C H A R G E T R A N S F E R R E A C T I O N S
A C R O S S
A D S O R B E D P H O S P H O L I P I D M O N O L A Y E R S
A T T H E M E R C U R Y / W A T E R I N T E R F A C E

by

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Abstract

Cyclic voltammetry using a hanging mercury drop electrode, (HMDE) was used to investigate charge transfer reactions of ubiquinone UQ_{10} across adsorbed phospholipid monolayers at the mercury/water interface.

The UQ_{10} molecule is thought to travel from the mercury surface to the water interface when reduced and to travel back to the mercury surface by a "flip-flop" motion, where it may be re-oxidized.

Voltammograms obtained suggested a quasi – reversible, surface reaction. The effects of incorporating UQ_{10} within different phospholipid environments, concentration of UQ_{10}, temperature, pH, and sweep rate/time dependence were investigated.

Second order kinetics for the decay of the reduced species suggest that the rate limiting step involves the disproportionation of the semiquinone radical, which may therefore be stable within the time span of the voltammetric experiments. The "flip-flop" motion of the UQ_{10} is therefore not involved in the rate determining step.

Contents

CHAPTER ONE

INTRODUCTION

1.1 Objectives of the work and research strategy

The aim of this project was to develop a novel mimetic system to gain insight into fundamental charge transfer reactions within biological systems. Electron transfer reactions involving ubiquinone UQ_{10} incorporated in lipid monolayers adsorbed at the mercury/aqueous interface have been used for this purpose.

The ubiquinone UQ_{10} molecule naturally resides within the inner mitochondrial membrane, whose basic structure is that of a mixed phospholipid bilayer. Other species such as cytochrome molecules, other proteins and cholesterol are embedded within the bilayer. This is the basis of the fluid mosaic hypothesis which is the currently accepted structure of the unit membrane (1).

Ubiquinones play an important role in the respiratory chain within mitochondria. Originally, coupled electron and proton transfer across the inner mitochondrial membrane formed the basis of the chemi-osmotic theory of charge transfer, although the mediator was not known (2). This model was developed to form the "Q-Cycle" in which ubiquinone (3) was regarded as the mediating intermediate. The vectorial character of the proton transfer across the membrane probably depends on the asymmetric character of the mitochondrial membrane.

Factors that may affect the physical nature of the phospholipid bilayers such as pH, temperature, or incorporation of other molecules, may consequently affect the way in which the ubiquinone molecule resides within a phospholipid bilayer.

In order to study the elementary electron transfer steps due to biomolecules incorporated in membranes, model systems are of

considerable interest. A fundamental problem here is the potential dependence of electron transfer reactions, and for this reason biomimetic systems that allow close control of the applied potential are advantageous.

In previous work the electrochemical behaviour of various adsorbed phospholipids were studied using cyclic and AC voltammetry at the mercury/water interface (4). It was shown that phospholipid monolayers adsorbed on the mercury surface in contact with electrolyte solutions, could be subjected to continuous cycling for several hours whilst remaining stable. Monolayers at the air/water interface are stable for longer periods (4) and therefore may be transferred to additional mercury drops by the process described later (Chapter two). The present work makes use of these systems to produce structures which are equovilent to half a bilayer. The formation of these layers has been achieved by transfer from a phospholipid monolayer adsorbed at the air/aqueous interface to a mercury droplet. The preparation method is simple and highly reproducible (4).

The present system has been developed so that ubiquinone UQ_{10} may be incorporated into adsorbed phospholipid monolayers in accurately known ratios.

Phospholipids undergo phase transitions at temperatures characteristic of the lipid, between a liquid and a liquid-crystalline phase. Thus the fluidity of a phospholipid layer is strongly dependent on the lipid structure and in particular, it's degree of unsaturation. For this reason, three different lipids have been studied in this work :

a) Dioleoyl phosphatidyl choline (PC), or di-o-PC.

b) Dipalmitoyl PC or di-p-PC.

c) Egg Lecithin.

The structure of dioleoyl PC and dipalmitoyl PC are shown in Fig (1.1).

Dioleoyl PC has two unsaturated hydrocarbon chains, whereas dipalmitoyl PC has two fully saturated hydrocarbon chains. Egg lecithin is composed of a mixture of phospholipids, whose main composition is that of dioleoyl PC and dipalmitoyl PC.

Studies of the electrochemical behaviour of the adsorbed monolayer have been undertaken to show the effects of pH, temperature and incorporation of cholesterol (5). Incorporation of cholesterol into the phospholipid monolayer gives increased rigidity to the monolayer when the lipid is above the phase transition temperature (5).

The objective of the present work was to use a membrane mimetic system to investigate the mechanism and nature of charge transfer across the inner mitochondrial membrane.

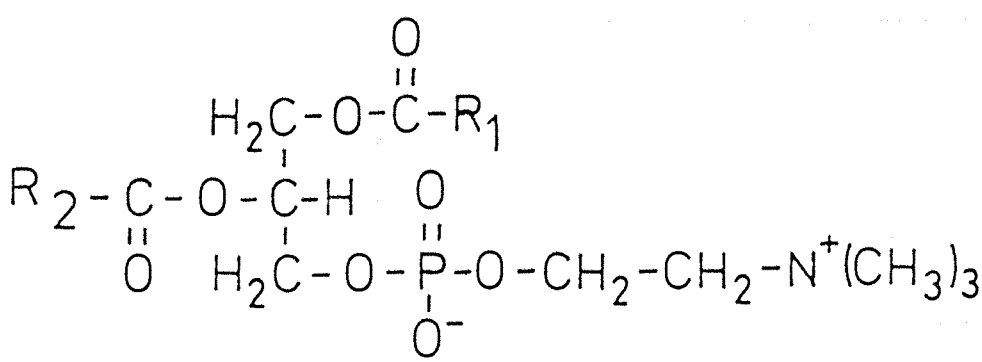
Structures of di-o-PC and di-p-PC

Dioleoyl PC ($R_1 = R_2$)

$R = CH_3(CH_2)_7CH{=}CH(CH_2)_7-C(=O)-O-$

Dipalmitoyl PC ($R_1 = R_2$)

$R = CH_3(CH_2)_{14}-CO-$



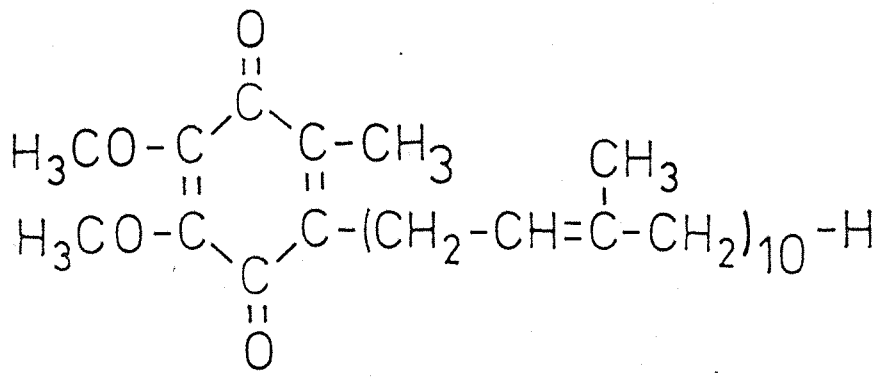
1.2 The biological role, significance and electrochemistry of ubiquinones.

In 1955 Morton's group in Liverpool, and Green's group in Wisconsin simultaneously isolated a lipid soluble compound from human kidney with an absorption maximum at 272 nm, the reduced form of which had an absorption maximimum at 290nm (6,7). Crane et al (8,9) discovered a compound shortly afterwards, which later proved to be identical to that of Morton's, and Green's. The structure was determined by oxidative degradation (8) and confirmed by synthesis in vitro (10,11). The structure was found to be as indicated in Fig (1.2.1), (9). Due to the ubiquitous distribution of the co-enzyme molecule within nature it is commonly known as ubiquinone. A subscript is used to signify the number of isoprene units of the hydrocarbon chain.

The molecule has a quinone moiety and is termed co-enzyme Q. The number of isoprene units is variable, although one hydrocarbon chain length is usually characteristic of the species from which it is found. Nearly all vertebrates possess UQ_{10}, although UQ_9 is found in fish. UQ_9 and UQ_{10} are found in species of rat. Some species of bacteria do however utilize co-enzyme Q molecules possessing different numbers of isoprene units, from UQ_1 to UQ_{10} (12).

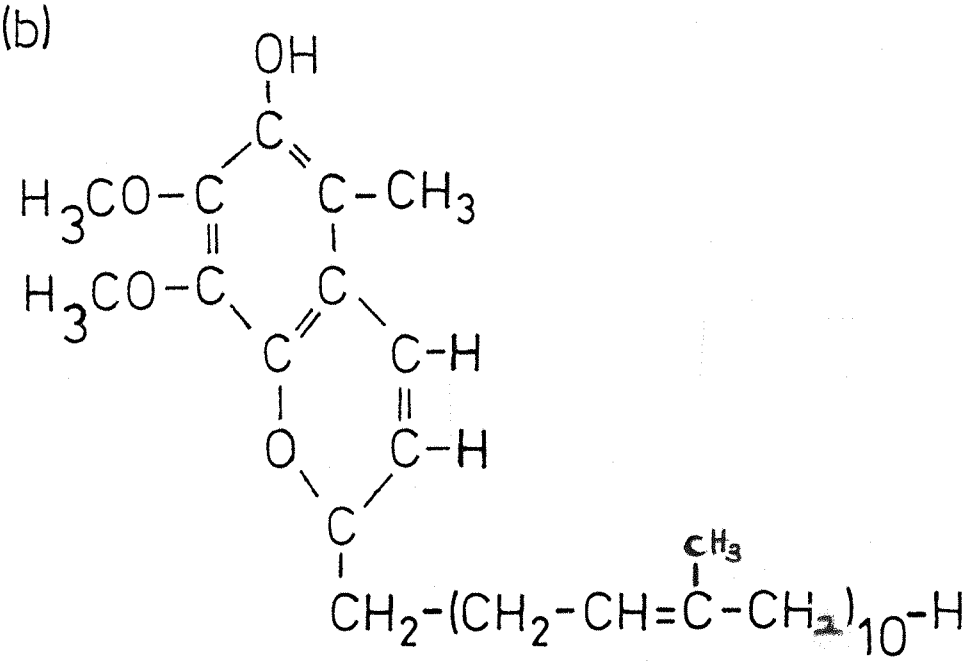
It was realized that the ubiquinone molecule resided mainly within the inner mitochondrial membrane and that it underwent successive oxidation and reduction reactions during respiration. The ubiquinone molecule was found to be present in varying concentrations within mitochondria taken from different sources, a typical value being of the order of 7 μ mol/g of mitochondrial protein. This

(a)



Ubiquinone Q_{10}

(b)



Ubichromanol

represents a concentration approximately five times higher than values quoted for cytochrome c (13). The inner mitochondrial membrane, in which ubiquinone is embedded, for example, for Ox heart has a lipid/protein composition (w/w) of around 0.3. 97% of the lipid consists of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, and cardiolipin (1).

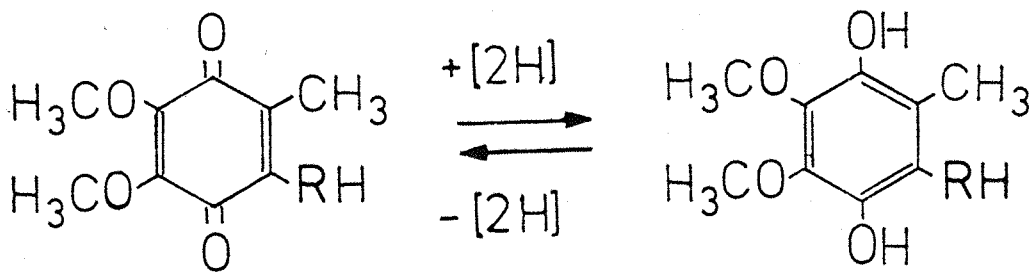
In vivo the ubiquinone molecule was observed to be present in the reduced state when respiration was inhibited by cyanide, and could be re-oxidized Fig (1.2.2). In addition, the extraction of ubiquinone by pentane or acetone resulted in loss of succinate oxidase activity (1).

The general role of ubiquinone has been identified as oxidising flavoproteins of the inner mitochondrial membrane and reducing the cytochrome b complex within the respiratory chain (1) as shown in Fig (1.2.3). Within biological systems, mitochondria are the site of phosphorylation of ADP coupled to electron transport, Fig (1.2.4). This process was found to be coupled to electron transfer by the pH gradient across the inner mitochondrial membrane. Some species such as dinitrophenol which can shunt a pH gradient were found to be uncoupling agents, inhibiting phosphorylation. Mitchell suggested that these effects indicated that the presence of the pH difference could replace the need for an intermediate "high energy" species, (2).

Hatefi et al (14) isolated NADH-ubiquinone reductase and ubiquinone-cytochrome c reductase around 1962. Work at the insitute for enzyme research at the university of Wisconsin, (1) resolved the respiratory chain within beef heart mitochondria into four complexes (see Fig 1.2.5) :

Fig (1.2.2)

Reduction and oxidation of ubiquinone.



Simple scheme for the respiratory chain.

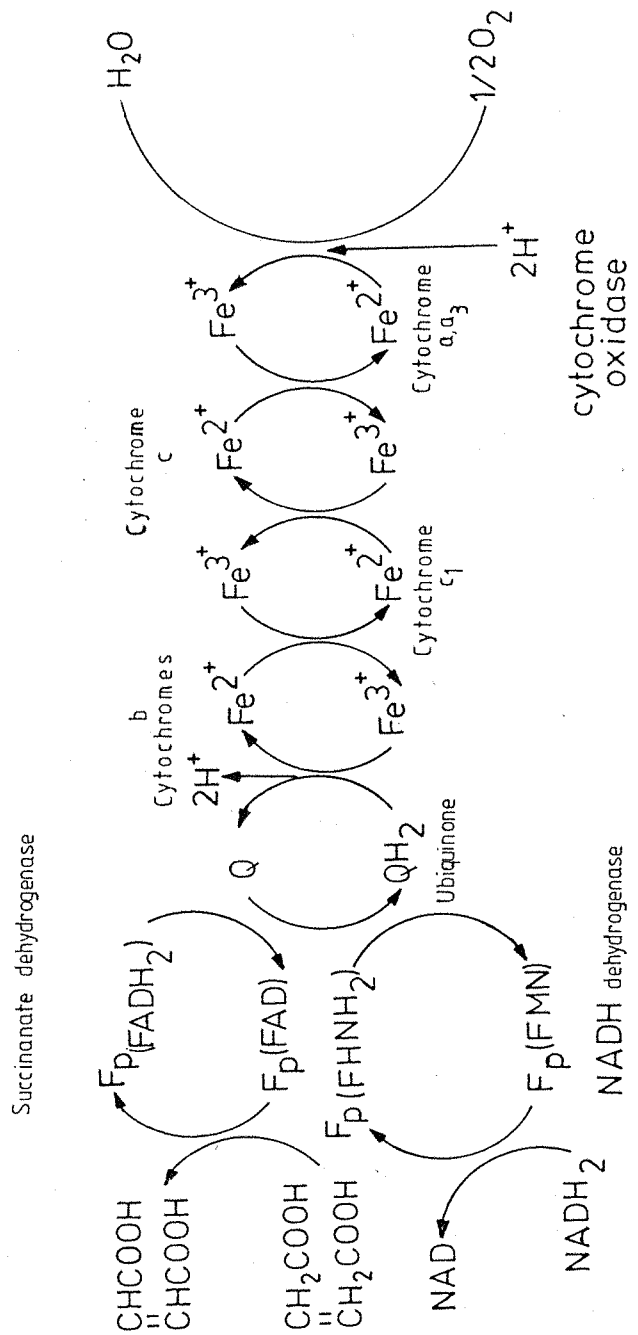
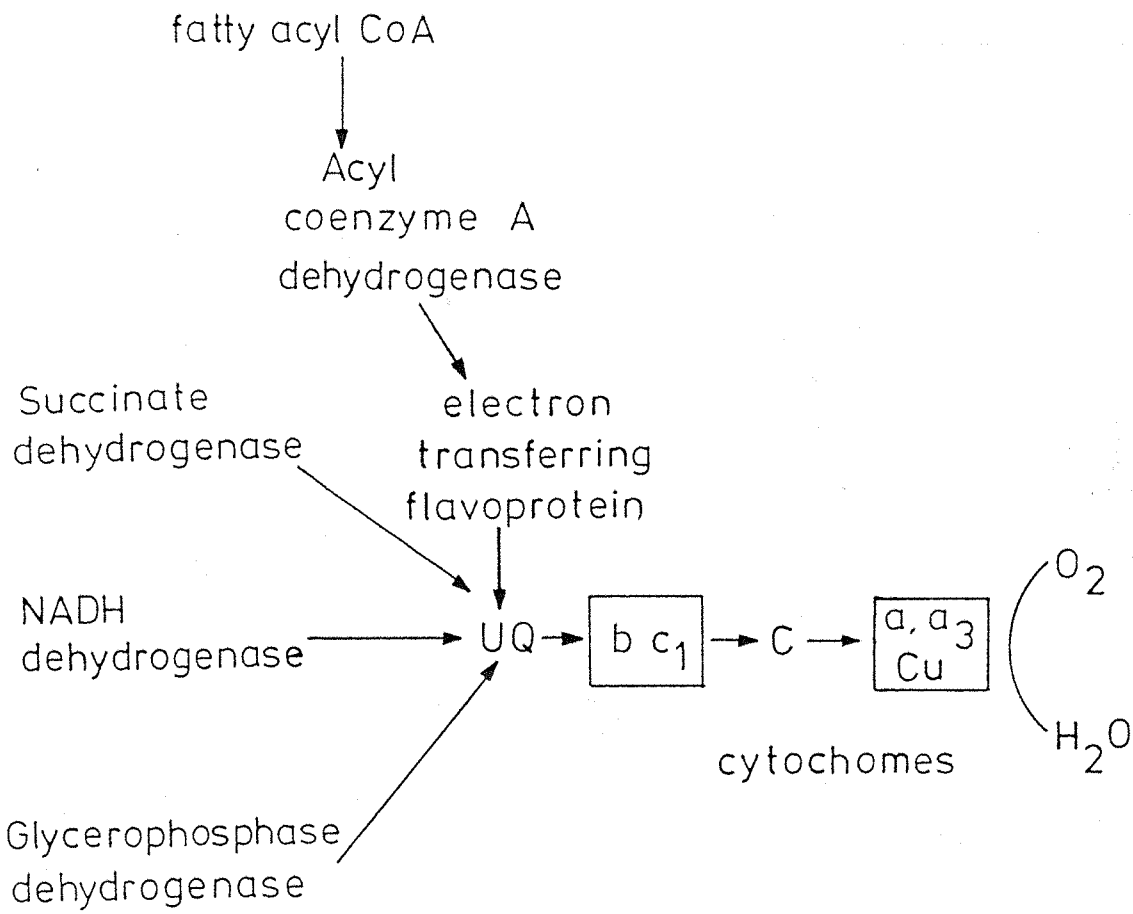
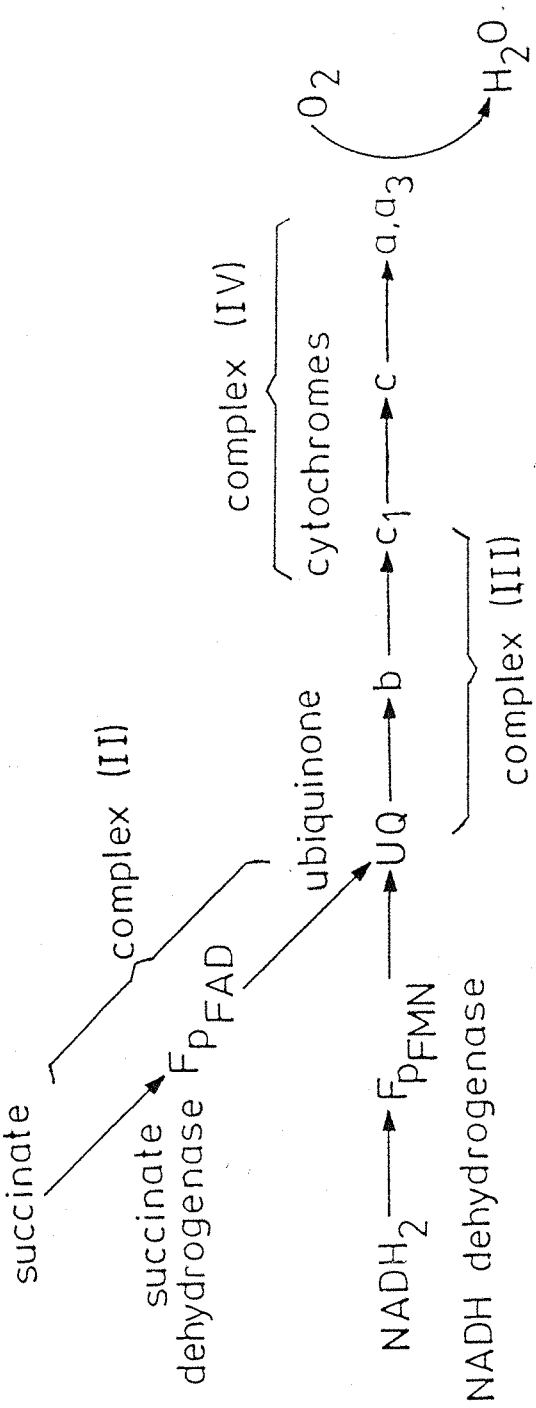


