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# The Partial Dissociation of Phosphorylation from Oxidation in Plant Mitochondria by Respiratory Chain Inhibitors*

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It is generally assumed that the specific inhibition of respiratory chain oxidations does not lead to any dissociation of phosphorylation from electron transfer. Support for this view has come from the demonstration that respiration in isolated animal mitochondria can be partially blocked by carbon monoxide (1), low concentrations of azide (2), or antimycin A (3) without affecting the ratio of phosphate esterified to oxygen consumed (P:O). On the other hand, many higher plant tissues respond to inhibitors in such a way as to suggest that effects on respiration and phosphorylation may differ markedly: oxygen uptake by these tissues may be unaffected, or even promoted, by relatively high concentrations of cyanide and carbon monoxide, whereas energy-requiring processes are readily inhibited (4). This type of effect suggests that an inhibition of cytochrome oxidase can cause an uncoupling of phosphorylation from oxidation. Support for this possibility has recently been provided by the demonstration that cyanide lowers the P:O ratio measured during the oxidation of organic acids by some isolated plant mitochondria (5, 6). Since cyanide can affect a number of different reactions, it was of interest to know whether more specific inhibitors might have a similar effect. The present report describes a study of the influence of a variety of respiratory chain inhibitors on the phosphorylations linked to the oxidation of citrate and succinate by sweet potato mitochondria. It has been found that interference with electron transfer within the cytochrome system does in fact lower the P:O ratio. Several explanations for the results, including the possibility that an alternate, nonphosphorylating respiratory pathway is functional in the presence of inhibitors, are considered.

A preliminary note describing some of the findings has been published (7).

## EXPERIMENTAL PROCEDURE

Sweet potatoes (*Ipomea batatas*) were purchased weekly at local markets and stored at room temperature. Respiratory rates of tissue slices were determined in small volumeters, as previously described (8). A method similar to that developed by Lieberman and Biale (9) was used for the isolation of the particulate fraction (mitochondria). Chilled tissue, 300 g, was passed through a meat grinder and then macerated at low speed in a Waring Blendor for 2 minutes with 300 ml of medium containing 0.5 M sucrose, 0.05 M Tris (pH 7.0), 0.01 M neutralized ethylenediaminetetraacetic acid, and 0.01 M neutralized cysteine·HCl. The homogenate was centrifuged at 1,000 × *g* for 5 minutes, the residue discarded, and the supernatant centrifuged at 10,000 × *g* for 20 minutes. The pellet was resuspended in 20 ml of the isolation medium (minus cysteine), the particles resedimented at 10,000 × *g*, and the final residue suspended in 5.0 ml of 0.5 M sucrose-0.05 M Tris (pH 7.0). All operations were carried out at roughly 0°. The protein content of the mitochondrial suspension, determined by the method of Lowry *et al.* (10), varied between 2 and 8 mg of protein per ml.

The oxidations of organic acids were carried out in Warburg vessels in duplicate or triplicate, with the use of standard manometric techniques. The reaction was started, after thermal equilibration, by tipping in the substrate from the side arm; it was stopped by mixing with 0.25 ml of 40% trichloroacetic acid. The initial and final phosphate concentrations were measured by the method of Bernhart and Wreath (11), and the difference between these values represents the phosphate uptake. Hexokinase (approximately 50 units per vessel), together with glucose, was included as a phosphate trap; the control P:O ratio was not lowered when one-half of this amount of hexokinase was used. Anaerobic experiments were carried out in Thunberg tubes, which had been evacuated (water aspirator) and then filled with either $N_2$ or CO; the extent of the reaction was calculated from the amount of ferricyanide reduced, as determined by the decrease in optical density at 420 mμ. Absorption spectra were obtained with the use of a Cary model 14 spectrophotometer.

