On the Mechanism of Respiratory Control

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ABSTRACT Control of oxidation is the key mechanism in the regulation of energy metabolism. In glycolysis the oxidation of glyceraldehyde-3-phosphate is controlled by DPNH, which inhibits glyceraldehyde-3-phosphate dehydrogenase. In oxidative phosphorylation the inhibition of electron flow from DPNH to oxygen, called "respiratory control," is the subject of this paper. After a discussion of the physiological significance of the "tight coupling" between phosphorylation and oxidation, studies on "loosely coupled" submitochondrial particles are reported. These particles are capable of oxidative phosphorylation in the presence of a suitable phosphate acceptor system, but in contrast to controlled, intact mitochondria they oxidize DPNH in the absence of phosphate and ADP. The addition of *o*-phenanthroline to submitochondrial particles gives rise to an inhibition of respiration, which is partly reversed by phosphate and ADP or by dinitrophenol. The properties of this model system of respiratory control will be described.

The great efficiency of respiration as compared with fermentation in energy metabolism was clearly recognized by Pasteur. He observed that yeast cells that are deprived of oxygen consume much larger quantities of sugar than do actively respiring cells in order to produce the same amount of growth. This phenomenon, now well known as the Pasteur effect, holds the key to the economy of energy metabolism and its regulation. We shall not dwell on the Pasteur effect, but will only pause to point out that an essential feature of the regulation of carbohydrate metabolism is the control of the oxidation steps in glycolysis and in mitochondrial respiration. Since these oxidation steps proceed only very slowly in the absence of P_i and ADP, we say they are "tightly coupled" to phosphorylation. When all available P_i and ADP are converted to ATP, the rates of glycolysis and respiration are markedly decreased. When ATP is utilized for biosyntheses, or other work, ADP and P_i are regenerated, and glycolysis and respiration are maintained. This ingenious linkage of energy utilization to energy production, this simple device of regulating the supply by the demand, is the essence of the control of energy metabolism.

How is this regulation achieved? In the case of glycolysis which is catalyzed by soluble enzymes, the phenomenon of tight coupling between oxidation and phosphorylation can be explained as follows: The oxidation of glyceraldehyde-3-phosphate is catalyzed by glyceraldehyde-3-phosphate dehydrogenase with DPN as hydrogen acceptor. The products of this oxidative step are DPNH and phosphoglyceryl enzyme. The latter reacts with phosphate to yield 1,3-diphosphoglycerate which in a subsequent step catalyzed by phosphoglycerate kinase transfers its phosphate to ADP. For a tight coupling, *i.e.* a dependency of the oxidation process on P_i and ADP, it should be necessary that (*a*) H_2O cannot substitute for phosphate; and (*b*) the phosphorylated intermediate is not readily hydrolyzed. The first condition is fulfilled by the relative stability of the acyl enzyme. Its reactivity with phosphate is over 10^5 times as rapid as with water. On the other hand, the second condition is not fulfilled, since the acyl phosphate intermediate is relatively unstable chemically and is also susceptible to hydrolysis by acyl phosphatases. Because of this, another control mechanism is superimposed: The oxidation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate is strongly inhibited by DPNH. This is due to an allosteric property of glyceraldehyde-3-phosphate dehydrogenase which was described several years ago (1, 2). We shall not elaborate on this control of the oxidative step in glycolysis, but we wish to emphasize that it depends on the metabolic stability of DPNH. The glycolytic process continues only when DPNH is oxidized either by a suitable acceptor such as pyruvate or by an electron transport chain either mitochondrial or extramitochondrial as, for example, in leucocytes (3) during phagocytosis.

