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Properties of Higher Plant Mitochondria. II. Effects of DNP, *m*-Cl-CCP, and Oligomycin on Respiration of Mung Bean Mitochondria[1]

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Summary. Effects of inhibitors of phosphorylation on the oxidation of succinate and of *l*-malate were investigated with tightly coupled mitochondria isolated from mung bean hypocotyls. When mitochondria were incubated with 2,4-dinitrophenol, or carbonyl cyanide *m*-chlorophenylhydrazone prior to the addition of substrate, the uncoupling effects of these chemicals were relatively small. This is probably caused by relative lack in these mitochondria of endogenous substrates, ATP, and/or "high-energy intermediates". The action of uncoupling agents is, therefore, revealed in a more striking manner when they are introduced during the second state 4. Of the 2 uncoupling agents tested, malate oxidation consistently required 1.5 to 2 times higher concentration of the agents for the half-maximal effects than succinate oxidation. From the comparison of the degree of uncoupling it is concluded that 2,4-dinitrophenol is a better uncoupler of succinate oxidation, whereas carbonyl cyanide *m*-chlorophenylhydrazone functions as a more complete uncoupler of malate oxidation.

Oligomycin does not inhibit state 4 rates, while the increment of respiration due to added ADP is completely inhibited by this antibiotic. Identical half-maximal effects are observed with the same concentration of oligomycin in both succinate and *l*-malate oxidation. The oligomycin effect depends on the mitochondrial concentration employed. The concentration of this chemical required for the half-maximal effect is 55 to 80 mμmoles per mg mitochondrial protein. It is suggested that this inhibitor of phosphorylation binds all of the phosphorylation sites regardless of whether the sites are functional or not.

The classical paper of Loomis and Lipmann (21), has been followed by a number of papers showing that DNP[3] uncouples phosphorylation from oxidation in mitochondria isolated both from plant tissues (1,2, 9, 10, 15, 25, 26) and from animal tissues (3, 4, 7, 13, 19, 23, 24). The uncoupling concentration of DNP is between 10^{-5} and 10^{-4} M. Recently, Heytler and his collaborators (8, 11, 12) showed that *m*-Cl-CCP was more potent than DNP as an uncoupler of animal and plant mitochondria. Tight coupling of oxidative phosphorylation is released at *m*-Cl-CCP concentrations between 10^{-7} and 10^{-6} M. Oligomycin has been shown to inhibit respiration by blocking a reaction involving the formation of a high energy intermediate (8, 11–13, 16–18, 20, 22, 24). In animal mitochondria, the oxidation of NAD-linked substrates is inhibited slightly by 1 μg of oligomycin, the inhibition is released by DNP (13, 16–18).

In the literature on plant mitochondrial respiration, emphasis has been placed on the similarities between plant and animal mitochondria (cf. 20). However, our first paper in this series (14) showed that mung bean mitochondria possessed several characteristics that were different from those of animal mitochondria. This paper further characterizes the respiratory properties of mung bean mitochondria and their responses to inhibitors and uncouplers of oxidative phosphorylation. The results from mung bean mitochondria are again compared with those from animal mitochondria.

Materials and Methods

The plant material, the mitochondrial preparation, the reaction medium, and the technique of respiratory measurements were identical with those described in the first paper of this series (14). Briefly, the mitochondria were isolated from hypocotyls of mung beans, which had been dark-grown for 4 to 5 days

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[3] Abbreviations: DNP = 2,4-dinitrophenol; *m*-Cl-CCP = carbonyl cyanide *m*-chlorophenylhydrazone; NAD = nicotine adenine dinucleotide; ADP = adenosine-5′-diphosphate; ATP = adenosine-5′-triphosphate; BSA = bovine serum albumin.

at 28°, in a medium consisting of 0.3 M mannitol, 0.1 % (w/v) BSA, 0.05 % (w/v) cystein, and 1 mM EDTA. They were then washed by means of 2 centrifugations, 250 × *g* for 10 minutes and 6000 × *g* for 15 minutes, and suspended in a medium composed of 0.3 M mannitol, 0.1 % BSA, and 0.1 mM EDTA. The reaction mixture contained 0.3 M mannitol, 10 mM KCl, 10 mM K-phosphate buffer (pH 7.2), and 5 mM $MgCl_2$. Respiration was measured polarographically with a Clark oxygen electrode; the definitions of Chance and Williams (4) are followed in describing the different mitochondrial steady state conditions.

In all respiratory measurements, the sequence of additions into the reaction cuvette (3 ml) was as follows: reaction medium, mitochondrial suspension, substrate, and repeated additions of ADP until anaerobiosis was reached. An inhibitor or uncoupler phosphorylation was introduced either during state 1 (2 mins before the addition of substrate), or during the second state 4 (2 mins before the second addition of ADP). These 2 methods of introduction are illustrated in figure 1 as method I and method II with succinate as substrate.