Fig (1.2.4)

Ubiquinone charge coupling within the respiratory chain



Four complexes of the respiratory chain



(I); NADH - ubiquinone reductase.

(II); Succinate - ubiquinone reductase.

(III); Ubiquinone - cytochrome c reductase.

(IV); Cytochrome c oxidase.

The incorporation of complex (III) from the mitochondrial respiratory chain into phospholipid vesicles by Leung and Hinkle (15), led them to link ubiquinone-cytochrome c reductase activity to the outward translocation of protons across the inner mitochondrial membrane. An $H^+/2e^-$ ratio of 1:0.9 was obtained, which clearly showed that complex (III) could translocate protons through a redox process, but the mechanism was not determined.

Ragan (16) showed that NADH-ubiquinone oxidoreductase (complex I), from pig brain mitochondria and the ubiquinol-cytochrome c oxidoreductase (complex III) from bovine heart mitochondria, combine in a 1:1 molar ratio to give the NADH-cytochrome c oxidoreductase (complex I - complex III). The reduction of NADH by complexes (I) and (III) was biphasic. The slow and fast phases of reduction were determined by the proportion of the total complex (III) associated with complex (I).

Mitchell in 1961 (2) proposed that the coupling process of phosphorylation could be via a "chemi-osmotic" mechanism. He suggested that the gradient of protons across the inner mitochondrial membrane acts in the place of a "high energy" intermediate. This gradient was the driving force for the synthesis of ATP by ATP synthetase, which then dissipates the proton gradient by translocating protons inwards.

The proton motive force across the membrane is the sum of the membrane potential and the hydrogen ion concentration gradient Eqn

(1.a).

$$\Delta p = \Delta\psi - \frac{2.303\ RT\ \Delta pH}{F} \qquad (1.a)$$

where Δp = proton motive force, $\Delta\psi$ = the membrane potential, and ΔpH = the proton gradient in pH units.

The matrix is the region of the mitochondria enclosed by the inner mitochondrial membrane. The cytosol is the region enclosed by the outer and inner mitochondrial membranes (see Fig 1.2.6).

The proton motive force that drives ATP synthesis is generated by the resiratory pathway. Each of the three proton translocating loops move protons in the outward, matrix to cytosol (m to c) direction, and electrons in the inward, (c to m) direction (17). This results in proton transport across the membrane, Eqn (1.b)

$$Ared + Box + 2H^{+}(m) \longrightarrow Aox + Bred + 2H^{+}(c) \qquad (1.b)$$

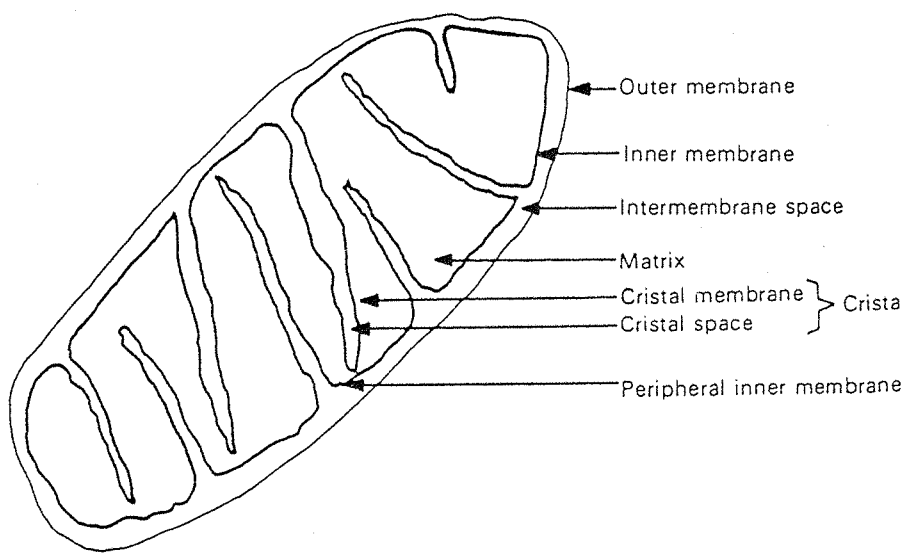
The absence of other components translocating protons in the mitochondria, led Mitchell to first propose the "Q-cycle" (3), Fig (1.2.7).

Ubiquinone is proposed to act as the proton carrier and involves the reduced and oxidized states, together with the semi-quinone radical. Electron paramagnetic resonance, E.P.R. studies have suggested the existence of the radical (18-22). In addition, recent work has suggested a mobile semiquinone radical that may move between sites within the cytochrome bc complex (23).

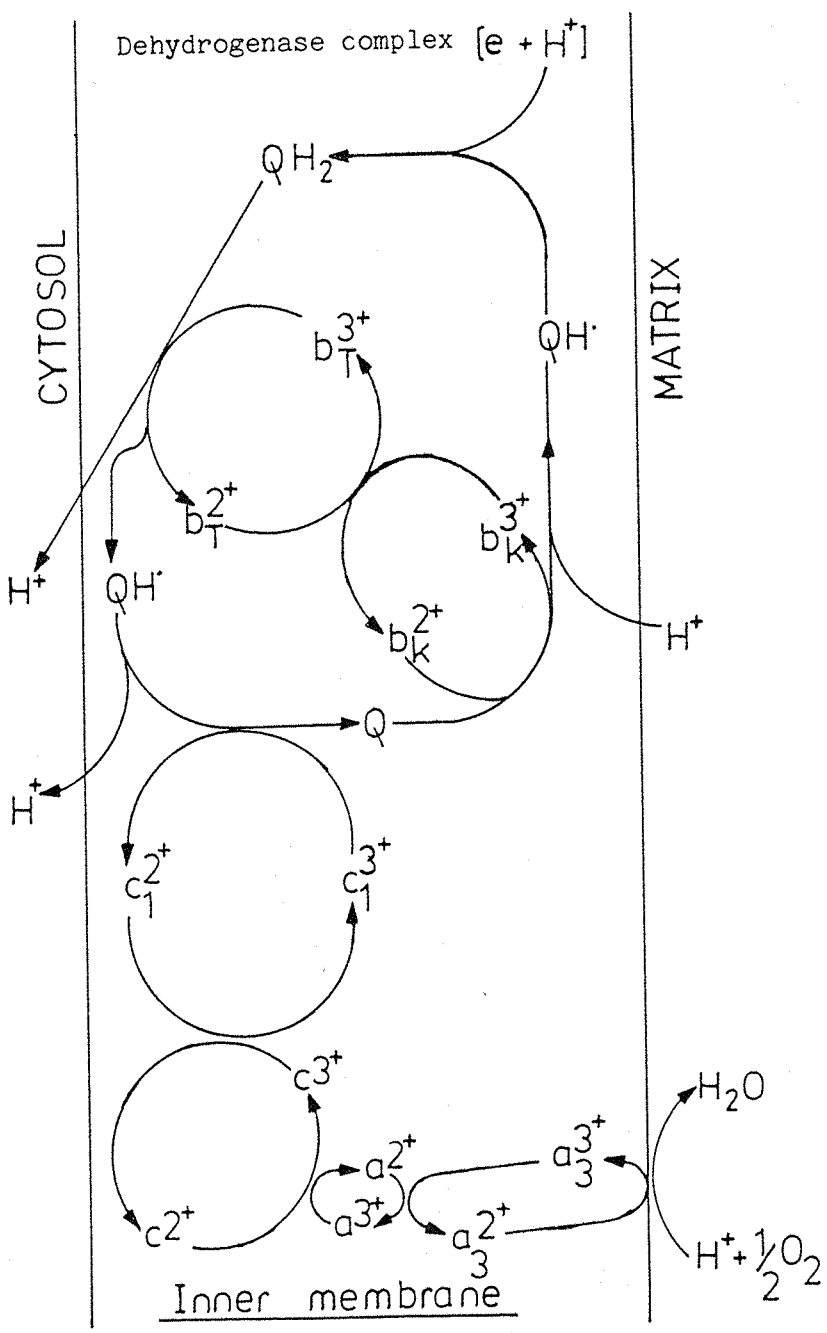
Prior to the present work, some electrochemical studies have been carried out with ubiquinones. The first study was reported in

Fig (1.2.6)

Structure of the mitochondrion



Q cycle


Dehydrogenase complex [e + H+]
QH2
CYTOSOL
MATRIX
bT3+
QH·
bT2+
bK3+
H+
QH·
bK2+
H+
Q
H+
c12+
c13+
c3+
c2+
a2+
a3+
a33+
a32+
H2O
H+ + ½O2
Inner membrane

1961 by V. Moret et al (24). This work did not reveal much information. The authors used a polarographic technique and found "reversible" electrochemical behaviour for several ubiquinones in ethanol/water mixtures. The authors also reported that no adsorption was observed, but this observation has been refuted by all subsequent work (25-27).

The next report of electrochemical studies on ubiquinone was in 1969 (26), on UQ_6 in aqueous methanol. A 90% methanol/water mixture was used so that the ubiquinone could be dissolved. Cyclic voltammetry was performed at a hanging mercury drop electrode (HMDE). For dilute UQ_6 solutions, the reaction occured initially in the adsorbed state, whereas for concentrations greater than approximately 0.04 mM, both the electrochemistry of the adsorbed ubiquinones and of the solution species could be observed. This was seen by the range of separation of the anodic and cathodic peak potentials which varied between 280 mV and 50 mV for different concentrations of ubiquinone. It was concluded by the authors that the overall oxidation/reduction involved a two electron step, and probably two protons.

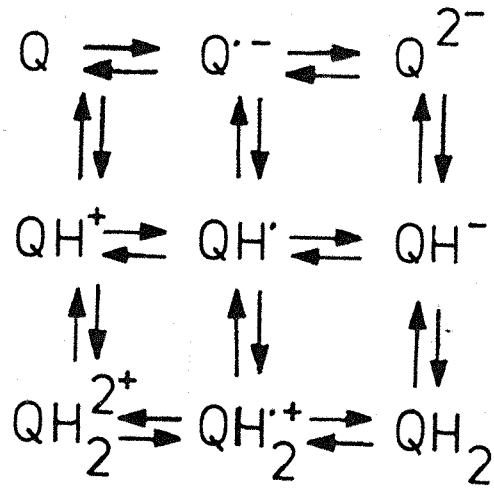
Electrochemical properties of UQ_1 and UQ_6 in an aprotic solvent, acetonitrile, with reference to the influence of various proton donors have also been investigated (25,26). The first study was on the electrochemical reduction of UQ_1, (25). The final product of the two electron reduction was shown to be the hydroquinone. No evidence was found for chromanol formation, Fig (1.2.1), either by cyclic voltammetry or double potential step chronoamperometry. Cyclic voltammetry showed that the reduction occurs in two discrete, one electron steps, both of which are reversible. The proposed reaction sequence Eqn was :

$$Q \underset{-e}{\overset{e}{\rightleftharpoons}} Q \underset{-e}{\overset{e}{\rightleftharpoons}} Q \qquad (1.c)$$

The second study was on UQ_6 in acetonitrile by cyclic voltammetry on a platinum electrode (26). The standard potential of ubiquinone was shown to shift to more positive potentials by the addition of acids as proton donors.

It was shown that a complete reaction pathway of all possible intermediates must be considered and this is shown in Fig (1.2.8). As can be seen, very little work has been done on the electrochemical properties of ubiquinones. It is expected that the properties of the compounds will differ somewhat from those of unsubstituted or hydrophilic quinones due to the presence of a very large hydrophobic residue in the former case, and as has been discussed before, this results in adsorption effects which are difficult to separate from electron transfer reactions to species in the bulk.

Possible reaction pathways and intermediates for the reduction and oxidation of UQ_{10}.



1.3 Adsorbed species at the mercury/water interface and other biomembrane mimetic systems.

Adsorbed phospholipid monolayers at the mercury/water interface have previously been recognized as having potential for mimetic modelling of biomembrane charge transfer reactions (4,5).

Other mimetic systems that have been studied previously have included phospholipids at the air/water interface, and particularly studies of lipid vesicles, by Hinkle (28,29), and later by other workers (30-32). The latter system relies on ferricyanide held within the liposomes being reduced by an external lipid insoluble species such as dithionate or ascorbate. It is assumed that the reduction of the ferricyanide may only occur by means of a lipid soluble species held within the lipid bilayer, acting as a mediator in a redox loop.

This system may yield useful information as to the relative efficiency of a species to act as a charge carrier across a lipid bilayer. However, there are limitations to the information that may be obtained from these systems. It is assumed that there is no mixing of the species held within the liposomes and the external reductant. In practice some liposomes will break-down, and so control experiments without the mediating species have to be performed. The extent of reduction of the internal species may then be calculated and subtracted from the results. The extent of reduction of the internal species is measured by U.V. spectroscopy typically at 420nm for ferricyanide, or via stop-flow apparatus for optimal time resolution (31). Little information may be obtained by these methods with respect to the mechanism of charge transfer across a membrane or of physical interaction of the mediating species with the

phospholipid layers.