All gases were obtained from commercial sources and the gas mixtures were made up by displacement. When a gas phase other than air was required in the manometric experiments, each Warburg vessel was flushed, while shaking, with at least 500 ml of the appropriate mixture. HCN was generated from the appropriate $Ca(CN)_2$-$Ca(OH)_2$ mixture added to the center well (12). Antimycin A was purchased from the Wisconsin Alumni Research Foundation, and 2-heptyl-4-hydroxyquinoline-*N*-oxide was kindly supplied by Dr. J. W. Lightbown.

## RESULTS

For the purposes of the present investigation, it was desirable to find a tissue which has a cyanide-resistant respiration and which also yields active mitochondria. Sweet potato roots were selected after a survey of a variety of readily available plant tissues. Thin (1 mm) slices of this storage root respire rapidly and the oxygen uptake is not inhibited by millimolar cyanide. In three experiments, the average $Q_{O_2}$ (μl of $O_2$ per hr per g fresh weight) was 132 in the air control and 160 in the presence of $10^{-3}$ M HCN, indicating that the respiration was actually

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promoted 21% by this "inhibitor." The particulate fraction prepared from sweet potatoes can carry out an active oxidation of Krebs cycle acids and a concomitant esterification of inorganic phosphate (9, 13). Table I shows that excellent P:O ratios can be obtained with these preparations, and the phosphate uptake is clearly dependent on an external supply of ADP. The fact that the oxygen uptake is doubled by the addition of ADP indicates some degree of "respiratory control." At relatively high concentrations, 2,4-dinitrophenol uncouples most of the phosphorylation from the oxygen uptake (Table II), and this fact indicates that the phosphate uptake is linked to electron transfer within the respiratory chain.

Low concentrations of cyanide inhibit both the oxygen and phosphate uptake by sweet potato mitochondria; however, the effect on phosphorylation is considerably greater, as can be seen in the data of Table III. In this experiment, $10^{-4}$ M HCN inhibited the oxidation of succinate 39%, whereas phosphorylation was reduced 64%, with the result that the P:O ratio was roughly halved; in four experiments the decrease in the P:O averaged 57%. The effect of cyanide on the citrate P:O was usually somewhat smaller and considerably more variable; in nine experiments the P:O ratio was decreased an average of 23% by $10^{-4}$ M HCN, with the individual values ranging from 7 to 75%. In general, the larger inhibitions were correlated with the lower control P:O values. Azide also reduces the P:O ratio, and again the effect is more marked with succinate than with citrate (Table III). Although it is well known that high concentrations of azide uncouple phosphorylation (14), the low concentrations (e.g. $3 \times 10^{-4}$ M) of azide which are effective here do not have a comparable effect on liver mitochondria (2). From the data in Table III, it is clear that there is no direct correlation between the inhibitions of oxygen and phosphate uptake, particularly at the higher concentrations of these agents.

Because carbon monoxide is a much more specific inhibitor of cytochrome oxidase, its effects were studied in more detail. Table III shows the results of typical experiments which illustrate the differential effect of CO on oxygen and phosphate uptake. Here, the P:O ratio for both substrates is decreased roughly 0.5 of a unit by CO-$O_2$ mixtures (4:1 and 9:1). The magnitude of this CO effect varied considerably with different preparations; thus, the inhibition of the P:O ratio by CO-$O_2$

Table I

*The ADP requirement for oxidation and phosphorylation*

Contents of reaction vessel, in μmoles: substrate, 60; sucrose, 1325; glucose, 60; $MgSO_4$, 20; diphosphothiamine, 0.2; DPN, 1.2; phosphate buffer (pH 7.0), 80; Tris buffer (pH 7.0), 12. To this was added 3 μmoles of ADP when required, 1 mg of hexokinase, 0.25 ml of mitochondrial suspension (2 mg of protein), and water to make a total volume of 3.0 ml. Oxygen consumption and disappearance of inorganic phosphate measured for incubation period of 60 minutes at 30°.