Succinate and *l*-malate were chosen as the primary substrates to examine succinoxidase and a NAD-linked oxidase system. DNP (recrystallized), *m*-Cl-CCP, and oligomycin were kindly provided by Dr. B. Pressman of the Johnson Foundation. These chemicals were prepared immediately before use in double-distilled water.

Mitochondrial concentrations were calculated in terms of nitrogen content in the same manner as in the previous paper (14).

Results

Effect of DNP. Figure 1 illustrates the effect of DNP upon the respiratory rates with succinate as substrate. When mitochondria were incubated with DNP for 2 minutes (fig 1A), the first state 4 rate doubles at a DNP concentration of 15 μM, but at higher concentrations (>30 μM) the rate is the same as the control. The first state 3 rate follows a pattern similar to the first state 4, but the increase in rate at 15 μM is about 30 % above the control and the rate does not reach the second state 3 level. At higher concentrations of this uncoupler, the rate becomes less than the control by about 35 %. The second state 4 rate increases with increasing concentrations of DNP and reaches maximum at 50 μM DNP. At higher concentrations it is no longer possible to separate this state from the first state 3. The ADP:O ratio for the first cycle of state 3 to 4 transition steadily decreased from 1.6 (without DNP) to 0.5 at 50 μM DNP.

When DNP is added during the second state 4, the tight coupling of succinate oxidation is released (fig 1B). The respiratory rate increases with increasing concentrations of DNP, reaches maximum,

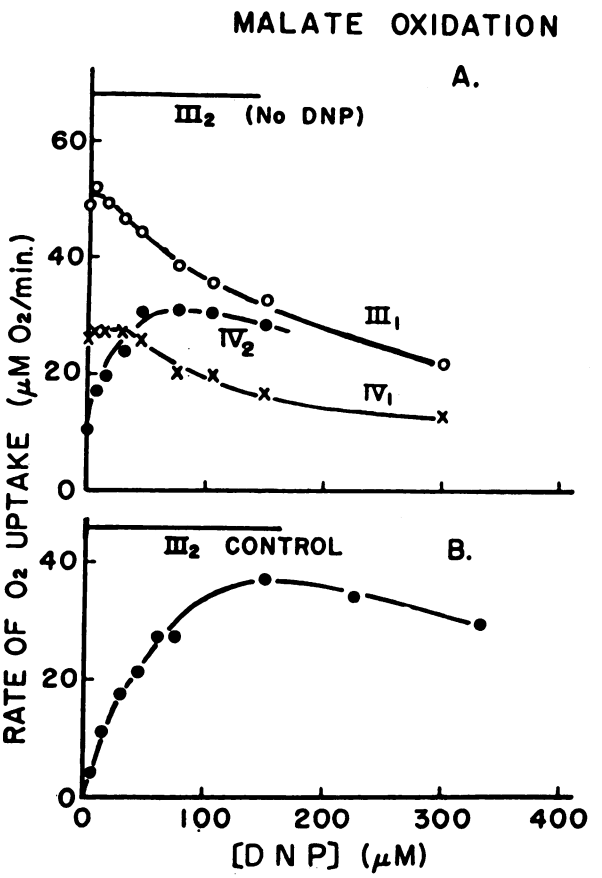

FIG. 1. Effect of DNP on malate oxidation. A) method I application; malate = 30 mM, ADP = 165 μM. B) method II application; malate = 30 mM, ADP = 140 μM. The rate before the DNP addition = 11.5 μM O_2/min.

and decreases gradually at higher concentrations. The concentration of DNP which causes 50 % stimulation of mitochondrial respiration is 20 μM. The maximum release is obtained at 80 μM, at which the respiratory rate is slightly above the second state 3 control. The concentrations of DNP higher than 250 μM inhibits the rate below the second state 3 control level.

Figure 2 shows a series of parallel experiments to the above with malate as substrate. The 2 minutes' incubation of mitochondria with DNP did not cause any stimulation of respiratory activity during both the first state 4 and the first state 3 with the range of concentrations tested (fig 2A). An inhibitory effect of DNP is detected above 30 μM. Similar to the effect on succinate oxidation, the second state 4 rate increases with increasing DNP concentrations and reaches maximum at 80 μM (fig 2A). The ADP:O ratio for the first cycle of state 3 to 4 transition decreased with increasing concentrations of this uncoupler; from 2.6 in control (without DNP), to less than 1 at 150 μM DNP. The maximum release of coupling of malate oxidation is reached at

