ACCEPTANCE ADDRESS, ELI LILLY AWARD IN BIOLOGICAL CHEMISTRY, 1961

Quinones in Lipoprotein Electron Transport Systems*

F. L. CRANE

From the Department of Biological Sciences, Purdue University, Lafayette, Indiana

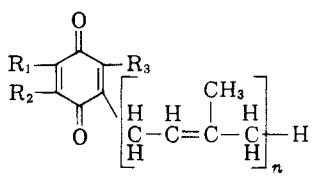
Received January 2, 1962

Coenzyme Q, vitamin K, and plastoquinone are lipophilic quinones which participate in electron transport reactions associated with oxidative or photosynthetic phosphorylation. Evidence for formation of the chromanol from vitamin K is consistent with mechanisms proposed for quinone function in phosphorylation reactions, but there is no clear evidence that the other quinones form chromanols. Further studies are indicated to determine the reactions of chromanols in biological systems as well as the distribution and site of function of quinones.

It is now clear that three lipophilic quinones are universally distributed as members of the phosphorylating electron transport systems in the great majority of living organisms. Furthermore, evidence is beginning to appear which would implicate these quinones as the initial phosphate acceptor site in the process of oxidative phosphorylation. The individual peculiarities of certain electron transport systems and of the quinones has led to a variety of approaches to the study of the function of the quinones. In some cases a direct approach to phosphorylation mechanisms has been possible, whereas in other systems study of quinone function has been successful only in following the electron transport function.

The three types of quinones are members of the coenzyme Q, plastoquinone, and vitamin K groups. As can be seen in formula (1), the basic features of these quinone structures are very similar in that each consists of a substituted quinoid ring with a long terpenoid tail. The long side-chain is in all cases unsaturated in the position β to the ring.

The only major structural difference among the compounds lies in the fact that the coenzyme Q and vitamin K groups have fully substituted quinoid rings whereas plastoquinone contains one unsubstituted position. Therefore reactions involving simple addition to the ring structure are unlikely except in the case of plastoquinone.



(1)

R_1	R_2	R_3		n
OCH_3	OCH_3	CH_3	coenzyme Q	6–10
CH_3	CH_3	H	plastoquinone	9
CH——	CH	CH_3	vitamin K_2	4–7
‖	‖			
CH	CH			
|	|			

* Supported in part by grants from the U.S.P.H.S. (RG 8025) and the National Science Foundation (G16083).

The unsaturated side-chain on each of the quinones provides the interesting possibility of reactions other than simple quinone-hydroquinone change in the functioning of the compounds in biological systems. This is especially pertinent in view of the demonstration by Russell and Brodie (1961) of conversion of vitamin K to the chromanol in phosphorylating systems from mycobacteria and the fact that Morton (1961) and others have found ubichromenol in biological material. Rowland (1958) has also found solanochromene, the chromenol of plastoquinone, in flue-cured tobacco, but the chromenol has not been found in fresh plant tissue as yet.

Clark and Todd (1961) have proposed that the oxidation-reduction of the chromanol provides a possible approach to the mechanism of oxidative phosphorylation. So far the only clear implication of the chromanol in phosphorylation has been in the mycobacteria system, where there has been found not only the chromanol but also evidence that phosphate attached to the chromanol may be transferred to ADP with formation of ATP by an enzyme-catalyzed process.

I think it is appropriate at this time to reexamine what is known about the distribution of these compounds in nature in order, first, to emphasize further their universal occurrence, as demonstrated by more extensive investigations, and, second, to eliminate certain discrepancies in our original survey work with regard to the occurrence of coenzyme Q in basidiomycetes.

If we consider the distribution of coenzyme Q as presented in Table I, it is apparent that quinones of this type are present in animals from protozoa to mammals as well as in most plants and in many microorganisms. The only area where they might be expected and have not been found is in certain lower animals such as lobster and snails. Certain changes in concentration of coenzyme Q under different growth conditions may be of interest in relation to the development of mitrochondrial systems. This type of change is exemplified by the change in coenzyme Q content in *Tetrahymena* from the second to the seventh day of culture or more

