The Role of Ubiquinone in the Respiratory Chain of *Acetobacter xylinum*

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1. Whole cells of *Acetobacter xylinum* were found to contain a quinone of the ubiquinone (coenzyme Q) group. The quinone was isolated from the cells and crystallized. It was identified by its physical, chemical and spectroscopic properties as a ubiquinone with 10 isoprene units (ubiquinone-10). No naphthaquinone was detected in the cells. 2. Cell-free extracts prepared by means of a French pressure cell were separated into three fractions by differential centrifugation. The ubiquinone was located predominantly in the particulate fraction sedimenting at 33 000g, which also contained most of the NADH oxidase and malate oxidase activities. The concentration of ubiquinone-10 in extracts was similar to that of the flavoproteins and about three times the concentration of the individual cytochromes. 3. Aerobic incubations of crude extracts with either NADH or malate resulted in reduction of the endogenous ubiquinone-10 to steady-state concentrations of 55 and 40% of the total quinone respectively. In the presence of cyanide more than 95% of the endogenous ubiquinone-10 was reduced by either NADH or malate. 4. The initial rate of reduction of endogenous ubiquinone-10 by malate and the rate of ubiquinol oxidation, in *A. xylinum* extracts, were found to be compatible with the overall rate of malate oxidation with oxygen. 5. The effects of various respiratory inhibitors on the oxidation-reduction reactions of the endogenous quinone indicate that its position on the respiratory chain is between the malate flavoprotein dehydrogenase and the cytochrome chain.

Though several lines of evidence for the participation of quinones as electron carriers in the respiratory chain of living organisms have been developed (Redfearn, 1961; Green & Brierley, 1965; Brodie, 1965), the question whether or not oxidoreduction of endogenous quinone is sufficiently rapid to be on the main path of electron transport from substrate to oxygen appears to be as yet unsolved (Chance, 1965). Previous studies on the FAD-linked malate oxidase system of *Acetobacter xylinum* have suggested that a quinone is involved in the respiratory chain of this organism (Benziman & Perez, 1965). The present paper describes the isolation and characterization of this quinone and the kinetics of the oxidation-reduction reactions of the endogenous quinone. It embodies evidence that strongly suggests that ubiquinone-10 is a functional component of the main respiratory chain of *A. xylinum*. A preliminary report of this work has appeared (Benziman & Goldhamer, 1967).

METHODS AND MATERIALS

Buffer. The buffer used throughout was tris-H₂SO₄ buffer, pH 7.4.

Cells. Succinate-grown cells of *A. xylinum* were grown

and harvested as described by Benziman & Burger-Rachamimov (1962).

Preparation of subcellular fractions. Cells were suspended in 0.05M-buffer to a concentration of 30–60mg. dry wt. of cells/ml. Portions (20ml.) of this suspension were treated in a cooled French pressure cell (American Instrument Co., Silver Springs, Md., U.S.A.) under 15000lb./in.². Cellular debris and unbroken cells were removed by centrifugation at 9000g for 10min. The supernatant is referred to as the crude extract.

The crude extract was centrifuged for 60min. at 33000g. The precipitate (heavy particles) was suspended in 0.05M-buffer to the original volume. The supernatant was further centrifuged for 60min. at 105000g in a Spinco model L preparative ultracentrifuge to yield the high-speed supernatant and the light particles, which were suspended to the original volume in the same buffer as above.

For certain experiments the crude extract was centrifuged at 105000g for 90min. and the precipitate suspended in 0.05M-buffer to the original volume. This preparation is referred to as the particulate fraction.

Isolation of the quinone. All the steps were carried out in dimmed light at room temperature.

Cell suspensions (170mg./ml.) were refluxed with 4 vol. of an iso-octane-propan-1-ol mixture (3:1, v/v) for 1hr. The organic phase was separated and the residue was then refluxed for 30min. with two further lots of equal volumes of iso-octane. The organic extracts were combined and concentrated under vacuum. The pale-yellow oily residue was

suspended in acetone, the acetone-insoluble material discarded and the solution concentrated to dryness under a stream of cold air. The residue was dissolved in iso-octane and applied as a streak parallel to the base of 10 cm. $\times$ 20 cm. silica-gel plates. The plates were developed in light petroleum (b.p. 40–60°)–ether (11:9, v/v) for 45–60 min., dried and scanned with ultraviolet light. Only one absorbing band was usually detected, with an R_F value quite different from that of any other component of the lipid fraction detected with H_2SO_4 spraying. The absorbing zone was scraped off and extracted with chloroform. The chloroform was evaporated by a stream of cold air and the residue was dissolved in ethanol. The ethanolic solution was cooled to 0°; after a few hours a white fluffy precipitate appeared and it was separated by centrifugation and discarded. Further cooling to –15° resulted in the appearance of yellow crystals, which were collected by centrifugation at –2° and recrystallized from ethanol at –15°. The yield was generally 0.4 mg. of quinone/g. dry wt. of cells.