Respiratory control in mitochondrial oxidation is more complex. A stimulation of respiration by adenine nucleotides was observed over 20 years ago by Ochoa (4). Lardy and Wellman (5) characterized the phenomenon in terms of its metabolic significance. Chance and his collaborators (6) made ingenious use of the phenomenon of respiratory control for measurements of oxidative phosphorylation without actually measuring oxidative phosphorylation. Instead of determining phosphate esterification or ATP formation by conventional methods, they simply determined how much respiration one can get for a certain amount of ADP. In carefully prepared mitochondria 3 moles of ADP were needed for each atom of oxygen consumed; therefore the P:O ratio was 3. This procedure cannot be used, however, in particles that do not have respiratory control.

Efforts in our laboratory to unravel the mysteries of the phosphorylation process have led to the preparation of various particles derived from mitochondria. These submitochondrial particles exhibit deficiencies in respect to different coupling factors, but they all have one property in common: They have little or no respiratory control. They are in Dr. Lipmann's ter-

minology "loosely coupled;" they respire without phosphate and ADP but can couple phosphorylation to the oxidative process, provided an appropriate phosphate acceptor system is present.

Let us examine what this means in the light of our present knowledge of oxidative phosphorylation. According to the current schemes shown below, the oxidative step (I) leads to the formation of a high energy intermediate $A \sim X$, which, analogous to the acyl enzyme of glycolysis, is not phosphorylated and which contains a member of the respiratory chain (scheme 1). According to scheme 2, $A \sim X$ is transformed into a second non-phosphorylated intermediate, $X \sim Y$, which does not contain a respiratory component. The non-phosphorylated intermediate is cleaved by phosphate to produce

Scheme 1

I $AH_2 + X + B \rightleftharpoons A \sim X + BH_2$

II $A \sim X + P_i \rightleftharpoons X \sim P + A$

III $X \sim P + \text{ADP} \rightleftharpoons X + \text{ATP}$

Scheme 2

I $AH_2 + X + B \rightleftharpoons A \sim X + BH_2$

I *a* $A \sim X + Y \rightleftharpoons X \sim Y + A$

II $X \sim Y + P_i \rightleftharpoons X \sim P + Y$

III $X \sim P + \text{ADP} \rightleftharpoons X + \text{ATP}$

$X \sim P$ (step II) which transfers the phosphate to ADP (step III). As mentioned before, respiratory control can be lost either at the site of the non-phosphorylated or at the site of the phosphorylated intermediate. Since submitochondrial particles obtained from sonically disrupted mitochondria respire in the absence of phosphate, we conclude that respiratory control is lost at the site of the non-phosphorylated intermediate; *i.e.*, that water can substitute for phosphate in step II.

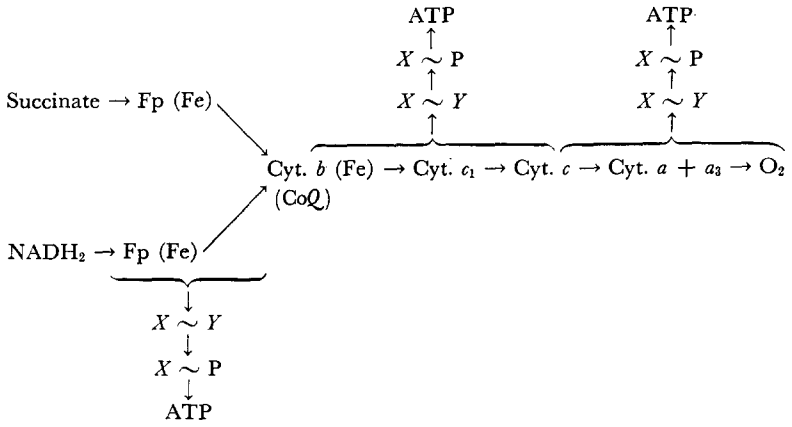
In order to understand how intact mitochondria exclude water from this step, we have made repeated attempts to impose respiratory control upon our submitochondrial particles. For example, the possible role of a mitochondrial ATPase inhibitor in respiratory control was tested (7). Although this inhibitor effectively blocked ATP hydrolysis in submitochondrial particles, it failed to stop respiration in the absence of P_i and ADP.