TABLE I
RANGE OF COENZYME Q DISTRIBUTION

Class or Phylum	Species	Type of Coenzyme Q	μmole Co Q/ g dry wt.	Reference
Animals				
Protozoa	*Tetrahymena pyriformis* 2-day	Q_8	0.45	
	7-day	Q_8	0.72	
Annelida	*Lumbricus terrestris*	Q_{10}?	0.28[a]	(Lester and Crane, 1959)
Insects	*Musca domestica*	Q_9	0.29	ditto
Insects	*Pieris rapae*	Q_9	0.27	ditto
Fish	Walleyed pike (muscle)	Q_9	0.09[a]	ditto
Mammals	Bovine heart	Q_{10}	0.38	(Linn *et al.*, 1959)
	Bovine liver	Q_{10}	0.29	ditto
Plants				
Blue-green algae	*Anacystis nidulans*		None	(Lester and Crane, 1959)
Green algae	*Cladophora* sp.	Q_9	0.018	ditto
Brown algae	*Fucus* sp.	Q_9	0.019	ditto
Red algae	*Polysiphonia* sp.		0.027	ditto
Spermatophytes	*Medicago sativa*	Q_{10}	0.23	ditto
Actinomycetes	*Streptomyces griseus*		None	ditto
Phycomycetes	*Mucor corymbifer*	Q_9	0.20	ditto
Ascomycetes	*Saccharomyces cerevisiae* aerobic	Q_6	0.35	ditto
Ascomycetes	*Saccharomyces cerevisiae* anaerobic		None	ditto
Basidiomycetes	*Agaricus campestris*	Q_9	0.05	(Erickson *et al.*, 1960)
Basiciomycetes	*Ustilago zea*	Q_{10}	0.20	ditto
Bacteria	*Azotobacter vinelandii*	Q_8	2.6	(Lester and Crane, 1959)
Bacteria	*Escherichia coli*	Q_8	0.31	ditto
Bacteria	*Pseudomonas fluorescens*	Q_9	0.94	ditto
Bacteria	*Bacillus mesentericus*		None	ditto
Bacteria	*Mycobacterium smegmatis*		None	ditto
Bacteria	*Clostridium perfringens*		None	ditto

[a] Estimated from fresh weight value.

dramatically by the disappearance of coenzyme Q in anaerobic yeast. These changes suggest that the amount of coenzyme Q present in a tissue is closely related to the mitochondrial concentration or to the number of mitochondria.

We still have not found coenzyme Q in certain lower animals which we have investigated, such as lobster, Portuguese man-of-war, oyster, and snail. Until the quinone is unequivocally identified in these animals, we cannot make an unqualified statement about its ubiquitous distribution. It is quite likely that the discrepancy is related to the presence of other lipid materials in these organisms which interfere with the spectrophotometric assay or that the quinone exists in a reduced form for which no assay is yet available. In our extracts from these materials we do find considerable absorption in the ultraviolet spectrum which is not clearly identifiable as due to known materials such as ubichromenol or ubichromanol, but which does interfere with the interpretation of changes induced by borohydride in the absorption spectra. This is an area which we continue to explore whenever suitable materials become available.

Plastoquinone (PQ) has been found consistently in significant amounts in all oxygen-producing photosynthetic tissues. It appears to be absent from all nonphotosynthetic tissue (Lester and Crane, 1959; Crane, 1961; Kofler *et al.*, 1959). Bishop (1959) has shown that the Hill reaction is stopped in chloroplasts from which plastoquinone has been extracted and this reaction can be restored by adding back plastoquinone. Krogman (1961) has further shown that extraction of plastoquinone leads to loss of photosynthetic phosphorylation and that activity is restored when plastoquinone is added back to the extracted chloroplasts. We have shown that the endogenous plastoquinone in chloroplasts undergoes oxidation-reduction changes when chloroplasts are incubated in light or dark (Crane *et al.*, 1960).

The distribution of plastoquinone in various photosynthetic tissues is shown in Table II. Plastoquinone is present in large amounts in most of these tissues except in certain photosynthetic bacteria which contain coenzyme Q or other quinones as yet unidentified.

We have also investigated the amount of plastoquinone present in various leaves in relation to the chlorophyll content and find that the ratio of plastoquinone to chlorophyll shows considerable variation (*cf.* Table III). Thus there is a variable pool of plastoquinone in chloroplasts in relation to the chlorophyll. It is also noteworthy that the amount of plastoquinone in chloroplasts is about twenty times the reported levels of cytochromes *f* and *b*. This is similar to the 10–20 fold stoichiometric excess of coenzyme Q over individual cytochromes in mitochondria. Therefore, a large pool of quinone in relation to

TABLE II
PLASTOQUINONE IN PLANTS

Group	Species	Plastoquinone Content (μmoles/g dry wt.)	Reference
Blue-green algae	*Anacystis nidulans*	0.11	(Lester and Crane, 1959)
Green algae	*Cladophora* sp.	0.07	ditto
	Chlorella	0.20	
Red algae	*Polysiphonia* sp.	0.11	(Lester and Crane, 1959)
Brown algae	*Fucus* sp.	0.08	ditto
Pteridophytes	*Equisetum arvense* (shoots)	0.41	
Spermatophytes	*Spinacea oleracea* (leaves)	1.35	
	Sorbus aucuparia (leaves)	4.25	
Euglenophyta	*Euglena*	1.49	(Fuller *et al.*, 1961)

