*REACTIONS OF COENZYME Q IN THE DPNH DEHYDROGENASE SEGMENT OF THE RESPIRATORY CHAIN**

By J. M. Machinist and Thomas P. Singer

EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH, HENRY FORD HOSPITAL, DETROIT, MICHIGAN

Communicated by Harland G. Wood, January 4, 1965

Despite extensive studies in many laboratories, neither the exact role of CoQ in electron transport nor its site of action are unambiguously established. Although it is generally agreed that CoQ is a functional component of the respiratory chain,[1, 2] pending the resolution of the question[3–6] whether or not the oxido-reduction of internal CoQ_{10} is sufficiently rapid to be on the main path of electron flow from flavoprotein to O_2, alternative functions,[5, 6] for example, electron transport between functionally interconnected respiratory chains,[7] cannot be precluded. At present there appears to be general agreement[6, 8] that the reduction of both endogenous and external CoQ_{10} by DPNH is inhibited by Amytal and their reoxidation is inhibited by antimycin A. This localizes the main reaction site of the natural homologue between flavoprotein and cytochrome c_1, but neither the identity of the immediate reductant nor that of the oxidant is firmly established. According to a recent report[9] short-chain CoQ homologues can also react in the cytochrome *c* region of the chain.

In one scheme[3] for the DPNH oxidase chain, CoQ is shown to accept electrons from DPNH dehydrogenase by way of nonheme iron. Since all the nonheme iron in this segment of the chain belongs to DPNH dehydrogenase[10, 11] and is functional in that enzyme,[12] it would follow that the dehydrogenase is the immediate reaction partner of CoQ. Evidence favoring this view is that a cytochrome-free particle, the DPNH-CoQ reductase complex[13] ("complex 1") catalyzes the Amytal-sensitive reduction of short-chain CoQ homologues, although not of external CoQ_{10}. This preparation, however, also contains components other than the flavoprotein (cf. below). Further, Pharo and Sanadi[14–16] have described a soluble preparation ("DPNH-ubiquinone reductase"), extracted from ETP_H by the acid-ethanol-heat procedure previously used[17–19] for the isolation of DPNH-cytochrome reductase from closely related particles. While ubiquinone reductase has been reported to catalyze the Amytal[15]- and rotenone[16]-sensitive reduction of CoQ homologues by DPNH, DPNH-cytochrome reductase is thought[16] to be devoid of such activity. This divergent behavior is puzzling in view of the great similarities in the extraction and isolation procedures and in starting materials. Retention of the Amytal- and rotenone-sensitive CoQ reduction through this extraction procedure is also paradoxical, since it has been shown[20] that the same method, whether applied to particles or to the purified DPNH dehydrogenase, fragments the flavoprotein, with attendant changes in the molecular and catalytic properties, including loss of its characteristic ferricyanide activity, substrate-inducible EPR signal[12] at $g = 1.94$, extensive loss of nonheme iron and of "labile" sulfide,[21] and the emergence of cytochrome *c* and DCIP reductase activities. The low molecular weight and low nonheme iron and "labile" sulfide content of ubiquinone reductase[14–16] and the absence of the $g = 1.94$ signal[22] indicate, in fact, extensive degradation. The observation[22] that the reduction of CoQ homologues by the purified dehydrogenase is very slow and is

Amytal- and rotenone-insensitive, although in all other respects explored its properties parallel those of the mitochondrial enzyme,[23, 24] seems to argue against a direct interaction between the dehydrogenase and CoQ.

In order to eliminate the possibility that the presumed differences between DPNH dehydrogenase and the DPNH-CoQ reductase complex and between DPNH-cytochrome reductase and ubiquinone reductase are methodological ones, it was decided to compare the respective preparations in the same laboratory. Another approach which seemed promising in characterizing the reactions of CoQ in the vicinity of the flavoprotein was the abolition and specific restoration of the DPNH-CoQ reaction in particles.

