the tris-buffered preparation resulted in a lower than normal yield of ubiquinone. This is what might be expected since ubiquinone is rather labile in alkaline solution. For the same reason the large increase in ubiquinone yield from the phosphate-buffered enzyme after treatment with sodium hydroxide was surprising. Presumably there exists in the phosphate-buffered enzyme suspension some agent which protects ubiquinone very effectively from destruction by alkali. Nothing is known about the nature of the bound form of ubiquinone.

SUMMARY

1. A method for determination of the ubiquinone concentration in small amounts of mitochondrial preparations is described.

2. Ubiquinone occurs in animal and plant mitochondria in relatively high concentrations. In heart-muscle preparations, for example, the concentration, on a molar basis, is four to five times those of the individual cytochromes.

3. A 'bound' form of ubiquinone appears to exist in phosphate-buffered heart-muscle prepara-

tions. This bound form, which amounts to about 25% of the total ubiquinone, can be extracted only after treatment of the preparation with alkali.

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The Kinetics of Ubiquinone Reactions in Heart-Muscle Preparations

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A great deal of evidence has been presented (Crane, Hatefi, Lester & Widmer, 1957; Lester & Fleischer, 1959; Pumphrey, Redfearn & Morton, 1958; Pumphrey & Redfearn, 1959; Redfearn, 1959) which suggests that ubiquinone (coenzyme Q) is a functional component of the respiratory chain in living organisms. It is widely distributed in animal and plant tissues, where it is located principally in the mitochondria in a concentration four to five times as great as those of the individual cytochromes. The endogenous ubiquinone is reduced to the quinol when the mitochondrial preparation is incubated with dihydrodiphosphopyridine nucleotide or succinate under anaerobic conditions, and is reoxidized when the preparation is aerated; the addition of ubiquinone to preparations in which the endogenous ubiquinone has been removed by acetone-extraction restores enzymic activity. Thus ubiquinone fulfils some of the criteria necessary for a substance to be considered as a functional component of the respiratory chain

(Slater, 1958). However, before ubiquinone can be assigned a position in the chain the kinetics of its oxidation and reduction must be known. The current concept of the electron-transport system for the oxidation of succinate and dihydrodiphosphopyridine nucleotide is a chain of cytochromes with branches at the 'substrate end' to link with dihydrodiphosphopyridine nucleotide and succinate through their specific flavoproteins (Chance, 1957; Slater, 1958). If ubiquinone is a member of such a system then its rates of oxidation and reduction must be at least equivalent to the overall rate of substrate oxidation.

In this paper the results of a study of the kinetics of the reactions of endogenous ubiquinone in mitochondrial preparations are described and they are discussed in relation to a possible position for ubiquinone in the respiratory chain. Part of this work has already been presented as a preliminary communication (Pumphrey & Redfearn, 1959).

MATERIALS AND METHODS

Dihydrodiphosphopyridine nucleotide (DPNH) was obtained from C. F. Boehringer und Söhne, Mannheim, Germany; antimycin A from the Kyowa Fermentation Co. Ltd., Tokyo; sodium amytal from Eli Lilly and Co., Basingstoke; and 2,3-dimercaptopropanol from L. Light and Co. Ltd. Cytochrome *c* was prepared from ox heart by the Keilin & Hartree (1952) procedure and dialysed against water. Phenazine methosulphate was prepared by the method of Singer & Kearney (1957).

Heart-muscle preparations. These were made from pig heart as described in the preceding paper (Pumphrey & Redfearn, 1960).