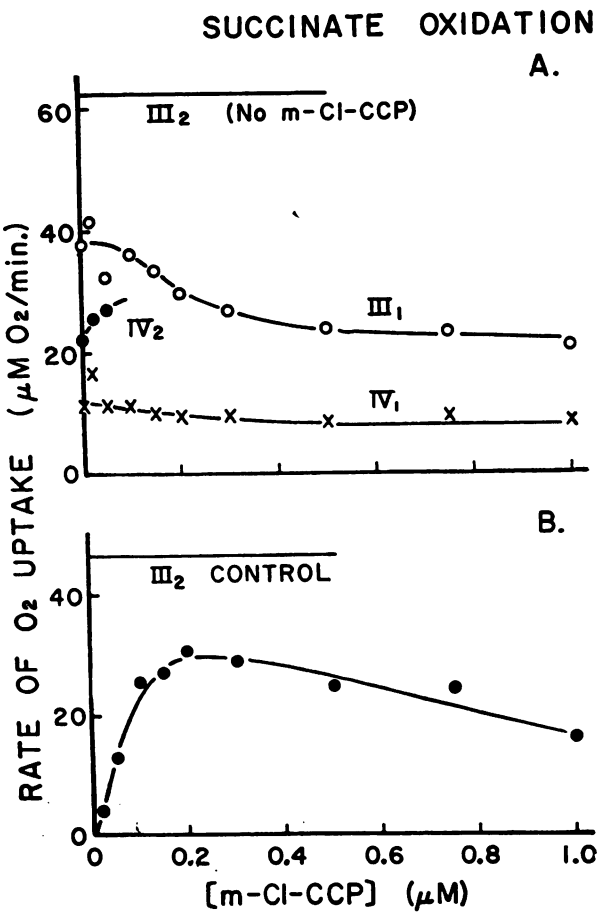

FIG. 2. (left) Effect of *m*-Cl-CCP on succinate oxidation. A) method I application, succinate = 7.5 mM, ADP = 165 μM, mitochondria = 112 μg N. The rate before the *m*-Cl-CCP addition = 25 μM O_2/min.

150 μM, but the stimulated rate is less by about 20 % than the second state 3 control (fig 2B). The half-maximum effect is seen at 35 μM.

Comparison of DNP effects on both succinate and malate oxidation brings about at least the following 2 points: 1) the stimulatory effect of low concentrations on both the first state 4 and the first state 3 rate, as seen with succinate as substrate, is not observed with malate as substrate (2). While the coupling of oxygen uptake with phosphorylation is released by this chemical on both succinate and malate oxidation, the concentration which brings about maximum release is different: 80 μM for succinate oxidation and 150 μM for malate oxidation. Furthermore, the maximum release of coupling in succinate oxidation equals, or is even slightly higher, than the second state 3 control, whereas that in malate oxidation is less by about 20 %. These differences point out that DNP is a better uncoupler for succinate oxidation than for malate oxidation.

It should be mentioned that DNP did not affect the state 1 respiration with either succinate or malate as substrates. These observations favor the view that the endogenous level of both adenosine nucleotides and substrates is low in the mung bean mitochondria.

Effect of m-*Cl-CCP*. Essentially the same experiments as with DNP were carried out with *m*-Cl-CCP (figs 3 and 4). As in the DNP experiments, uncoupling effect of this chemical was not detected in state 1 respiration.

With succinate as substrate, the primary effect of *m*-Cl-CCP is to inhibit the first state 4 and the first state 3 rates (fig 3A). There was no distinct stimulation of respiration detected in these respiratory states at the range of concentrations tested. The ADP:O ratio abruptly decreased from 1.5 for control to 0.8 at 0.05 μM *m*-Cl-CCP. Tight coupling is, nevertheless, broken by this compound at 0.2 μM, when it was applied during the second state 4 (fig 3B). It is noteworthy that the maximum release is only about 60 % of the second state 3 control. The half-maximal effect is observed at 0.06 μM of this uncoupling agent.

With malate as substrate, *m*-Cl-CCP stimulates the respiratory rates of both the first state 4 and the first state 3 more than with succinate (fig 4A).