TABLE III
QUANTITATIVE RELATION BETWEEN PLASTOQUINONE AND CHLOROPHYLL IN LEAVES OF VARIOUS PLANTS

Plant Species	μM Plastoquinone/ μM Chlorophyll	μM Plastoquinone/ g dry wt.
Equisitum arvense (horsetail)	0.06	0.41
Acer rubrum (swamp maple)	0.05	0.30
Spinacia oleracea (spinach)	0.10	1.35
Aesculus hippocastanum (horse chestnut)	0.42	3.66
Cercis canadensis (red bud)	0.47	1.53
Aesculus glabra (buckeye)	1.01	3.46

the cytochromes must be considered in any interpretation of electron transport kinetics both in chloroplasts and in mitochondria.

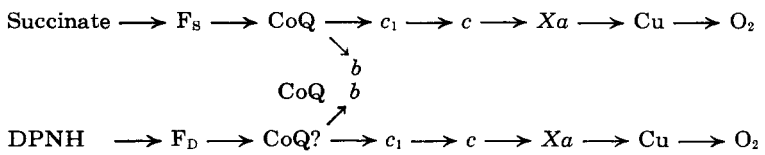
Other quinones may also be present in animals and plants or in specialized organisms. These other quinones may be harder to find because coenzyme Q and plastoquinone occur in relatively large amounts. Fuller *et al.* (1961) have reported evidence for a quinoid compound in chlorobium with an absorption maximum about 262 mμ. We have also identified an unknown quinoid material in lipids from spinach chloroplasts which has an absorption maximum at 254 mμ and is chromatographically different from coenzyme Q or plastoquinone, as well as a quinone which resembles tocopherol quinone.

Although vitamin K has been known for several years, rather little has been done by way of precise chemical definition of the amounts of this group of compounds in tissues. The members of the K series have been isolated from several bacteria, notably mycobacteria, and from alfalfa. Martius (1961) has presented some evidence for vitamin K in avian liver. Arnon (1961) has discussed possible function of vitamin K in photosynthesis, but there is no clear evidence as yet for vitamin K in chloroplasts. Of course the extraction, isolation, and identification of vitamin K compounds are in many ways more difficult than for the coenzyme Q or plastoquinone compounds. It would seem that a precise localization of the vitamin K or its functional derivatives (*e.g.* chromanols) is an essential preliminary to an evaluation of its function in oxidative or photosynthetic phosphorylation.

There is good evidence (Green, 1961) that coenzyme Q functions at a site in the electron transport chain immediately after the succinic dehydrogenase flavoprotein and just before cytochrome *b* or c_1, as shown in the scheme for electron transport in mitochondria.

Hatefi *et al.* (1960) have used short-chain analogues of coenzyme Q to isolate a DPNH coenzyme Q reductase from mitochondria; however, this system does not react with coenzyme Q_{10} and thus does not provide clear evidence for coenzyme Q_{10} function in the oxidation of DPNH. Other evidence for coenzyme Q_{10} function in the oxidation of DPNH is based on reduction of coenzyme Q_{10} by DPNH in particles containing the entire electron transport system. In these particles the coenzyme Q could be reduced because of interaction of the components in the DPNH pathway with the components in the succinate pathway rather than by direct interaction with coenzyme Q. We feel that clear evidence for the role of coenzyme Q in DPNH oxidase activity is still lacking. Other proposals for site of action

Scheme for Electron Transport in Mitochondria



F_S, succinic dehydrogenase; F_D, dihydrodiphosphopyridine nucleotide dehydrogenase; CoQ, coenzyme Q; cytochromes *b*, c_1, *c*, and *a* indicated by letter; Cu, Copper; *X*, site of oxidation of tetrachlorohydroquinone.

of coenzyme Q off the main electron transport pathway have been made (Redfearn, 1962; Chance, 1961), but evidence for this is scanty and primarily based on observations of slow reduction of a part of the coenzyme Q in mitochondria.

Extraction studies give the best evidence for a complete requirement for coenzyme Q in the main electron transport chain involved in succinate oxidation. No clear evidence for coenzyme Q function in DPNH oxidation has yet been obtained with extraction studies. Several solvent systems have been used to extract the quinone, and in all cases where the quinone is completely removed it is specifically required for restoration of succinoxidase activity. Unfortunately with some extraction procedures solvent-induced inhibitions must be considered. In extraction studies we also must watch for activation effects which lead to apparent restoration of activity at a low level when a new faster rate of electron flow has in fact been potentiated by the treatment, so that full recovery of activity would represent much more activity than was present in the original system.