Before turning to describe recent experiments which point to a role of non-heme iron in respiratory control, it may be appropriate to review briefly the relationship of the respiratory chain and non-heme iron (scheme 3).

Evidence for the existence and participation of non-heme iron in mitochondrial enzymes has been provided by a number of investigators. Mahler and Elowe (8, 9) showed that DPNH cytochrome *c* reductase, a flavoprotein isolated from mitochondria, contains iron which becomes reduced on addition of DPNH. Non-heme iron was found in mitochondrial succinate dehydrogenase (10), DPNH dehydrogenase (11), submitochondrial particles (12), and in complexes I, II, and III (13–16) of the electron transport chain. Recently Rieske *et al.* (17, 18) isolated from complex III a derivative protein which

contained non-heme iron. It was suggested that this protein mediates electron transfer between cytochromes *b* and c_1 ; an electron spin resonance signal at $g = 1.94$ was observed in mitochondria and submitochondrial particles on reduction of the electron transport chain and was ascribed to the presence of non-heme iron (19). The purified DPNH dehydrogenase gave a similar signal on reduction (20).

Scheme 3



During the course of studies in our laboratory on DPNH oxidation in submitochondrial particles, it was noted that *o*-phenanthroline, an effective iron-chelating agent, inhibited oxygen uptake. Similar observations were made independently by Redfearn *et al.* (21). Further analysis of this inhibition has led to a model system for respiratory control which will be described here.

As illustrated in the polarographic tracing shown in Fig. 1 *a*, addition of ADP had little effect on the oxidation of DPNH by ETP_H, as indicated by the respiratory control value of 1.15. This value represents the ratio of oxidative activity in the presence of a suitable phosphate acceptor over that without acceptor. These particles, however, catalyzed phosphorylation linked to DPNH oxidation with a P:O ratio sometimes approaching 3. In the experiment shown in Fig. 1 *b*, the addition of 1 mM *o*-phenanthroline to respiring particles resulted in an immediate and marked inhibition of respiration. Addition of ADP after *o*-phenanthroline elicited a reversal of this inhibition yielding a respiratory control ratio of 1.64.

It was thought that if this ADP reversal of the inhibition by *o*-phenanthroline is associated with oxidative phosphorylation, then a number of important criteria should be fulfilled. Among these are: (*a*) a dependency on P_i for acceptor release of respiration, (*b*) inhibition of respiration by ATP

(22), and (*c*) specificity of ADP as a phosphate acceptor. The requirement for P_i for maximal release by ADP of *o*-phenanthroline–inhibited DPNH oxidation is illustrated in Fig. 1 *c*. The addition of ADP increased respiration from 0.079 to 0.10 μmole O_2/min./mg and subsequent addition of P_i increased respiration still further to 0.144 μmole O_2/min./mg, resulting in a respiratory control ratio of 1.82 in the presence of both ADP and P_i.