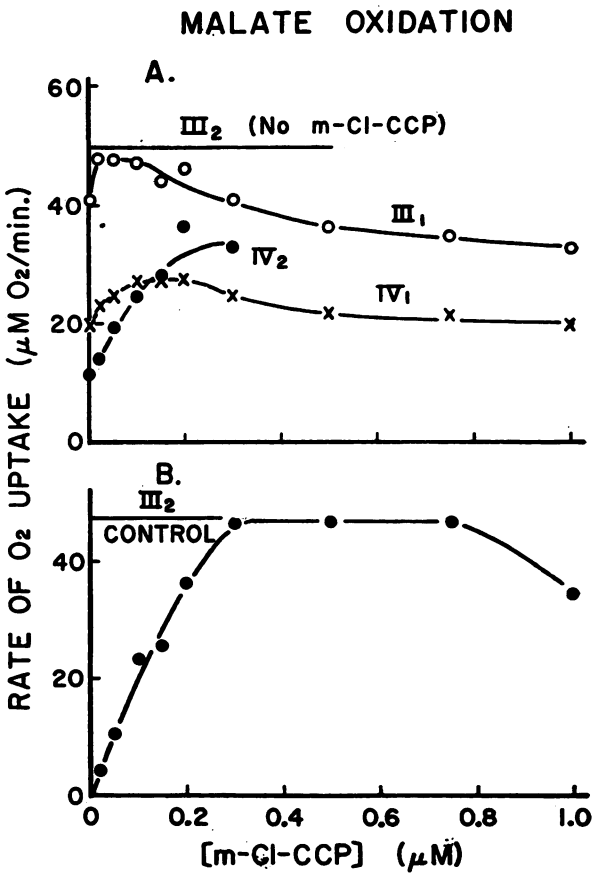

FIG. 3. (right) Effect of *m*-Cl-CCP on malate oxidation. A) method I application, malate = 30 mM, ADP = 165 μM, mitochondria = 120 μg N. B) method II application, malate = 30 mM, ADP = 165 μM, mitochondria = 112 μg N. The rate before the *m*-Cl-CCP addition = 13 μM O_2/min.

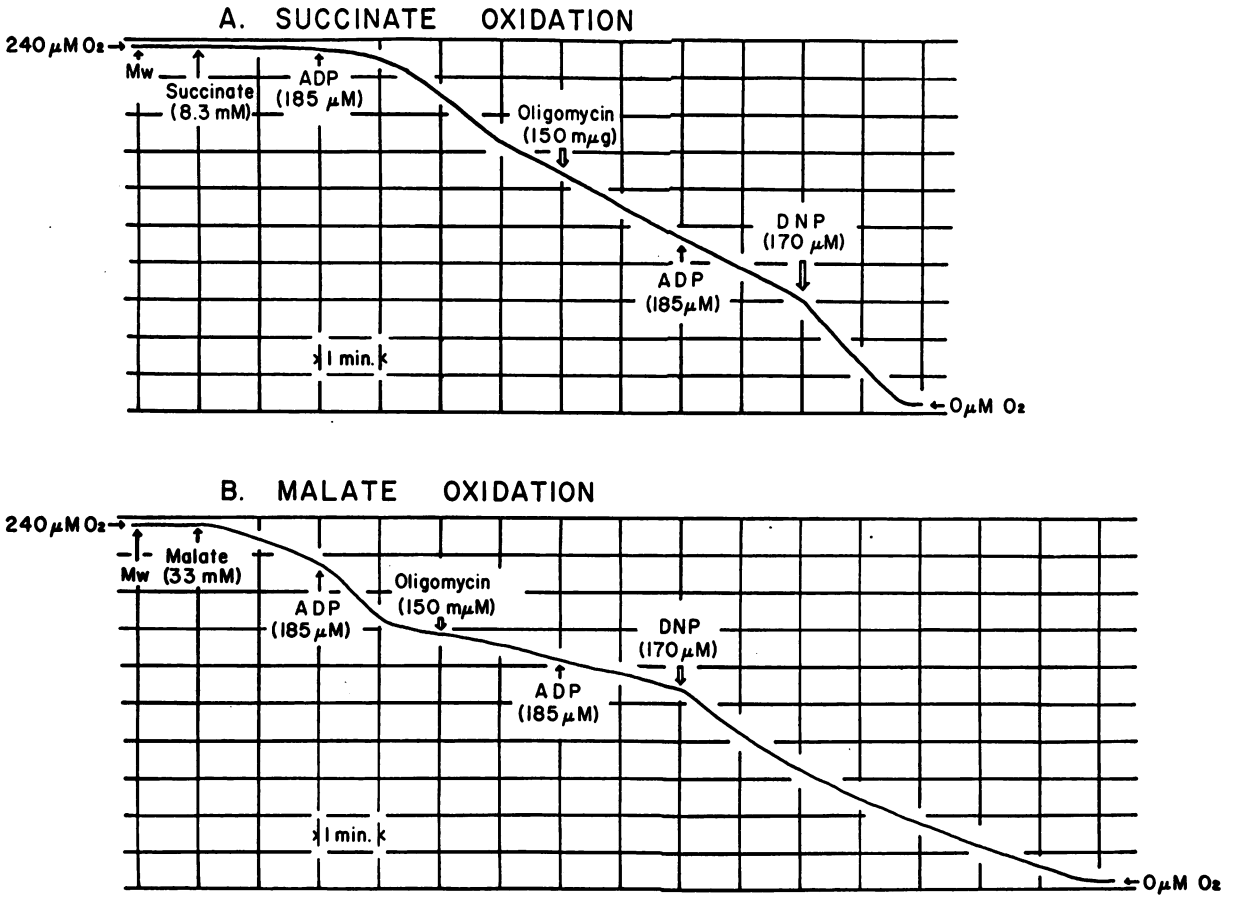

FIG. 4. Inhibition of state 3 respiration by oligomycin and reversal by DNP. A) succinate oxidation, B) malate oxidation.