Rate measurements

Unless otherwise stated, all measurements were carried out at room temperature (usually in the range 18–22°). The preparations and reagents were allowed to equilibrate to room temperature before the assays. In the ubiquinone-reductase and ubiquinol-oxidase assays, the room temperature was recorded for each incubation period. It was found that the temperature variation for each complete assay was not more than $\pm 0.5^\circ$. Any handling which was likely to cause temperature increases was avoided. Each ubiquinone-reductase and ubiquinol-oxidase rate was compared with the succinic-oxidase rate measured with the same preparation suspended in the same buffer at the same time and with the water bath set to the appropriate mean temperature. In the DPNH-oxidase assay, the temperature of the cell contents was measured immediately at the end of the reaction. It was usually slightly higher than that of the DPNH-ubiquinone-reductase assay. Where there was a temperature difference for the two assays to be compared, the DPNH-oxidase rate was corrected to the temperature of DPNH-ubiquinone-reductase assay assuming a temperature coefficient of 2.0 for a 10° difference.

Succinic-ubiquinone reductase. The heart-muscle preparation (1 ml.) containing 10–30 mg. of protein in 0.1 M- KH_2PO_4 - Na_2HPO_4 or 2-amino-2-hydroxymethylpropane-1:3-diol (tris) -HCl buffer, pH 7.4, was measured into a 15 ml. centrifuge tube. The tube was then shaken in air to ensure that all of the ubiquinone in the preparation was in the oxidized state. After addition of 0.2 ml. of 0.06 M-KCN (to inhibit cytochrome oxidase) the reduction was started by the rapid addition of 0.3 M-sodium succinate (0.2 ml.) from a spring-loaded 1 ml. pipetting syringe; a stopwatch was started simultaneously. After a suitable time interval the reaction was stopped by the rapid addition of cold (about -20°) methanol (4 ml.) containing pyrogallol (1 mg./ml.) from a 10 ml. pipetting syringe; the stopwatch was stopped simultaneously. The syringe, which was all glass and metal with no rubber connexions or valves, was filled with the methanol-pyrogallol mixture from a flask surrounded with crushed solid carbon dioxide. The addition of the cold methanol stopped the enzymic reaction practically instantaneously; thus when the substrate and the methanol-pyrogallol mixture were added simultaneously there was little or no reduction of the ubiquinone. The denatured enzyme preparation was then extracted with light petroleum and the ubiquinone estimated as described in the preceding paper (Pumphrey & Redfearn, 1960). The whole procedure was then repeated, different time intervals

being used between the addition of the substrate and the termination of the reaction.

By plotting ΔE_{275} against time a smooth curve was obtained and the rate of reduction could be calculated from the initial slope. In most cases, however, the maximum rate was calculated from the straight line obtained by plotting $\log [(\Delta E_{275})_t - (\Delta E_{275})_\infty]$ against time, where $(\Delta E_{275})_t$ is proportional to the concentration of oxidized ubiquinone after incubation for *t* sec. with substrate, and $(\Delta E_{275})_\infty$ is proportional to the concentration when all measurable reduction had ceased (usually after incubation for 1 min.). The concentrations of ubiquinone were calculated from the ΔE_{275} values as described by Pumphrey & Redfearn (1960) and the rates were expressed as μmoles of ubiquinone reduced/mg. of protein/hr.

The DPNH-ubiquinone-reductase assay was similar except that 0.2 ml. of 0.035 M-DPNH was used.

Ubiquinone reductase measured after preincubation of the enzyme preparation with the substrate. The ubiquinone-reductase assay measures reduction in the first few seconds after the addition of substrate, but the manometric succinic-oxidase assay measures oxygen uptake 5–10 min. after, and the spectrophotometric DPNH-oxidase assay measures oxidation of DPNH at least 30 sec. after substrate addition. Thus a comparison of the ubiquinone-reductase rate with the overall rate of oxygen uptake determined by these methods would be very misleading if there happened to be a lag period of even a few seconds after the addition of the substrate. In order to eliminate any error due to a possible lag period a modified ubiquinone-reductase assay was devised. The heart-muscle preparation (1 ml.) was mixed with the substrate (0.2 ml. of 0.3 M-sodium succinate or 0.035 M-DPNH) and left for a suitable time interval (usually 10 min.). The tubes were then shaken in air for 1 min. so that the endogenous ubiquinone reached its aerobic steady-state oxidation-reduction level. Potassium cyanide (0.2 ml. of 0.06 M) was then added rapidly from a spring-loaded pipetting syringe. The reaction was stopped after suitable time intervals (0–60 sec.) and the ubiquinone extracted and determined in the way previously described.