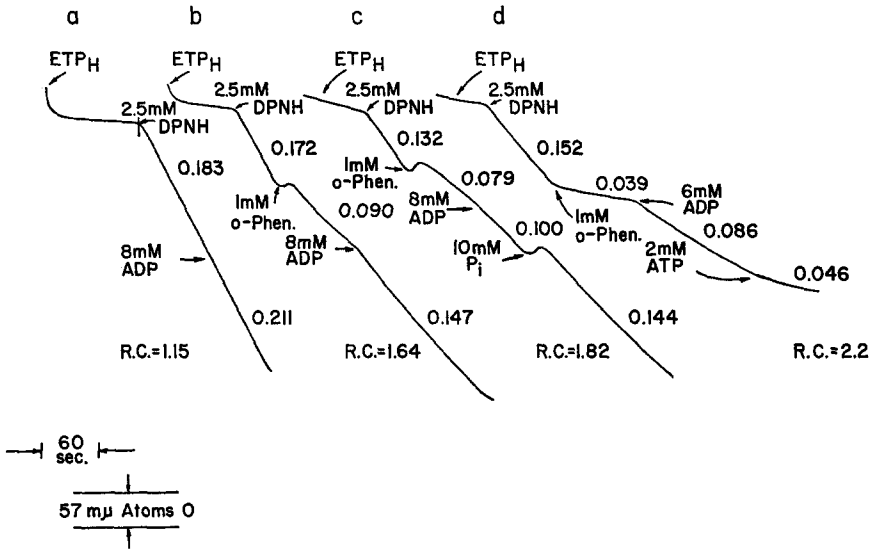


FIGURE 1. Induced respiratory control by *o*-phenanthroline. Rates of oxygen consumption were measured polarographically at room temperature with a Clark oxygen electrode, and calculated as μmole O_2/min./mg protein. The reaction mixture contained in a final volume of 1.0 ml: 100 mM tris-SO_4 pH 7.4; 5 mM $MgSO_4$; 10 mM KPO_4 pH 7.4 (added before particle additions in a, b, and d); and 0.33 mg ETP_H.[1] In d, ETP_H were incubated for 5 minutes at room temperature with 0.083 mg ATPase inhibitor/mg ETP_H before addition to the oxygen electrode. All other additions are indicated on the tracings.

The studies of Chance (23), Klingenberg (22), and their collaborators showed that electron flow in mitochondria is reversed by ATP. While the details of this phenomenon are complex, it is clear that in tightly coupled mitochondria ATP inhibits state 3 respiration (22) by a mass action effect on the electron transfer steps as depicted in schemes 1 and 2. It can be seen from the experiment shown in Fig. 1 *d*, that the addition of ATP to particles in the

[1] Abbreviations: SMP = submitochondrial particles
ETP_H = electron transport particles from heavy layer beef heart mitochondria
PMS = phenazinemethosulfate
Me = divalent metal ion
F = oxidized flavin
$\dot{F}H$ = flavin radical

presence of *o*-phenanthroline and ADP resulted in an inhibition of DPNH oxidation.

Finally, the specificity of ADP in stimulating respiration should be consistent with its specificity as a phosphate acceptor in oxidative phosphorylation. The data presented in Table I show that this is the case. Of the dinu-

TABLE I

NUCLEOTIDE SPECIFICITY OF RELEASE OF *o*-PHENANTHROLINE-INHIBITED DPNH OXIDASE

Rates of oxygen consumption were measured polarographically as described in Fig. 1. The reaction mixture contained in a final volume of 1.0 ml: 100 mM tris-SO_4 pH 7.4; 20 mM KPO_4 pH 7.4; 5 mM $MgSO_4$; 2.5 mM DPNH; 1 mM *o*-phenanthroline; 15 mM nucleotide; and 0.31 mg SMP. The release ratio with ADP in the absence of *o*-phenanthroline varied from 1.10 to 1.15.

Nucleotide	+ *o*-phenanthroline	+ Nucleotide	Release ratio
	μmoles O_2/min.	*μmoles O_2/min.*	
ADP	0.045	0.063	1.40
GDP	0.035	0.033	0.94
UDP	0.045	0.050	1.10
IDP	0.053	0.045	0.85

cleotides tested, only ADP was effective in releasing the inhibition by *o*-phenanthroline of DPNH oxidation.

In the experiments thus far described, relatively high concentrations of ADP (8 mM) were used to elicit maximal release of respiration. It was observed, however, that much less ADP was needed when the DPNH concentration was lowered. This is illustrated by the data presented in Fig. 2. In this experiment the effect of ADP on DPNH oxidation by ETP_H in the presence and absence of *o*-phenanthroline was followed fluorometrically. At a DPNH concentration of 50 μM and 0.5 mM *o*-phenanthroline, half-maximal release of respiration was obtained at approximately 150 μM ADP. This is considerably less than that required for half-maximal release of respiration in the O_2 electrode experiments (about 1 mM ADP).