If we consider some of the procedures which have been used to remove coenzyme Q from the electron transport particle of beef heart mitochondria, we find a requirement for coenzyme Q proportional to the removal of the quinone. Four procedures for extraction of the electron transport particle of mitochondria have been studied rather extensively. These procedures are: short extraction periods (1 minute) with iso-octane at 20°, long extraction periods (30 minutes) with vigorous shaking with iso-octane at 0°, long extraction periods with vigorous shaking with iso-octane at 25°, and acetone extraction. The short-time 20° extraction technique is rather inefficient for removal of coenzyme Q and, as pointed out by Redfearn *et al.* (1960), in studies on other mitochondrial material does not give clear evidence for a specific coenzyme Q requirement.

When the electron transport particle from beef heart mitochondria is extracted with iso-octane at 0° for longer periods, the maximum amount of coenzyme Q removed always approaches 50% of the total in the particle, as shown in Table IV. When an aqueous solution of cytochrome *c* is added to this system full recovery of activity is obtained, and very often an excess over the original activity appears. When lipid cytochrome *c* is added, however, only one half of the activity is restored. If coenzyme Q is also added with the lipid cytochrome *c*, then full restoration of activity is approached. Coenzyme Q without lipid cytochrome *c* will only partially restore activity in this system. When this evidence is taken in conjunction with our evidence that lipid cytochrome *c* is in fact the form in which cytochrome *c* is present in the electron transport particle, I feel we have a rather clear demonstration of the function of both coenzyme Q and lipid cytochrome *c* in these particles.

TABLE IV

RESTORATION OF SUCCINOXIDASE ACTIVITY IN ISO-OCTANE-EXTRACTED ELECTRON TRANSPORT PARTICLES

	Unextracted	Extracted
Coenzyme Q content (mg/g)	4.3	2.0
Additions	Succinoxidase (μM/min. × mg protein)	
None	0.50	0.03
Lipid cytochrome *c*	0.48	0.22
Coenzyme Q_{10}	0.50	0.14
Lipid cytochrome *c* + coenzyme Q_{10}	0.50	0.52
Aqueous cytochrome *c*	0.58	0.68

Electron transport particles extracted with iso-octane for 2 hours at 0° as previously described (Crane *et al.*, 1959).

Extraction with iso-octane at room temperature leads to almost complete removal of coenzyme Q and complete loss of succinoxidase activity. Lipid cytochrome *c* is not as effectively removed by this procedure as by the 0° treatment. As we have previously described (Crane *et al.*, 1959), other lipid materials in the extract (NL II) are needed along with coenzyme Q to restore activity in these extracted particles, but lipid cytochrome *c* is not required (*cf.* Table V). An unresolved problem still remains in the fact that aqueous cytochrome *c* will often restore full activity to these particles without coenzyme Q. Either the small amount of residual coenzyme Q acts with very high efficiency in these particles, or we must postulate a two-pathway system of electron transport, as we have previously discussed, one of them requiring coenzyme Q and another in which cytochrome *c* can fill in any gaps created by the solvent treatment.

TABLE V

EFFECT OF NEUTRAL LIPID ON SUCCINOXIDASE RESTORATION IN ISO-OCTANE-EXTRACTED ELECTRON TRANSPORT PARTICLES[a]

Addition[b]	Succinoxidase (μM/min. × mg)
None	0.16
Coenzyme Q_{10} 0.1 mg	0.20
Coenzyme Q_{10} 0.1 mg + BHMPL 0.03 mg	0.29
Coenzyme Q_{10} 0.1 mg + BHMPL 0.03 mg NL II 0.03 mg	0.72
BHMPL 0.03 + NL II 0.03	0.13

[a] Electron transport particles extracted with iso-octane for 2 hours at room temperature 25° as previously described (Crane *et al.*, 1959). [b] BHMPL, beef heart mitochondria phospholipid; NL II, accessory lipid material from beef heart mitochondria (Crane *et al.*, 1959).

We can make the following points with regard to extraction with nonpolar solvents for purposes of demonstration of a coenzyme Q requirement. First, solvent inhibition effects must be eliminated by repeated high-speed centrifugation of the particles or long exposure under vacuum. Second, very vigorous extraction is required to remove coenzyme Q, and, finally, when coenzyme Q is removed other lipid materials will also be removed which may be required along with coenzyme Q to restore full activity.