| Substrate | ADP | $O_2$ | $P_i$ | P:O |
|---|---|---|---|---|
| | | *μatoms* | *μatoms* | |
| Succinate | − | 6.3 | 3.2 | 0.51 |
| | + | 12.3 | 21.8 | 1.77 |
| Citrate | − | 3.1 | 4.2 | 1.36 |
| | + | 6.5 | 17.8 | 2.74 |

Table II

*The uncoupling of oxidative phosphorylation by 2,4-dinitrophenol*

Reaction conditions as described in Table I.

| 2,4-Dinitrophenol | Succinate | | | Citrate | | |
|---|---|---|---|---|---|---|
| | $O_2$ | $P_i$ | P:O | $O_2$ | $P_i$ | P:O |
| *M* | *μatoms* | *μatoms* | | *μatoms* | *μatoms* | |
| 0 | 14.4 | 21.7 | 1.51 | 12.7 | 30.9 | 2.44 |
| $5 \times 10^{-5}$ | 15.3 | 19.1 | 1.25 | 12.4 | 24.4 | 1.97 |
| $1 \times 10^{-4}$ | 13.8 | 14.7 | 1.07 | 11.4 | 19.6 | 1.72 |
| $5 \times 10^{-4}$ | 5.8 | 3.6 | 0.62 | 11.9 | 4.7 | 0.40 |

Table III

*Effects of cytochrome oxidase inhibitors on oxidation and phosphorylation*

Reaction conditions as described in Table I. KCN or $NaN_3$ added to main compartment; $Ca(CN)_2$-$Ca(OH)_2$ mixture added to center well. For CO experiments, air in gas phase replaced by flushing with appropriate mixture before starting reaction

| Inhibitor | Concentration | Succinate | | | Citrate | | |
|---|---|---|---|---|---|---|---|
| | | $O_2$ | $P_i$ | P:O | $O_2$ | $P_i$ | P:O |
| | | *μatoms* | *μatoms* | | *μatoms* | *μatoms* | |
| Cyanide | 0 | 18.3 | 32.2 | 1.76 | 14.8 | 38.2 | 2.58 |
| | $4.6 \times 10^{-5}$ M | 14.8 | 23.7 | 1.60 | 7.8 | 20.8 | 2.66 |
| | $1 \times 10^{-4}$ M | 11.2 | 11.6 | 1.04 | 7.2 | 14.7 | 2.04 |
| Azide | 0 | 14.5 | 19.9 | 1.37 | 12.7 | 34.0 | 2.68 |
| | $1 \times 10^{-4}$ M | 10.6 | 12.2 | 1.15 | 8.8 | 22.1 | 2.52 |
| | $3 \times 10^{-4}$ M | 10.0 | 8.8 | 0.88 | 8.5 | 17.5 | 2.06 |
| | $1 \times 10^{-3}$ M | 9.4 | 2.5 | 0.27 | 7.8 | 13.3 | 1.70 |
| CO | 0 | 13.5 | 19.3 | 1.43 | 10.2 | 28.0 | 2.74 |
| | CO-$O_2$ (4:1) | 9.0 | 8.2 | 0.91 | 9.6 | 21.5 | 2.24 |
| | 0* | 10.6 | 12.5 | 1.18 | 8.7 | 21.6 | 2.48 |
| | CO-$O_2$ (9:1) | 7.0 | 5.1 | 0.73 | 7.1 | 14.0 | 1.97 |

* Gas phase = $N_2$:$O_2$ (9:1) at 1 atmosphere.