The relative contribution of the three phosphorylation sites to the *o*-phenanthroline—induced respiratory control in submitochondrial particles was tested with different substrates. As shown in Table II, *o*-phenanthroline did not inhibit the oxidation of succinate or ascorbate plus PMS,[1] and ADP did not stimulate, suggesting that the second and third phosphorylation sites were not involved in the inhibition phenomenon. These results were consistent with previous work which suggested that respiratory control in intact mitochondria appeared to be operative primarily at the first phosphorylation site (24, 25).

In tightly coupled mitochondria, specific uncoupling agents release respiration in the absence of P_i and ADP (26). It was of interest therefore to examine the effects of some of these uncouplers on DPNH respiration inhibited by

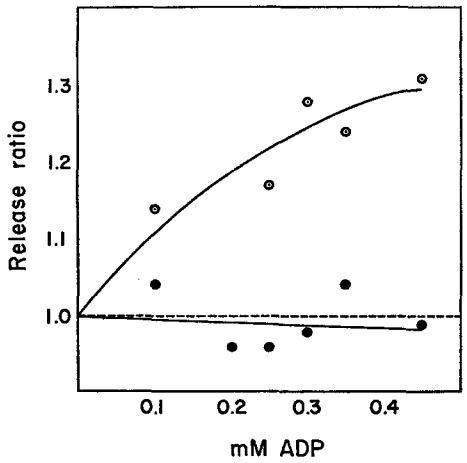


FIGURE 2. Effect of ADP on respiration in the presence and absence of *o*-phenanthroline. Rates of DPNH oxidation were measured in an Eppendorf fluorometer by the decrease in fluorescence intensity. The reaction mixture contained in a final volume of 2.0 ml: 85 mM tris-SO_4 pH 7.4; 4.3 mM $MgSO_4$; 17 mM KPO_4 pH 7.4; 0.05 mM DPNH; 0.5 mM *o*-phenanthroline; 0.34 mg ETP_H; and varying amounts of ADP as indicated in the figure. All rate measurements were made at room temperature with ADP in the incubation mixture prior to addition of particles and compared in duplicate experiments to the rates obtained in the absence of ADP. Open circles, plus 0.5 mM *o*-phenanthroline; filled circles, no *o*-phenanthroline.

TABLE II

EFFECT OF VARIOUS SUBSTRATES ON *o*-PHENANTHROLINE-INDUCED RESPIRATORY CONTROL

Rates of oxygen consumption were determined polarographically as described in Fig. 1. The reaction mixture contained in a final volume of 1.0 ml: 100 mM tris-SO_4 pH 7.4; 10 mM KPO_4 pH 7.4; 5 mM $MgSO_4$; 20 mM ADP; 1 mM *o*-phenanthroline. Substrates added were: 1 mM DPNH; 10 mM Na succinate; 20 mM ascorbate plus 4 μM PMS. The reaction vessel contained 0.66 mg SMP for determination of DPNH respiration and 0.17 mg SMP for determination of succinate and ascorbate-PMS respiration. The rates of oxygen consumption in the ascorbate-PMS system were corrected for non-enzymatic oxidation.

Substrate	− *o*-phenanthroline	+ *o*-phenanthroline	ADP	Release ratio
	μmoles O_2/min.	*μmoles O_2/min.*		
DPNH	0.069	0.027	0.044	1.63
Succinate	0.120	0.136	0.117	0.86
Ascorbate + PMS	0.044	0.062	0.038	0.61

o-phenanthroline. As shown in Table III, of the uncouplers tested, dinitrophenol, trifluorocarbonyl cyanide phenylhydrazone,[2] desaspidin,[2] and pentachlorophenol were found to be most effective in releasing *o*-phenanthroline–

[2] Trifluorocarbonyl cyanide phenylhydrazone (Tri F-CCP) was a gift from Dr. P. Heytler, and desaspidin was a gift from Dr. L. Runeberg.

inhibited respiration. Maximal release of respiration by the uncouplers was obtained at concentrations which completely uncouple oxidative phosphorylation. In the absence of *o*-phenanthroline little effect on respiration by the uncouplers was observed. The effect of these uncouplers was found to be competitive with *o*-phenanthroline at concentrations of the chelator below 0.5 mM.