This method is possible only in the tris-buffered enzyme preparations where the ubiquinone in the aerobic steady state is predominantly in the oxidized form. When KCN is added to inhibit oxidation of ubiquinol, the ubiquinone is reduced by the excess of substrate present. In phosphate buffer, only about 25 % of the reducible ubiquinone is in the oxidized form at the steady state and the concentration of ubiquinone is therefore too small for accurate rate measurements.

Ubiquinol oxidase. The heart-muscle preparation (1 ml.) was mixed with water (0.2 ml.) or 0.3 M-cytochrome *c* (0.2 ml.) in a 15 ml. conical centrifuge tube. Sodium succinate (0.2 ml. of 0.3 M) was then added and mixed, followed immediately by light petroleum, b.p. 40–60° (0.3 ml.), to form a layer on the surface. The tube was then left to stand at room temperature for 3 min., during which time the oxygen in the solution was depleted and the respiratory carriers became reduced. A small amount of oxaloacetic acid (0.5–1.0 mg.) was then added to the solution with a glass rod and mixed very carefully to avoid aeration. The mixture was allowed to stand for another 3 min. to allow the oxaloacetic acid to dissolve and effectively inhibit succinic-dehydrogenase activity. Oxidation

was initiated by bubbling a fine stream of oxygen through the mixture. The reaction, which was timed, was stopped after a suitable interval by the rapid addition of cold methanol-pyrogallol mixture as described above. The procedure was repeated 10–12 times, at different time intervals (1 sec.–5 min.). Ubiquinone concentration was plotted against time, and ubiquinol-oxidase rate, calculated from the initial slope, was expressed as μ moles of ubiquinol oxidized/mg. of protein/hr. The ubiquinol-oxidase reaction is not first-order.

Succinic oxidase. This was determined manometrically. The reaction mixture consisted of KH_2PO_4 - Na_2HPO_4 or tris-HCl buffer, pH 7.4, 0.07 M; sodium succinate, 0.043 M; cytochrome *c*, 0.043 mM; heart-muscle preparation, approx. 2 mg. of protein. Final reaction volume of the flask contents was 1.4 ml. The centre well contained 0.1 ml. of 20 % (w/v) NaOH and filter paper. The gas phase was air. The curve relating phosphate concentration and succinic-oxidase activity had been determined for this preparation and the optimum concentration found to be 0.07 M. Also, it had been found that 0.043 mM-cytochrome *c* was sufficient for maximum succinic-oxidase activity.

Dihydrodiphosphopyridine nucleotide oxidase. This was determined spectrophotometrically with a Unicam SP. 500. The reaction mixture consisted of KH_2PO_4 - Na_2HPO_4 or tris-HCl buffer, pH 7.4, 0.067 M; DPNH, 0.1 mM; cytochrome *c*, 0.02 mM; heart-muscle preparation, 0.08–0.15 mg. of protein, in a total volume of 3 ml. The reaction was started by adding the enzyme preparation, and extinction readings at 340 $m\mu$ were taken at 30 sec. intervals. The rate was calculated as μ moles of DPNH oxidized/mg. of protein/hr., a molecular extinction coefficient for DPNH at 340 $m\mu$ of 6.22×10^3 being used.

Succinic dehydrogenase. Phenazine methosulphate was used as the electron acceptor in the manometric assay described by Giuditta & Singer (1959).

Steady-state measurements

The enzyme preparation (1 ml.) was mixed with the substrate (0.2 ml. of 0.3 M-succinate or 0.035 M-DPNH) and 0.3 mM-cytochrome *c* (0.2 ml.) or water (0.2 ml.). The mixture was left to stand at room temperature for 5 min. and was then shaken rapidly in air for 1 min. to allow the respiratory-chain components to attain their steady-state oxidation-reduction levels. The mixture was denatured with cold methanol and the ubiquinone-ubiquinol mixture extracted and estimated as already described.