Table IV

*Reversal of the CO inhibition of phosphorylation by either oxygen or light*

Reaction conditions as described in Table I. Substrate = citrate. Light from three 300-watt tungsten reflector flood lamps passed through a window in the Warburg bath and was reflected up through the bottoms of the vessels. Dark experiment carried out simultaneously in a second Warburg bath.

| Gas phase | Phosphate uptake | |
|---|---|---|
| | Dark | Light |
| | *μatoms* | *μatoms* |
| Air | 22.7 | |
| 30 $N_2$-50 CO-20 $O_2$ | 13.6 | |
| 50 CO-50 $O_2$ | 24.4 | |
| 90 $N_2$-10 $O_2$ | 10.1 | 9.9 |
| 90 CO-10 $O_2$ | 6.0 | 10.4 |

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TABLE V

*Effects of CO on ferricyanide reduction and phosphorylation under anaerobic conditions*

Reaction mixtures in Thunberg tubes as described in Table I, except for inclusion of 0.5 ml of mitochondrial suspension and 50 μmoles of potassium ferricyanide in each tube. Following evacuation, refilling with $N_2$ or CO, and thermal equilibration, reaction started by tipping in mitochondria from side arm. Incubated for 30 minutes at 30° and reaction stopped by immersing tubes in boiling water. In the absence of mitochondria or substrate, there was essentially no ferricyanide reduction (estimated at 420 mμ), and there was no phosphate uptake in the absence of ferricyanide or substrate.

| Gas phase | Succinate | | | Citrate | | |
|---|---|---|---|---|---|---|
| | Ferricyanide | $P_i$ | P:2e | Ferricyanide | $P_i$ | P:2e |
| | *μmoles* | *μatoms* | | *μmoles* | *μatoms* | |
| $N_2$ | 47.2 | 5.8 | 0.25 | 23.4 | 10.8 | 0.92 |
| CO | 46.0 | 6.0 | 0.26 | 26.8 | 11.2 | 0.84 |

TABLE VI

*Effects of inhibitors which act between cytochromes b and c on oxidation and phosphorylation*

Reaction conditions as described in Table I. Antimycin added in 95% ethanol solution, but equivalent amounts of alcohol did not have any inhibitory effect on the controls.

| Inhibitor | Concentration | Succinate | | | Citrate | | |
|---|---|---|---|---|---|---|---|
| | | $O_2$ | $P_i$ | P:O | $O_2$ | $P_i$ | P:O |
| QO* | 0 | 14.1 | 19.0 | 1.35 | 7.1 | 15.7 | 2.22 |
| | $1 \times 10^{-6}$ M | 9.0 | 7.7 | 0.86 | 4.8 | 8.6 | 1.79 |
| | $3 \times 10^{-6}$ M | 7.5 | 4.5 | 0.60 | 3.9 | 3.2 | 0.82 |
| | $1 \times 10^{-5}$ M | 5.0 | 2.5 | 0.50 | 2.9 | 2.2 | 0.76 |
| Antimycin | 0 | 14.2 | 20.5 | 1.44 | 15.0 | 39.5 | 2.64 |
| | 0.1 μg per ml | 6.0 | 5.5 | 0.92 | 9.8 | 16.8 | 1.72 |
| | 0.3 μg per ml | 5.9 | 1.6 | 0.27 | 9.0 | 8.5 | 0.94 |

* QO, 2-heptyl-4-hydroxyquinoline-*N*-oxide.

TABLE VII

*The nonadditive nature of the inhibitions caused by CO and QO*

Reaction conditions as described in Table I.

| Inhibitor | Succinate | | | Citrate | | |
|---|---|---|---|---|---|---|
| | $O_2$ | $P_i$ | P:O | $O_2$ | $P_i$ | P:O |
| | *μatoms* | *μatoms* | | *μatoms* | *μatoms* | |
| 0 | 13.5 | 19.3 | 1.43 | 10.2 | 27.7 | 2.72 |
| $CO-O_2$ (4:1) | 9.0 | 8.2 | 0.91 | 9.6 | 21.4 | 2.23 |
| $1 \times 10^{-6}$ M QO | 5.4 | 2.5 | 0.46 | 8.0 | 15.4 | 1.93 |
| $CO-O_2$ (4:1) + $10^{-6}$ M QO | 6.0 | 4.1 | 0.68 | 7.9 | 14.9 | 1.89 |

(4:1) when succinate was the substrate ranged from 15 to 67%. In ten experiments with $CO-O_2$ (4:1), the average lowering of the P:O ratio, relative to the control, was 30% for succinate and 18% for citrate. Similar values were obtained with $CO-O_2$ mixtures, 9:1.