Hinkle developed the model system of a vesicle in 1970 (28), for use as a biomimetic system of the inner mitochondrial membrane. His aim was to model the electron transport across the inner mitochondrial membrane as proposed in the chemi-osmotic theory (2). Previous work had shown that the uncoupling agents of oxidative phosphorylation could increase the proton permeability in both natural and artificial membranes (33-35). Hinkle reasoned that uncouplers should increase the rate of transmembrane electron transfer by preventing the formation of a retarding membrane potential. Hinkle prepared liposomes by the sonication of soybean lecithin in excess ferricyanide solution, and 1mM EDTA for an hour at $4^{o}C$. Ferricyanide was used as the internal species, with ascorbate as the external reductant.

The results obtained showed that the uncoupling agents of oxidative phosphorylation increased the rate of ferricyanide reduction five fold, which was accompanied by an uptake of protons into the vesicles.

Hinkle showed, using the same model system, that the translocation of protons accompanies ATP hydrolysis (36). Two protons were translocated for each pair of electrons traversing a coupling region of the respiratory chain. Hinkle used the vesicle model to show that the electrochemical gradient of protons couples to the oxidative phosphorylation pathway across the mitochondrial membrane (29), by the incorporation of cytochrome oxidase into the model membrane so that energy conservation was retained.

Deamer et al (30) used fluorescent probes for measuring pH gradients across membranes in order to test the postulates of the

chemi-osmotic theory (2). It was shown that a difference of four pH units could arise, when dyes were used as mediators in vesicles which accepted hydrogen ions upon reduction. Liposomes could therefore be used as a biomembrane system in which controlled pH gradients could be established.

Light induced proton uptake and transmembrane pH differences in lipid vesicles were studied by Hauska et al (31) by the measurement of light induced quenching of 9-amino-fluoroscein in chloroplasts. These authors used various redox compounds and compared the results to when no light pulses were applied.

Hill et al (32) quantitatively compared the rates of proton translocation across lipid membranes by the use of lipid vesicles with different dyes.

Ubiquinones and benzoquinones with variable chain length have been incorporated into lipid vesicles to investigate their relative efficiency as electron translocating agents (37). Again external ascorbate and internal ferricyanide were used as the oxidant and reductant. Hauska concluded that the longer chain homologues proved to be more efficient as electron translocating agents than the shorter chain homologues.

Langmuir-Blodgett films of lipids have been utilized as biomembrane mimetic systems (38,39) as well as being used in electronic devices (40). Investigations have been carried out to elucidate the function of various species within the photosynthetic pathway (41,42). Langmuir-Blodgett films may be formed by the placing of layers of lipid between electrodes. Layers of varying thickness may be formed by the succesive building up of monolayers. The Langmuir-Blodgett film technique may be used to form monolayers,

bilayers or thin films up to five hundred layers thick between metal electrodes without imperfections or holes in the molecular arrangements. The Langmuir-Blodgett technique has been used to investigate several biological processes in which charge transfer occurs. For example, Gershfield (43), studied the influence of anaesthetics on lipid monolayers. The Langmuir-Blodgett technique has also been developed to form mimetic bilayers to model the complete biological membrane (44).

Recently phospholipid monolayers have been studied at the interface between two immiscible electrolyte solutions (ITIES) (45). The presence of the adsorbed phospholipid layers at the "liquid/liquid" interface was found to be potential dependent (46), and the theory of ion transport across the layers has been developed (47).

Phospholipid layers adsorbed on a mercury electrode surface have been studied in a variety of ways, the present work being a further extension of these techniques.

The first work at a mercury electrode surface was performed on a dropping mercury electrode. Adsorption of the phospholipid on the mercury surface taking place as each drop formed at the nitrogen(g)/water interface (48). Investigations were carried out to determine the characteristics of a monolayer of egg lecithin or phosphatidyl serine (from bovinespinal chord). Large adsorption/desorption pseudo-capacitance peaks were observed at high positive or negative polarization potentials. It was concluded that the relaxation time for the reformation of the monolayer was of the order of 0.5 to 1 ms, depending on the sweep rate of the voltammetry. In addition, it was shown that polypeptides could penetrate the

monolayer of phosphatidyl serine and, in some cases, the monolayer of phosphatidyl choline. This was deduced from the frequency by which polar groups, by lateral diffusion or flipping motions, could gained access to the mercury surface. This in turn was estimated from the shift of the potential of the reduction peaks of an electroactive dinitrophenol label, which was introduced to a small percentage of the polar groups. In addition, an estimation of the penetration of the polypeptides could was made by the size of the pseudo-capacitance reduction peaks of the oligiopeptide disulphide S-S bond. This technique was further developed by the use of a hanging mercury drop electrode.

The present work is a development of the technique described in the recent investigation of the adsorption of phospholipid monolayers on a hanging mercury electrode (4). A variety of adsorbed phospholipids were studied including dipalmitoyl PC, dioleoyl PC and egg lecithin - the three lipids studied in the present work. The work was aimed at establishing the electrochemical properties of the adsorbed monolayers.

If the lipid (in a solution of pentane) was spread in excess of the collapse pressure on the surface of the base electrolyte, complete coverage could be reproducibly obtained. Spreading in excess of the collapse pressure showed no further adsorption on the mercury surface (DME). This is in ag reement with previous work by Miller et al on the dropping mercury electrode (48-50), and later by Nelson on the HMDE (4).

The HMDE could be lowered from the oxygen free atmosphere through the air/water monolayer interface and thus into the bulk electrolyte solution (KCl) (as described in chapter two). It was

deduced that the adsorbed monolayers are solvent free. Due to the volatility of pentane, it was assumed that after a period of several hours, no solvent remains within the monolayer at the water/air interface. Cyclic volatamograms obtained by passing mercury drops through the interface several hours after spreading, and those obtained after a few minutes, proved to be identical. Therefore the pseudo-capacitance peaks obtained are not due to artifacts of solvent molecules within the phospholipid.

The results obtained (4) showed electrochemical properties that were characteristic of each lipid. Due to the stability of the monolayers over a period of several hours, a system had been developed where the electrochemical properties of adsorbed monolayers could easily and consistently be recorded.

Pseudo-capacitance values were measured from both ac and dc cyclic voltammetry. The results agreed to within 2% of each other. This suggests nearly total capacitive current was observed, so justifying estimations of capacitance from cyclic voltammetry on this system. Repeated scans between +0.06 and -1.5V versus Ag/AgCl were reproducible and no net desorption on the mercury surface was observed. In addition, the drop was held within the bulk electrolyte and so was not in dynamic equilibrium with the air/water/phospholipid interface.

A large region of low capacitance was found between the approximate potentials of +0.25 to -0.7 V versus Ag/AgCl. The value of this capacitive minimum varied between lipids but was always close to 2 $\mu F/cm^2$. This layer appeared to be insulating so inhibiting ionic faradaic processes. It was proposed that the lipid resided with the hydrocarbon moiety pointing towards the mercury. A theoretical

calculation of the capacitance, assuming a hydrocarbon chain length of 2.5nm, yielded a value of around 0.7 $\mu F/cm^2$. Nelson (4), found that drop expansion caused a thinning of the monolayer, and gave an increase in the value of the capacitance in the minimum region. It has been suggested that the values obtained can be explained in terms of overlapping or twisting of the hydrocarbon chains, so causing a thinning of the monolayer. In addition, continuous scanning over a period of hours caused a small increase in the value of the capacitance in this region. It must be assumed that some small desorption must occur from the mercury electrode surface under these conditions over a period of time.

This low capacative insulating region is of importance for the present study involving the incorporation of ubiquinone UQ_{10} to the system. Ubiquinone UQ_{10} has a mid point potential within the respiratory chain of approximately -202 mV with respect to the saturated calomel electrode (S.C.E.) (1).

Nelson et al (4) also reported peaks negative to the low capacitive region, that were attributed to re-orientations of the lipid on the mercury surface (4). The peaks showed little temperature dependence up to $45^{o}C$, and showed only small changes in capacitance, with variation of sweep rate. It was suggested that the reorientation peaks represent a thinning of the monolayer by compression of the monolayer.

Croxley has predicted a quadratic dependence of the capacitance measured on the voltage applied. He concluded that the electroconstrictive force (in $dynes/cm^2$) is of the order of eight times that of the Young's modulus for the same lipid. This could therefore account for electroconstriction overcoming the

elasticity of the lipid (51).

It should also be noted that capacitance peaks at around -0.2 V (versus Ag/AgCl) are also attributed to reorientation peaks of the lipid (4).

1.4 Kinetic studies of ubiquinone

Several studies to deterime the kinetics of ubiquinone reactions within mimetic models have been performed.

As mentioned before, Mitchell originally suggested that coupled electron and proton transport occurs across the inner mitochondrial membrane by a chemi-osmotic mechanism (2). This was later developed to form the proposed Q-cycle, where ubiquinone acts as the redox mediator (3). A similar mechanism was proposed for the electron and proton translocation across the inner chloroplast membrane by means of plastoquinone (5).

Kroger et al (52) showed that UQ_{10} would have to flip from one surface of the inner mitochondrial membrane to the other, at least four times per second, to function in the Q-cycle, if it was assumed that the process involved the translocation of two electrons.

The rate of the possible diffusion of ubiquinone across the membrane has to be considered. Other factors such as interactions by hydration forces or electrostatic forces could also affect the rate of movement of the ubiquinone molecule across a membrane.

Ubiquinone possesses a polar quinone ring, and a hydrocarbon isoprene chain. The structure therefore possesses structural similarities to a lipid molecule. It would therefore be expected that the diffusion coefficient of ubiquinone would be of a similar magnitude to that of a lipid. A typical value for the diffusion coefficient of a lipid is 10^{-8} cm^2s^{-1} (53). If the inner mitochondrial membrane is assumed to have the cross section of a lipid bilayer and that each hydocarbon chain length is 2.5nm (4), then the ubiquinone molecule would be able to diffuse across the

membrane and back again 400 times per second. Kornberg et al (54) studied lateral diffusion of phospholipids within phoshatidyl choline vesicles by spin labelling N.M.R. techniques. They concluded that the frequency of the molecular translation step was considerably in excess of $3x10^3$ s^{-1}. Miller et al (48) studied the electrochemical behaviour of adsorbed monolayers on the dropping mercury electrode (D.M.E.), and the effects of adding polypeptides. They concluded that a significant number of indentations existed within adsorbed monolayers. The number of these indentations increased when polypeptides were added to the monolayer. Direct adsorption of the polypeptide was therefore allowed to occur on the mercury surface. This has kinetic implications for the present work, as adsorption of the ubiquinone on the mercury surface within adsorbed monolayers could present a possible rate determining factor for it's redox behaviour.

Considerable interest has been shown towards the interactions of quinones of varying hydrocarbon chain length and the lipids in which they are incorporated. Until the present work was undertaken all work had been directed towards investigating the effects of changing functional groups of the quinone molecule and in particular the length of the isoprene chain.

Hauska et al (37,55,56) and Ohnishi et al (57), investigated the efficiency of quinones as redox mediators for different hydrocarbon chain lengths. The investigations were performed by trapping ferricyanide within sonicated vesicles of phophatidyl choline, as previously described (Section 1.3). The external reductant was dithionite. The results showed that the longer chain homologues were more efficient as redox mediators than the quinones

of shorter chain length. This clearly showed that molecular interactions between the quinones and the lipid or between the quinone with further quinone molecules, affected the rate of electron/proton transport across the vesicle membrane. Using spectroscopic stop-flow measurements, Hauska et al (37,55) showed that the rate limiting step was not that of the flipping motion of the quinone/quinol ring.

Cain et al (58) used X-ray diffraction techniques on disk membranes to show that UQ_3, strongly affects the packing shown by dipalmitoyl PC when it is reduced and oxidised. Their results suggest that the ubiquinol (reduced form of ubiquinone) may gain access to an aqueous phase, where hydration forces and counter ion interactions occur. This holds possible kinetic implications for the present project, as it is expected that the ubiquinol would try to gain access to an aqueous phase, (See discussion). Kornberg and McConnell (54) showed, using N.M.R. techniques, that UQ_3 caused a more regular packing of lecithin hydrocarbon chains, when incorporated in bilayers of egg lecithin. This was explained by the possible interaction of hydroquinone protons with the polar headgroup region of the lecithin producing a specific reduced UQ_3 lecithin interaction, thus inducing a more regular chain.

If it is assumed that the electron/proton translocation occurs principally by the physical movement of some oxidation state of UQ_{10}, then three possible species may be responsible for transport across the membrane ; the ubiquinone, the ubiquinol, or the semiquinone radical. On reduction of the ubiquinone or oxidation of the ubiquinol, the semiquinone radical $QH^{\bullet}$ might be expected to be formed in a one electron reduction or oxidation step respectively. Evidence

has been found for the formation of the semiquinone radical using E.P.R. techniques by Ohnishi et al (18-22, 57) and by Futami and Hauska (57). Futami and Hauska only found evidence for the formation of the semiquinone radical with quinones lacking the isoprenoid chain. The authors suggested that isoprenoid quinones build domains within the membranes where the life-time of the semiquinone radical might be decreased by fast disproportionation.

Recently, Rich and Wikstrom (23) provided evidence to suggest that the semiquinone may be able to cross the inner mitochondrial membrane and act as the proton/electron translocating species. This had originally been suggested by the formation of the "b" cycle (59,60), that the formation of a semiquinone radical could act as an oxidant of the cytochrome b. Rich and Wilstrom (23) suggested that the principle of a mobile semiquinone could be combined with the main concept of the Q-cycle, and that the redox sites for the coupling of oxidative phosphorylation are situated across the inner mitochondrial membrane. This cycle has been termed the SQ cycle to distinguish it from the Q cycle or the b cycle. Mitchell (61) showed that any combination of the b and Q cycles could occur. Rich and Wilstrom (23) developed this concept to show that account had to be taken of a possible electrogenic semiquinone transfer from the centres of oxidation of quinol and the centre of reduction of cytochrome b. They realized that such a transfer must be localized thereby not allowing the equilibration of semiquinone with the Q pool.

These studies are useful in gaining a qualitative concept of the redox behaviour of UQ_{10} which governs the kinetics of such reactions. Work however has been performed to gain quantitative measurements of rate constants governing the redox behaviour of UQ_{10}.

Chance and Ericinska (62) used fluorescent probes within mitochondria to determine the relative rates of flipping of UQ_{10} within the inner mitochondrial membrane. With the use of this probe they found that by pulsing anaerobic mitochondria with oxygen, ubiquinone was shown to have a mobility that is considerably greater when the vesicle membrane is oxidized, than when it is in the reduced state. It was not possible to calculate values for the rate constants.

Futami et al (56), measured rate constants for the cyclic reduction and reoxidation of various quinones within vesicle systems. A value of 1.277 s^{-1} was measured for UQ_{10}. This value allows UQ_{10} to act in the Q-cycle, as do the values quoted for for all ubiquinones possessing two or more isoprene units. Futami concluded that the flipping motion of UQ_{10} was not the rate limiting step. Kingsley and Feingenson (63), measured rate constants for the transmembrane flip-flop motion of UQ_{10} within dimyristoyl lecithin vesicles, when ubiquinones of varying isoprene chain length were used. The values they obtained varied between 23 s^{-1} for UQ_{10} and 216 s^{-1} for UQ_1. The values measured were minimum values for the transmembrane flipping of UQ_{10}, due to limits imposed by the instrumentation. The values are approximately twelve times higher than the rate constants quoted for the electron/proton translocation across the vesicle membrane by Futami (56) and it was therefore concluded by Kingsley and Feigenson that the rate determining step was not the flip-flop motion of UQ_{10}.

Rich (64) investigated the pathways or redox equilibration of quinols and quinones in aqueous and aprotic solvents. They found that the rate determining step involved the couple $UQH^-/UQH^\bullet$ of the reducing quinol and the couple $UQ^{\bullet -}/UQ$ of the oxidizing quinone. It

was suggested that the reduction and oxidation reactions could therefore have separate rate constants with values of significant difference.

CHAPTER TWO

EXPERIMENTAL

2.1 Introduction.

Cyclic voltammetry has been used throughout the course of these investigations to study charge transfer reactions of ubiquinone UQ_{10} across an adsorbed phospholipid monolayer at the mercury/water interface.

A purpose built cell and hanging mercury drop electrode (HMDE) were constructed, which were used thoughout all the investigations (Fig 2.1).

The effects of concentration of ubiquinone within the lipid monolayer, the use of different lipids, sweep rate, the potentials of upper and lower sweep limits, consecutive cyclic sweeps, variations of time intervals between succesive sweeps, and pH were investigated.

2.2 Chemicals, preparation, and storage of solutions

Three lipids were used throughout the present work;

a) Dioleoyl phosphati dylcholine: (dioleoyl P.C. or dioleoyl Lecithin).

b) Dipalmitoyl phosp hatidylcholine: (dipalmitoyl P.C. or Dipalmitoyl Lecithin).

c) Egg phosphatidylcholine : (egg P.C. or egg Lecithin). All were Grade A quality and were obtained from Lipid Products, Nutfield nurseries, Nutfield, Surrey.

All the lipids were supplied in solution in a methanol/chloroform mixture within sealed ampules.

The lipids were stored at $-25^{\circ}C$, in glass flasks, with glass stoppers. The remaining air space was flushed with high purity Argon (BOC Ltd), prior to storage and each time after the flasks were opened.

Ubiquinone was supplied from the Sigma corporation, and subsequently stored at $-25^{\circ}C$.

During preliminary investigations it was found that lipids were extremely sensitive to oxygen. This was in accordance with results, by Nelson (4). Traces of oxygen were found to be present in the nitrogen origi nally employed (BOC Ltd), so Argon (BOC Ltd) was used for all further work. The lipid solutions were only opened to the atmosphere for the minimum possible periods of time.