RESULTS

Substrate-oxidase activities

The succinic-oxidase and DPNH-oxidase activities depended on the buffer used (phosphate or tris) and on the presence or absence of added cytochrome *c*. The succinic-oxidase values were in the range 9.6–26.9 μ moles of succinate oxidized/mg. of protein/hr. for the phosphate-buffered preparations at room temperature (18–22°) in the presence of added cytochrome *c*. In the absence of added cytochrome *c* values were only 30–50 % of these activities. In the tris-buffered preparation, addition of cytochrome *c* had little or no effect on the

succinic-oxidase activities; these were 50–70 % of the maximal activities in the phosphate-buffered preparations. Succinic-dehydrogenase activities of the heart-muscle preparations were equivalent to those of succinic oxidase measured in the presence of added cytochrome *c*. This finding agrees with the work of Giuditta & Singer (1959).

The DPNH-oxidase activities, which showed a similar dependency on the buffer and addition of cytochrome *c*, were approximately twice those of the succinic oxidase.

The preparations usually retained good enzymic activities for 7–10 days when stored at 2°.

Steady-state oxidation-reduction levels of ubiquinone

The aerobic steady-state oxidation-reduction level of the endogenous ubiquinone depended on the medium used to suspend the enzyme particles. In phosphate buffer, at the steady state, 60–80 % of the total reducible ubiquinone was in the reduced state, whereas in tris buffer the corresponding figure was 30–40 %. Addition of cytochrome *c* decreased the value for the phosphate-buffered preparation but had little effect on the tris-buffered preparation (Table 1). This is to be expected since added cytochrome *c* usually resulted in a marked increase in succinic-oxidase activity in the former preparations but had less effect on the latter.

Ubiquinone reductase

In the presence of cyanide, about 90 % of the extractable ubiquinone is reduced by succinate, and about 70 % by DPNH. The difference is not due to the different concentrations of substrate used in the succinic- and DPNH-ubiquinone-reductase assays, since 0.2 ml. of 0.015 M-succinate was found to reduce ubiquinone to the same extent as the same volume of 0.3 M-succinate.

The reduction of endogenous ubiquinone in the presence of cyanide and an excess of substrate is a first-order reaction; i.e. by plotting $\log[(\text{ubiquinone concn.})_t - (\text{ubiquinone concn.})_\infty]$ against time of

Table 1. *Steady-state oxidation-reduction levels of ubiquinone in pig-heart-muscle preparations*

Details of method, including concn., temp. etc., are described in the text. Substrate used was succinate. Numbers in parentheses indicate number of experiments.

Preparation	Cytochrome <i>c</i> addition (final concn. 0.043 mM)	Ubiquinone (oxidized) as a percentage of the total reducible ubiquinone $\pm$ S.D.
Phosphate-buffered	–	19 $\pm$ 4 (9)
Phosphate-buffered	+	40 $\pm$ 8 (4)
Tris-buffered	–	62 $\pm$ 9 (4)
Tris-buffered	+	65 $\pm$ 4 (5)

incubation, a straight line is obtained (Fig. 1). Similar results were obtained when DPNH was used as substrate. Also, when preparations suspended in tris buffer were used, similar results were obtained with either substrate.

The rate of reduction of ubiquinone by succinate was compared with the succinic-oxidase rate measured manometrically at the same temperature. The main difference in the conditions between the ubiquinone-reductase and the succinic-oxidase assays is the concentration of the enzyme preparation. In the former the enzyme is present in about five times the concentration used in the latter. The concentration of the enzyme preparation (varied within the limits imposed by assay procedure), however, did not affect the specific ubiquinone-reductase activity. Also, increasing the concentration of the preparation in the succinic-oxidase assay to a value equivalent to that used in ubiquinone-reductase assay had little effect on the rate of oxygen uptake per mg. of protein, provided that oxygen was used as the gas phase. When air was used the rate of solution of oxygen became a limiting factor and there appeared to be a decrease in the succinic oxidase with increasing concentrations of enzyme preparation. It is possible therefore to make valid comparisons between succinic-ubiquinone-reductase and succinic-oxidase rates.