Acetone extraction of the electron transport particle or of various mitochondria has been very successful as an approach to a demonstration of a specific coenzyme Q requirement in electron transport associated with succinate oxidation. When coenzyme Q is extracted from mitochondrial particles with acetone under various conditions, a specific requirement for coenzyme Q is induced in the system and no other material, including other quinones (*e.g.* plastoquinone) or cytochrome *c*, can restore activity. Only members of the coenzyme Q group with an isoprenoid side-chain containing 10 or more carbon atoms are effective is this system (Ambe and Crane, 1960). Other lipid materials (such as NL II) are also found in the acetone extract and are required along with the long isoprenoid side-chain members of the coenzyme Q group (coenzyme Q_6 to Q_{10}) for full restoration of activity, presumably because they facilitate the entry of the quinone into the proper position in the particle (Crane and Ehrlich, 1960). Since there seems to be good agreement among workers in the field concerning the success of acetone extraction, a detailed discussion of the system does not seem necessary. It should be pointed out that the acetone extraction procedure is also the only really successful procedure for demonstration of a coenzyme Q requirement for succinic cyctochrome *c* reductase activity (Ambe and Crane, 1960). In the face of all this success a few words of caution concerning the acetone procedure may be appropriate. According to the observations of Lester and Fleischer (1961), the cytochrome *c* in the acetone-treated particles is converted to an inactive state, so that both coenzyme Q and cytochrome *c* are required for full restoration of activity. Thus, as suggested by Redfearn (1962), acetone treatment may lead to a rather extensive displacement of mitochondrial components. We may summarize the degree of alteration of electron transport according to the various solvent extraction procedures in the following terms. The cold iso-octane treatment releases bound cytochrome *c* and one half of the coenzyme Q, with subsequent restoration by lipid cytochrome *c* together with coenzyme Q or by aqueous cytochrome *c*. Room temperature iso-octane treatment induces a requirement for coenzyme Q alone plus lipid accessories or cytochrome *c* alone. Acetone treatment induces a requirement for both cytochrome *c* and coenzyme Q with lipid accessories. Which of these systems more closely represents the original electron transport in the untreated particles depends on an understanding of the coupling of phosphorylation to the electron transport system. Unfortunately, all solvent treatments of phosphorylating mitochondria have consistently led to loss of phosphorylation even though electron transport is often greatly improved. This uncoupling effect in which loss of phosphorylation is accompanied by faster electron transport is often observed after solvent extraction of whole mitochondria rather than particles such as electron transport particles. An example of the effect is shown in Table VI. Similar effects have been reported by Lester and Fleischer (1961). These stimulatory effects of solvent extraction make studies of coenzyme Q restoration in mitochondria often quite inconsistent and difficult to interpret, but more significantly they may be a clear indication that the uncoupled, solvent-treated systems are somewhat short-circuited in their electron flow as compared to the intact mitochondrion. Therefore, we can say that coenzyme Q is clearly a member of the electron transport system, but we have a lot to learn about its relation to a phosphorylating system.

TABLE VI

RESTORATION OF ELECTRON TRANSPORT AFTER ACETONE EXTRACTION OF HEAVY BEEF HEART MITOCHONDRIA

Additions	Succinoxidase	
	Original	Extracted
None	0.04	0.00
Cytochrome *c* 0.5 mg	0.09	0.02
Coenzyme Q_{10} 0.2 mg	0.03	0.00
Coenzyme Q_{10} + cytochrome *c*	0.08	0.16

Heavy fraction of beef heart mitochondria in sucrose suspension extracted with ten volumes of acetone for 5 minutes at 5°.

The role of accessory lipid factors in the functioning of coenzyme Q_{10} in the mitochondrial membrane system still remains to be fully explored. The fact that coenzyme Q_{10} can easily be removed by solvent extraction procedures indicates that the coenzyme is not tightly bound to any protein as is the case with water-soluble coenzymes. The fact that additional lipid or detergent materials are required in the treated mitochondria for coenzymatic activity indicates that coenzyme Q functions as a free component of a lipid matrix. The lipid matrix is presumably attached to the mitochondrial structural protein by hydrophobic bonding.

In iso-octane extraction procedures a phospholipid fraction is required along with coenzyme Q to restore full activity (Crane *et al.*, 1959). We have further examined the specificity of various phospholipid fractions in their ability to supplement coenzyme Q in this system and find maximum activity produced by addition of small amounts of the cephalin fraction, consisting mostly

of phosphatidyl ethanolamine and ethanolamine plasmalogens, as shown in Figure 1. The inositide and lecithin fractions are considerably less active. I think it is important to emphasize here that the phospholipid requirement described is specific to phospholipid and is not duplicated by other compounds such as tocopherol, beef serum albumin, or *n*-butyl stearate, which have been implicated as restorative agents in other solvent treatments of electron transport systems.

More drastic iso-octane extraction or acetone extraction produces a requirement for another lipid fraction as an accessory to coenzyme Q which we have referred to as NL II (Crane *et al.*, 1959; Hendlin and Cook, 1960). As we have discussed previously, this compound is required in conjunction with long-chain members of the coenzyme Q group (coenzyme Q_6 to Q_{10}) but not with coenzyme Q_2 or Q_3 (Crane and Ehrlich, 1960a). The NL part of the original designation, indicating a neutral lipid fraction, is perhaps unfortunate, since in highly purified fractions of this material we find that an unsaturated fatty acid is the predominant component. Thus a certain degree of detergent effect can be expected with this compound, and in some cases Triton X-100 can replace it. The effects of the Triton X-100 are not always consistent in replacing the activity. As indicated in Table V, a very small amount of NL II is required for restoration of activity, but further discussion of its mode of action should await complete characterization of the active material.