Flasks together with all the glassware were cleaned by soaking in a 98% $H_2SO_4/KMnO_4$ solution overnight, followed by rinsing with high purity water, and then placed in an atmosphere of steam for two hours.

All solutions of ubiquinone and lipids were prepared in HPLC grade pentane (Fisons Ltd) so that 100 µl of the lipid/pentane or lipid/ubiquinone/pentane solutions were of the required concentration to form a complete monolayer surface of the base electroyte solution.

Initial investigations showed that solutions prepared in pentane of both lipids, and of ubiquinone could be kept for periods of weeks without degradation. However solutions containing both a lipid and ubiquinone in pentane, suffered degradation. This was seen by the appearance of new fractions in thin layer chromatography, and a corresponding irreproducibility in the electrochemical behaviour. Solutions containing lipid and ubiquinone in chloroform (HPLC grade - BDH chemicals Ltd.), also suffered a similar degradation.

It is suggested that catalytic cleavage of double bonds within the lipid in the presence of ubiquinone could be a possible explanation for this effect. This was inferred from the disruption of the peaks of the lipid, previously assigned to lipid reorientations (4), as seen from cyclic voltammetry. The effect was also most pronounced when dioleoyl P.C. is used.

All solutions were tested by thin layer chromatography on alumina using a (70/25/5); methanol/chloroform/water relative percentage carrier solvent mixture.

Water was prepared by re-distillation of Millipore Q water from a potassium permanganate/sodium hydroxide solution. The alkaline/permanganate was used in order to ensure the elimination of organic impurities, which could cause unwanted adsorption. $KMnO_4$ and NaOH were of "Analar" grade - (BDH chemicals Ltd).

The base electrolyte used throughout the experiments was a phosphate buffer, prepared from high purity water, and "Analar" grade

di-potassium hydrogen orthophosphate trihydrate, and potassium dihydrogen orthophosphate - (BDH chemicals Ltd). Buffers were prepared (0.1 M) from di-potassium hydrogen orthophosphate trihydrate, and then adjusted in pH by the addition of potassium dihydrogen orthophosphate. pH values were measured to two decimal places, using a Beckman research pH meter.

"Aristar" grade mercury - (BDH Chemicals Ltd), was used without further purification.

2.3 Design of Cell and Electrodes.

The cells used throughout the present work were purpose designed. Four hanging mercury drop electrodes (H.M.D.E.) and two cells were made, and used in rotation due to the lengthy cleaning and preparative processes.

The cell design is shown in Fig (2.1). The design of the cell allows the raising and lowering of the electrode, whilst passing Argon over the electrolyte surface. This prevents the entry of oxygen from the atmosphere to the cell, whilst lowering or raising the H.M.D.E. The H.M.D.E. may be lowered or raised through the modified syringe fitting, by a vernier screw clamp. The glass cell was designed to fit a Metrohm polagraphic cell lid, through which the electrodes and Argon bubbler were designed to fit. The Argon bubbler was designed so that Argon could be passed, through the electrolyte solution prior to spreading, and then over the surface of the electrolyte, after spreading and for the duration of the experiment. The flow of gas was regulated by an external two-way gas tap and screw constriction clamp.

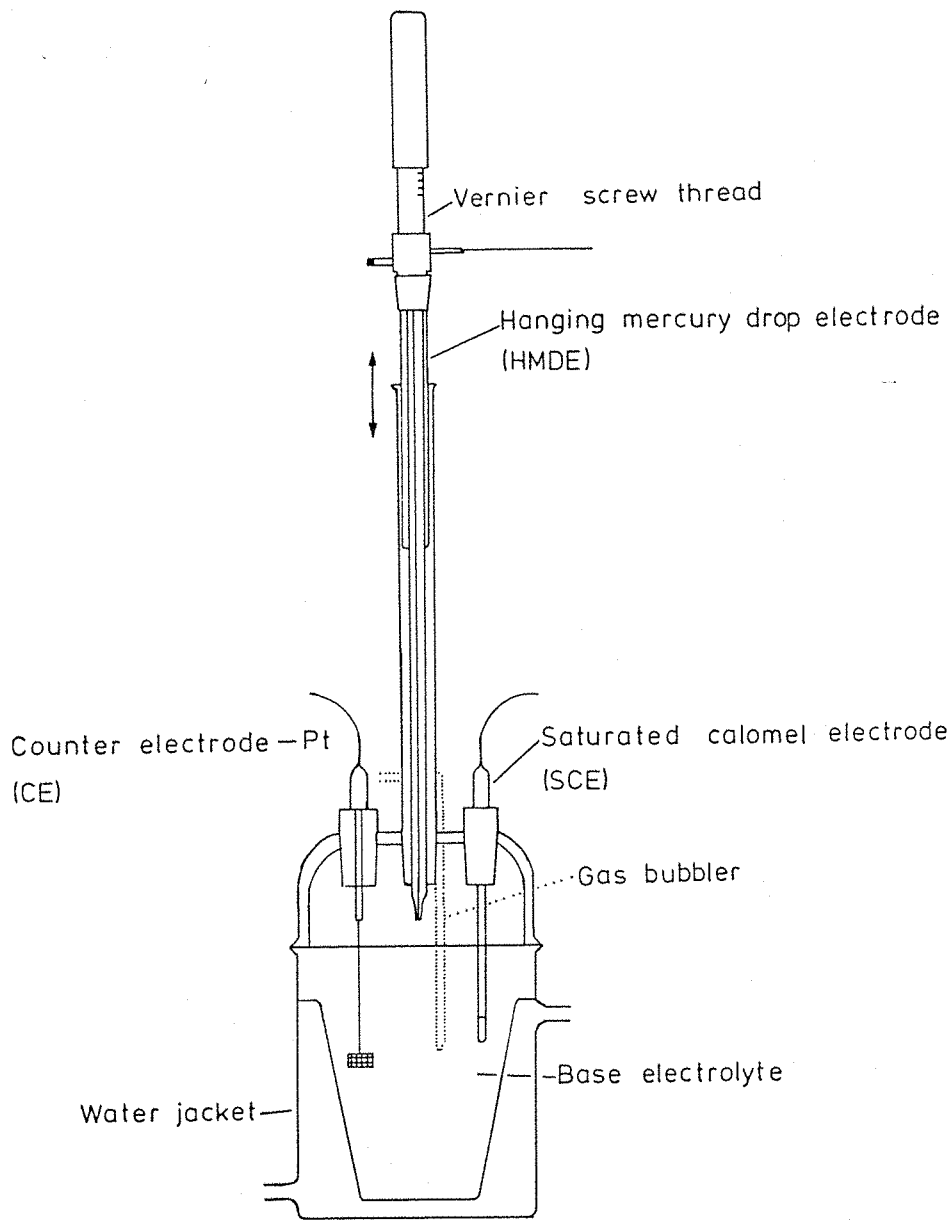
The cell was surrounded by a water jacket, for thermostatic temperature regulation.

The reference electrode used was a saturated calomel electrode. The secondry electrode was made from a piece of platinum gauze (approximatly one square cm), welded to a length of platinum wire which was mounted in a glass holder.

Mercury drops were extruded from the H.M.D.E. by an accurate calibrated vernier screw plunger. The H.M.D.E. was formed from a glass bore drawn to a finer capillary, the end being ground to a

Fig (2.1)

Electrochemical cell for HMDE cyclic voltammetry



smooth surface, perpendicular to the bore of the capillary. Before use the ends of the electrodes were examined under a microscope. Due to the accurately known extrusion of mercury by the vernier screw thread, small differences of the end capillary bore did not effect the calibration of drop sizes.

The H.M.D.E. was lightly siliconized prior to use by immersing for a few seconds in siliconising solution, and placing in an oven overnight, at 60^{o}C followed by rinsing in high purity water.

2.4 Instrumentation.

Potentials are quoted versus the saturated calomel electrode. A Hi-Tec PPR1 waveform generator was used to generate the scanning potential between potentials of -1.3 to + 0.1V, and at sweep rates between $2Vs^{-1}$ and $500Vs^{-1}$. Three poentiostats were used for different scan rates. For scan rates between $500Vs^{-1}$ and $50Vs^{-1}$, Hi-Tec "DT11005" and "DT2101" potentiostats were used. For scan rates lower than $50Vs^{-1}$, a "low-noise" potentiostat built within the University run from a regulated power supply was employed.

Cyclic voltammograms were recorded and stored in a Gould OS4100 digital storage oscilloscope, and subsequently recorded by a Bryans 2600 A4 X-Y recorder.

All experiments were performed with the cell housed in an aluminium Faraday cage, grounded to a true earth.

Temperatures were regulated by use of a thermostated Grant SE15 water bath, for temperatures at or above room temperatures, and together with an MK refrigeration unit, for temperatures below room temperature.

2.5 Methodology.

Argon was passed through the electrolyte for two hours before lipid/Ubiquinone solutions were spread. Argon was then blown over the surface of the electrolyte by reversal of the two-way gas tap.

Lipid/Ubiquinone solutions were spread onto the surface of the electrolyte by injection of a 100 µl aliquot from an Oxford sampler micropipette above the surface of the electrolyte through a socket in the lid of the cell. The area of the electrolyte surface was calculated to be $28cm^2$.

The solvent was allowed to evaporate for at least fifteen minutes before a mercury drop was passed through the lipid monolayer. A mercury drop was formed above the surface of the electrolyte. The drop was formed with a diameter of 0.902 mm, corrosponding to a surface area of 2.56×10^{-6} m^2, by turning of the vernier screw plunger. Calculations were performed by the formation, subsequent collection, and weighing of one hundred drops, and then further repeating the results. Agreement with capacitance results of Nelson to within one percent, for adsorbed dioleoyl P.C. monolayer gave further confirmation of the correctness of calibration of drop size, (4).

The mercury drop was lowered gently through the monolayer, into the bulk electrolyte. Cyclic voltammetry was then carried out in the normal way.

Successive drops could be formed above the surface of the electrolyte, by raising and lowering of the H.M.D.E.

Aliquots containing both lipid and ubiquinone in solution were

prepared by mixing 400 µl aliquots from this mixture for spreading. The mixing was carried out immediatly before spreading, to prevent degradation of the lipid, as already described (section 2.2).

The lipid was spread in excess of 0.3 µg cm^{-2}, corresponding to spreadings greater than the collapse pressure. Percentage incorporations of Ubiquinone were calculated from these values. In accordance with earlier observations (4), coverages greater than 0.3 $\mu g/cm^2$ of the electrolyte surface, did not result in increased adsorption on the mercury drop.

CHAPTER THREE

RESULTS AND DISCUSSION

Results.

3.1 Introduction.

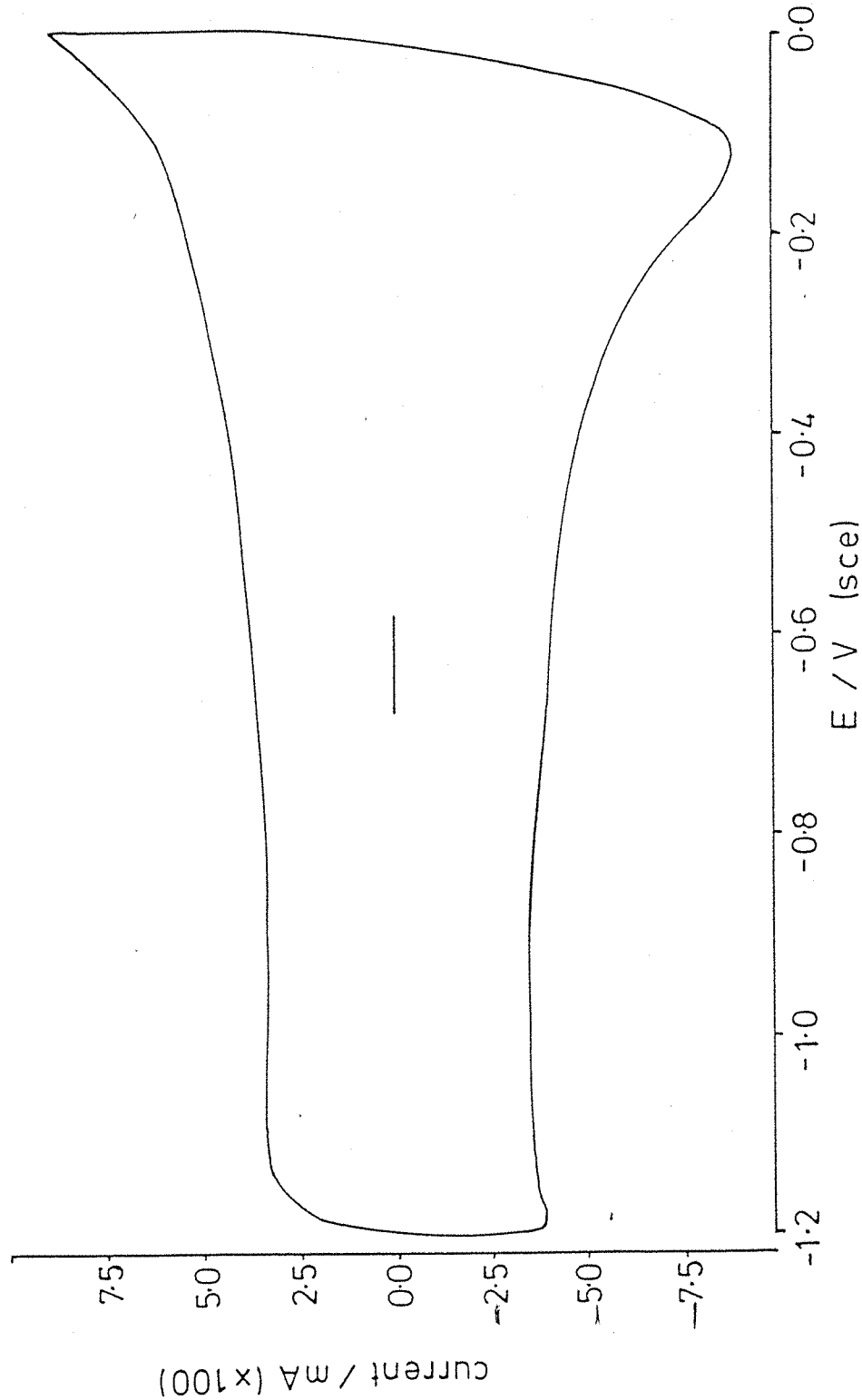
Experiments were performed using cyclic voltammetry, to investigate the electrochemical state of UQ_{10}, incorporated within monolayers of phospholipids adsorbed at the mercury/water interface. The comparative redox behaviour of UQ_{10} within monolayers of different lipids, the effect of the upper and lower limits of potential sweep, the incorporation of time delays whilst under potential control before starting a new sweep, the concentration of UQ_{10}, the pH of the base electrolyte, and the temperature, were investigated. In addition rate constants were measured for the reduction and oxidation of UQ_{10}, within three different phospholipids.

3.2.1 Voltammetry of base electrolyte.

The base electrolyte used throughout this work was K_2HPO_4/KH_2PO_4 (pH 8.1), which as expected showed no electroactive behaviour between the potentials of 0.0 and -1.2V (Fig 1.2.1).

Fig (3.2.1)

Current vs potential by cyclic voltammetry
scan rate 500 Vs^{-1} 0.0 to -1.2 V
at 22°C, pH 8.1 base electrolyte (K_2HPO_4/KH_2PO_4)



3.2.2 Voltammetry of adsorbed lipid monolayers on mercury.

The potential dependence of the pseudo-capacitance/current at $500Vs_{-1}$, for the three lipids, dioleoyl PC, dipalmitoyl PC, and egg lecithin between 0.0 and -1.2V are shown ; (Fig 3.2.2).