Methods.—ETP and DPNH dehydrogenase were isolated, and DPNH-ferricyanide, DPNH-cytochrome *c*, and menadione activities, "labile" sulfide, nonheme iron, and flavin were determined as in previous papers.[10, 11, 20, 21, 24] ETP_H was isolated according to Hansen and Smith.[25] DPNH-CoQ reductase complex, made according to Hatefi *et al.*,[13] was the preparation characterized in the preceding paper.[21] DPNH-cytochrome reductase and DPNH-ubiquinone reductase were extracted by DeBernard's modification[18] of Mahler's method[17] from ETP and ETP_H, respectively. The two preparations behaved identically throughout fractionation.[20] The DPNH-CoQ_1 reaction was measured according to Hatefi *et al.*,[13] except that the Asolectin was prepared by the method of Wharton and Griffiths.[26] Antimycin A (10^{-6} *M*) was present only in assays of particles. The reduction of CoQ_6 was usually measured by the procedure of Pharo and Sanadi[16] ("Tris-$SO_4^=$ assay"), starting the reaction with DPNH. For comparative purposes samples were also assayed by starting the reaction by the addition of CoQ_6 from a 20 mM solution in methanol to give a final concentration of 0.2 mM. Occasional samples were also assayed in the system described by Hatefi *et al.*[13] for CoQ_1 reduction, except for the omission of Asolectin ("phosphate assay"). In particulate samples either cyanide (1 mM) or azide (2 mM) + antimycin A (10^{-6} *M*) was used to prevent reoxidation of CoQ_6. While assays involving CoQ_1 gave linear initial rates and good reproducibility, considerable difficulty was encountered with CoQ_6 assays. In both particles and soluble samples, regardless of the component used to start the reaction, initial rates were nonlinear, but while in particles high rates of recording (6–12 inches/min) with suitable scale expansion afforded approximately linear initial rates and replication within a few per cent error, with soluble samples deviation from linearity could not be overcome; agreement between duplicates was poor (occasionally over 100% difference), regardless of the assay system or whether the results were calculated from pseudo-first-order rate constants or from linearized initial velocity. Hence all results given represent the averages of at least 5 determinations. The phosphate assay gave lower activities in soluble preparations than the Tris-$SO_4^=$ system, in accord with Pharo and Sanadi,[16] but no material difference was detected in ETP or ETP_H (Table 3).

All assays were performed with the fast recording spectrophotometer previously described,[27] and results are expressed as μmoles of DPNH oxidized/min at 30° under the conditions of the particular assay.

Results.—DPNH dehydrogenase and the DPNH-CoQ reductase complex: Table 1 compares the most important properties of the highly purified soluble enzyme with those of the simplest particulate system known to catalyze the Amytal-sensitive oxidation of DPNH by CoQ homologues. The similarities between the two preparations are quite apparent. Ferricyanide is the best electron acceptor known for both; they contain about the same amount of iron, and the iron is catalytically active (i.e., substrate-reducible) in both, as judged by the EPR signal[12, 28] at $g = 1.94$. Both are thermolabile; neither reduces cytochrome *c* or CoQ_{10} at significant rates in the unmodified state, but both are fragmented to DPNH-cytochrome reductase by acid-ethanol at 42–44°.

The specific activity of the complex in the ferricyanide assay, calculated from the data of Hatefi *et al.*[13] to V_{max} or directly determined[21] is much lower than that of

TABLE 1

COMPARISON OF DPNH DEHYDROGENASE AND DPNH-CoQ REDUCTASE

Property	DPNH deh.	DPNH-CoQ reductase
Form	Soluble	Particulate
Nonheme iron: FMN ratio	16–18	17–18*
"Labile" S/Fe ratio	1.5–1.6†	1.0†
Substrate-reducible EPR signal at $g = 1.94$	+	+
Reactivity with cyt. *c*	Trace	Very low*
Fragmentation and conversion to cytochrome reductase by heat or acid-ethanol	+	+
Turnover no., $Fe(CN)_6^{=}$	$1.3–1.5 \times 10^6$†	6.5×10^5
Turnover no., CoQ_1	$0.5–0.7 \times 10^3$	$9.5 \pm 0.3 \times 10^3$* 11.7×10^3†
Turnover no., CoQ_1, in presence of rotenone	$0.5–0.7 \times 10^3$	$0.7–1 \times 10^3$
Turnover no., CoQ_{10}	<100	<100*
CoQ_{10} content	0	4.5 mμmoles/mg*
Lipid content	0	0.22 mg/mg*

* From Hatefi *et al.*[13]
† From Lusty *et al.*[21] All turnover numbers are at 30° per mole of FMN.

the best preparations of the dehydrogenase,[21] and the turnover number of the complex in the ferricyanide assay (per mole of FMN) is about half the value determined for the dehydrogenase after two gradient centrifugations (Table 1). These differences probably reflect the presence of FMN-containing impurities in the complex. The probable presence of iron-flavoprotein impurities[21] in the DPNH-CoQ reductase complex may also account for its apparently lower "labile" S/Fe ratio.