Enzymic assays. All the reactions were carried out at 25° unless otherwise stated. Reactions involving the endogenous quinone were assayed essentially as described by Redfearn & Pumphrey (1960).

Steady-state measurements. Portions (1 ml.) of the particulate fraction containing 10–15 mg. of protein in 0.1 M-buffer were incubated for 5 min. with 10 μ moles of L-malate or 7 μ moles of NADH. The mixture was shaken for 1 min. to bring the respiratory carriers to their steady-state level, and then denatured by the addition of 4 ml. of methanol at –60° containing 0.1% pyrogallol. The quinone was extracted and determined as described by Pumphrey & Redfearn (1960).

Malate-ubiquinone reductase. Conical centrifuge tubes containing 1 ml. of the particulate fraction (10–15 mg. of protein) in 0.1 M-buffer and 0.05 ml. of 0.2 M-L-malate were left for 10 min. with occasional shaking to allow the endogenous quinone to reach its aerobic steady-state level. The reaction was started by injecting into the mixture 0.1 ml. of 0.15 M-KCN. The reaction was terminated in each tube after different time-intervals (1–30 sec.) by the addition of 4 ml. of a cold (–60°) methanol-pyrogallol mixture. The quinone was then extracted and determined as described above. The rate of the reaction was calculated from the initial slope of the curve obtained by plotting quinone concentration versus time.

Ubiquinol oxidase. To each of a series of conical centrifuge tubes containing 1 ml. of the particulate fraction was added 0.1 ml. of 0.2 M-L-malate followed immediately by light petroleum (b.p. 40–60°) to form a layer on the surface. Each tube was left for 10 min. (until the mixture was depleted of its oxygen) and 0.05 ml. of 0.03 M-Amytal was introduced into the lower phase. The mixture was left for 5 min. and oxidation started by bubbling a fine stream of air through the mixture. The reaction was stopped in each tube after different time-intervals (1–60 sec.) by the addition of the cold methanol-pyrogallol mixture. The quinone was then extracted and estimated and the rate of the reaction calculated as above.

Malate oxidase. This activity was determined at 25° and pH 7.4 as described by Benziman & Galanter (1964), with 5–10 mg. of the particulate fraction protein.

Malate dehydrogenase. This activity was determined at pH 7.4 with ferricyanide as acceptor as described by Karniely & Benziman (1966).

Analytical methods. Individual cytochromes and total

flavoprotein in cell-free extracts were assayed spectrophotometrically as described by Jones & Redfearn (1966). Ultrasonically treated crude extract preparations containing 15 mg. of protein/ml. were used for the assay. Measurements were made with a Cary model 11 recording spectrophotometer. Quinone concentrations of cells and of cell-free extracts were determined as described by Pumphrey & Redfearn (1960). Ubiquinone-10 was determined spectrophotometrically from the change in extinction at 275 m μ of an ethanolic solution of the quinone after reduction with $NaBH_4$. Readings were made in a Beckman DB recording spectrophotometer and ϵ 12250 was used for the calculations. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Catalytic hydrogenation. Ethanolic solutions of the quinone were hydrogenated with palladium on charcoal as catalyst as described by Lester, Hatefi & Crane (1959).

Infrared spectra. The infrared spectra of quinone samples in KBr disks were measured with a Perkin-Elmer model 337 grating infrared spectrophotometer.

Nuclear-magnetic-resonance spectra. All the nuclear-magnetic-resonance (n.m.r.) data were obtained with a Varian Associates model 4300B high-resolution n.m.r. spectrometer, operating at 60 Mc/sec. The quinone was dissolved (50 mg./ml.) in carbon tetrachloride. Proton shifts were measured relative to tetramethylsilane.