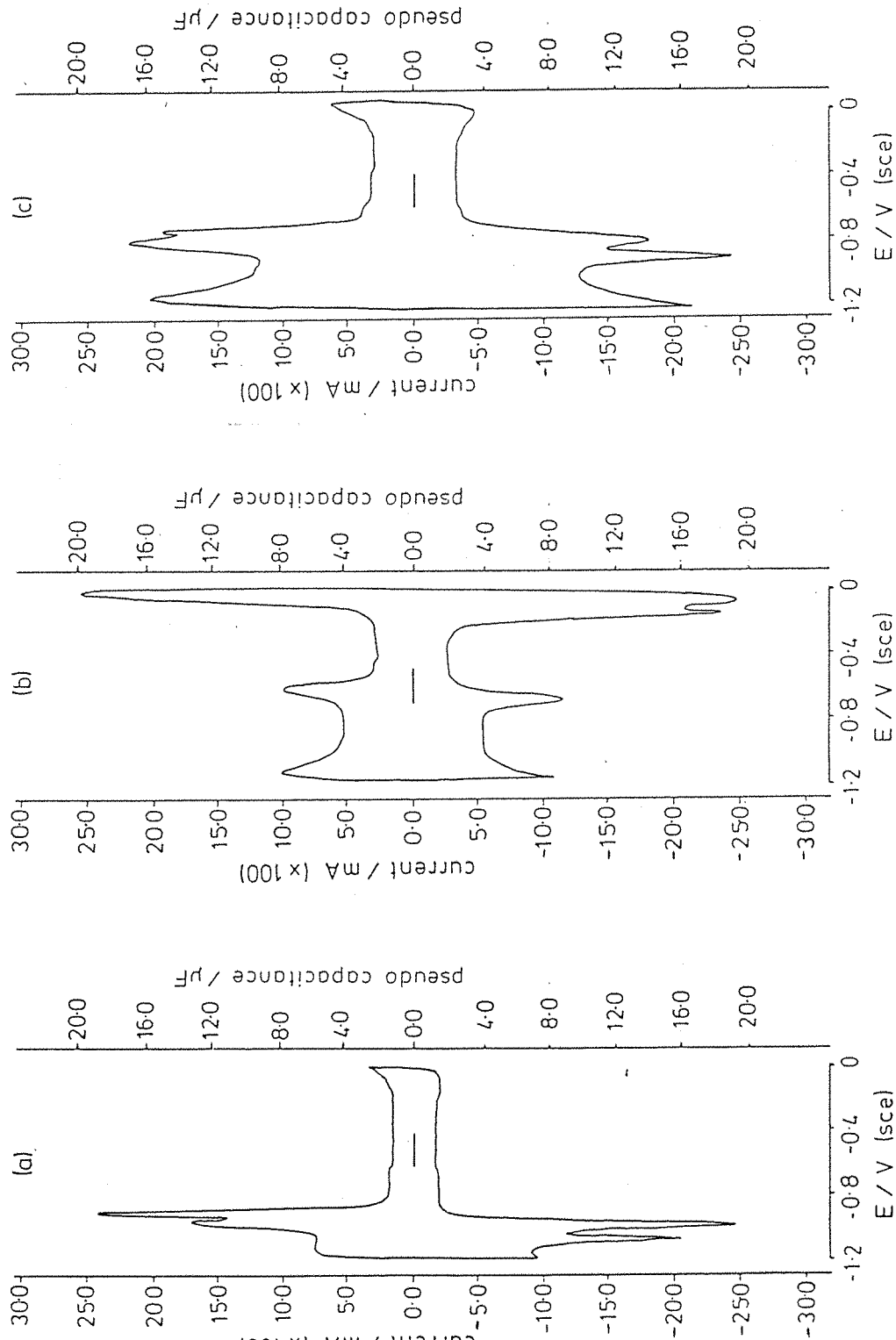
All three lipids show capacitance peaks between the potentials of -1.2 and -0.0V. The existence of these peaks has previously been reported for dioleoyl PC, and egg lecithin (4). Dipalmitoyl PC in addition shows peaks on the cathodic and anodic sweeps between the potentials of -600mV and -680mV, together with peaks extending from 0.0V to -200mV on the cathodic sweep, coupled to one peak at -10mV on the reverse sweep.

Dioleoyl PC shows a low capacitive region extending from about -200mV to -840mV, egg lecithin shows a low capacitive region between the potentials of -100mV and -700mV. The minimum capacitance values for this region for dioleoyl PC and egg lecithin respectivly are 1.57 and 1.95 $\mu F/cm^2$. The minimum capacitive value for dipalmitoyl PC is 1.94 $\mu F/cm^2$ at a potential of -400mV.

Volat mmograms at sweep rates below $50Vs^{-1}$ show no further change in capacitance values. The capacitive minimum value of dioleoyl PC increases to a value of 2.0 $\mu F/cm^2$, whilst that of egg lecithin increases to a value of 2.14 $\mu F/cm^2$ at low sweep rates.

pseudo-capacitance / current v potential by cyclic voltammetry
scan rate 500 V s^{-1} at 22° C, scan 0.0 -1.2, pH 8.1
0.05 ratio (w/w) UQ_{10}

a) Dioleoyl PC
b) Dipalmitoyl PC
c) Egg lecithin



3.2.3 Incorporation of 0.05 ratio UQ_{10} (w/w) in adsorbed lipid layers.

Phospholipid monolayers of dioleoyl, dipalmitoyl and egg lecithin subjected to continous cyclic voltammetry at $500Vs^{-1}$ with incorporated ubiquinone,(0.05 ratio w/w), all showed behaviour that could not be distinguished from that of lipid monolayers alone. Reduction peaks for ubiquinone only appear at or below sweep rates of $50Vs^{-1}$, (Table 1). No oxidation peaks were observed.

TABLE 1

Table of reduction potentials, and peak pseudo-capacitance values, under continuous cyclic voltammetry, at pH 8.1, 22°C, between 0.0, and -1.2V, at $50Vs^{-1}$ for UQ_{10}, in different lipid monolayers.

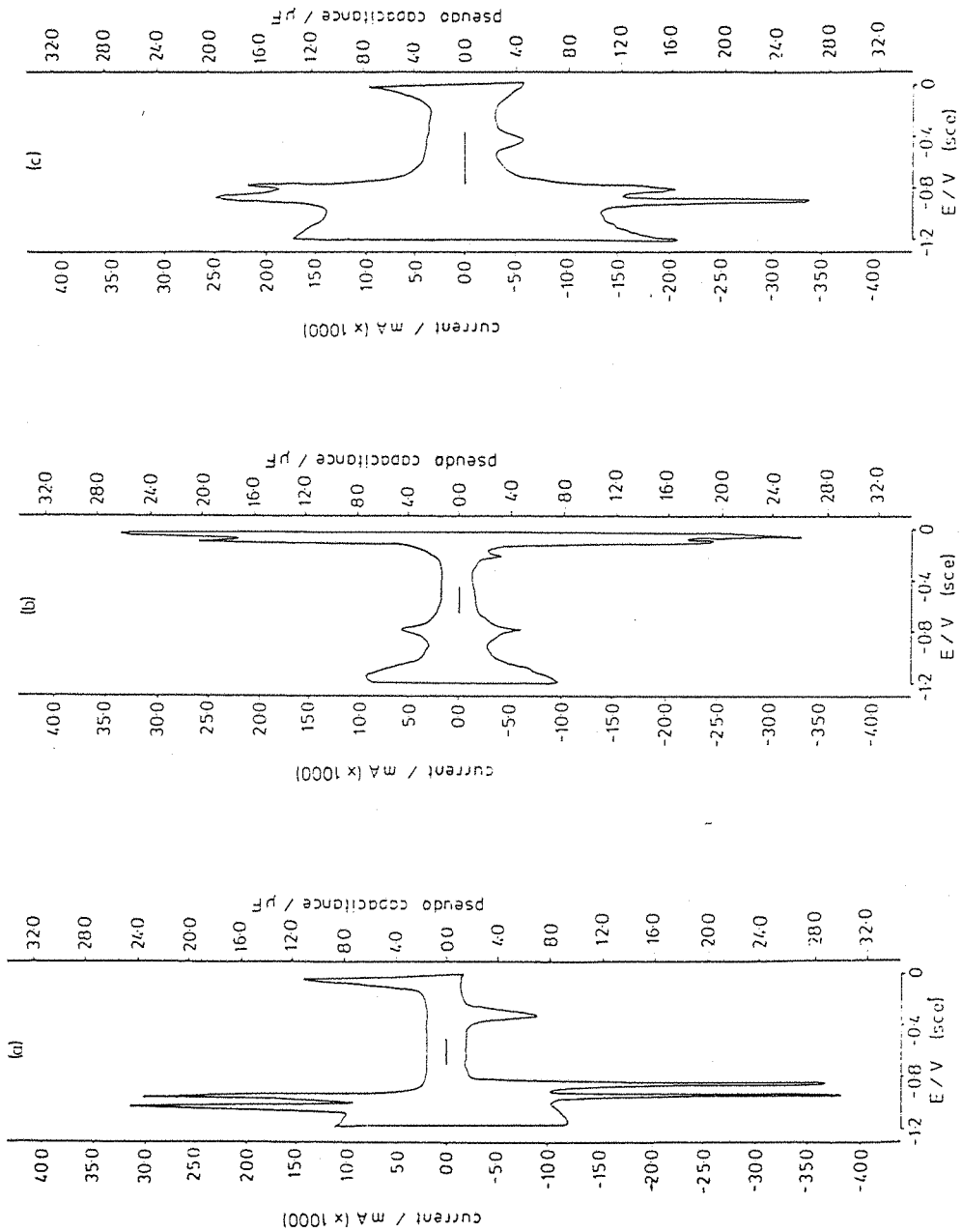
Lipid	Reduction potential(mV)	peak pseudo-capacitance (uF/cm^2)
Dioleoyl PC	-460	2.54
Dipalmitoyl PC	-220	4.69
Egg Lecithin	----	----

Oxidation together with reduction peaks can be seen at sweep rates at or below $5Vs^{-1}$ in dioleoyl PC and dipalmitoyl PC monolayers, (Fig 3.2.3). A reduction wave was only seen for UQ_{10}, within an egg lecithin monolyer at $5Vs^{-1}$. For a two electron reduction/oxidation process under bulk diffusion control, a peak separation of 29.8 mV would indicate reversibility in the system. However a more analogous system for comparison is a polymer modified electrode, (see discussion). For a reversible surface redox reaction, the oxidation

Fig (3.2.3)

pseudo-capacitance / current v potential by cyclic voltammetry
scan rate 5 V s^{-1} at 22° C : scan 0.0 -1.2 V , pH 8.1
0.05 ratio (w/w) UQ_{10}

a) Dioleoyl lecithin
b) Dipalmitoyl PC
c) Egg lecithin



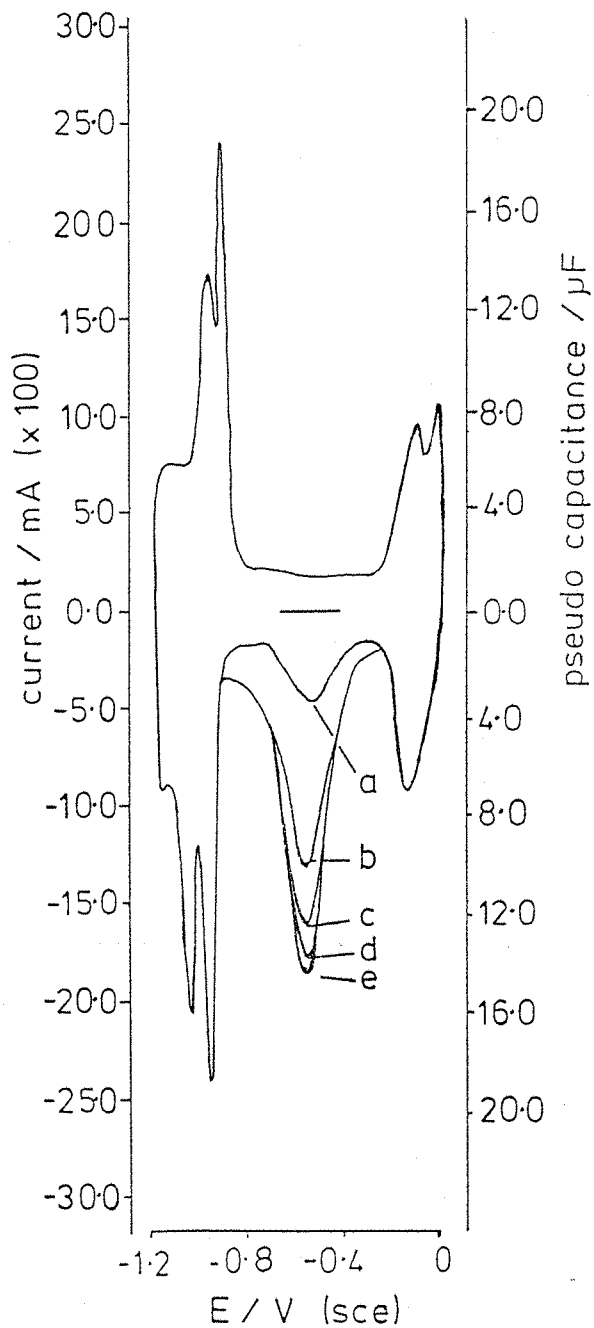
and reduction waves should occur at the same potential. However the observed peak separation for dioleoyl and dipalmitoyl PC monolayers indicate that the reduction and oxidation of UQ_{10} is highly irreversible under the conditions investigated. The reduction/oxidation peak potentials of ubiquinone were sweep rate dependent, and this effect was more apparent at higher sweep rates (Section 3.2.7). The peak separation was dependent on the nature of the lipid in which ubiquinone was incorporated and the reduction/oxidation process became more reversible in the order ; egg lecithin, dioleoyl PC, dipalmitoyl PC.

3.2.4 Effect of a time delay on the current/potential response for monolayers containing 0.05 ratio (w/w) UQ_{10}

Voltammetry at fast continuous sweep rates yields no electroactive behaviour of UQ_{10} (Section 3.2.4) within a dioleoyl PC monolayer between 0.0 and -1.2V. Irreversible reduction and re-oxidation of UQ_{10} was observed upon the first sweep when a time delay of 1ms or greater was introduced whilst under potential control at the positive limit . Reduction was observed at a potential of -480mV and the reverse oxidation of the reduced species formed occurred at a potential of -100mV. The peak separation was 380mV. The oxidation peak current and potential remains constant at 0.093mA/7.22 $\mu F/cm^2$ and in the potential range of -100mV. It occurs however in the same potential range of a molecular re-orientation process of dioleoyl PC (4). This re-orientation peak increases in magnitude upon the introduction of a time delay at the positive limit, and moves to a more negative potential of approximately -50mV. The reduction peak is unchanged in potential when a 1ms or greater time delay is introduced at the positive limit under potential control. However the UQ_{10} peak current/pseudo-capacitance value depends on the time delay introduced at the positive limit (Fig 3.2.4)(a). The pseudo-capacitance reaches a plateau of around 0.175mA/13.67 $\mu F/cm^2$, for time delays of 1s or greater (Fig 3.2.4)(b). As the time delay was increased, greater reduction of UQ_{10} occured during the first sweep, whilst the current of the re-oxidation wave remained unchanged. Neither reduction or oxidation waves of UQ_{10} were observed on succesive sweeps. The decay of the reduced species with time is shown (Fig 3.2.4)(c).

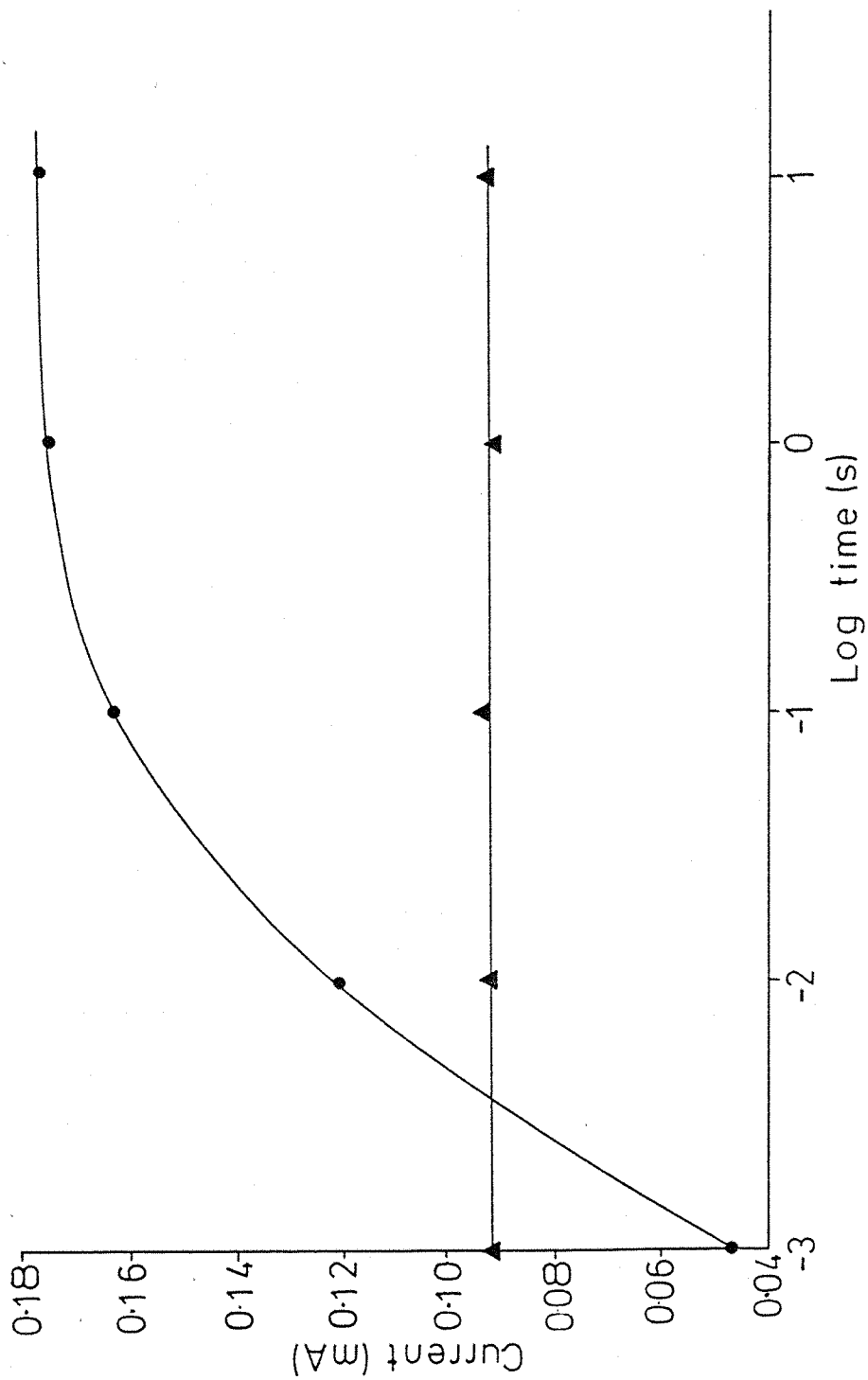
Cyclic voltammograms of 0.05 ratio (w/w) $^{UQ}_{10}$ in dioleoyl PC adsorbed monolayer at 500Vs^{-1} pH 8.1, 22°C between 0.0 and -1.2V