TABLE III

RELEASE OF *o*-PHENANTHROLINE INHIBITION OF DPNH OXIDASE BY VARIOUS UNCOUPLING AGENTS

Rates of oxygen consumption were measured polarographically as described in Fig. 1. The reaction mixture contained in a final volume of 1.0 ml: 100 mM tris-SO_4 pH 7.4; 2.5 mM DPNH; 1 mM *o*-phenanthroline; 0.33 mg ETP_H ; and the uncoupling agent at a final concentration indicated in the table. In each experiment the uncoupler was added 1 minute after the addition of *o*-phenanthroline.

Uncoupler	Final concentration	+ *o*-phenanthroline	+ Uncoupler	Release ratio
	M	*μmoles O_2/min.*	*μmoles O_2/min.*	
Dinitrophenol	10^{-4}	0.040	0.062	1.55
Arsenate	2×10^{-3}	0.040	0.044	1.10
Ca^{++}	10^{-4}	0.032	0.027	0.84
Cd^{++}	10^{-6}	0.040	0.036	0.90
Desaspidin	10^{-6}	0.040	0.072	1.80
Penta-Cl phenol	10^{-5}	0.040	0.080	2.00
Tri F-CCP	5×10^{-7}	0.040	0.080	2.00

It appeared from these experiments that non-heme iron, which we assume to be the target of *o*-phenanthroline action, may play a key role in the phenomenon of respiratory control and in addition, may be the site at which dinitrophenol and other uncouplers interact. We attempted, therefore, to remove non-heme iron from submitochondrial particles under relatively mild conditions. Massey (27) found that non-heme iron in succinate dehydrogenase gave a characteristic color reaction with *o*-phenanthroline which was enhanced in the presence of dithionite. The effect of *o*-phenanthroline and dithionite on the $^{32}P_i$–ATP exchange reaction, a partial reaction of oxidative phosphorylation catalyzed by mitochondria and submitochondrial particles, is shown in Table IV. In this experiment there was no significant effect on exchange rate by dithionite or *o*-phenanthroline alone, but a marked inhibition was obtained when both were added together.

When the particles were reisolated by centrifugation after such treatment, essentially the same effects on the $^{32}P_i$–ATP exchange were observed, and the DPNH oxidase was found to be similarly inhibited (Table V). Moreover, removal of non-heme iron by *o*-phenanthroline was considerably increased in the presence of dithionite.

TABLE IV

EFFECT OF *o*-PHENANTHROLINE AND DITHIONITE ON THE $^{32}P_i$–ATP EXCHANGE REACTION IN ETP_H

The reaction mixture containing in a final volume of 0.3 ml: 10 mM tris-SO_4 pH 7.4; 1 mM *o*-phenanthroline; 0.5 mM neutralized and freshly prepared dithionite; and 1.65 mg ETP_H was incubated for 5 minutes at 30°C. Following this preincubation, 0.2 ml of a mixture containing 8 μmoles $MgSO_4$; 20 μmoles KPO_4 pH 7.4; 10 μmoles ATP; and $^{32}P_i$ (specific activity equal to 2380 CPM/μmole P_i) was added and the mixture incubated for 10 minutes at 30°C. The reaction was terminated with 0.5 ml of a charcoal–trichloroacetic acid mixture containing 100 mg of washed charcoal per ml in 10 per cent trichloroacetic acid and 50 mM KPO_4 . The charcoal was collected on a filter paper disc in a precipitation apparatus (Tracerlab), washed 10 times with 2.0 ml of 5 per cent trichloroacetic acid, and counted in a gas flow counter (Nuclear Chicago).