Experiments were next designed to determine whether this uncoupling action of CO is in fact due solely to the reaction between CO and cytochrome oxidase, or whether some other component involved in phosphorylation also combines with CO. The inhibition of phosphate uptake depends on the $CO-O_2$ ratio, rather than on the concentration of CO alone, and can be completely reversed by light (Table IV). Some phosphorylation can be demonstrated during the anaerobic reduction of ferricyanide by succinate and citrate, but this is not significantly inhibited by CO (Table V). Spectrophotometric observations support the view that CO combines only with the terminal oxidase: when CO is bubbled through a mitochondrial suspension which has been reduced enzymatically with DPNH, the difference spectrum, (CO + DPNH) − (DPNH), shows a single trough at 443 mμ and a single peak at 430 mμ (cytochrome $a_3$-CO). (When hydrosulfite is used to reduce the respiratory components, the peak of the CO complex shifts to 420 and 425 mμ, suggesting that CO can combine with an additional component, possibly a peroxidase, which is not in the respiratory chain.) The above findings support the view that all of the inhibition of phosphorylation by CO is the result of a specific reaction with cytochrome oxidase.

It would be of interest to know whether interference with electron transfer at other loci within the cytochrome system can affect the P:O ratio. At least two inhibitors, antimycin A and QO,[1] are known to act specifically between cytochromes *b* and *c*. Low concentrations of these compounds inhibit the oxidation of succinate and citrate by sweet potato mitochondria, but they have an even greater effect on the associated phosphorylations (Table VI). In every case the P:O ratio is lowered, and at the higher inhibitor concentrations the absolute decrease in the ratio is greater than 1.0. The average lowering of the P:O caused by $10^{-6}$ M QO was 35% for succinate and 18% for citrate. In the presence of $10^{-6}$ M QO, replacing the air in the gas phase with a $CO-O_2$ (4:1) mixture did not cause any further decrease in either oxygen or phosphate uptake, suggesting that the effects of these inhibitors are not additive (Table VII).

A number of inhibitors which act on the respiratory chain between the substrate and the cytochrome system were also examined. Amytal normally blocks the oxidation of DPNH by flavoprotein, but in nonphosphorylating preparations it can also act between flavoprotein and cytochrome *b* (15). Table VIII shows that the oxidations by sweet potato mitochondria are only slightly inhibited by concentrations of Amytal which completely block oxidation in mammalian mitochondria; in this respect they resemble skunk cabbage particles (16). The fact that relatively high concentrations cause some decrease in the oxygen uptake and the P:O ratio with both citrate and succinate, suggests that this is not a specific effect on DPNH reoxidation.

Malonate was used to inhibit succinate oxidation at the substrate level. In marked contrast to the effects obtained with other inhibitors, the oxygen uptake was more sensitive than the phosphate uptake, with the result that the P:O ratio was actually increased (Table VIII). In four experiments, $3 \times 10^{-3}$ M malonate increased the P:O for succinate an average of 48%, while the oxidation was being inhibited 62%. This result indicates clearly that a decrease in the rate of electron flow to oxygen *per se* does not lead to a decrease in the P:O ratio.

Since isocitric dehydrogenase contains an essential —SH group which combines with iodoacetate (17), this agent was used as a substrate-level inhibitor of citrate oxidation. At $1 \times 10^{-4}$ M, iodoacetate inhibits citrate oxidation by sweet potato mitochon-

[1] The abbreviation used is: QO, 2-heptyl-4-hydroxyquinoline-*N*-oxide.