In the case where DPNH was used as the substrate the ubiquinone-reductase rate was compared

with that of DPNH oxidase measured spectrophotometrically, and here the differences in the concentration of the enzyme preparation were even greater. Since it was not possible to increase substantially the concentration used in this DPNH-oxidase assay it has been assumed that the latter behaves similarly to the succinic oxidase in this respect.

In a series of determinations (Table 2) made on different heart-muscle preparations it was found that the maximal rate of reduction of endogenous ubiquinone by succinate or DPNH was always much less than the overall rate of substrate oxidation; e.g. succinic-ubiquinone-reductase rates ranged from 13 to 52 % of the succinic-oxidase rates in the phosphate-buffered preparations. In order to eliminate any error due to a lag period in the reaction after the addition of the substrate, the ubiquinone-reductase rates were also determined by the second method, in which the substrate is preincubated with the enzyme preparation. This method can be used satisfactorily only with the tris-buffered preparations, where the aerobic steady-state level of ubiquinone is 60–70 %. The rates obtained (Table 2), calculated for an initial concentration of 100 % of ubiquinone, were higher than those obtained by the standard-assay procedure but they were still less than the overall substrate-oxidation rates.

The ubiquinone-reductase rates shown in Table 2 have been calculated from the logarithmic graphs for an initial concentration of 100 % of ubiquinone. A more valid rate for comparison with the substrate-oxidase rate would be the rate of reduction where the initial ubiquinone concentration is at its steady-state level. This rate can be calculated for the appropriate steady-state ubiquinone concentration. Thus when succinate is the substrate, the steady-state rates will be 40 % of the rates given for the phosphate-buffered enzyme in Table 2, and 65 % for the tris-buffered enzyme.

A possible criticism of the ubiquinone-reductase assay is the use of cyanide to inhibit cytochrome oxidase, since it has been shown (Tsou, 1951) that cyanide, besides inhibiting cytochrome oxidase, has an inhibitory effect at other points in the chain. In order to eliminate the possibility of a partial inhibition of ubiquinone reductase by cyanide, rate measurements were made in the presence of antimycin A instead of cyanide. Antimycin A is an effective inhibitor of ubiquinol oxidase but it does not affect ubiquinone reduction (Pumphrey & Redfearn, 1959). Rate measurements were made on preparations where ubiquinol oxidase was inhibited with antimycin A and compared with those obtained with cyanide as the inhibitor. The succinic-ubiquinone-reductase rates were identical in the two cases.

Fig. 1. Logarithmic plot of rate of reduction of ubiquinone by succinate at 20.5° in a pig-heart-muscle preparation suspended in phosphate buffer.

Table 2. Comparison of maximum succinic- and dihydrodiphosphopyridine nucleotide-ubiquinone-reductase rates with those of succinic and dihydrodiphosphopyridine nucleotide oxidase in pig-heart-muscle preparations. Methods are described in the text. The substrate-oxidase activities were measured in the presence of added cytochrome *c*.