Additions	μmoles ATP^{32} formed/mg /10 min.
None	0.273
o-Phenanthroline	0.339
Dithionite	0.268
o-Phenanthroline + dithionite	0.126

TABLE V

EFFECT OF *o*-PHENANTHROLINE AND DITHIONITE ON THE $^{32}P_i$–ATP EXCHANGE REACTION AND DPNH OXIDASE IN ETP_H . REISOLATION OF PARTICLES

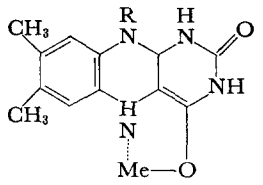
ETP_H (21 mg) were incubated with 0.5 mM neutralized and freshly prepared dithionite and 1 mM *o*-phenanthroline where indicated in the table. The reaction mixture contained in a total volume of 5.0 ml: 20 mM tris-SO_4 pH 7.4. After 15 minutes of incubation at 30°C, the contents of the tube were centrifuged at 105,000 × *g* for 30 minutes. The pellet was resuspended in 0.3 ml of 0.25 M sucrose containing 0.01 M tris acetate pH 7.4. Aliquots were removed for assay of the $^{32}P_i$–ATP exchange reaction as described in Table IV and for DPNH oxidase by measuring the disappearance of absorbance at 340 mμ in a 1.0 ml reaction mixture containing 0.125 mM DPNH; 200 mM KPO_4 pH 7.4. The reaction was carried out at room temperature.

The amount of iron removed from the particle was determined as Fe^{++} by measuring the absorbancy at 510 mμ of the Fe^{++}–*o*-phenanthroline complex in the supernatant solution from the 105,000 × *g* spin. A few milligrams of dithionite were added to the sample before the measurements were made to convert all of the extracted iron to the Fe^{++} form.

Additions	$^{32}P_i$–ATP exchange	DPNH oxidase	Fe removed
	μmole ATP^{32}/mg/ 10 min.	*μmole/mg/min.*	*mμmole/mg*
None	0.252	0.240	—
Dithionite	0.245	0.212	—
o-Phenanthroline	0.322	0.193	0.95
o-Phenanthroline + dithionite	0.152	0.132	1.50

DISCUSSION

The formation of chelates between riboflavin and several metals (Me) was demonstrated by Albert (28) and a general structure for the chelate was suggested (9), as indicated below:



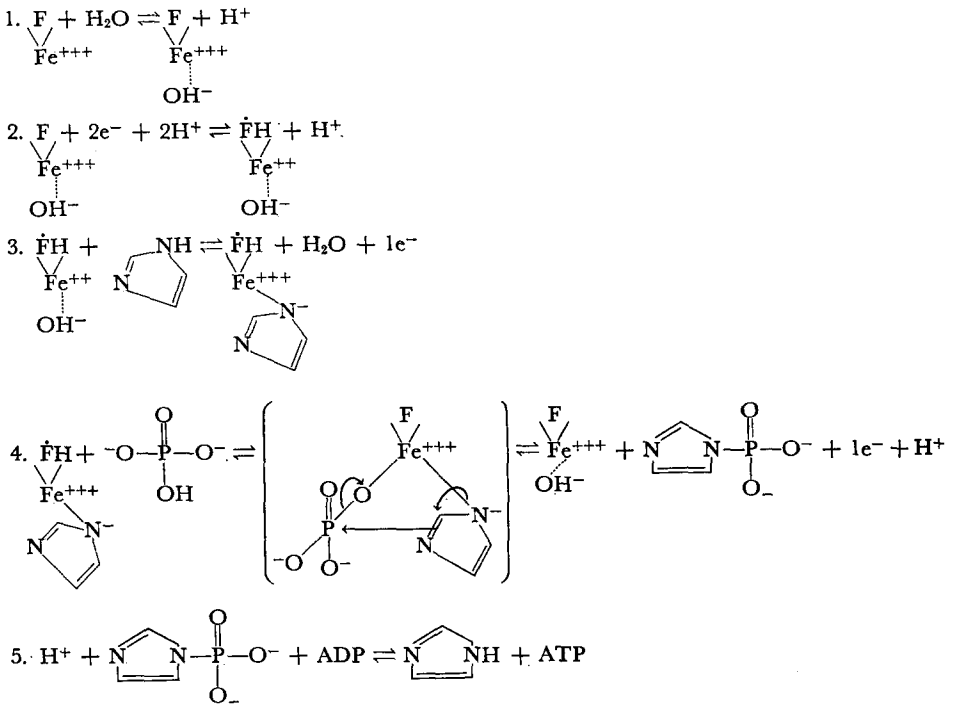
Oxidation of DPNH in mitochondria involves hydrogen transfer followed by successive steps of electron transfer through the cytochromes. It was proposed by Mahler (29) that a *d*-metal such as iron could play a key role in the transition from two to one electron transfer. The role of transition metals in stabilizing free radical systems has been described (30) and may involve spin—spin interaction of the paramagnetic metal with the flavin radical (31). The experiments which we have described in this paper suggest the possibility that non-heme iron is involved in the site at which the energy of DPNH oxidation is produced and conserved. We wish to present a hypothesis (scheme 4) which explains many of the experimental observations, but it should be emphasized that there is no direct evidence for any of the proposed steps or for the participation of imidazole.