The highest stimulation was caused by this chemical at 0.02 to 0.2 μM. Furthermore, the stimulated first state 3 rate approaches the level of nontreated second state 3 rate. The ADP:O ratio decreased from 2.4 without *m*-Cl-CCP to less than 1 at 0.75 μM of this chemical. The release of tight coupling is also more markedly demonstrated with malate as substrate (fig 4B) than with succinate. The maximum release is attained in the concentration range of 0.3 to 0.7 μM. It should be noted that the maximally released rate equals the second state 3 control. The half-maximal effect is shown at 0.12 μM.

These results point out that *m*-Cl-CCP does not act identically on the oxidation of succinate and of malate: (1) In malate oxidation, the respiratory rates of the first state 4 and the first state 3 are stimulated at low concentrations of this chemical, while a similar observation is not obtained with succinate as substrate; (2) The release of coupling is complete in malate oxidation, whereas the efficiency is about 60 % in succinate oxidation; and (3) The range of concentrations which give the maximal release of coupling is different between the oxidation of the 2 substrates.

From the comparison of the data obtained with DNP effects, it can be concluded that *m*-Cl-CCP functions as uncoupler more completely in malate oxidation, whereas DNP is a better uncoupler of succinate oxidation.

Effect of Oligomycin. The inhibition of the state 3 respiratory rate by oligomycin and its reversal by DNP (13, 15–18, 24–26) is illustrated in figure 5. The second addition of 185 μM did not cause any appreciable increase in the respiratory rates over preceding state 4 rates in the presence of oligomycin alone. The addition of DNP, however, releases the inhibition caused by oligomycin. It should be noted that oligomycin did not inhibit state 4 with the concentration used here.

Using the same addition sequence as above, the oligomycin inhibition of state 3 and state 4 rates was examined with both succinate and malate as substrates. The same preparation of mitochondria was used for these studies and the results are shown in figure 6. The second state 3 rates of both malate and succinate are clearly inhibited. The half-maximal inhibition for both succinate and malate oxidation was caused by an oligomycin concentration of about 80 mμg when the mitochondrial concentration was 150 μg N. Oligomycin does not bring about the complete inhibi-

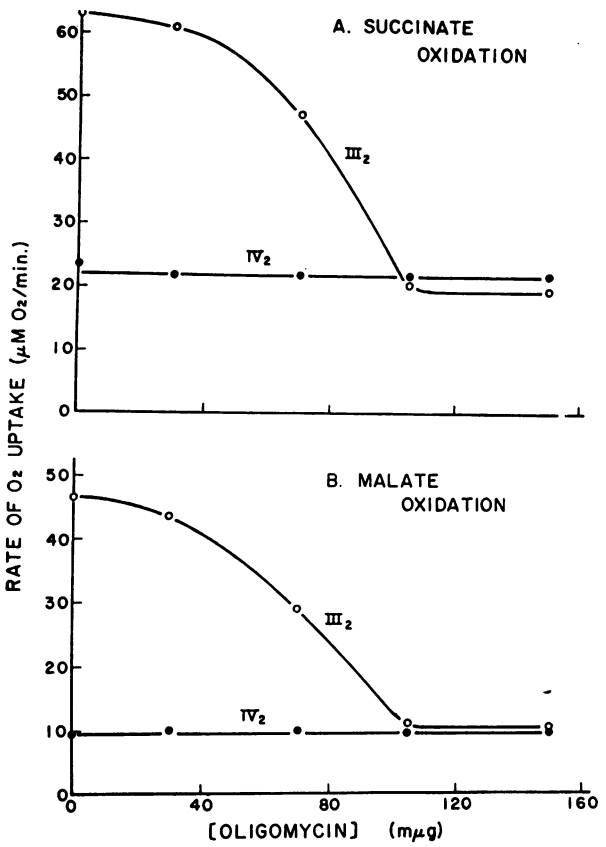

FIG. 5. Effect of oligomycin on the oxidation of succinate (A) and malate (B). Oligomycin applied during the second state 4; effects examined on the second state 4 and the second state 3. Succinate = 8.3 mM, malate = 33 mM, ADP = 185 μM, mitochondria = 150 μg N.

tion of state 3 rates, as noted by Huijing and Slater (13) with rat liver mitochondria: about 30 % of the succinate oxidation and about 20 % of the malate oxidation rate in this state remained uninhibited, even at high concentrations of this inhibitor. The uninhibited rates coincide with the state 4 rates which are not at all inhibited throughout the range of concentrations studied. These findings confirm closely the results obtained with animal mitochondria (13, 17, 18).