time delays under potential control at 0.0V
a) 1ms b) 10ms c) 100ms d) 1s e) 10s



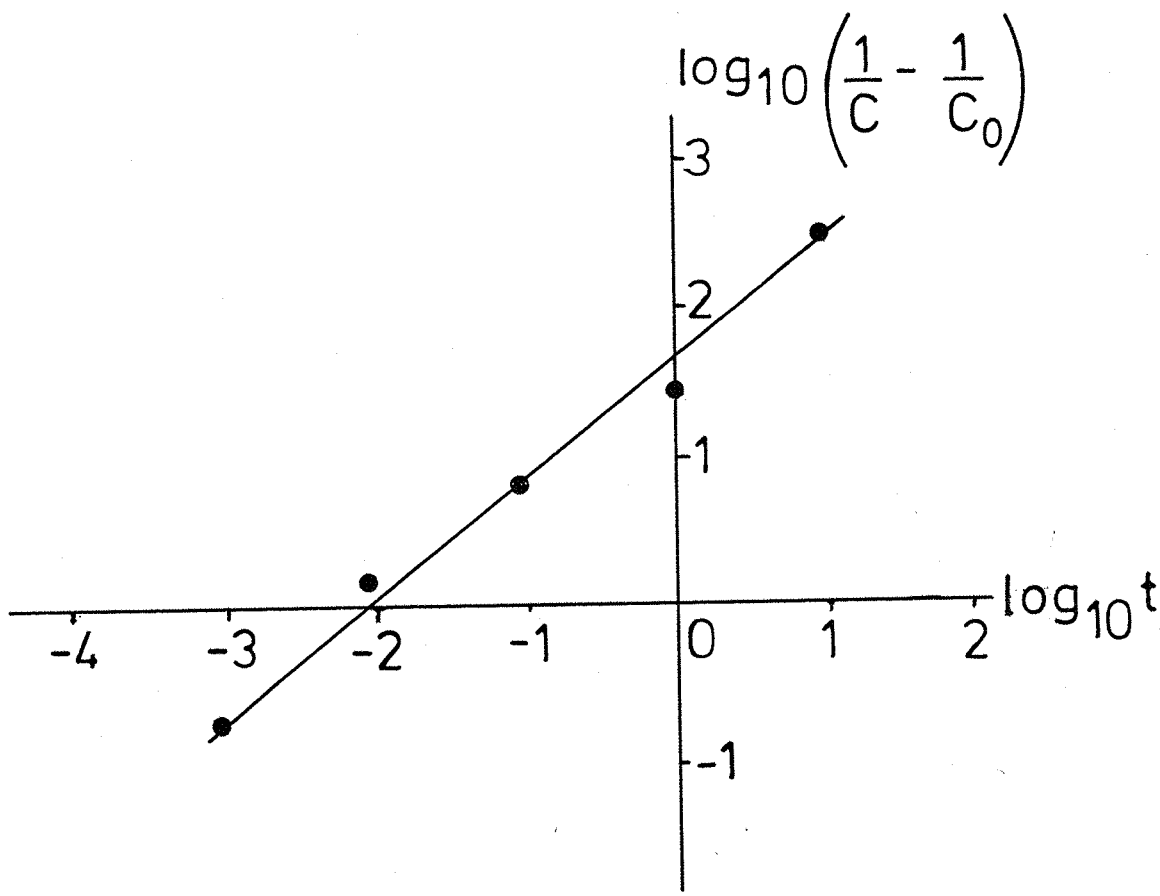
Graph of reduction and oxidation peak currents of UQ_{10} vs $\log_{10}$ time delay introduced at the positive limit. 0.05 ratio (w/w) UQ_{10} incorporated in dioleoyl lecithin at 22 °C, pH 8.1 buffer.

• Reduction
▲ Oxidation.



Graph of $\log_{10}\left(\frac{1}{C} - \frac{1}{C^{o}}\right)$ vs $\log_{10} t$,

(from peak reduction currents vs time - Fig (3.2.4) (a)

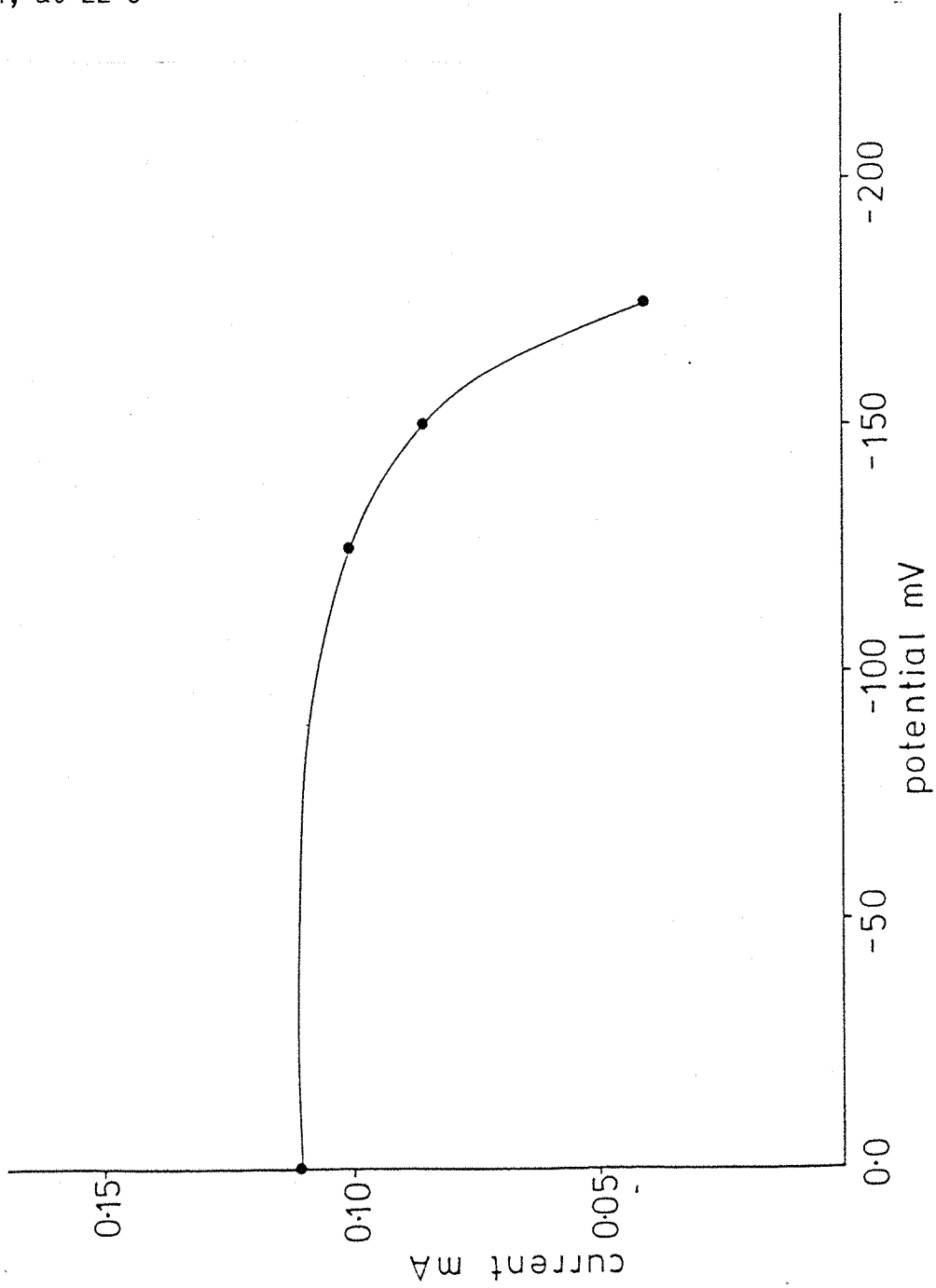


3.2.5 Effect of potential limits for monolayers containing 0.05 ratio (w/w) UQ_{10}

Voltammetry at $500Vs^{-1}$, whilst maintaining a delay of 100ms or greater at the positive limit showed irreversible reduction and re-oxidation of UQ_{10} within a dioleoyl PC monolayer during the first potential sweep (Section 3.2.4).

Variation of the negative limit to potentials ranging from -1300mV to -800mV using the same time delay showed no effect on the reduction or re-oxidation of UQ_{10}, when a time delay of 100ms at the positive limit was included. Increasing the positive limit to a potential of +100mV under similar conditions showed no effect on either the reduction or oxidation peaks of ubiquinone. The positive limit was not increased to potentials more positive than +100mV due to the onset of the mercury oxidation. When the positive potential limit, was set at potentials negative to -100mV, the re-oxidation peaks of UQ_{10} disappeared. Changing the positive limits between 0.0mV and -200mV, caused a decrease of the peak current/pseudo-capacitance for the reduction of UQ_{10} (Fig 3.2.5). Qualitatively, similar results were observed when a 1 ms delay was introduced at the positive limit of the sweep. Variation of the negative limit to potentials ranging from -1300mV to -800mV, or increasing the positive limit to a potential of +100mV, did not affect the reduction or re-oxidation of UQ_{10}. Lowering the positive limit from a potential of 0V to -50mV with a 1 ms delay caused the total loss of the the reduction peak of UQ_{10}.

Graph of the reductive peak current v potential of the positive limit (100 ms delay at positive limit), at 500 V s^{-1}
0.0 to -1.2 V
0.05 ratio (w/w) UQ_{10} in dioleoyl PC
pH 8.1, at 22°C



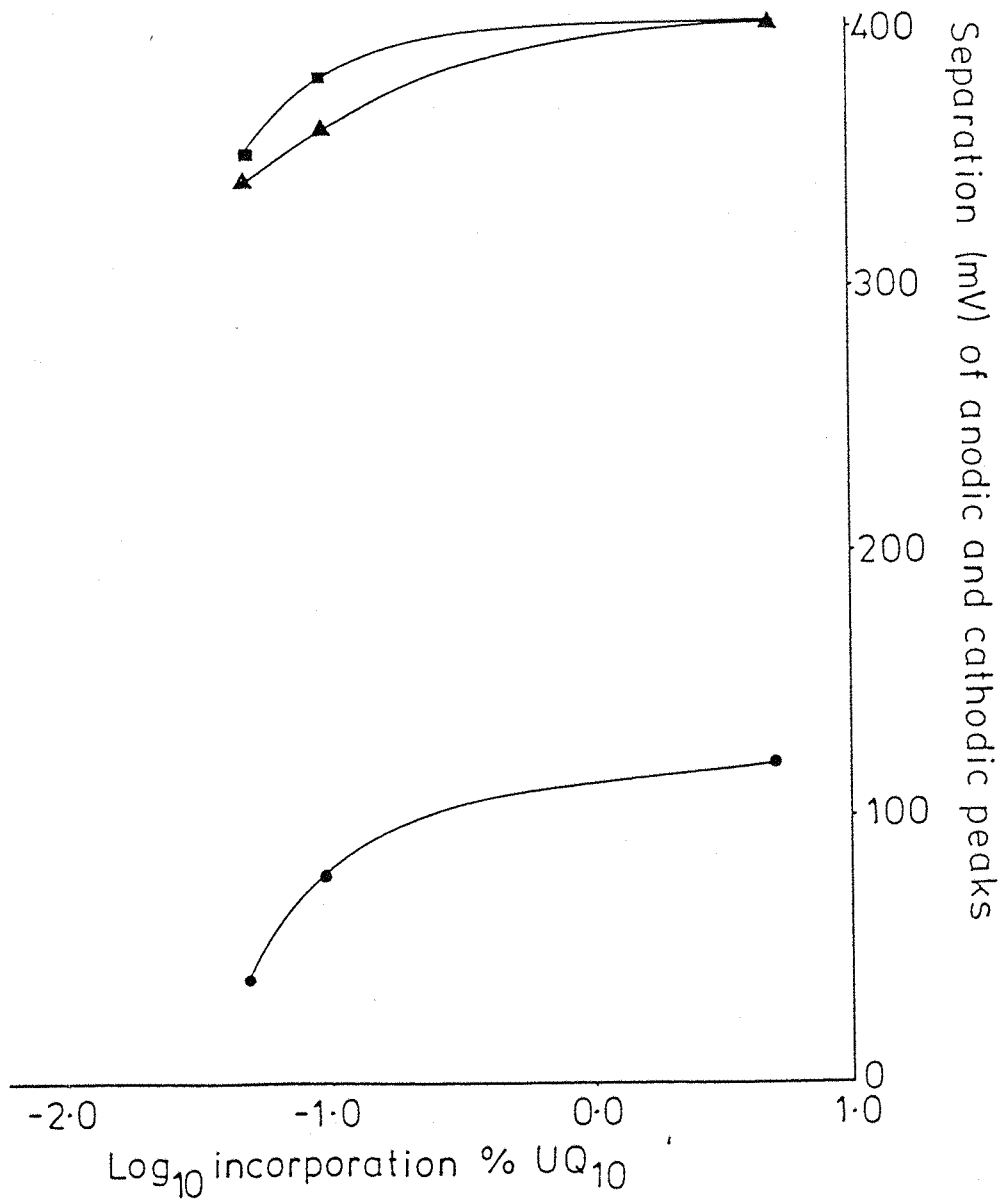
3.2.6 Effect of UQ_{10} concentration within phospholipid monolayers.

Reduction and oxidation of UQ_{10} occurs under conditions of continuous voltammetry at $5Vs^{-1}$, between potentials of 0.0V and -1.2V within monolayers of dioleoyl PC, dipalmitotyl PC, and egg lecithin. For the three lipids studied, the separation in potentials of the reduction and the oxidation waves of UQ_{10} is inversely related to the concentration of UQ_{10} (Fig 3.2.6). It is interesting to notice that UQ_{10} was found to be reduced and re-oxidized with greater reversibility in the order egg lecithin, dioleoyl PC, dipalmitoyl PC.

Fig (3.2.6)

Graph of peak separation v Log_{10} % incorporation UQ_{10} (w/w)
at 5 V s^{-1}
pH 8.1 at 22°C, 0.0 to -1.2V

▲ : Dioleoyl PC
● : Dipalmitoyl PC
■ : Egg lecithin



3.2.7 Effect of sweep rate

At or below a sweep rate of $50Vs^{-1}$, at room temperature, UQ_{10}, at a concentration ratio of 0.05 (w/w), undergoes both reduction and re-oxidation on continuous cycling between the potentials of 0.0V and -1.2V at $22^{o}C$.

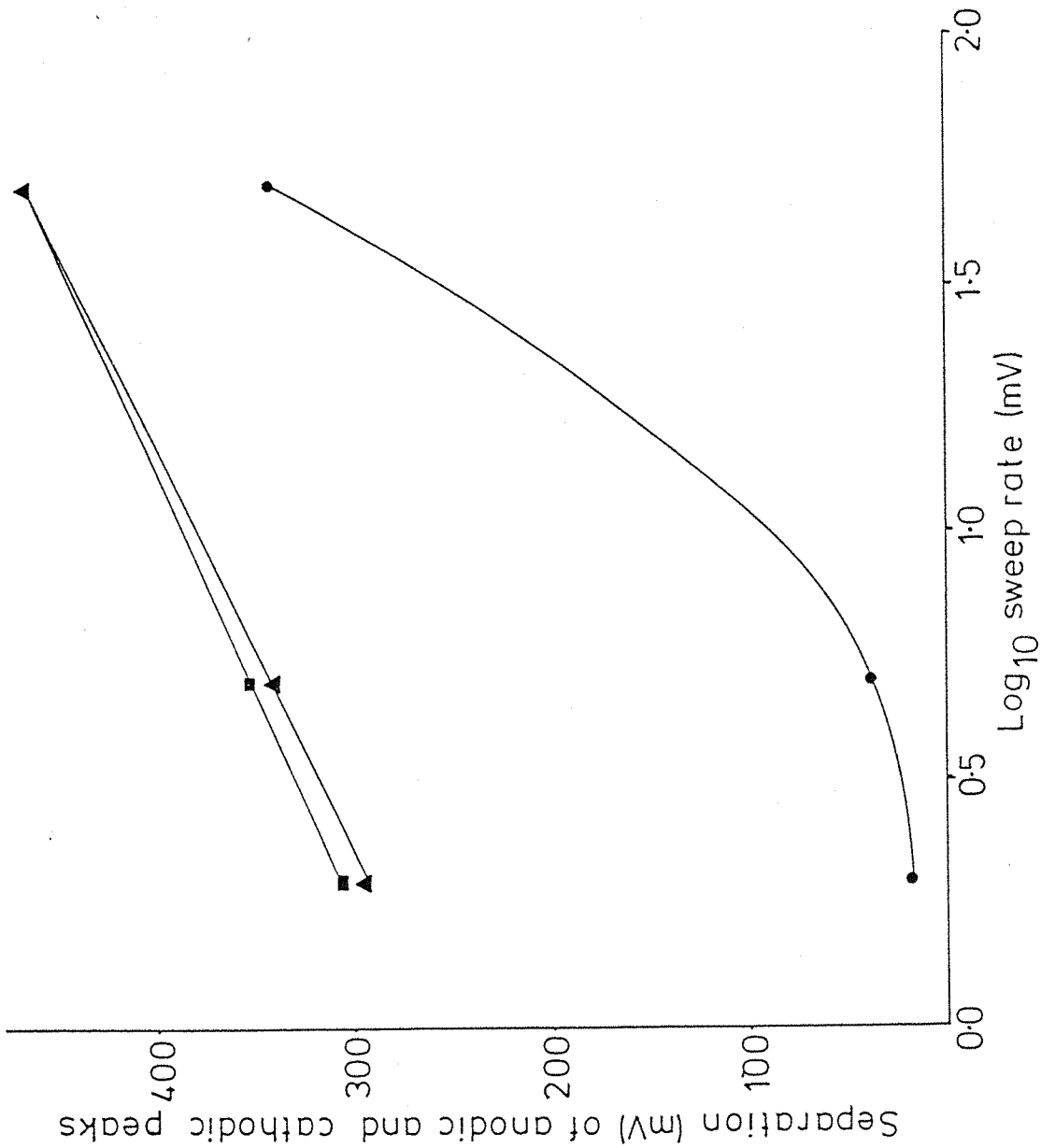
The separation of the cathodic and anodic peaks of UQ_{10}, increased with increasing sweep rate, when UQ_{10} was incorporated in monolayers of all three lipids (Fig 3.2.7). The system therefore became more reversible with a decrease in sweep rate.