Preparation no.	Age of preparation (days)	Suspending buffer	Substrate pre-incubated	Temp. of assays	Maximum ubiquinone-reductase rate (μmoles of ubiquinone reduced/mg. of protein/hr.)	Substrate-oxidase rate (μmoles of substrate oxidized/mg. of protein/hr.)	Ubiquinone-reductase rate as percentage of substrate-oxidase rate
19	6	Phosphate	No	18.1°	2.6	11.4	23
20	2	Phosphate	No	20.7	3.6	26.9	13
23	3	Phosphate	No	20.5	4.9	18.7	26
27	2	Phosphate	No	19.7	5.0	9.6	52
28	2	Phosphate	No	18.5	4.0	10.9	37
31	2	Phosphate	No	22.5	4.5	23.4	20
23	3	Tris	No	20.5	2.9	10.6	26
27	3	Tris	No	19.7	2.8	5.8	48
30	3	Tris	Yes	21.2	3.0	4.2	72
31	1	Tris	Yes	20.7	3.9	5.3	74
25	3	Phosphate	DPNH	19.0	4.2	31.3	13
32	1	Phosphate	DPNH	22.1	5.9	39.9	15
32	2	Tris	No	22.0	3.6	16.5	22
32	4	Tris	Yes	21.9	4.6	13.0	35

Ubiquinol oxidase

The method used for measuring ubiquinol-oxidase rates was found to be rather unsatisfactory and the results were not as consistent as those obtained by the ubiquinone-reductase assay. In many cases the points on the $\Delta E_{275}/\text{time}$ graphs were scattered and reliable rate measurements were not possible. This scattering was attributed mainly to the layer of light petroleum used to exclude air from the preparation; it is possible that during the subsequent passage of oxygen the light petroleum becomes thoroughly mixed with the enzyme, causing some deactivation and premature extraction of lipid.

The ubiquinol-oxidase rates in phosphate-buffered preparations were too fast to be measurable at room temperature in the presence of cytochrome *c* but measurable rates could be obtained in the absence of added cytochrome *c*, and a typical result is shown in Fig. 2. The maximal ubiquinol-oxidase rates were always lower than the succinic-oxidase rates measured under the same conditions (Table 3).

Addition of cytochrome *c* accelerated both the ubiquinol-oxidase and succinic-oxidase rates in most phosphate-buffered preparations. Although it was difficult to obtain reliable ubiquinol-oxidase rates in the presence of added cytochrome *c*, it seems probable that the two rates were increased proportionately because addition of cytochrome *c* increased the steady-state level of ubiquinone and succinic-oxidase activity in about the same proportion. Ubiquinone reductase is not accelerated by addition of cytochrome *c*.

Fig. 2. The rate of oxidation of endogenous ubiquinol at 21° in a pig-heart-muscle preparation suspended in phosphate buffer. Cytochrome *c* was not added in this experiment.

Table 3. *Comparison of maximum ubiquinol-oxidase rates with those of succinic oxidase in pig-heart-muscle preparations*

Details of methods are described in the text. Activities were measured in the absence of added cytochrome *c*.

Preparation no.	Age of preparation (days)	Suspending buffer	Temp.	Maximum ubiquinol-oxidase rate (μ moles of ubiquinol oxidized/mg. of protein/hr.)	Succinic oxidase (μ moles of succinate oxidized/mg. of protein/hr.)
29	1	Phosphate	21.0°	0.8	3.5
34	1	Phosphate	21.0	1.7	6.7
34	3	Phosphate	21.5	1.4	6.9
35	1	Tris	1.5	1.8	1.2

Table 4. *Effects of inhibitors on the oxidation-reduction reactions of ubiquinone*

Details of methods are described in the text.

Inhibitor	Final concn. (mM)	Reaction inhibited
KCN (neutralized)	9	Ubiquinol oxidase
Antimycin A	71 μ g./ml.	Ubiquinol oxidase
2:3-Dimercaptopropanol	7	Ubiquinol oxidase
Oxaloacetic acid	5	Succinic-ubiquinone reductase
Sodium amytal	1.4	DPNH-ubiquinone reductase

Ubiquinol-oxidase rates in tris-buffered preparations were too fast to measure at room temperature even in the absence of added cytochrome *c*, but by reducing the temperature to 1.5° reasonable rates were obtained. These rates were 50% higher than that of the succinic oxidase measured under the same conditions (Table 3). The steady-state level of the ubiquinone approached 100% under these conditions.