Figure 6 makes it possible to calculate the stoichiometric relationship of oligomycin to mitochondrial protein. Assuming that the ratio of mitochondrial nitrogen to protein is 0.16, we obtain a value corresponding to 80 mμg oligomycin per mg mitochondrial protein for the half-maximal inhibition. This stoichiometric relationship is further examined by varying both the mitochondrial and oligomycin concentrations in the reaction cuvette, as illustrated in figure 7. Oligomycin was introduced during the second state 4 and the second state 3 rates were recorded. In view of the previous results, the second state 4 rate in the presence of oligomycin is considered to be the maximal inhibition. The oligomycin concentration to cause half-maximal inhibition is computed from figure 7A and plotted in figure 7B in terms of mitochondrial concentration. The mitochondrial concentration is defined as that concentration which gives one-half the rate of the second state 3 minus the second state 4.

It is clear from the results that the amount of oligomycin needed for half-maximal inihibition increases proportionately with the mitochondrial concentration in the reaction cuvette. From the slope of figure 8B, this amount is calculated to be 340 mμg oligomycin per mg mitochondrial N, or, on the basis of 16 % nitrogen per protein, 55 mμg oligomycin per mg protein.

Discussion

The method I application of either DNP or *m*-Cl-CCP caused little or no stimulation of both the state 1 and the first state 4 respiration rate, whereas a marked stimulation was observed with the method II application (figs 1-3). These findings indicate that the presence of both substrate and ATP in the mitochondria is necessary for an uncoupling agent to stimulate respiration and uncouple oxidative phosphorylation.

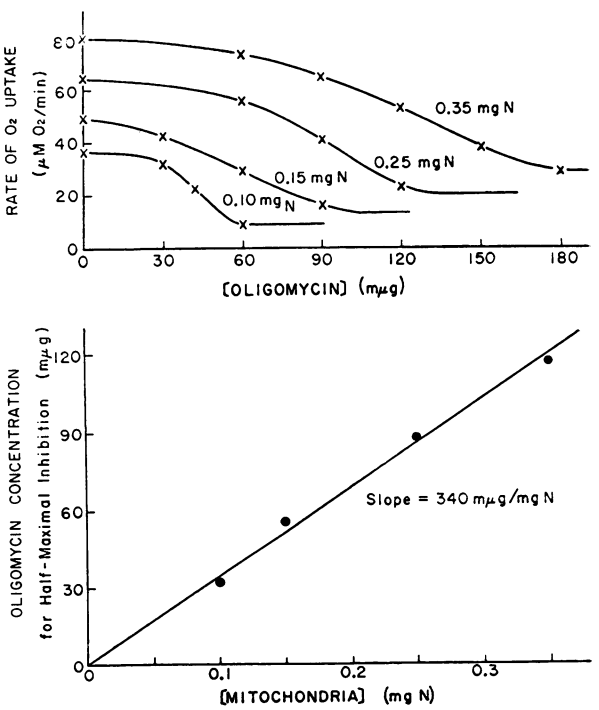

FIG. 6. Titration of oligomycin effect on the second state 3 respiration with various concentrations of mitochondria. Numbers on each curve denotes mitochondrial concentration in terms of mg N/3 ml reaction mixture. Substrate = 33 mM malate, ADP = 150 μM. A (upper): rate of oxygen uptake versus oligomycin concentration. B (lower): oligomycin concentration for half-maximal effect plotted in terms of mitochondrial concentration.

The maximum stimulation of the first state 4 respiration was observed at a DNP concentration of 15 μM when the mitochondria were incubated with the uncoupler prior to the addition of succinate (fig 1A). In order to release tight coupling, however, 80 μM is needed (fig 1B). In case of malate oxidation, 0.1 to 0.2 μM *m*-Cl-CCP stimulates the first state 4 rate with the method I application (fig 4A), whereas a concentration range of 0.3 to 0.75 μM was necessary to break the coupling (fig 4B). The difference in the effective concentrations of these uncouplers between methods I and II applications is about 4-fold.