For a given sweep rate, the cathodic and anodic peak separation of UQ_{10}, within the three lipid monolyers, increased in the order : dipalmitoyl PC, dioleoyl PC, egg lecithin.

Fig (3.2.7)

Graph of peak separation v Log_{10} (sweep rate) V s^{-1}
1 part in 2000 (w/w) incorporation UQ_{10}, pH 8.1

▲ : Dioleoyl PC
● : Dipalmitoyl PC
■ : Egg lecithin



3.2.8 Effect of pH

An increase in pH of the base electrolye to a value of 9.1 (a rise in pH of one unit) for 0.05 ratio (w/w) incorporation of UQ_{10} within a dioleoyl PC, monolayer resulted in a decrease in peak separation of the reduction and oxidation peaks of UQ_{10} (Table 2) when a 100ms time delay was introduced under potential control at the positive potential at a sweep rate of 500 Vs^{-1}.

TABLE 2

Table of reduction and oxidation potentials for UQ_{10}, within a dioleoyl PC monolayer - variation of pH of base electrolyte. 100ms delay at the positive limit. Scan 0.0V to -1.2V at $500s^{-1}$,

pH	Reduction potential(mV)	Oxidation potential(mV)	Peak separation(mV)
8.1	-600	-100	500
9.1	-640	-40	600

Peak capacitances for both the reduction and oxidation of UQ_{10} remained unchanged at 10.55 uF/cm^2 and 9.37 uF/cm^2 respectively.

3.2.9 Effect of Temperature

Contrary to the results obtained at $22^{o}C$ UQ_{10} was reduced at $0.5^{o}C$ on continuous voltammetry at $500Vs^{-1}$ between the potentials of 0 and -1.2V when incorporated in dioleoyl PC at a 0.05 ratio (w/w). The reduction of UQ_{10} occurred at a potential of -560mV, with a peak current/pseudo-capacitance of 0.068 mA/5.25μF/cm^{2}. No oxidation peak was observed. Continuous voltammetry at $0.5^{o}C$ and 50 Vs^{-1} resulted in both reduction and subsequent re-oxidation of UQ_{10} at potentials of -440mV and -40mV respectively. A decrease in temperature therefore introduces greater reversibility (Table 3). This is an interesting result which is in contrast with the usual expectation of a greater irreversibility when the temperature is decreased.

TABLE 3

Table of reduction potentials, oxidation potentials, and peak separations for UQ_{10}, within a dioleoyl PC monolayer, with variation of temperature. pH 8.1 base electrolyte, 0.0V to -1.2V, at $50Vs_{-1}$.

Temperature degrees(C)	Reduction potential(mV)	Oxidation potential(mV)	Peak separation(mV)
0.5	-440	-40	400
22.0	-460	---	---

3.2.3 Discussion

The effects of the experimental conditions on the redox behaviour of UQ_{10} incorporated into an adsorbed phospholipid monolayer on mercury have been presented in Section 3.2. A model will be presented of the redox behaviour of UQ_{10} in such monolayers, showing how it is affected by the environment in which it resides, both in vitro (in the present mimetic system), and in vivo.

The maximum reduction charge observed during the reduction sweep was of $2.8\mu C/cm^2$ for the 0.05 ratio system. The surface concentration of compressed monolayers of phospholipids at the air-solution interface varies between $4.5x10^{-10}$ and $2.4x10^{-10}$ moles/cm^2 (74). From these values, the surface composition of the layers adsorbed on mercury is between 8.7 and 4.3% (w/w). These results would appear in aggreement with the nominal surface composition of the mixture placed at the gas-solution interface. However there is very strong evidence for a preferential adsorption of UQ_{10} compared with the phospholipids. When the normal concentration was reduced 100 times, only approximately 20% decrease in the reduction wave was observed. This indicates that there is strong specific adsorption of the quinone on mercury, as compared with the lipid. This question needs further investigation.

Information regarding the environments in which different oxidation states of UQ_{10} reside may be drawn from the results of sections 3.2.4 and 3.2.5. Reduction of UQ_{10} (within dioleoyl PC) at 5% (w/w) concentration, together with some re-oxidation of UQ_{10}, was observed at a potential scan rate of $500Vs^{-1}$ on the first cyclic potential sweep, when a time delay was introduced at a potential of

0.0V. The peak reduction current was dependent on the time delay, (Fig 3.2.4a , Fig 3.2.4b) and also on the value of the positive limit (Fig 3.2.5). No reduction or oxidation of UQ_{10} occurred when repetitive cycling was employed. When a one second or greater delay was introduced at the positive limit the peak reduction current reached a maximum corresponding to 13.67 $\mu F/cm^2$. It may therefore be inferred that if a one second delay or greater is introduced at a positive limit of 0.0V, then all of the UQ_{10} is present in the oxidised state at the mercury surface. Qualitatively, only a small proportion of the ubiquinol was re-oxidized during the reverse anodic sweep. No further reduction or oxidation of UQ_{10} was observed during succesive potential sweeps due to the UQ_{10} still being present in the reduced form. When the negative limit was altered from -1.2 to -1.1V, the re-oxidation of the ubiquinol could not be observed on the anodic sweep. Therefore, a characteristic time was required during the anodic sweep to allow any re-oxidation of UQ_{10} to be observed. It may also be deduced that a time delay of greater than 100ms was required, at a potential of 0.0V, to allow all of the ubiquinol to re-oxidize.

These results show that the oxidized form of UQ_{10} could be reduced during a cathodic potential sweep at $500Vs^{-1}$, but that the re-oxidation was highly irreversible.

Two explanations could account for these effects. The first requires that the ubiquinone molecule must possess a value of the diffusion coefficient within the monolayer that allowed the reduction and re-oxidation of UQ_{10} to be under diffusion control. In this situation the "flip-flop" motion of the UQ_{10} would be the rate limiting step. The "flip-flop" motion would involve the quinone ring

residing at the mercury surface, reduction to give a quinol form and moving to the lipid/aqueous interface. The reverse process would occur on re-oxidation. The second possible explanation is that although the "flip-flop" mechanism of the UQ_{10} is not the rate limiting step, a characteristic time is required for the UQ_{10} to be in a position with respect to the metal surface where it can be re-oxidized. This could be a consequence of the need to overcome interactions between the UQ_{10} redox moiety with the aqueous phase. All of the experiments performed suggest the latter case to be true. The following discussion will explain how these conclusions were drawn.

The reduction of ubiquinone yields the ubiquinol as the final product (Fig 1.2.2). This is supported by the work of Marcus and Hawley (25) who found no evidence for the formation of the chromanol (Fig 1.2.1) when UQ_{10} was reduced. De Pierre et al (65), together with Robertson et al (66), proposed that the UQ_{10} molecule crosses the inner mitochondrial membrane by a "flip-flop" motion of the quinone ring. The quinone ring is highly hydrophobic, but on reduction becomes hydrophilic. It was proposed that the quinol ring then flips across the inner mitochondrial membrane under the influence of the proton motive force, to the matrix side. A pH gradient of around 1.4 units is present across the membrane, the lower pH existing on the cytosol (outer) side of the membrane (Fig 1.2.6). Upon re-oxidation the ubiquinol flips back to the cytosol side of the membrane, due to the increased hydrophobic nature of the quinone ring.

Work has been performed to determine the rates of flipping of the UQ_{10} molecule and to compare these to rates of reduction and

oxidation of UQ_{10}, within mimetic systems of the inner mitochondrial membrane (see introduction 1.4). Kroger et al (52) showed that for the UQ_{10} to function in the Q-cycle as suggested by Mitchell, the UQ_{10} molecule must cross the membrane with a frequency of at least four times a second (see introduction 1.4). Values calculated by Kingsley and Feigenson (63) using ^{1}H N.M.R. spectroscopy within vesicles suggested that a minimum rate of flipping of the UQ_{10} molecule was 23 s^{-1}. It was also stressed by the authors that the actual rate of flipping could be considerably higher, and that the rate quoted was calculated within limitations imposed by instrumentation. The rate constant measured for the reduction/oxidation of UQ_{10} within the vesicles was 1.277 s^{-1} (63). This represented the rate constant for the rate limiting step, and therefore, it was concluded that the rate limiting step was not that of the "flip-flop" motion of the quinone ring.

Hauska showed that varying the isoprene chain length of ubiquinone affected the efficiency of the molecule as a redox mediator within a vesicle (31,37). The longer chain quinones showed a greater efficiency than their shorter chain homologues. If the rate of the "flip-flop" motion is not the rate limiting step then the length of the isoprene chain must have altered the availability of the quinone ring to be reduced/oxidized in some other manner. Although these previous studies had concentrated on the ubiquinone molecules, before the present work, no comparison had been made of the redox behaviour of ubiquinone within different lipid environments.

Imposing the following conditions on the UQ_{10}/phospholipid adsorbed monolayer gave a higher degree of reversibility as inferred

from the peak separation :

A) Incorporation of UQ_{10} (at the same concentration), in the order egg lecithin > dioleoyl PC, > dipalmitoyl PC (Section 3.2.2)

B) Lowering of the concentration of UQ_{10} within the monolayer (Section 3.2.6),

C) Lowering of the sweep rate (Section 3.2.7),

D) Lowering of the temperature (Section 3.2.9)

In addition a decrease in reversibility was observed with an increase in pH (Section 3.2.8).

There is a clear relationship between the observed reversibility and the known phase behaviour of the lipids studied. Each of the lipids studied possesses a characteristic phase transition temperature above which the lipid has a fluid structure. At temperatures below the phase transition, the lipid has a liquid crystalline structure. The phase transition temperatures for the the three lipids are $-15^{o}C$, $-22^{o}C$ and $39^{o}C$ respectively (67,68). Dipalmitoyl PC exists in the liquid crystalline state at $22^{o}C$, whereas dioleoyl PC and egg lecithin exist in the fluid state at the same temperature. The values quoted for the phase transition temperature of egg lecithin may vary by as much as $\pm 8^{o}C$ according to the source from which the lipid is obtained, due to the lecithin being a mixture of a number of lipids, the consituent ratios of which may vary from one source to another. UQ_{10} within the dipalmitoyl lecithin monolayer showed redox behaviour that approached the reversible situation more closely, showing approximately half the

peak separation of the other two lipids, at 22°C (Section 3.2.2) and (Fig 3.2.2). This implies that the rigidity of the lipid affects the ease by which the UQ_{10} molecule may be reduced and oxidized. The rigidity of the lipid must therefore be a contributing effect for the ability of the ubiquinol to gain access to the metal surface, by some means other than by controlling the rate of the "flip-flop" motion.

The increased reversibility shown at lower sweep rates implies that as greater time is allowed for the ubiquinol to gain access to the mercury surface more ubiquinol may be re-oxidized during the anodic potential sweep. If the rate limiting step is not the rate of the "flip-flop" motion of UQ_{10}, then the slowest step must be in overcoming the interaction of the UQ_{10} with either the lipid head groups or the aqueous phase at some distance from the mercury surface.

As an increase in reversibility was shown by the incorporation of UQ_{10} into layers of increased rigidity, it may be inferred that a monolayer of greater rigidity reduces in some way access of the more hydrophobic ubiquinol to the region where it can interact with the aqueous phase side of the adsorbed monolayer. A higher percentage of reduced UQ_{10} would remain at the mercury surface where it can be re-oxidized during the anodic sweep thus resulting in a more reversible behaviour.

It would be expected that lowering the temperature from 22 to 0.5°C would increase the rigidity of the monolayer of dioleoyl PC. Indeed a decrease in the peak separation of the oxidation and reduction waves of UQ_{10} was observed indicating an increase in reversibility of the system with a decrease in temperature. It is again suggested that the increase in rigidity of the monolayer

reduces access of the hydrophilic ubiquinol to the aqueous phase. A greater percentage of ubiquinol therefore remains at the mercury surface, where it can be reduced during the cathodic sweep.

At the pH of the solution studied, reduction of a quinone occurs through the intermediate formation of the corresponding semiquinone radical (23) (see Fig 1.2.8) ie Eqn (3.a):

$$Q + e^- \longrightarrow Q^- \qquad (3.a)$$

The overall reaction pathway depends on the decay mechanism of the semiquinone.

In the case of the ubiquinones, the intermediate formation of the semiquinone radical ($UQ^{\cdot -}$) has been observed (23), and it has been suggested that this radical may be sufficiently mobile to act as the main mediating species. $UQ^{\cdot -}$ can decay by a chemical-electrochemical mechanism or by disproportionation. In the first case, the reaction would be :

$$UQ^{\cdot -} + H^+ \longrightarrow UQ.H \qquad (3.b)$$

followed by

$$UQH + H^+ + e^- \longrightarrow UQH_2 \qquad (3.c)$$

The disproportionation reaction involves the overall stoichiometry :

$$2UQ^{\cdot -} + 2H_2O \longrightarrow UQ + UQH_2 + 2OH^- \qquad (3.d)$$

If the $UQ^{\cdot -}$ is sufficiently stable with respect to disproportionation (within the time scale of the voltammetric experiments), it would be possible to treat the reaction in the immobilized layer as equivalent to a thin film redox polymer electrode. In the latter case rate constants were calculated following the theore tical voltammograms reported by Aoki et al (67) assuming that the reduction and oxidation of UQ_{10} occured as a surface reaction within a layer acting as a finite diffus ional space. The model used by these authors has been based on redox polymer electrodes (67). The rate constants were calculated for the reduction and the oxidation of UQ_{10} when UQ_{10} was incorporated in the lipid monolayer of a concentration of 0.05% (w/w).

The equation reported by Aoki et al, is shown below for a reductive surface reaction.

$$\ln (\Lambda \sqrt{w}) = -\alpha_a [0.294 - 6.12 \exp(-4.53\alpha_a) + 0.188 \gamma_p] + (2.20 - 0.169\alpha_a)/\gamma_p \qquad (3.e)$$

where $\gamma_p = (nF/RT)(E_p - E^\circ)$

$w = nFd^2v/RTD$, and

$\Lambda = k'(RT/nFDV)^{1/2}$

d = the thickness of the diffusion space, taken to be 1.1 nm (2).
v = the potential sweep rate, and
D = the diffusion coefficient of ubiquinone, assumed to be 1×10^{-8}

The rate constants calculated are shown in table 4.

TABLE 4

Table of rate constants calculated at different sweep rates for incorporation of UQ_{10} in one part in two thousand (w/w), in dioleoyl PC, dipalmitoyl PC, and egg lecithin.

		Rate constants (s^{-1})	
	Sweep rate(Vs^{-1})	Reduction	Oxidation
Dioleoyl PC	50.0	0.03	0.09
	5.0	0.05	0.10
	2.0	0.09	0.10
Dipalmitoyl PC	50.0	0.09	0.06
	5.0	0.09	0.06
	2.0	0.09	0.06
Egg lecithin	50.0	0.03	0.09
	5.0	0.05	0.10
	2.0	0.09	0.11

The results in table 4 must be regarded as approximate due to experimental inaccuracies. The formal rate constant should be the same if the anodic and cathodic sweep is considered. Also it can be seen that the value of k' for the reduction process is not constant for different sweep rates for dioleoyl PC and egg lecithin. It is interesting to notice though, that for the lipid probably giving a more rigid structure, the values of k' are sweep rate independent and agree within the expected experimental errors. For instance the choice of a different value of E^{o} would make them coincide. The value chosen is simply based on biochemical evidence and is somewhat approximate. For this lipid, disproportionation or subsequent chemical reactions could be sufficiently hindered so as to make the redox polymer model applicable. For the other two more fluid lipids, this is obviously incorrect and a mechanism involving either an EC sequence or a disproportionation step should be considered. It is

unfortunate that the theory for an EC mechanism at redox film electrodes has not ben developed.

It is interesting to speculate on the possible nature of the "chemical" step above mentioned.

The ubiquinol ring might be expected to undergo interactions with both the aqueous phase, by hydration forces, or interactions with solute ions and also with the polar head groups of the lipid. The forces of interaction of the ubiquinol with the aqueous side of the lipid/water interface could therefore provide a step, which from the point of view of the Gibbs energy of the reaction, would act as the equivalent of a chemical step in an EC mechanisn. The translocation of UQ_{10} across the adsorbed phospholipid monolayer fulfills the conditions of the second postulate above, that the rate limiting step for the reduction involves the limitation of the access of the ubiquinol to the mercury surface, without the rate of the flipping motion of UQ_{10} controlling the overall kinetics. It should be stressed that the ability of the ubiquinol to overcome the hydration forces at the lipid/aqueous interface is proposed as the rate limiting step and not the rate of the flipping motion of the ubiquinone or ubiquinol.