We can summarize the evidence for coenzyme Q function in electron transport in the following areas. (1) There is a significant amount of quinone in all mitochondria examined. (2) Extraction of the quinone by solvents leads to loss of the capacity of the particles to oxidize succinate, and addition of coenzyme Q specifically restores antimycin-sensitive succinoxidase activity (Crane *et al.*, 1959; Hendlin and Cook, 1960). (3) Coenzyme Q in mitochondria undergoes oxidation in the presence of oxygen and reduction in the presence of substrate and cyanide, as determined both by extraction measurements (Hatefi *et al.*, 1959) and by direct spectrophotometric observation of intact mitochondria (Chance, 1961). (4) Added coenzyme Q is reduced in the presence of mitrochondria plus substrate, and the hydroquine is oxidized when incubated aerobically with mitochondria (Hatefi *et al.*, 1959). (5) Enzyme systems with coenzyme Q reductase activity and reduced coenzyme Q oxidase activity have been isolated from mitochondria (Green, 1961). The only indication of coenzyme Q function in phosphorylating electron transport comes from stimulation of photosynthetic phosphorylation in chromatophores from *Rhodospirillum rubrum* deficient in coenzyme Q, as observed by Rudney (1961).

Evidence for plastoquinone function in photosynthetic electron transport in chloroplasts is based on evidence similar to that listed above for

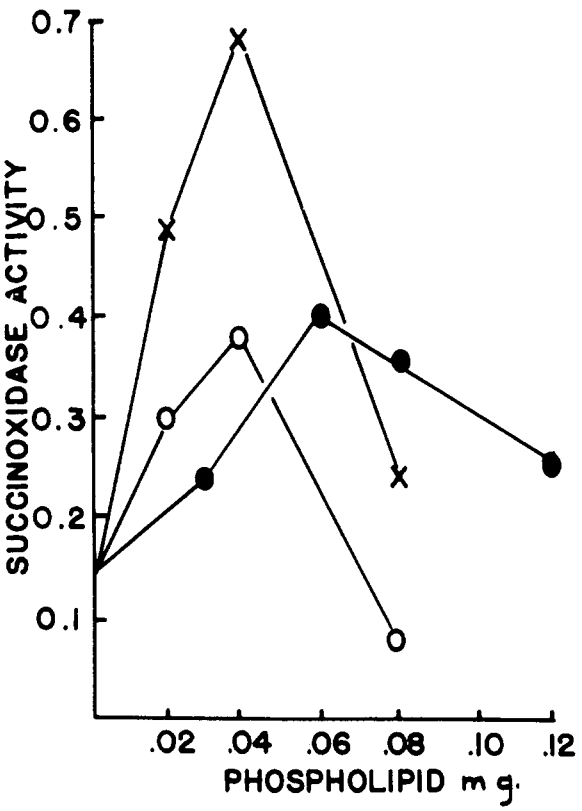


FIG. 1.—Effect of phospholipids from heart mitochondria on the restoration of succinoxidase activity in iso-octane-extracted electron transport particles. Electron transport particles extracted with iso-octane for 1 hour at room temperature (Crane *et al.*, 1959). —×—, Phosphatidyl ethanolamine fraction (cephalin); -O-, lecithin fraction; -●-, mixed phospholipids from mitochondria.

coenzyme Q. The quinone is present in large amounts in chloroplasts. Solvent extraction of the quinone interrupts electron transport, and the quinone specifically restores activity. The oxidation-reduction state of the quinone changes when chloroplasts are exposed to light or to Hill reaction electron acceptors. It may be even more significant that Krogman (1961) has shown that photosynthetic phosphorylation can be restored in extracted chloroplasts by addition of plastoquinone.

The elegant studies of Brodie (1961) and his co-workers have laid a very firm foundation for the function of vitamin K in a phosphorylating electron transport system in mycobacteria. They have provided all the types of evidence discussed above for the function of the quinone except that the oxidation of DPNH rather than succinate has been studied. In addition they have presented evidence for conversion of the vitamin K to the chroman form (naphthotocopherol) during the phosphorylation process. This work has added an entirely new dimension to studies of the function of the three quinones. Unfortunately, no clear evidence has yet been found for chroman

formation involving coenzyme Q or plastoquinone.