The concentration of DNP or *m*-Cl-CCP which inhibits oxidative phosphorylation is capable of stimulating both latent ATPase activity and respiratory rate of mitochondria (8, 11, 12, 19, 21–23). Furthermore, a current hypothesis is that the primary action of these uncoupling agents is to hydrolyze "high-energy intermediates" which are formed during oxidative phosphorylation (4–7, 19, 20, 22, 23). Taken together, these considerations and our experimental results suggest that the mung bean mitochondria, as isolated, contain relatively little endogenous substrate, ATP, and/or "high-energy intermediates". With these mitochondria, therefore, the effect of uncoupling agents is revealed when the agent is introduced during the second state 4, at which point the mitochondria have been able to accumulate "high-energy intermediates" on which the uncouplers can act.

With rat liver mitochondria, the maximal rate of respiration caused by uncoupling agents is equal, or nearly so, to the control state 3 rate when glutamate is used as substrate (5, 7, 19), while the DNP stimulated rates of oxidation of other substrates, such as succinate and malate, are greater than the control state 3 rate (7). Our results indicate that DNP uncouples succinate oxidation more effectively than malate oxidation (figs 1, 2), whereas with *m*-Cl-CCP a complete uncoupling is obtained only in malate oxidation (figs 3B, 4B). The action of an uncoupling agent thus appears to depend on the kind of substrate used in the experiment. These results do not support Parker's view that all uncouplers produce their effects by a similar mechanism (23).

Table I summarizes the results from mung bean mitochondria and compares them with the data from rat liver mitochondria. In this table, effects of *m*-Cl-CCP and oligomycin are expressed in terms of mμmoles per mg mitochondrial protein, since stoichiometric relationship between the number of inhibitor molecules and mitochondrial protein have been reported from the studies on animal mitochondria (8, 13, 17, 22–24). The same concentration range of DNP uncouples both mung bean and rat liver mitochondria, but with *m*-Cl-CCP this is not so. A 10 to 20 fold higher concentration of *m*-Cl-CCP is required to produce the half-maximal stimulation on the respiration of mung bean mitochondria, as compared with that of rat liver mitochondria. With oligomycin, on the other hand, a 3 to 5 fold lower concentration is required to produce the half-maximal inhibition of state 3.

Table I further points out that 1.5 to 2 times higher concentrations of DNP and *m*-Cl-CCP are needed for stimulation of malate oxidation than for the oxidation of succinate, with mung bean mitochondria. This difference can be fortuitous, judging from the complicated nature of the effects of these agents. Nevertheless, in view of 2 and 3 phosphorylation sites in the oxidation of succinate and malate, respectively, it is tempting to speculate that these uncoupling agents act at the functional phosphorylation sites.

The effects of oligomycin on the respiration of mung bean mitochondria agree qualitatively with those on the respiration of animal mitochondria: (1) the inhibition of respiration in a phosphorylating medium is usually not complete, (2) the inhibited respiration rate corresponds to the state 4 respiration rate, and (3) the same concentration of oligomycin produces the half-maximal effect whether the effect is examined on succinate or on the oxidation of NAD^+-linked substrate. It appears, therefore, that if oligomycin inhibits phosphorylation stoichiometrically with the

Table I. *Effective Concentrations of Phosphorylation Inhibitors on the Respiration of Mung Bean and Rat Liver Mitochondria*

Inhibitor	Substrate	Conc. of inhibitor for half-maximal effect*	References
A. Mung bean mitochondria			
DNP	Succinate	20 μM	fig 1B
	Malate	35 μM	fig 2B
m-Cl-CCP	Succinate	0.25 mμmoles/mg P	fig 3B
	Malate	0.5 mμmoles/mg P	fig 4B
Oligomycin	Succinate	80 mμg/mg P	fig 6A
	Malate	80, 55 mμg/mg P	fig 6A, 7
B. Rat liver mitochondria			
DNP	Glutamate	10–20 μM	6,19
	Pyruvate-fumarate	30 μM	23
m-Cl-CCP	Malate-glutamate	0.05 mμmoles/mg P	8
Oligomycin	Succinate	270 mμg/mg P	13
	Glutamate	270 mμg/mg P	13

* Calculated from the data in the References column.

number of sites, this antibiotic binds with all the sites in the mitochondria regardless of whether the sites are functional or not.