On the other hand, there are clear-cut differences in lipid and CoQ_{10} content and in rotenone-sensitive CoQ_1 activity. The rotenone- (and Amytal-) insensitive CoQ_1 turnover number is the same for the two preparations and this reaction may represent a direct interaction between the flavoprotein and short-chain CoQ homologues. It may be noted that the maximal activity of the complex toward CoQ_1 is only 1–2 per cent of the potential catalytic activity of DPNH dehydrogenase as determined[28] by the ferricyanide assay or the rate of appearance of the EPR signal at $g = 1.94$. Since the only unambiguous difference between the catalytic properties of the two preparations involves the inhibitor-sensitive CoQ_1 reduction, the question arises whether its absence reflects preparative modification of the dehydrogenase. The mild procedure used in its isolation[10] and the parallelism in all of its other known properties with the particle-bound enzyme[23] would argue against this. An alternative possibility is that the inhibitor-sensitive reduction of CoQ_1 requires the presence of bound phospholipid and/or of CoQ_{10} (in fact, the reduction of external CoQ_1 might proceed via the internal CoQ_{10}). Removal of these factors during solubilization of the flavoprotein with phospholipase A would abolish this reaction. Evidence for this view is presented later in this paper.

Thermal degradation of the flavoprotein to DPNH-CoQ_1 reductase: It is known that exposure of DPNH dehydrogenase to temperatures over 30° results in fragmentation to low molecular weight products, changes in substrate specificity, labilization of the flavin, loss of nonheme iron and of "labile" sulfide, parallel loss of DPNH-ferricyanide activity and of substrate-inducible EPR signal at $g = 1.94$, and secondary emergence of cytochrome *c* and DCIP reductase activities.[12, 20, 29] Table 2 shows that, under the conditions previously used,[12, 21] exposure to 35° also causes a 5-fold increase in DPNH-CoQ_1 activity. For purposes of comparison the time course of the destruction of ferricyanide activity and of the formation of

TABLE 2

EMERGENCE OF CoQ_1 REDUCTASE ACTIVITY ON THERMAL DEGRADATION OF DPNH DEHYDROGENASE

Time at 35° (min)	μMoles DPNH Oxidized/Min/Ml at 30°		
	CoQ_1	Cyt. *c*	$Fe(CN)_6^=$
0	4.37	2.47	4500
20	8.55	14.7	2000
40	12.9	27.6	1000
60	20.6	39.3	537
120	24.6	74.8	342
160	25.0	69.8	270

DPNH dehydrogenase (specific activity = 417) was incubated at 35° in 0.03 *M* phosphate, pH 7.8, at 10.9 mg protein/ml in air.

cytochrome reductase activity is included. Although the increase in cytochrome reductase activity is greater than the rise in CoQ_1 activity, both processes reach a maximum at about the same time and lag behind the rate of destruction of ferricyanide activity. CoQ_1 reductase activity created by heat is rotenone-insensitive.

It appears that the direct reaction of the dehydrogenase with CoQ_1, like the reactions with DCIP and cytochrome *c*, is hindered in the native enzyme and this steric hindrance may be removed by suitable conformational changes. Although this finding in no way negates the metabolic significance of Amytal- and rotenone-sensitive CoQ reactions (particularly those involving the natural homologue), it points to a need for caution in interpreting all reactions of CoQ as being necessarily physiological or as being more reliable than so-called artificial acceptors.[30]

DPNH-cytochrome reductase and DPNH-ubiquinone reductase: Several DPNH-cytochrome reductase preparations derived from heart mitochondria have been described in the literature,[17-19, 31] but a systematic comparison failed to reveal significant differences among them.[20] This type of DPNH-cytochrome reductase is a fragmentation product of DPNH dehydrogenase, formed by the extraction procedure.[20] Three of these preparations[17-19] were extracted by minor modifications of the acid-ethanol-heat procedure of Straub.[32] DeBernard's variant[18] of this method has also been used for the extraction of DPNH-ubiquinone reductase.[16] Purification of the latter[16] also follows substantially the procedure[20] elaborated for the isolation of DPNH-cytochrome reductase. The only known difference is that while DPNH-cytochrome reductase is extracted from ETP, in the extraction of ubiquinone reductase ETP_H, a closely related particle is used. It was of interest, therefore, to compare ETP and ETP_H with regard to certain catalytic properties, particularly those involving CoQ derivatives.