Failure to detect chroman derivatives of coenzyme Q or plastoquinone may be ascribed to the fact that the study of these quinones has been confined to nonphosphorylating mitochondria or chloroplasts, or it may be a consequence of our deficient knowledge of the chemistry of chromans in biological systems. For example, we have incubated the 6-chromanol of hexahydrocoenzyme Q_4 with beef heart mitochondria and find that the compound disappears without formation of a corresponding mount of the quinone or chromenol, as shown in Table VII. The disappearance of the chromanol is inhibited by cyanide and requires several minutes of incubation. Until we know what products chromanols are converted to in mitochondria, we may have little success in a search for derivatives of coenzyme Q involved in phosphorylation. We also find that under conditions most suitable for photosynthetic phosphorylation a considerable portion of the plastoquinone usually found in chloroplasts disappears. It is clear that consideration of other reactions besides simple oxidation and reduction of the quinones is required in mitochondria and chloroplasts.

TABLE VII

DESTRUCTION OF HEXAHYDRO Q_4 CHROMANOL BY INCUBATION WITH BEEF HEART MITOCHONDRIA

Incubation Time (min.)	Treatment	Chromanol Present (by 290 mμ absorbancy)
0	None	0.260
10	None	0.140
10	10^{-4} M KCN	0.260
10	No chroman added	0.020
10	No mitochondria	0.360

Incubation of 0.2 mg chromanol with 40 μmoles phosphate buffer, *p*H 7.0, and 5 mg beef heart mitochondrial protein in a total volume of 1.0 mg. Reaction stopped by addition of 5 ml of ethanol.

As a final point I would like to consider an area of investigation initiated by Dr. Earl Jacobs in relation to possible quinone function in oxidative phosphorylation. There are three sites for coupling of electron transport to electron flow in mitochondria, and the phosphorylation reactions at these sites show fairly similar properties, as in their sensitivity to dinitrophenol. The first site is in the area of flavoprotein, and these compounds with certain similarities to quinone structure have been implicated in phosphorylation by Low *et al.* (1958). Martius (1961) has also presented evidence for the presence of vitamin K in mitochondria, and Beyer (1959) has shown an effect of vitamin K on restoration of phosphorylation coupled to oxidation of DPNH but not of succinate in irradiated mitochondria. Thus, the first site may be related to flavin or vitamin K, the second site is in the area of coenzyme Q, and the third site is in the area of cytochrome *c*. We find quinoid compounds at the first two sites, but nothing of this type has been recognized in the area of cytochrome *c* or of cytochrome oxidase. We cannot postulate a general mechanism of phosphorylation based on quinone derivatives without a quinone or its equivalent at this third site.

By using a series of quinones as external electron donors with ascorbate as the reducing agent, Jacobs and Crane (1961) have shown antimycin-sensitive oxidation of reduced menadione with a P/O ratio of 2, antimycin-sensitive oxidation of tetramethylhydroquinone with a P/O ratio of 1, antimycin-insensitive oxidation of tetramethylphenylenediamine with a P/O ratio of 1, and antimycin-insensitive oxidation of tetrachlorohydroquinone without accompanying phosphorylation even after cytochrome *c* is removed from mitochondria. It would appear that the first two quinones are oxidized at the flavoprotein and coenzyme Q sites, with loss of phosphorylation at the site of entry but with continued phosphorylation at subsequent sites in the chain. The quinoneamine compounds seem to enter the chain in the region of cytochromes *c* or c_1 and potentiate a phosphorylation at the cytochrome oxidase region. Jacobs postulates by analogy that tetrachlorohydroquinone may interact at a quinoid site near cytochrome oxidase in a manner which precludes phosphorylation at this quinoid site. We have purified cytochrome oxidase and find that tetrachlorohydroquinone oxidase activity parallels reduced cytochrome *c* oxidase activity quite well during purification. This new enzymatic activity of cytochrome oxidase does not require cytochrome *c* and is specific for tetrahalogen-substituted benzoquinones. It is also possible to remove cytochrome *c* from mitochondria and use the tetrachlorohydroquinone to restore succinoxidase activity. Thus the reduced cytochrome *c* oxidase system is no longer specific for the oxidation of reduced cytochrome *c*, and the discovery of this enzymatic activity opens up new possibilities in the investigation of the cytochrome oxidase portion of the electron transport chain.

We have gone from well-established facts concerning the existence of quinones in electron transport systems to hypotheses concerning their function in phosphorylation reactions. In many ways more new problems have arisen from the discovery of these compounds than have been solved. The facts are that rather large pools of each of the quinones are present in the respective electron transport systems and that they undergo oxidation reduction changes. If the proposals for their function as primary intermediates in phosphorylation are right, then evidence for the appropriate phosphorylated intermediates should be forthcoming, and these or similar quinoid structures should be found associated with each phosphorylation site.