It is not possible at present to distinguish between an EC mechanism of the type above described, from one in which the electrochemical step is followed by disproportionation. Of course in the latter case, the flipping over of the ubiquinol molecule towards the aqueous phase would still occur. It is quite clear that the re-oxidation process in the more fluid lipids is highly irreversible, strongly indicating the validity of the flipping model proposed. There is however, some evidence pointing towards a disproportionation

mechanism for the re-oxidation reaction. Figure 3.2.4 shows the effect on the rate of re-oxidation of the ubiquinol at a constant potential. The decay of UQH_2 is not first order but results fit approximatly second order kinetics. This is given by (70), Equ (3.f) :

$$\frac{1}{c} - \frac{1}{c_o} = kt \qquad (3.f)$$

where c_0 is the initial concentration,

Figure 3.2.4b shows a test of the data in Fig (3.2.4). Although there is a limited amount of reliable information available at present, the results do point to a second order mechanism. Thus the oxidation of the ubiquinone appears to follow the mechanism :

$$UQH_2 \longrightarrow UQH^{\bullet} + H^+ + e^- \qquad \text{(fast)} \qquad (3.g)$$

$$2\ UQH^{\bullet} \longrightarrow UQ + UQH_2 \qquad \text{(R.D.S)} \qquad (3.h)$$

As previouslu discussed the alteration of the degree of reversibility of the redox behaviour of UQ_{10} when incorporated in monolayers of different lipids, must suggest that the interaction of the lipid with UQ_{10} affects the ability of the ubiquinol to interact with the aqueous side of the lipid. The site of interaction of UQ_{10}, as proposed in the second explanation, is at the lipid/aqueous interface and is due to hydrophilic/hydration forces.

Work in which the ubiquinone had been altered in some way gave similar conclusions that interactions between the ubiquinone and the lipid are involved in the rate limiting step of the redox behaviour of UQ_{10}. Hauska et al showed that the length of the isoprene chain of the ubiquinone molecule affected the efficiency of the molecule as a

redox mediator within a vesicle (37,56). Kinsley and Feigenson (63) showed that the length of the isoprene chain length altered the positioning of the quinone head within outer monolayers of vesicles. Longer isoprene chain lengths caused the quinone rings to be embedded deeper within the outer monolayer of the vesicle. Various suggestions to explain the increased efficiency to electron transfer of the longer chain quinones have been made (58). These involve suggestions such as the formations of molecular clusters of UQ_{10} heterogeneously distributed within different vesicles and the action of the isoprene chain acting as an analogue of an electrically conducting wire within nature. These suggestions have now been refuted (55).

The dependence of reversibility on the concentration of UQ_{10} may be explained by the formation of molecular clusters of UQ_{10} at higher concentrations, so retarding the motion of the UQ_{10} molecule and providing an alternative route for electron transfer, by means of electron tunnelling across the monolayer. Similar findings were shown by Andrieux et al (71), on voltammetry of a redox polymer film. The electrode was coated in poly(p-nitrostyrene) and it was shown that the electron transfer in the polymer film was much slower than when the electroactive species was present in solution. It was suggested by the authors that the current-potential curves could be rationalized in terms of a linear diffusion process occuring within a finite space. However the low order of the observed kinetics could be explained in terms of solvent/counter ion interactions, preventing the reaction showing normal diffusional control.

To conclude it is suggested that two rate limiting steps may exist. The first involves overcoming the forces of interaction of the reduced ubiquinol ring or an intermediate with the mercury surface,

so that the ubiquinol ring may flip to the lipid/aqueous interface. The second for the re-oxidation involves overcoming the forces of interaction of the quinol head with the lipid/aqueous interface so that it may flip back to the mercury surface. The rate of the flipping motion is not the rate limiting step in either case. For the fluid lipids the electrochemical reduction and oxidation of UQ_{10} is therefore a surface reaction and is not under diffusion control. presented. All of the rate constants are below a third of the value

All the rate constants calculated are more than two orders of magnitude lower than the results obtained for vesicles (63) indicating that the "flip-flop" mechanism is not the rate limiting step but rather, adsorption at the interfaces.

The findings of this work are in agreement with the results obtained by Rich who suggested that the redox behaviour of various quinones within vesicles was determined by rate limiting steps involving the reduction of a $Q^{\bullet -}$, and the oxidation of a $QH^{-}/QH^{\bullet}$ couple (64). This represents a C.E.C reaction mechanism ; a chemical step, an electrical event and a further chemical event. This could be shown by a chemical reaction occuring at the mercury surface, a "flip-flop" event, and possibly an electrical disproportionation of a semi-quinone radical within the membrane, (23,63), followed by another chemical reaction at the mercury surface.

The existence of the semiquinone radical may further complicate the explanation of the redox behaviour observed. This has already been discussed in section 1.4. The disproportionation could contribute to the explanation of the irreversible redox behaviour observed. Further work is planned to investigate the possible contribution of disproportionation to the influence of the redox

behaviour of UQ_{10} (see suggestions for further work).

Increasing the pH which leads to an increase in the peak separation of the reduction and oxidation waves of UQ_{10} (Section 3.2.7). A decrease in reversibility is therefore introduced to the system with an increase in the pH. A pK value for UQ_{10} was not available. However comparisons may be made with other molecules. The UQ_{10} has a structure of a benzene ring with six functional groups. The acid/base behaviour would be expected to be govered by by the two carbonyl groups at the 1 and 4 positions in the oxidized form. The isoprene chain, the methyl and the two methoxy groups would all be expected to be weak electron donors to the phenolic ring. The results of section 3.2.7 suggest this to be true. The small percentage of UQ_{10} that would be in the unassociated form would not account for the change in peak separation of 100mV (Section 3.2.7), however further work is planned to investigate more comprehensively the effects of pH on the redox behaviour of UQ_{10}, (see suggestions for further work).

The pK of the hydroquinone is 10.35 (72). The change of pK by substituent groups in aromatic compounds may be predicted by means of the Hammett equastion (73). Using this analysis it is predicted that the UQ_{10} molecule would have a pK value of approximatly 10.2 although it must be recognized that this analysis is difficult due to the highly substituted nature of the benzene ring. The substituent functional groups in UQ_{10} have therefore have little effect on the pK value of the molecule. A small percentage of the ubiquinone might be expected to exist in the dissociated form, and therfore would be stabilized by an environment with a higher OH^- concentration and a lower H^+ concentration.

Conclusion

This study has shown that phospholipids adsorbed at the mercury/water interface may be used as mimetic sytems of charge transfer reactions across biological membranes.

Evidence has been presented suggesting that charge transfer mediated by UQ_{10}, is controlled by a rate limiting step involving the disproportionation of the semiquinone radical. It is hoped in conjunction with other techniques to gain a further insight to the mechanism of charge transfer across adsorbed lipid monolayers by UQ_{10}, which in turn may give a greater understanding of the charge transfer reactions of UQ_{10} within vivo. In future studies, the system may also help gain an insight to the charge transfer reactions of other membrane bound mediating species involved in fundamental charge transfer reactions.

Suggestions for further work

The preliminary work to date has allowed an insight to be made to the biomimetic system being studied. However further work is needed in order to gain a true understanding both of the model, and the implications it holds for charge transfer reactions within the inner mitochondrial membrane. The present work has generated many questions some of which possibly may be answered by the work suggested below.

a) Voltammetry using potential step techniques.

Rate constants may be calculated for the oxidation and reduction of UQ_{10}, with a greater degree of accuracy.

b) Investigation of the effect of pH on the UQ_{10}/lipid monolayer at the mercury interface over a wide range of pH values.

c) Investigation of the effect of temperature over a range of temperatures.

d) Voltammetry using further lipids such as dimysterol.

e) Fourier transform infra-red spectroscopy (FT-IR).

It is hoped to obtain infrared absorption spectra corresponding to oxidation states of UQ_{10} existing at different potentials.

f) Electroreflectance spectroscopy.

It is hoped to detect spectral changes within the UV/visible region corrosponding to different redox states whilst under potential control.

Both the FT-IR and the electroreflectance tecniques may show spectral changes at differing potentials corresponding to the structural differences of different oxidation states of ubiquinone.

g) Incorporation of other charge transfer molecules into adsorbed phospholipid monolayers.

A "flip-flop" mechanism has been suggested for the inner chloroplast membrane bound species plastoquinone, which operates within the photosynthetic chain in plants, as a charge transfer species. As an example it hoped that the present mimetic system may give insight into the charge transfer reactions in which plastoquinone acts as a mediating species.

References

(1) J.N. Prebble, Mitochondria, chloroplasts, and bacterial membranes, Longman, 1981, p.24-102.

(2) P. Mitchell, Nature (London), 191, (1961), p.144.

(3) P. Mitchell, FEBS lett, 59, (1975), p.137.

(4) A. Nelson and A. Benton, Phospholipid monolayers at the mercury/water interface, J. Electroanal Chem, 202, (1986), p.256.

(5) A. Nelson and N.A. Auffret, Phospholipid monolayers of Dioeyl lecithin at the mercury/water interface, J. Electroanal Chem. 244, (1988) p.99.

(6) R.A. Morton, Nature (London), 182, (1958), p.1764.

(7) R.H. Thomson, in Naturally Occuring Quinones, p.174-178.

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

(9) F.L. Crane in Prog. Chem. Fats, Lipids 7, No2, Hollman Edition, Pergamon Press, 1964, p.267.

(10) O. Isler, H.Mayer, R.Ruegg, and J.Wursch, Vitamins and Hormones, 24, (1960), p.331.

(11) O.Schindler, Prog.Chem.Org.Nat.Prods, 20, (1962), p.73.

(12) G.D. Daves, R.F. Muraca, J.S.Whittick, P.Friss, and K.Falkers, Biochemistry, 6, (1967), p.2861.

(13) A.M. Pumphrey, and E.R. Redfearn, Biochem. J, 76, (1960), p.61.

(14) Y. Hatefi, A.G. Haavik, L.R. Fowler, and D.E. Griffiths, J.Biol Chem, 273, (1962), p.1676, and p.1681.

(15) K.H. Leung and P. Hinkle, J. Biol Chem, 250, (1975), p.8467.

(16) C. I. Ragan, and C. Heron, Biochem. J, 174, (1978), p.783.

(17) P. Mitchell, Bioenergetics, 3, (1972) p.5.

(18) T. Chnishi, J.C. Salerno, H.Blum, J.S.Leigh, and W.J.Ingledew, Bioenergetics of Membranes and Boimembranes - Volume 1, Elsevier/North Biomedical press (1977), p.209.

(19) S. De Vries, S.P.J. Albracht, J.A. Berden, and E.C. Slater, J. Biol Chem, 256,(1981), p. 11996.

(20) S. De Vries, J.A. Berdon, and E.C. Slater, FEBS Lett, 122, p.144.

(21) A.A. Konstantinov, and E.K. Ruuge, FEBS Lett, 81. (1977),p.137.

(22) T. Ohnishi and B. Trunpower, J. Biol. Chem, 255, p.3278.

(23) P.R. Rich, and M. Wikstrom, FEBS 194, (1986), p.176.

(24) V. Moret, S.Pinamonti, and E. Fornasary, Biochem. Biophys. Acta, 54, (1961), p.381.

(25) M.F. Marcus, and M.D. Hawley, Biochimim. Biophys. Acta, 226, (1971), p.234.

(26) F.L. O'Brien and J.W. Olver, Analytical Chemistry, 41,(1969), p.1810.

(27) G. Cauquis and G. Marbach, Biochim. Biophys. Acta, 283, (1972), p.239.

(28) P. Hinkle, Boichem. Biophys Res Comm, 41, (1970), p.1375.

(29) P. Hinkle, Fed Proc 32, (1973), p.1988.

(30) D.W. Deamer, R.C. Prince and A.R. Croft, Biochim. Biophys Acta 274 (1972), p.323.

(31) G.Hauska and R.C. Prince, FEBS lett 41 (1974), p.35.

(32) R. Hill, A.R. Crofts, R.C. Prince, E. Hilary-Evans, N.E. Good and D.A. Walker, New Phytologist, 77, (1976), p.1.

(33) J. Bielawski, T.E. Thompson, and A.L. Lehninger, Boichem. Biophys. Res. Comms, 24, (1966), p.948.

(34) V.P. Skulachev, H.H. Sharaf and E.A. Liberman, Nature, 216, (1967), p.718.

(35) P. Mitchell in, Theroctical and Experimental Biophysics Vol 2, A. Cole Ed, Marcel Dekker, p.159.

(36) P.C. Hinkle, and L.L. Horstman, Journal Biol Chem, 246, (1971), p.6024.

(37) G. Hauska in, Bioenergetics of Membranes, Developments in Bioenergetics and Biomembranes Volume one, (1977) Elsevier/North, - Holland biomedical press, p.177.

(38) P.S. Vincent and G.G.Roberts, Thin Solid Films, 68, (1980), p.135.

(39) P.S. Vincett, W.A. Barlow, F.T. Boyle, J.A. Finney, and C.G. Roberts, Thin Solid Films, 60, (1979), p.265.

(40) J. Holoyda, C.R. Kannewarf and J.W. Kaufman, Thin Solid Films, 69, (1980), p.205.

(41) M. Suki, K. Nembach, and D. Mobius, Thin Solid Films, 27, (1975), p.205.

(42) M. Calvin, Science, 184, (1974), p.375.

(43) N. L. Gershfield, J. Phys. Chem, 66, (1962), p.1923.

(44) M. Montal, and P. Muller, Proc. Natl Acad Sci USA, 69, (1972), p.3561.

(45) H.H.J. Girault and D.J. Schiffrin in M.J. Allen and P.N.R. Usherwood (Eds), Charge and Field Effects in Biosystems, Abacus press, England, (1984), p.171.

(46) S. Ohki, C.B Ohki, and N. Duzgunes, Adv. Colloid Interface Sci, 5, (1976), p.271.

(47) H.J. Girault, and D.J. Schiffrin, J. Electroanal Chem, 179 (1984), p.277.

(48) I.R. Miller, J. Rishpon, and A. Tenbaum, Bioelectrochem. Bioenerg, 3, (1976), p.528.

(49) M.F. Lecompte and I.R. Miller, Biochemistry, 19, (1980), p.3439.

(50) I.R. Miller in, Topics in Bioelectrochemistry and Bioenergetics, Wiley Chichester, (1981), p. 161.

(51) J.M. Croxley, Biophys J. 13, (1973), p.711.

(52) A. Kroger, M. Klingenberg, and S. Schweidler, Eur. J. Biochem, 34, p.358.

(53) Stryer in Biochemistry, W.H. Freeman and company, San Fransisco, p.114.

(54) R. Kornberg, and H.M. McConnell, Proc. Nat. Acad. Sci. USA, 68, No 10, (1971) p.2564.

(55) A. Futami, H.Eduart, and G. Hauska, Biochim. Biophys. Acta, 547, (1979), p.583.

(56) A. Futami, and G. Hauska, Biochim. Biophys. Acta, 547, (1979), p.597.

(57) T. Ohnishi, J.C. Salerno, H. Blum, J.S. Leigh, and W.J. Ingledew : Bioenergetics of membranes, (1977), L. Packer et al ed, Elsevier/North Biomedical Press.

(58) J. Cain, G. Santillan, and J.K. Blasie, Molecular motion in membranes as indicated by X-ray diffraction, in Biological Membranes, Plenum, (New York) (1981).

(59) M. Wilstrom, and K. Krab, Curr. Topp. Bioener, 10, p.51. Membranes, Plenum (1981).

(60) M. Wilstron, and M. Saraste in Bioenergetics. Elsevier/North Biomedical press (1984), p.49.

(61) P. Mitchell, J. Theor. Biol, 62, (1976), p.327.

(62) B. Chance and M. Erecinska, Eur J. Biochem, 54, (1975) p.529.

(63) P.B. Kingsley, and G.W. Feigenson, Biochim. Biophys. Acta, 635, (1981), p.602.

(64) P.R. Rich, Biochim. Biophys. Acta, 637, (1981), p.23.

(65) J.W. DePierre, and L. Ernster, Annu. Rev. Biochem, (1977), 46, p.201.

(66) R.N. Robertson, and N.K. Boardman, FEBS Lett, 60, p.1.

(67) The physical chemistry of lipids, Handbook of lipid research (Plenum), - appendix IV, p.629.

(68) R. Harrison, and G. G. Lunt in, Biological Membranes, Blackie and Sons, London, (1980), p.268.

(69) K. Aoki, K. Tokuda, and H. Matsuda, J. Electroanal. Chem, 160, (1984), p.33.

(70) A.A. Frost and R.G. Pearson, in kinetics and mechanism, p.13 Wiley International edition, New York.

(71) C.P. Andrieux, and J.M. Saveant, J. Electroanal. Chem, 111, (1980), p.337.

(72) CRC Organic compound identification handbook, 52nd Edition, p.D120.

(73) G.B. Barlin and D.D. Perrin, Quarterly reviews, 20, (1966), p.75.

(74) V.J. Cunnane, D.J. Schiffrin, M. Fleischman, G. Geblewicz, and D. Williams, J. Electroanal. Chem, 243, p.455.

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