Table 3 shows that no material difference exists between ETP and ETP_H in the relative rates of oxidation of DPNH with different acceptors. As shown in Table 4, acid-ethanol treatment of both ETP and ETP_H results in extensive and comparable losses of ferricyanide, CoQ_6, and of rotenone-sensitive CoQ_1 activities, accompanied by major increases in cytochrome reductase and rotenone-insensitive CoQ_1 reductase activities. The differences between ETP and ETP_H are not significant, since the values given are averages and the data for individual preparations overlap for ETP and ETP_H. (Thus the increase in cytochrome reductase ranged from 237 to 1000% for ETP, from 352 to 2100% for ETP_H; for rotenone-insensitive CoQ_1 reduction from 129 to 400% for ETP, 138 to 538% for ETP_H. The reason for this scatter is that these activities are both created and destroyed in the De-

TABLE 3

COMPARISON OF ETP AND OF ETPH PREPARATIONS

Criterion	ETP*	ETPH*
DPNH-$Fe(CN)_6^=$ act.	32.9	47.8
DPNH-CoQ_1 act.	1.02	1.10
DPNH-CoQ_6 act.	0.39†	0.40†
	0.29‡	0.38‡
	0.42§	0.43§
Ratio: $Fe(CN)_6^=$:CoQ_1:CoQ_6	100:3.1:0.88‡	100:2.3:0.80‡
DPNH-cyt. *c* act.	0.146	0.093
Inhibition of CoQ_1 act. by		
10^{-6} *M* rotenone	93%	90%
3.5 mM Amytal	85%	86%

All activities are μmoles DPNH oxidized/min/mg protein at 30° and are V_{max} values in the case of $Fe(CN)_6^=$ and cyt. *c*.
* Average of 6 experiments for ETP and of 7 experiments for ETPH, except as noted.
† Determined in phosphate buffer, reaction started with Q_6, average of 3 experiments.
‡ Determined in Tris-$SO_4^=$ buffer, reaction started with DPNH, single experiment.
§ Determined in Tris-$SO_4^=$ buffer, reaction started with Q_6, average of 2 experiments.

TABLE 4

YIELDS ON ACID-ETHANOL TREATMENT OF ETP, ETPH, AND OF PURIFIED DPNH DEHYDROGENASE BY DEBERNARD'S METHOD

	Yield (%)		
Reaction	ETP	ETPH	DPNH deh.*
DPNH-$Fe(CN)_6^=$	2.2	2.1	1–2*
DPNH-cyt. *c*	450	543	3000
DPNH-menadione		201	
Rotenone-sens. DPNH-CoQ_1	0–1	0–2	
Rotenone-insens. DPNH-CoQ_1	216	270	406
DPNH-CoQ_6†	8.7	8.9	33

The yields represent average values for 6 ETP and 7 ETPH preparations. Activities were compared in particles and in extracts after acid-ethanol treatment, centrifugation, and lyophilization. Assays were performed under optimal conditions for each reaction and were V_{max} values for $Fe(CN)_6^=$, cyt. *c*, and menadione.
* Values from ref. 20. Acid-ethanol treatment at 42°.
† Completely rotenone-sensitive in the particles; sensitivity small and variable in extracts. Rotenone, 1 μM.

Bernard procedure, and the yield depends on the time required to reach 44° and hence on the volume of enzyme worked up.)

That exposure to acid-ethanol at 42–44° indeed creates rotenone-insensitive CoQ_1 activity (rather than converts a rotenone-sensitive reduction to an insensitive one) is best seen from the last column which shows that the fragmentation of highly purified DPNH dehydrogenase (which lacks a rotenone-sensitive CoQ reductase activity) by this procedure creates a 400 per cent increase in total CoQ_1 activity. Thus the emergence of reactivity with short-chain CoQ homologues is another consequence of the acid-ethanol degradation of this enzyme.[20]