Thin-layer chromatography. The silica gel used for coating the plates was washed with light petroleum (b.p. 40–60°)–ether (1:1, v/v) before use. The developing solvent used during the chromatographic purification of the quinone [light petroleum (b.p. 40–60°)–ether (11:9, v/v)] proved to be the most suitable one for separating mixtures of ubiquinones and naphthaquinones and also gave the best separation between the endogenous quinone and other components of the lipid extract. Quinone spots were located by their extinction or fluorescence with light at 360 m μ or 240 m μ . Other lipid components were detected by spraying the plates with H_2SO_4 .

Chemicals. NADH, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide, ubiquinone-10 and ubiquinone-6 were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. Other quinone homologues and various naphthaquinones were a generous gift from Hoffmann-La Roche and Co. Ltd., Basle, Switzerland. Absorbents for thin-layer chromatography were obtained from Camag, Muttenz, Switzerland.

RESULTS

Nature of the quinone in *A. xylinum*. The fraction obtained by lipid extraction of *A. xylinum* cells contained material with a benzoquinone ultraviolet-absorption spectrum. No naphthaquinone could be detected either chemically or spectrophotometrically in this fraction. Similar results were obtained when the cells were extracted by the method of Bishop, Pandya & King (1962), which involves saponification of cells in the presence of pyrogallol before solvent extraction. Fractionation of the lipid extracts by thin-layer chromatography or by column chromatography (Bishop *et al.* 1962) resulted in the isolation of only one quinone of the ubiquinone type. All the fractions obtained during

Table 1. *Ultraviolet spectra of the quinone of A. xylinum and of known quinones*

Values given are for quinones dissolved in ethanol. Q-AX, quinone isolated from *A. xylinum*. The data for other quinones are from Lester *et al.* (1959).

	Extinction coefficient			
	Q-AX	Q-10	Q-9	Q-8
Ubiquinone . . .				
Oxidized: Max. 275 m μ	167	165	185	206
Min. 236 m μ	29.1	28.4	33.1	38
Max. 405 m μ	6.0	5.9	6.9	7.3
Reduced: Max. 290 m μ	45.5	46.4	51.7	60.0
Difference: Ox. - Red. 275 m μ	142	142	158	176

the different isolation and purification procedures were examined for the presence of naphthaquinones by the sensitive tests of Irreverre & Sullivan (1941) and Schilling & Dam (1958); none was detected.

Characterization of the quinone. An ethanolic solution of the recrystallized quinone showed the characteristic ubiquinone absorption spectrum in the ultraviolet region, with a peak at 275 m μ and a broader band in the visible region at 405 m μ . On reduction with borohydride the resultant quinol was characterized by a single band at 290 m μ . The extinction coefficients of the quinone on a weight basis (extinction of a 1% solution) agreed satisfactorily with those of a ubiquinone with 10 isoprene units (ubiquinone-10) (Table 1). The close structural relationship between the isolated quinone and ubiquinone-10 was further indicated from the similarity between their infrared spectra.

Catalytic hydrogenation of the quinone revealed that 1.31 moles of hydrogen were required/100 g. of quinone. This value, which is compatible with a ubiquinone with 10 double bonds in the side chain, indicates that the isolated quinone is a member of the homologous series of ubiquinones with regular isoprene side chains.

The quinone of *A. xylinum* had m.p. 47°, similar to that of ubiquinone-10. The same melting point was observed for a mixture of the quinone and authentic ubiquinone-10.

Reversed-phase thin-layer chromatography of the isolated quinone together with ubiquinone-10 and ubiquinone-9 resulted in R_F values identical with those of ubiquinone-10. The R_F values for the isolated quinone, ubiquinone-10 and ubiquinone-9 were respectively 0.16, 0.16 and 0.25 with acetone-water (19:1, v/v) and 0.13, 0.13 and 0.24 with propan-1-ol-water (9:1, v/v).

The identity of the isolated quinone with ubiquinone-10 was finally confirmed by studying their n.m.r. spectra. For the isolated quinone the following values of the integration were observed (the

Table 2. *Concentration of respiratory carriers in A. xylinum*

Assays were carried out as described in the Methods and Materials section. The values represent the results of four determinations on four different preparations of ultrasonically treated crude extract.

Carrier	Content (μ moles/g. of protein)
Ubiquinone-10	1.33
Flavoprotein	1.18
Cytochrome <i>c</i>	0.42
Cytochrome <i>b</i>	0.45
Cytochrome <i>a</i>	0.35

Table 3. *Steady-state oxidation-reduction levels of ubiquinone in A. xylinum*

Determinations were carried out with the particulate fraction as described in the Methods and Materials section.