Acknowledgment

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Literature Cited

1. Akazawa, T. and H. Beevers. 1957. Mitochondria in the endosperm of the germinating castor bean: a developmental study. Biochem. J. 67: 115–18.
2. Bonner, J. and A. Millerd. 1953. Oxidative phosphorylation by plant mitochondria. Arch. Biochem. Biophys. 42: 135–48.
3. Borst, P. and E. C. Slater. 1961. The site of action of 2,4-dinitrophenol on oxidative phosphorylation. Biochem. Biophys. Acta 48: 362–79.
4. Chance, B. and G. R. Williams. 1956. The respiratory chain and oxidative phosphorylation. Advan. Enzymol. 17: 65–134.
5. Chance, B., G. R. Williams, and G. Hollunger. 1963. Inhibition of electron and energy transfer in mitochondria. III. Spectroscopic and respiratory effects of uncoupling agents. J. Biol. Chem. 238: 439–44.
6. Chappell, J. B. 1963. The effect of alkylguanidines on mitochondrial metabolism. J. Biol. Chem. 238: 410–17.
7. Chappell, J. B. 1964. The effect of 2,4-dinitrophenol on mitochondrial oxidations. Biochem. J. 90: 237–48.
8. Goldby, R. A. and P. G. Heytler. 1963. Uncoupling of oxidative phosphorylation by carbonyl cyanide phenylhydrazones. II. Effects of carbonyl cyanide *m*-chlorophenylhydrazone on mitochondrial respiration. Biochem. 2: 1142–47.
9. Hackett, D. P. 1959. Respiratory mechanisms in higher plants. Ann. Rev. Plant Physiol. 10: 113–46.
10. Hackett, D. P., B. Rice, and C. Schmid. 1960. The partial dissociation of phosphorylation from oxidation in plant mitochondria by respiratory chain inhibitors. J. Biol. Chem. 235: 2140–44.
11. Heytler, P. G. 1963. Uncoupling of oxidative phosphorylation by carbonyl cyanide phenylhydrazones. I. Some characteristics of *m*-Cl-CCP action on mitochondria and chloroplasts. Biochem. 2: 357–61.
12. Heytler, P. G. and W. W. Prichard. 1962. A new class of uncoupling agents—carbonyl cyanide phenylhydrazones. Biochem. Biophys. Res. Commun. 7: 272–75.
13. Huijing, F. and E. C. Slater. 1961. The use of oligomycin as an inhibitor of oxidative phosphorylation. J. Biochem. Tokyo 49: 493–501.
14. Ikuma, H. and W. D. Bonner, Jr. 1967. Properties of higher plant mitochondria. I. Isolation and some characteristics of tightly coupled mitochondria from dark-grown mung bean hypocotyls. Plant Physiol. 42: 67–75.
15. Lance, C., G. E. Hobson, R. E. Young, and J. B. Biale. 1965. Metabolic processes in cytoplasmic particles of the avocado fruit. VII. Oxidative and phosphorylative activities throughout the climacteric cycle. Plant Physiol. 40: 1116–23.
16. Lardy, H. A. 1962. Reactions involved in oxidative phosphorylation as disclosed by studies with antibiotics. In: Biological Structure and Function. First IUB/IUBS Symposium, Stockholm. Academic Press, Inc., New York, Vol. II. p 265–68.
17. Lardy, H. A., D. Johnson, and W. C. McMurray. 1958. Antibiotics as tools for metabolic studies. I. A survey of toxic antibiotics in respiratory, phosphorylative and glycoltic systems. Arch. Biochem. Biophys. 78: 587–97.
18. Lardy, H. and W. C. McMurray. 1959. The mode of action of oligomycin. Federation Proc. 18: 269.
19. Lardy, H. A. and H. Wellman. 1953. The catalytic effect of 2,4-dinitrophenol on adenosine phosphate hydrolysis by cell particles and soluble enzymes. J. Biol. Chem. 201: 357–70.
20. Lieberman, M. and J. E. Baker. 1965. Respiratory electron transport. Ann. Rev. Plant Physiol. 16: 343–82.
21. Loomis, W. F. and F. Lipmann. 1948. Reversible inhibition of the coupling between phosphorylation and oxidation. J. Biol. Chem. 175: 807–08.
22. Margolis, S., G. Lenaz, and H. Baum. Stoichiometric aspects of the uncoupling of oxidative phosphorylation by carbonyl cyanide phenylhydrazones. In press.
23. Parker, V. H. 1965. Uncouplers of rat liver mitochondrial oxidative phosphorylation. Biochem. J. 97: 658–62.
24. Slater, E. C. 1963. Uncouplers and inhibitors of oxidative phosphorylation. In: Metabolic Inhibitors. R. M. Hochster and J. H. Quastel, eds. Academic Press, New York-London, Vol. II. p 503–16.
25. Wiskich, J. T. and W. D. Bonner, Jr. 1963. Preparation and properties of sweet potato mitochondria. Plant Physiol. 38: 594–604.
26. Wiskich, J. T., R. E. Young, and J. B. Biale. 1964. Metabolic processes in cytoplasmic particles of the avocado fruit. IV. Controlled oxidation and coupled phosphorylations. Plant Physiol. 39: 312–22.