Table 5 compares the properties of soluble preparations derived from ETP and ETPH by the DeBernard procedure. Activities with different electron acceptors were examined at all stages of purification with comparable results. The cytochrome-reducing fragment isolated from the two sources has essentially the same properties and the data agree with published values[20] for Mahler's cytochrome reductase. The two preparations also show similar "labile" sulfide: iron ratios.[21] The activity ratios in Table 5 differ somewhat from those published by Pharo and Sanadi,[16] but this is not unexpected since their assays were not V_{max} values and it is known[20] that the apparent K_m for dyes is not constant in this type of product, and, further, since considerable variation in the creation and destruction of the various activities will occur depending on the exact conditions of the acid-ethanol-heat treat-

TABLE 5

COMPARISON OF PRODUCTS OF ACID-ETHANOL FRAGMENTATION DERIVED FROM ETP AND ETPH

Property	Products Derived from ETP	Products Derived from ETPH
Ratio; cyt. *c*: $Fe(CN)_6^{=}$:menadione:CoQ_1:CoQ_6* activities	100:99:21:29:9.1	100:102:24:28:9.1
Rotenone inhib. of CoQ_1 act. (%)	0	0
Amytal inhib. of CoQ_1 act. (%)	0	0
Rotenone inhib. of CoQ_6 act. (%)*	0–20	0–20
Amytal inhib. of CoQ_6 act. (%)*	38	39
K_m for DPNH†	7.5×10^{-5} *M*	7.3×10^{-5} *M*
K_m for TNDPNH†,‡	2.1×10^{-4} *M*	2.8×10^{-4} *M*
V_{max} TNDPNH/V_{max} DPNH†	0.3	0.5
s_0	5.6	5.7§
Flavin linkage	Labile	Labile
Elution from hydroxylapatite at pH 7.4	0.05 *M* $PO_4^{=}$	0.05 *M* $PO_4^{=}$

Extraction of the particles was by DeBernard's method;[18] the extracts were clarified, lyophilized, fractionated at 0.4–0.6 sat. $(NH_4)_2SO_4$, and chromatographed on hydroxylapatite.[20]

* The data refer to 6 ETP and 7 ETPH preparations, examined at various stages of purification, except those noted (*) where single preparations were isolated simultaneously from ETP and ETPH under identical conditions of volume, protein concentration, etc. The concentration of Amytal was 3.5 mM; inhibition by rotenone was studied in the range 0.1–1 μM.

† Kinetic data from cytochrome reductase assays.

‡ TNDPNH, thionicotinamide analogue of DPNH.

§ Data from Sanadi.[14]

ment. The scatter of data was particularly great in the assay of CoQ_6 activity. For this reason comparison with regard to this assay is limited to samples which were isolated side-by-side under the same conditions of volume, protein concentration, etc.

Since molecular weights based on flavin content and Fe/FMN ratios are not reliable for cytochrome reductases because of the lability of the flavin,[20] the only major difference between DPNH-cytochrome reductase and the preparation from ETPH in the literature[16] appears to be in rotenone-sensitivity. As a result of the difficulties of the CoQ_6 assay (cf. *Methods*), a precise value could not be established in the present study, but in no case was over 25 per cent inhibition observed. In contrast, Pharo and Sanadi[16] reported up to 77 per cent inhibition at presumably comparable rotenone/enzyme ratios. However, the inhibition observed by these workers disappeared completely at higher rotenone concentrations,[16] while rotenone inhibition of DPNH dehydrogenase in intact particle preparations remains complete even at high concentrations.

Inactivation and restoration of rotenone-sensitive DPNH-CoQ activity: Although lipid requirements for electron transfer have been known for several components of the respiratory chain,[33] direct evidence for the role of lipids in the initial steps of DPNH oxidation has been lacking. Fleischer *et al.*[34] reported that DPNH-CoQ_1 activity is abolished by brief digestion with phospholipase A and is restored by removing fatty acid inhibitors and that after longer digestion phospholipids are also required for reactivation. It has been known that brief contact with low amounts of phospholipase A (1–2 μg/mg ETP protein) interrupts electron flux from DPNH dehydrogenase to the respiratory chain and extracts considerable lipid and protein without solubilizing the dehydrogenase and this treatment has been used as the initial step in the isolation of the flavoprotein.[11] Digestion of ETP with purified phospholipase[11] at the level mentioned abolishes all rotenone-sensitive DPNH-CoQ_1 activity in 5–10 min at 30°. In extending these observations we found that after brief (1–3 min) contact with phospholipase, although inactivation of CoQ_1