Substrate	Cyanide (5 mm)	Reduction of total quinone (%)
Malate	—	40
Malate	+	96
NADH	—	55
NADH	+	95

corresponding theoretical values for ubiquinone-10 are shown in parentheses): 10 (10) olefinic protons, τ 4.97; 6 (6) methoxy protons, τ 6.07; 2 (2) allylic methylene protons, τ 6.97; 38 (39) methylene and ring methyl protons, τ 8.00; 3 (3) side-chain methyl (first) protons, τ 8.27; 31 (30) side-chain methyl protons, τ 8.35.

Quinone content of cells. Whole cells of *A. xylinum* were found to contain between 0.6 and 0.8 μ mole of quinone/g. dry wt. of cells. To determine the localization of the quinone in the cell, cell-free extracts were subfractionated by differential centrifugation, each fraction being assayed for its quinone content. The quinone was exclusively located in the particulate fractions of the cells with more than 95% in the heavy particles sedimented at 33000g. This fraction contained also most of the malate- and NADH-oxidizing activities.

Concentration of respiratory carriers. The concentrations of electron carriers in *A. xylinum* are given in Table 2. The values for the cytochromes and flavoprotein were calculated from the difference spectrum resulting from the reduction of crude extracts by dithionite, assuming that the extinction coefficients of the carriers in *A. xylinum* are similar to those of other micro-organisms. It appears from the results that cytochromes *c*, *b* and *a* are present in the cells in approximately equimolar amounts whereas the concentrations of ubiquinone and of the

Table 4. *Ubiquinone oxidation-reduction and malate oxidase activity in A. xylinum extracts*

Assays were carried out with the particulate fraction as described in the Methods and Materials section.

Reaction	Activity (μ mole/min./mg. of protein)
Malate oxidase	0.027
Malate dehydrogenase	0.040
Malate-ubiquinone reductase	0.026
Ubiquinol oxidase	0.035

flavoproteins are three times that of the individual cytochromes.

Steady-state concentration of the quinone. When cell-free extracts of *A. xylinum* were incubated with malate or NADH under anaerobic conditions the endogenous quinone was reduced to ubiquinol. On aeration of the mixture the ubiquinol was oxidized back to ubiquinone. As shown in Table 3, the steady-state concentration of the endogenous ubiquinol under aerobic conditions was higher when NADH was oxidized than when malate was the oxidizable substrate. Phosphate had no effect on the steady-state ubiquinone/ubiquinol ratio. In the presence of cyanide, as under anaerobic conditions, more than 95% of the quinone could be isolated in its reduced form after the incubation of extracts with either one of the substrates tested.

Oxidation-reduction reactions of the quinone. The enzymic reduction of the endogenous quinone by malate was studied by preincubating the enzyme preparation with the substrate and stopping the reaction at suitable time-intervals after addition of cyanide to inhibit the reoxidation of the quinol by cytochrome oxidase. The rate of reduction was calculated from the initial slope of the curve obtained by plotting ubiquinol concentration at each time-interval against time. The rate of ubiquinone reduction given in Table 4 represents the rate at the steady-state concentration of oxidized quinone and was not corrected to 100% oxidized quinone. Such a correction should result in higher reduction rates since the reaction appears to be of first-order (cf. Redfearn & Pumphrey, 1960). To measure ubiquinol oxidase activity the endogenous quinone was reduced by malate under anaerobic conditions and the reaction initiated by bubbling air through the mixture. Further reduction of the quinone by malate was inhibited by Amytal. Table 4 summarizes the rates of the different reactions occurring during the oxidation of malate by cell-free extracts. It appears that both the rate of ubiquinone reduction by malate and the rate of ubiquinol oxidation are compatible with the overall rate of malate oxidation. On the other hand, the malate dehydrogenase activity is considerably

higher than the malate oxidase activity. These results were obtained in many experiments with different preparations and, though the absolute rate values differed from preparation to preparation, the relative rates were similar to those given here.

Effect of respiratory inhibitors. Sodium Amytal (3mM) inhibited by 75% the reduction of ubiquinone by malate and the oxidation of malate by oxygen. Potassium cyanide (5mM) and 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (0.04mM) inhibited by 95% and 80% respectively both the ubiquinol oxidase and malate oxidase activities, but had no effect on the ubiquinone reductase activity of extracts.

