitors has been demonstrated spectroscopically between cytochromes b and cytochrome c in animal mitochondria (4, 5, 8, 14, 20, 26). With plant mitochondria, the antimycin A or NOQNO effect is spectroscopically resolved into 3 cytochrome b type peaks, but similar to animal mitochondria, cytochromes a and c peaks disappear (2, 3, 23). It is thus quite probable that the nature of electron transport inhibition by this third group of chemicals is quite similar to that in animal mitochondria. This similarity is further strengthened by the ability of antimycin A or NOQNO to titrate, with identical titers. The titer values, 0.22 μmole/mg protein for mung bean mitochondria (fig 7B) and 0.47 μmole/mg protein for rat liver mitochondria (calculated from the data in ref 14) reveal, however, that the ratio of protein content of animal mitochondria to plant mitochondria is about 5. Earlier, we have compared a similar ratio in terms of QO2 values (table I of ref 18) and found the ratio to be at least 4. A sizeable portion of state 3 respiration (20-30%) leaks out before the site of action of these inhibitors (cf. 16). The difference in the action of the 2 chemicals is that NOQNO causes uncoupling of phosphorylation, whereas the uncoupling effect is negligible with antimycin A.

The fourth inhibitor group includes the inhibitors of cytochrome oxidase (15, 16, 21, 22, 31, 34-36, 38). The half-maximal inhibition of state 3 respiration with mung bean mitochondria is at 4 to 5 μM and 30 to 40 μM of cyanide and azide, respectively (table I). With tightly coupled rat liver mitochondria, the half-maximal inhibition was observed at 40 to 60 μM with azide (35). The Keilin-Hartree heart muscle preparation and yeast cells gave rise to values in the same order of magnitude (21, 31, 36). These observations suggest that plant mitochondria contain an electron transport system similar to the animal system. It is, however, of interest to notice that 20 to 40% of state 3 respiration remained uninhibited and this amount corresponds closely to antimycin A or NOQNO insensitive respiration. It appears that this amount of state 3 respiration is carried out before the site of antimycin A or NOQNO inhibition (cf. 16). Furthermore, it is highly probable that this leak of electrons to oxygen contributes to the slightly lower ADP:O ratio observed previously (18).

Nature of Succinate and Malate Oxidation. Malonate does not inhibit malate oxidation at all (fig 1), while the malonate inhibition of succinoxidase follows a classical type of competitive inhibition (fig 2). This indicates that the succinoxidase path of electron transport does not contribute to the oxidation of malate. In contrast to the above, the results with amytal and rotenone, inhibitors of NADH-cytochrome c reductase, show that they do partially inhibit the oxidation of succinate (figs 3B and 4B), suggesting strongly that the NADH oxidase path of electron transport contributes to the second state 3 oxidation of succinate at a 20% level. This contribution is presumably due to the energy-linked reduction of intramitochondrial NADH by succinate during the second state 4 (6, 7). Furthermore, the first state 4 rate of malate oxidation is brought by amytal and rotenone to the same final level as the maximally inhibited first state 3, while the second state 4 rate is practically not inhibited at all (figs 3 and 4). These observations suggest that the first state 4 and the state 3 of malate oxidation is carried out through the intramitochondrial NADH oxidase path, but the second (and later) state 4 is not. Furthermore, the fact that only 40 to 50% of state 3 rate of malate oxidation is inhibited by rotenone while the effect of amytal is near complete suggests that only one-half of malate oxidation is channeled through the rotenone-sensitive NADH path and the other half probably through an amytal-sensitive path.

We have previously noted that in succinate oxidation, the first state 4 is about 50% and the first state 3 rate about 25% less than the subsequent rates of respective states (18). From the study of phosphorylation inhibitors, it was concluded that mung bean mitochondria lack endogenous substrates, ATP, and/or "high energy intermediates" (19). In view of all the points discussed above, it can be concluded that both the first states 4 and 3 of succinate oxidation are carried out through the succinoxidase proper of electron transport, but the subsequent state 3 and state 4 involve the NADH oxidase path as well. This is apparently the case, since the final level of malonate inhibition approaches the second malate state 4 level (fig 1B).

Differences Between Plant and Animal Mitochondria. Mung bean mitochondria resemble in many respects animal mitochondria. There are, however, distinct differences between the 2 types of mitochondria (18, 19, this paper): (I) mung bean mitochondria are capable of oxidizing malate at rapid rates; (II) plant mitochondria oxidize externally applied NADH rapidly; (III) the respiratory pattern of mung bean mitochondria differs from that of rat liver mitochondria during the early respiratory states of our standard assay; (IV) respiratory rates of
phosphorylating state by mung bean mitochondria are at least 4 times faster than that by rat liver mitochondria; (V) while tightly coupled rat liver mitochondria give ADP:O ratios very close to the theoretical values, the ratios with mung bean mitochondria are consistently less than the theoretical values; (VI) the action of uncoupling agents is apparently not the same in the 2 types of mitochondria; (VII) respiratory inhibitors acting in the middle or at the end of the electron transport system do not completely inhibit state 3 respiration by mung bean mitochondria; (VIII) the titers of antimycin A, NOQNO, and carbonyl cyanide m-chlorophenylhydrazone on mung bean mitochondria are about 5 times higher than those reported with rat liver mitochondria, while the titer value of oligomycin is about 5 times less on mung bean mitochondria than on rat liver mitochondria; and (IX) inhibition by rotenone on mung bean mitochondria is about 50 times less effective than that on rat liver mitochondria. In addition to these, spectroscopic studies have revealed differences between plant and animal mitochondria (2, 3, 23).

Similarities and dissimilarities between the 2 types of mitochondria so far compared, lead to a conclusion that mung bean mitochondria contain not only an electron transport chain of the animal type, but also another oxidation pathway as well (cf. 16).

Literature Cited