On the other hand, the large pool of quinone which is apparently localized in one part of the electron transport chain, at least in mitochondria,

may suggest a redox poising system in the lipid phase of the membrane structure with hitherto unsuspected significance to our understanding of energy conversion in biphasic electron transport systems containing layers of lipid and protein.

Acknowledgment

I wish to express my appreciation to Dr. D. E. Green, for his encouragement and support of the early studies on coenzyme Q at the Enzyme Institute, University of Wisconsin, and to my colleagues there, Drs. R. L. Lester, Y. Hatefi, C. Widmer, and K. S. Ambe. Dr. Karl Folkers of Merck, Sharp and Dohme Research Laboratories has kindly supplied various derivatives of coenzyme Q. Mr L. P. Kegel has been primarily responsible for studies of plastoquinone, and Mr. D. H. Moury has investigated the reactions of the hexahydrocoenzyme Q chromanol.

References

Ambe, K. S., and Crane, F. L. (1960), *Biochim. et Biophys. Acta 43*, 30.

Arnon, D. I. (1961), *Fed. Proc. 20*, 1012.

Beyer, R. E. (1959), *J. Biol. Chem. 234*, 688.

Bishop, N. I. (1959), *Proc. Nat. Acad. Sci. U. S. 12*, 1696.

Brodie, A. F. (1961), *Fed. Proc. 20*, 995.

Chance, B. (1961), *in* Ciba Symposium on Quinones in Electron Transport, London, J. & A. Churchill, p. 327.

Clark, V. M., and Todd, A. (1961), *in* Ciba Symposium on Quinones in Electron Transport, London, J. & A. Churchill, p. 190.

Crane, F. L., Widmer, C., Lester, R. L., and Hatefi, Y. (1959), *Biochim. et Biophys. Acta 31*, 476.

Crane, F. L., and Ehrlich, B. (1960), *Arch. Biochim. Biophys. 89*, 134.

Crane, F. L., Ehrlich, B., and Kegel, L. P. (1960), *Biochem. Biophys. Research Communs. 3*, 37.

Crane, F. L. (1961), *in* Ciba Symposium on Quinones in Electron Transport, London, J. & A. Churchill, p. 36.

Erickson, R. E., Brown, K. S., Wolf, D. E., and Folkers, K. (1960), *Arch. Biochem. Biophys. 90*, 314.

Fuller, R. C., Smillie, R. M., Rigopoulos, N., and Yount, V. (1961), *Arch. Biochem. Biophys. 95*, 197.

Green, D. E. (1961), *in* Ciba Symposium on Quinones in Electron Transport, London, J. & A. Churchill, p. 130.

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

Hatefi, Y., Haavik, A. G., and Jurtshuk, P. (1960), *Biochem. Biophys. Research Communs. 3*, 281.

Hendlin, D., and Cook, T. M. (1960), *J. Bioi. Chem. 235*, 1187.

Jacobs, E. E., and Crane, F. L. (1961), *in* Abstracts of Proceedings of the Fifth International Biochemical Congress, London, Pergamon, p. 462.

Kofler, M., Langemann, A., Ruegg, R., Chopard-dit-Jean, L. H., Rayroud, A., and Isler, O. (1959), *Helv. Chim. Acta 42*, 1283.

Krogman, D. W. (1961), *Biochem. Biophys. Research Communs. 4*, 275.

Lester, R. L., and Crane, F. L. (1959), *J. Biol. Chem. 234*, 2169.

Lester, R. L., and Fleischer, S. (1961), *Biochim. et Biophys. Acta 47*, 358.

Linn, B. O., Page, A. C., Jr., Woug, E. L., Gale, P. H., Shunk, C. H., and Folkers, K. (1959), *J. Am. Chem. Soc. 81*, 4007.

Low, H., Siekevitz, P., Ernster, L., and Lindberg, O. (1958), *Biochim. et Biophys. Acta 29*, 392.

Martius, C. (1961), *in* Ciba Symposium on Quinones in Electron Transport, London, J. & A. Churchill p. 312.

Morton, R. A. (1961), *in* Ciba Symposium on Quinones in Electron Transport, London, J. & A. Churchill, p. 5.

Redfearn, E. R. (1962), *in* Proceedings of the Fifth International Biochemical Congress, Symposium V, London, Pergamon, in press.

Redfearn, E. R., Pumphrey, A. M., and Fynn, G. H. (1960), *Biochim. et Biophys. Acta 44*, 404.

Rowland, R. L. (1958), *J. Am. Chem. Soc. 80*, 6130.

Rudney, H. (1961), *J. Biol. Chem. 236*, PC 39.

Russell, P. J., Jr., and Brodie, A. F. (1961), *in* Ciba Symposium on Quinones in Electron Transport, London, J. & A. Churchill, p. 205.