DISCUSSION

In previous studies (Benziman & Perez, 1965) with extracts of *A. xylinum* that were irradiated with ultraviolet light, it was suggested that a vitamin K-like compound is involved in the malate oxidase system of this organism. However, as reported in the present paper, no naphthaquinone could be detected in any of the lipid fractions extracted from *A. xylinum* cells by different extraction procedures involving both simple solvent extractions and extraction after previous hydrolysis. The only quinone present in these extracts in physiologically significant amounts (Redfearn, 1961) was of the ubiquinone type. Thus it seems that *A. xylinum* belongs to the group of organisms that contain ubiquinone as their sole quinone moiety (Jones & Redfearn, 1966; Brodie, 1965).

The presence in the quinone of *A. xylinum* of the 2,3-dimethoxy-5-methylbenzoquinone nucleus is evident from its characteristic ultraviolet spectra before and after reduction with borohydride and from its infrared spectrum. The close relationship between the quinone and ubiquinone-10 with regard to the nature of the side chain is indicated from the similarity of their extinction coefficients in the ultraviolet region and from their identical chromatographic mobilities. The existence in biological systems of ubiquinones with partially reduced side chains (Diplock, Edwin, Green, Bunyan & Marcinkiewicz, 1960; Gale, Arison, Trenner, Page & Folkers, 1963; Hatefi, 1963) necessitated some additional evidence for the final characterization of the quinone. The evidence obtained from n.m.r.-spectral analysis, from m.p. determinations and from catalytic hydrogenation experiments makes it possible to identify the quinone of *A. xylinum* as ubiquinone-10, i.e. an ubiquinone with 10 isoprene units in its side chain. Such a ubiquinone is relatively rare in microbial species, in which ubiquinones with side chains of 6-8 isoprene units occur most frequently (Lester *et al.* 1959; Crane, 1965).

Fractionation of *A. xylinum* extracts into large

particles, small particles and a high-speed supernatant revealed that the quinone is located predominantly in the heavy particulate fraction, which also contains most of the NADH oxidase and malate oxidase activities. Such an association of the quinone with oxidative enzymes suggests a possible role for the quinone in the electron-transport system of the cell.

Three criteria that must be met to include a compound as an obligatory member of the electron-transport system have been proposed by Slater (1958). These are: (1) the compound must be present in amounts commensurate with other respiratory-chain components such as flavines and haems; (2) the compound should be reduced and oxidized at rates consistent with the overall activity; (3) removal of the compound must result in loss of activity, and addition of the component to the depleted system should restore activity.

That the quinone in *A. xylinum* fulfils the first of these criteria is evident from the finding that its molar concentration in the cells is three times that of the individual cytochromes and equivalent to that of the flavoproteins. The quinone content of the cells (0.6–0.7 $\mu\text{mole/g. dry wt.}$) agrees favourably with that generally found in aerobic bacteria (Crane, 1965), though several micro-organisms with a higher quinone content have been reported (Cook & Umbreit, 1963; Lester & Crane, 1959).

To test the second of the above-mentioned criteria in our system, we have used the approach of Redfearn & Pumphrey (1960) of studying the oxidation–reduction of the endogenous quinone rather than that of an externally added quinone, to avoid the limitations arising from the low solubility of ubiquinone-10 under physiological conditions (Hatefi, 1963; Ramasarma & Lester, 1960). By using the extraction methods of Redfearn & Pumphrey (1960), which provide rapid means of stopping enzymic reaction and for separating the endogenous quinone into its oxidized and reduced forms, it was shown that more than 95% of the quinone of *A. xylinum* underwent reduction by either malate or NADH when reoxidation of the quinol was arrested by anaerobiosis or by cyanide. In mammalian mitochondria it has been reported (Redfearn & Pumphrey, 1960; Chance, 1965; Redfearn & Whittaker, 1966) that 10% or more of the endogenous quinone could not be reduced by substrate in the presence of cyanide. The results of the present study on the steady-state oxidation–reduction level of the quinone during malate oxidation are similar to those reported by Redfearn & Pumphrey (1960) for the quinone of non-phosphorylating pig heart mitochondria during succinate oxidation. However, the effect of phosphate in lowering the steady-state ubiquinone/ubiquinol ratio reported by Redfearn & Pumphrey

(1960) for the above-mentioned preparations and by Hatefi (1959) for phosphorylating ox heart mitochondria could not be demonstrated in our system.