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TABLE VIII

*Effects of inhibitors which act between the substrate and the cytochrome system on oxidation and phosphorylation*

Reaction conditions as described in Table I.

| Inhibitor | Concentration | Succinate | | | Citrate | | |
|---|---|---|---|---|---|---|---|
| | | $O_2$ | $P_i$ | P:O | $O_2$ | $P_i$ | P:O |
| Amytal | 0 | 20.2 | 32.4 | 1.61 | 12.8 | 32.3 | 2.52 |
| | $1 \times 10^{-3}$ M | 17.4 | 28.8 | 1.66 | 12.4 | 29.1 | 2.35 |
| | $3 \times 10^{-3}$ M | 15.5 | 16.3 | 1.05 | 10.6 | 20.5 | 1.94 |
| Malonate | 0 | 13.8 | 17.8 | 1.29 | | | |
| | $1 \times 10^{-3}$ M | 9.0 | 13.5 | 1.50 | | | |
| | $3 \times 10^{-3}$ M | 4.9 | 8.5 | 1.74 | | | |
| Iodoacetate | 0 | | | | 10.6 | 24.2 | 2.28 |
| | $1 \times 10^{-4}$ M | | | | 7.3 | 20.2 | 2.77 |
| *p*-Chloromercuribenzoate | 0 | | | | 10.6 | 24.2 | 2.28 |
| | $1 \times 10^{-5}$ M | | | | 9.2 | 20.5 | 2.23 |

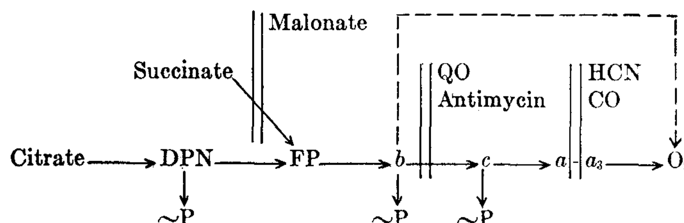
dria, but at the same time it increases the P:O roughly 20% (Table VIII). *p*-Chloromercuribenzoate, at a concentration of $1 \times 10^{-5}$ M, inhibited citrate oxidation slightly without causing a significant change in the P:O ratio (Table VIII); at higher concentrations the P:O was lowered. Although it is difficult to assign a single specific site of action to any —SH combining agent, the results indicate clearly that it is possible to decrease the rate of citrate oxidation without lowering the P:O ratio.

## DISCUSSION

The results show that interference with electron transport in the cytochrome system can partially dissociate phosphorylation from oxidation in sweet potato mitochondria. This effect, which is the consequence of specific interactions with the electron-transferring components, appears to differ fundamentally from the uncoupling action of those agents which interfere with the reactions linking phosphorylation to oxidation. The possibility that the differential inhibition of phosphate uptake is due simply to the decrease in rate of electron flow is eliminated by the experiments with substrate-level inhibitors. It might be argued that these preparations oxidize the substrates via two entirely separate respiratory pathways, and that only one of these is inhibitor-sensitive and coupled to phosphorylation. However, the relative amounts of phosphate and oxygen uptake eliminated by the inhibitors indicate that the P:O ratio of the blocked respiratory pathway would have to be greater than the theoretical maximum. It also seems improbable that malonate, on the one hand, and CO and QO, on the other, react with different respiratory chains. What then is the mechanism whereby the P:O ratio is lowered?

One possible explanation is that phosphorylation is more sensitive than oxidation to changes in the steady-state levels of the respiratory components. This would imply that the free energy change available for phosphorylation depends upon the steady-state concentrations of the carriers, and as yet there is no experimental evidence to support this view (2). There is some evidence that the oxidation state of the carriers in liver mitochondria can influence the partial reactions of oxidative phosphorylation (18), but this possibility remains to be tested with the sweet potato preparations.

Another possible explanation is that there is a second respiratory pathway to oxygen and that electron flow can be diverted by certain inhibitors into this nonphosphorylating pathway. The uncertainty as to the exact contribution of each of the three phosphorylation sites to the over-all P:O ratios makes it difficult to localize such a bypass precisely. The fact that the percentage reduction of the P:O is generally greater with succinate than with citrate suggests that the three sites are not equally sensitive and that the extra step which is unique to citrate is the least sensitive. On the basis of the available data, the most likely locus of such a bypass to oxygen would appear to be at the level of cytochrome *b*, as suggested in the following diagram of the respiratory chain.