TABLE 6

REVERSIBLE INACTIVATION OF DPNH-CoQ_1 ACTIVITY BY PHOSPHOLIPASE A

Expt.	Components	DPNH-CoQ_1 activity (μmoles/min/mg) No rotenone	With rotenone
1	ETP	0.71	0.058
	ETP + 0.15 mg Asolectin	0.91	0.041
2	ETP after 3 min with p-lipase	0.20	0.056
3	Same as 2, with 1.5 mg Asolectin	0.56	0.054
4	Same as 2, after 3 washes	0.19	
5	Same as 4 + 1.5 mg Asolectin	0.64	0.054
6	ETP	0.64	0.057
	ETP + 0.15 mg Asolectin	0.88	0.054
7	ETP after 1 min with p-lipase	0.35	0.050
8	Same as 7 + ML* (7.5 μg P)	0.65	0.050
9	Same as 7, after washing with ML* and sucrose	0.56	0.050
10	Same as 9 + ML* (7.5 μg P)	0.89	0.054

ETP was incubated with 2 μg purified phospholipase A[11]/mg protein at 30°. Washing in expt. 4 was by 7 min centrifugation at 144,000 × g twice with 0.5% serum albumin in 0.25 M sucrose, pH 7.4, then once with sucrose. In expt. 9 phospholipase action was stopped by adding 3 ml of cold 0.25 M sucrose containing dialyzed mitochondrial lipid (1.5 mg P) to 1 ml (25.7 mg) ETP. After centrifugation the residue was washed twice with 9 ml sucrose and finally resuspended in sucrose. Assays were at 30° and were started with DPNH. Incubation with lipids was for 5 min at 30°. Rotenone concentration, 10^{-6} M (2–5 μg/mg protein).

* Dialyzed preparation of mitochondrial lipids from beef heart.[35]

reduction was only partial, most of the activity could be restored by the addition of phospholipids to the unwashed preparation but only incompletely or not at all by washing with serum albumin (Table 6). When the reaction was stopped by the addition of phospholipids, following removal of phospholipase by repeated centrifugations, all of the original Amytal- and rotenone-sensitive CoQ_1 reductase activity was restored by a preparation[35] of mitochondrial lipids (Table 6).

The report of Fleischer *et al.*[34] and these observations establish an absolute requirement for lipids in the DPNH-CoQ reaction. Hence this reaction cannot be viewed as a simple, direct interaction between the nonheme iron of the flavoprotein and external CoQ_1. The remaining disparity between the purified dehydrogenase and the DPNH-CoQ reductase complex (inhibitor-sensitive CoQ_1 reduction) may then revolve around the presence of lipids in the former but not in the latter.

Summary.—The properties of DPNH dehydrogenase and of the DPNH-CoQ reductase complex were compared. The only difference in catalytic activity detected was the ability of the latter to catalyze the rotenone- and Amytal-sensitive reduction of external, short-chain CoQ homologues. As judged by the full reactivation of this reaction by mitochondrial lipids, following inactivation by phospholipase A, the reduction of CoQ by DPNH requires lipids which are not present in the purified flavoprotein. DPNH-cytochrome *c* and DPNH-ubiquinone reductases from heart mitochondria were compared, and no major difference in properties was detected. The reactivity of DPNH dehydrogenase with CoQ_1, like its cytochrome reductase activity, is very low. On thermal or acid-ethanol modification of the enzyme, however, a large increase in CoQ_1 reductase activity occurs. The acid-ethanol fragmentation product also catalyzes a partially Amytal- and rotenone-sensitive reduction of CoQ_6.

The abbreviations used are CoQ, coenzyme Q; ETP and ETP_H, electron transport particles; DCIP, 2,6-dichlorophenolindophenol.

* This study was aided by grant HE 01995 from the National Heart Institute, by grant G 20457 from the National Science Foundation, and by contract Nonr 1656 (00) between the Office of Naval Research and the Edsel B. Ford Institute for Medical Research.

[1] Hatefi, Y., R. L. Lester, F. L. Crane, and C. Widmer, *Biochim. Biophys. Acta*, **31**, 490 (1959).

[2] Redfearn, E. R., in *Quinones in Electron Transport*, ed. G. E. W. Wolstenholme and C. M. O'Connor (London: J. and A. Churchill, 1961), p. 346.

[3] Green D. E., *Vth Int. Congress Biochem., Moscow, 1961*, Plenary Lecture (London: Pergamon Press, 1961), Reprint No. 176.

[4] Green, D. E., Y. Hatefi, and W. F. Fechner, *Biochem. Biophys. Res. Commun.*, **1**, 45 (1959).