Effects of inhibitors

The effects of various respiratory-chain inhibitors on the oxidation-reduction reactions of ubiquinone are summarized in Table 4.

As pointed out in an earlier report (Pumphrey & Redfearn, 1959), the finding that antimycin A inhibited ubiquinol oxidation but not ubiquinone reduction was contrary to that of another group of workers (Hatefi, Lester, Crane & Widmer, 1959), who claimed the converse. This group have now repeated their earlier work with a different technique and have obtained results in complete agreement with the present results (Green, Hatefi & Fechner, 1959).

2:3-Dimercaptopropanol also inhibited the oxidation of ubiquinol but had no effect on the reduction reaction. Spectrophotometric examination of the lipid extract after treating the preparation with either antimycin A or 2:3-dimercaptopropanol indicated that neither substance had combined chemically with ubiquinone or ubiquinol.

DISCUSSION

The results have shown that the rate of reduction of ubiquinone by either substrate was less than the rate of oxidation of that substrate. Also, the rate of oxidation of ubiquinol was less than that of succinic oxidase, except in one experiment carried out at 1.5° with a tris-buffered enzyme. The ubiquinone-reductase and ubiquinol-oxidase rates used in these comparisons are maximal rates which have been calculated for initial concentrations of 100% of ubiquinone and 100% of ubiquinol respectively. In the aerobic steady-state conditions of the succinic- and DPNH-oxidase assays, the concentrations of ubiquinone and ubiquinol will, of course, be less than 100%. Since neither the ubiquinone reduction nor ubiquinol oxidation are zero-order reactions, their rates at the steady state will be less than the maximal rates. Thus the rates of the reduction and reoxidation of ubiquinone at the steady state as percentages of the substrate-oxidase rates would be even less than those given in Tables 2 and 3.

The experiments with inhibitors show that the endogenous ubiquinone in heart-muscle preparations is reduced by the succinate- and DPNH-specific flavoproteins and reoxidized by way of the antimycin A-sensitive factor and cytochrome oxidase. Thus it lies between the flavoproteins and the antimycin A-sensitive factor. However, the kinetic data indicate that electron transfer through

ubiquinone does not account for the total electron flux through the system as measured by the succinic- or DPNH-oxidase activities. These activities are governed by the rate-limiting reactions in the system. It was found that the succinic-dehydrogenase activity, measured by the phenazine methosulphate method, was equivalent to the succinic-oxidase activity; the latter was measured in the presence of added cytochrome *c* so that the concentration of endogenous cytochrome *c* would not be limiting. Thus it seems likely that the rate of flavoprotein reduction is the rate-limiting reaction in succinate oxidation. The succinic-ubiquinone-reductase rate therefore cannot be greater than the rate-limiting reaction, because ubiquinone acts after the flavoprotein, but the rates would be equivalent if ubiquinone was in the chain. The fact that the rates are not equivalent means that a position for ubiquinone on a branch pathway must be postulated. This raises the question of a possible relationship with cytochrome *b*, which has also been shown to lie off the main respiratory chain in non-phosphorylating preparations (Chance, 1952, 1958). There are, however, a number of reasons why ubiquinone cannot be considered as part of the cytochrome *b* pathway. These are: (a) in non-phosphorylating particles the rate of reduction of cytochrome *b* is only 2% of the overall oxidase rate (Chance, 1952), whereas ubiquinone reductase is about 25% of the overall rate; (b) addition of antimycin A results in a tenfold increase in the rate of reduction of cytochrome *b* (Chance, 1958) but ubiquinone reduction is unaffected; (c) the oxidation-reduction potential of ubiquinone is calculated to be +120 mv at pH 7.4 (Joel, Karnovsky, Ball & Cooper, 1958), whereas the highest value for cytochrome *b* at the same pH has been given as +77 mv (Colpa-Boonstra & Holton, 1959); on this basis ubiquinone is more likely to act after cytochrome *b*, which would be inconsistent with the kinetic data.