This scheme has a number of attractive features in relation to the problems of oxidative phosphorylation. The entrance of water shown in reactions 1 to 4 accounts for the well known $H_2{}^{18}O$–phosphate exchange reaction associated with oxidative phosphorylation. The reversible and rapid hydroxylation of the oxidized flavin-ferric complex (reaction 1) allows for the exchange of water oxygen with phosphate oxygen (reaction 4). Following the 2 electron reduction of the hydroxylated flavinferric chelate (reaction 2), successive one electron transfer steps shown in reactions 3 and 4 are facilitated by the interaction of the *d*-orbitals of iron with the π electron system of flavin.

The choice of an imidazole group as a reactant in these steps is based on its well known ability to coordinate with iron and the demonstration by Boyer and his associates (32, 33) of the participation of a protein-bound histidine phosphate in a phosphate transfer reaction catalyzed by succinate thiokinase. A phosphohistidine protein is also involved in bacteria in transfer reactions from phosphoenolpyruvate to sugars (34). The imidazole in scheme 4 is regenerated in step 5 by the transphosphorylation of the phosphoryl group to ADP.

Assuming that *o*-phenanthroline inhibits electron flow by interacting with non-heme iron, how do we visualize the reversal of the inhibition by ADP or

Scheme 4



uncoupling agents? Imidazole, representing X in schemes I and II, together with P_i displaces the *o*-phenanthroline from its site of attachment to non-heme iron. In the absence of ADP this process ceases when free X is no longer available. Electron flow is reestablished on addition of ADP since X is regenerated from $X \sim P$ (scheme 1). In contrast, the uncoupling agents may displace *o*-phenanthroline directly from the iron complex as indicated by the kinetics of competitive inhibition.

In order to obtain an inhibition by *o*-phenanthroline of the $^{32}P_i$–ATP exchange reaction, a reducing agent such as dithionite was required. This is in line with the observation of Massey (27) that phenanthroline can effectively interact with the non-heme iron of succinate dehydrogenase only in the presence of dithionite. We have made similar observations with purified DPNH dehydrogenase. The mercurial sodium mersalyl was found to replace dithionite in facilitating, under appropriate conditions, the inhibition of the $^{32}P_i$–ATP exchange reaction by *o*-phenanthroline. This finding is of particular interest in connection with the observation of the labilization by mersalyl of non-heme iron in ferredoxin (35) and the proposed association of non-heme iron with labile sulfide groups, both in ferredoxin (36, 37) and DPNH dehydrogenase (38).

In conclusion, we should like to reemphasize that we have described here

a model of respiratory control. Obviously, *o*-phenanthroline is not the physiological regulator of respiration. But these studies have pointed to non-heme iron as a likely site for the regulation of electron flow and possibly as a component of the non-phosphorylated high energy intermediate of oxidative phosphorylation.

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