[5] Chance, B., in *Quinones in Electron Transport*, ed. G. E. W. Wolstenholme and C. M. O'Connor (London: J. and A. Churchill, 1961), p. 327.

[6] Redfearn, E. R., and A. M. Pumphrey, *Biochem. J.*, **76**, 64 (1960).

[7] Kimura, T., and T. P. Singer, *Nature*, **184**, 791 (1959).

[8] Ramasarma, T., and R. L. Lester, *J. Biol. Chem.*, **235**, 3309 (1960).

[9] Whittaker, P. A., and E. R. Redfearn, Scientific Memo # 153, IEG # 1, National Institutes of Health, circulated May 28, 1964.

[10] Ringler, R. L., S. Minakami, and T. P. Singer, *J. Biol. Chem.*, **238**, 801 (1963).

[11] Cremona, T., and E. B. Kearney, *J. Biol. Chem.*, **239**, 2328 (1964).

[12] Beinert, H., G. Palmer, T. Cremona, and T. P. Singer, *Biochem. Biophys. Res. Commun.*, **12**, 432 (1963).

[13] Hatefi, Y., A. G. Haavik, and D. E. Griffiths, *J. Biol. Chem.*, **237**, 1676 (1962).

[14] Sanadi, D. R., T. A. Andreoli, R. L. Pharo, and S. R. Vyas, in *Energy-Linked Functions in Mitochondria*, ed. B. Chance (New York: Academic Press, 1963), p. 26.

[15] Sanadi, D. R., T. E. Andreoli, R. L. Pharo, and S. R. Vyas, *Federation Proc.*, **22**, 405 (1963).

[16] Pharo, R. L., and D. R. Sanadi, *Biochim. Biophys. Acta*, **85**, 346 (1964).

[17] Mahler, H. R., N. K. Sarkar, L. P. Vernon, and R. A. Alberty, *J. Biol. Chem.*, **199**, 585 (1952).

[18] DeBernard, B., *Biochim. Biophys. Acta*, **23**, 510 (1957).

[19] Mackler, B., *Biochim. Biophys. Acta*, **50**, 141 (1961).

[20] Watari, H., E. B. Kearney, and T. P. Singer, *J. Biol. Chem.*, **238**, 4063 (1963).

[21] Lusty, C. J., J. Machinist, and T. P. Singer, *J. Biol. Chem.*, in press.

[22] Singer, T. P., in *Comprehensive Biochemistry*, ed. M. Florkin and E. H. Stotz (Amsterdam: Elsevier), vol. 14, in press.

[23] Singer, T. P., in *The Enzymes*, ed. P. D. Boyer, H. A. Lardy, and K. Myrbäck (New York: Academic Press, 1963), vol. 7, p. 345.

[24] Minakami, S., T. Cremona, R. L. Ringler, and T. P. Singer, *J. Biol. Chem.*, **238**, 1529 (1963).

[25] Hansen, M., and A. L. Smith, *Biochim. Biophys. Acta*, **81**, 214 (1964).

[26] Wharton, D. C., and D. E. Griffiths, *Arch. Biochem. Biophys.*, **96**, 103 (1962).

[27] Minakami, S., R. L. Ringler, and T. P. Singer, *J. Biol. Chem.*, **237**, 569 (1962).

[28] Beinert, H., G. Palmer, T. Cremona, and T. P. Singer, *J. Biol. Chem.*, **240**, 475 (1965).

[29] Cremona, T., E. B. Kearney, M. Villavicencio, and T. P. Singer, *Biochem. Z.*, **338**, 407 (1963).

[30] Hatefi, Y., A. G. Haavik, and P. Jurtshuk, *Biochem. Biophys. Res. Commun.*, **2**, 281 (1960).

[31] King, T. E., and R. L. Howard, *J. Biol. Chem.*, **237**, 1686 (1962).

[32] Straub, F. B., *Biochem. J.*, **22**, 787 (1939).

[33] Green, D. E., and S. Fleischer, in *Biochemical Problems of Lipids*, ed. A. C. Frazer (Amsterdam: Elsevier, 1963), p. 325.

[34] Fleischer, S., A. Casu, and B. Fleischer, *Federation Proc.*, **23**, 486 (1964).

[35] Fleischer, S., and H. Klouwen, *Biochem. Biophys. Res. Commun.*, **5**, 378 (1961).