Two schemes for a position for ubiquinone in the non-phosphorylating heart-muscle preparation can be put forward (Fig. 3). In scheme (I) ubiquinone is part of a branch pathway of the chain linking flavoprotein with the antimycin A-sensitive factor and directly concerned in electron transport. In scheme (II) it is in equilibrium with either the flavoprotein or some unknown component so as to form a blind alley; in this case it would not be directly involved in electron transport. It is not possible at present to decide which of these two alternatives is more likely, except that Lester & Fleischer (1959) claim, on the basis of extraction-reactivation experiments, that ubiquinone is an essential hydrogen carrier, a conclusion which would appear to favour the first.

With regard to the function of ubiquinone in the respiratory chain two possibilities may be suggested. The first is that it acts as an electron conductor for linking together the chains of respiratory carriers. Evidence has been advanced by Kimura & Singer (1959) in support of earlier work (Wu & Tsou, 1955) that the respiratory chains are interlinked to form a common system for the various dehydrogenases. The relatively high concentration of ubiquinone with respect to the other carriers, the fact that most of it appears to be accessible to both the DPNH- and succinate-specific flavoproteins and its lipophilic properties make it a suitable candidate for an interchain carrier in a system in which lipids undoubtedly play a structural role. Such a function would be consistent with scheme (I) in Fig. 3.

The other possible function is in oxidative phosphorylation. Hatefi (1959) has shown that adenosine diphosphate and inorganic phosphate affect the steady-state levels of ubiquinone in mitochondria and has suggested that it functions in oxidative phosphorylation by the mechanism proposed by Harrison (1958). In the preceding paper (Pumphrey & Redfearn, 1960), evidence was pre-

Fig. 3. Two possible schemes for a position for ubiquinone in the non-phosphorylating respiratory chain. UQ, Ubiquinone; X, antimycin A-sensitive factor; FP, flavoprotein.

sented for the existence of a bound form of ubiquinone when the heart-muscle preparation was suspended in a medium containing inorganic phosphate. It is possible that part of the oxidative-phosphorylation process is still intact in these preparations and that this bound form is a phosphate-containing derivative of ubiquinone or ubiquinol and is an intermediate in the process. Such a role would be consistent with scheme (II) in Fig. 3.

SUMMARY

1. Methods for studying the kinetics of endogenous ubiquinone in heart-muscle preparations are described.

2. The rate of reduction of ubiquinone was not equivalent to the steady-state rate of electron transfer as measured by the rate of oxidation of the substrate.

3. Studies with inhibitors indicated that ubiquinone was reduced by the flavoproteins and oxidized by way of the antimycin A-sensitive factor and cytochrome oxidase.

4. It is suggested that ubiquinone is a branch pathway of the respiratory chain, and that it may function either as an interchain carrier or as an intermediate in oxidative phosphorylation.

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Studies on the Biosynthesis of Proteins in the Silkworm, *Bombyx mori* (L.)

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Although extensive studies have been made on the biosynthesis of proteins in animals, plants and micro-organisms, relatively little is known about similar mechanisms in insects. The use of labelled amino acids for studying the synthesis of silk by the giant silkworm, *Hyalophora (Platysamia) cecropia* (L.), was first reported by Zamecnik, Loftfield, Stephenson & Williams (1949), who showed that silk spun 24 hr. after injection of a mixture of carboxy-

labelled glycine and DL-alanine was radioactive. They also provided evidence for the incorporation of [1-¹⁴C]glycine *in vitro* by incubating excised silk glands aerobically in Krebs-Ringer phosphate medium. Shimura, Koide, Itabashi & Fukai (1955) demonstrated net fibroin synthesis in perfused silk glands and studied the effects of various metabolic intermediates added to the medium. In a later study, Shimura, Fukai, Suto & Hoshi (1958) showed that [1-¹⁴C]glycine injected into the silkworm was incorporated into the proteins of the silk gland, gut and dermal tissues, and that 4 hr. after injection,

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