Previous spectrophotometric studies have shown that the cytochrome *b* component of some plant mitochondria remains largely oxidized even when most of the *c*-*a*-$a_3$ components have been reduced by inhibitors, and a "cytochrome *b* oxidase hypothesis" has been proposed to account for inhibitor-resistant plant respiration (see (4)). The present findings lend some support to this hypothesis and to the view that the inhibition of various energy-requiring processes in plant tissues, in the absence of any inhibition of oxygen uptake, results from a preferential effect of inhibitors on phosphorylation.

There is reason to believe that the type of modified respiratory system considered here may be found in other tissues. The fact that CO inhibits the Pasteur effect in some animal tissues without affecting the respiratory rate (19) is suggestive. Recently, Kimura and Singer (20) have provided evidence for the existence of an inhibitor-resistant pathway from choline to oxygen in isolated liver mitochondria; they suggest that the inhibitor causes a "leak" to develop in the respiratory chain at the level of cytochrome *b*. Keilin (21) originally observed the autoxidizability of cytochrome *b*, but definite proof that this reaction with oxygen can proceed at an appreciable rate is still lacking. Probably the best illustration of the ability of inhibitors to influence the pathway of electron flow is provided by the demonstration that in bacteria containing two or more terminal oxidases, the relative contribution of each to the total respiration is altered by the addition of CO (22).

## SUMMARY

The influence of respiratory chain inhibitors on oxidative phosphorylation in sweet potato mitochondria has been examined. With the use of succinate and citrate as substrates, it has been shown that phosphorylation is more sensitive than oxidation to the inhibitory effects of cyanide, azide, carbon monoxide, antimycin, and 2-heptyl-4-hydroxyquinoline-*N*-oxide, all of which lower the P:O ratio. The evidence indicates that this results from a specific interference with electron transport within

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the cytochrome system, either at the oxidase level or between cytochromes *b* and *c*. In contrast, inhibition of oxidation at the substrate level does not lower the P:O ratio. The results are compatible with the hypothesis that inhibitors acting between cytochrome *b* and oxygen can divert electrons to an alternate, nonphosphorylating respiratory pathway.

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Oxidative phosphorylation is the metabolic pathway in which cells use enzymes to oxidize nutrients, thereby releasing energy which is used to produce adenosine triphosphate (ATP). In most eukaryotes, this takes place inside mitochondria. Almost all aerobic organisms carry out oxidative phosphorylation. This pathway is so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis. A computational model for the mitochondrial respiratory chain that appropriately balances mass, charge, and free energy transduction is introduced and analyzed based on a previously published set of data measured on isolated cardiac mitochondria. The basic components included in the model are the reactions at complexes I, III, and IV of the electron transport system, ATP synthesis at F1F0 ATPase, substrate transporters including adenine nucleotide translocase and the phosphateâ€"hydrogen co-transporter, and cation fluxes across the inner membrane including fluxes through the K+/H+ antiporter and Plant respiration has long been described to be resistant to cyanide, since the first observation was made in 1929 by Genevois on sweet pea (Lathyrus odora-tus) seedlings. Soon after, Van Herk and Badenhuizen (1934) and Van Herk (1937 a, b, c) showed that the respiration of the spadix of the Sauromatum guttatum was highly resistant to cyanide. Respiration in this group of plants (Araceae) is known to be extremely high and linked to heat production, particularly during pollination (see Lance 1972, Meeuse 1975).Â  Hackett DP, Rice B, Schmid C (1960b) The partial dissociation of phosphorylation from oxidation in plant mitochondria by respiratory chain inhibitors. J Biol Chem 235:2140â€"2144.PubMedGoogle Scholar.