In agreement with the second criterion it was found that in *A. xylinum* extracts the rates of the malate–ubiquinone reductase and ubiquinol oxidase reactions are compatible with the overall rate of malate oxidation. These results are strikingly different from those reported for the ubiquinone of mammalian systems. Thus Redfearn & Pumphrey (1960), using the same extraction method, reported that the rate of reduction of mitochondrion-bound ubiquinone by succinate or NADH and the rate of oxidation of bound ubiquinol were consistently lower than the overall rate of substrate oxidation. These findings, which were later verified by direct spectrophotometric measurements of the kinetics of ubiquinone reduction (Chance & Redfearn, 1961), led Redfearn & Pumphrey (1960) to postulate that ubiquinone functions outside the main pathway of electron transfer in mitochondria. However, it has been pointed out (Hatefi, Jurtshuk & Haavik, 1961) that since ubiquinone is present in mitochondria in high excess over other electron carriers (10:1 over cytochrome c_1) the portion of ubiquinone in excess of other carriers may not undergo rapid oxidation–reduction. In line with the latter reasoning it could be suggested that since the amount of quinone in excess of other carriers is significantly lower in *A. xylinum* than in mitochondria (Hatefi *et al.* 1961; Green & Wharton, 1963) a much greater portion of the quinone could be expected to turn over during substrate oxidation in *A. xylinum* than in mitochondria, thus explaining the better correlation between the kinetics of quinone oxidation–reduction and substrate oxidation observed here as compared with that reported for the mitochondrial system. In addition, since the rate of malate oxidation in our system appears to be much lower than the rate of succinate oxidation in mitochondria (3.1 versus 16.9 $\mu\text{moles/mg. of protein/hr. at } 20^\circ$) (Redfearn & Pumphrey, 1960), the errors in rate determinations due to any time-lag during the mixing and denaturation of the protein might be expected to become much more pronounced in the rather rapid oxidizing system of the mitochondria than in our system.

The malate dehydrogenase activity of *A. xylinum* extracts as measured by ferricyanide reduction is considerably higher than that of the malate oxidase activity. Therefore it seems likely that the rate-limiting reaction in the overall oxidation of malate is not the reduction of the primary dehydrogenase, but rather the reduction of ubiquinone, which is situated on the respiratory chain, probably after the flavoprotein as discussed below.

Previous studies (Benziman & Perez, 1965) have

revealed that irradiation of *A. xylinum* extracts with light at 360m μ resulted in considerable loss of their malate oxidase activity. The activity could be restored by the addition of vitamin K derivatives, especially vitamin K₃. The possibility exists that these quinones might be replacing ubiquinone, especially since the kinetics of reduction of vitamin K₃ and of oxidation of reduced vitamin K₃ by the extracts was compatible with the kinetics of malate oxidation in the presence of vitamin K₃. Also, exposure of *A. xylinum* extracts to light at 360m μ for 90 min. was found to result in the destruction of up to 50% of their endogenous ubiquinone. However, all attempts to reactivate the irradiated extracts by the addition of exogenous ubiquinone-10 failed (H. Goldhamer & M. Benziman, unpublished work). This could be due to the inadequate solubility of the added quinone in the enzymic system (cf. Kashket & Brodie, 1963; Weber, Rosso & Noll, 1963). The sensitivity of the ubiquinone in *A. xylinum* to irradiation is especially noteworthy in view of the report that the ubiquinone of rat liver mitochondria was resistant to ultraviolet light (Redfearn, 1961).

It appears from the results presented thus far that the quinone in *A. xylinum* can be considered as an obligatory component of the respiratory chain of this organism at least with regard to malate oxidation.

The experiments with inhibitors might indicate the position of the quinone in the respiratory chain. Amytal inhibits the malate oxidase reaction in *A. xylinum* at a site in the respiratory chain that is on the oxygen side of the flavoprotein, presumably before cytochrome *b* (Benziman & Galanter, 1964; Benziman & Perez, 1965). The inhibition by Amytal of the malate-quinone reductase activity and the lack of inhibition of ubiquinol oxidation would place the quinone at a site on the respiratory chain after the site of Amytal inhibition. On the other hand, the inhibition of ubiquinol oxidation by 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide, which is believed to block electron transport between cytochromes *b* and *c* (Lightbown & Jackson, 1956), indicates that the quinone acts before cytochrome *c* and possibly before cytochrome *b*. Thus it appears that in *A. xylinum* the position of ubiquinone on the respiratory chain lies between the primary flavo-protein dehydrogenase and the cytochrome chain, similar to the position assigned for it in other oxidative systems (Redfearn & Pumphrey, 1960; Green & Wharton, 1963; Redfearn & Whittaker, 1966).

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