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Microwave Hall Mobility Measurements on Heavy Beef Heart Mitochondria

D. D. Eley, R. J. Mayer* and R. Pethig†

Department of Chemistry, University of Nottingham
** Department of Biochemistry, The Medical School, University of Nottingham*

Abstract

The observed initial microwave Hall mobility values at 1·21 tesla of heavy beef heart mitochondria is at least six times greater than that observed for bovine serum albumin at similar resistivity values. The respiratory inhibitor cyanide significantly reduces the initial Hall mobility values for HBHM and for a preparation of HBHM cytochrome oxidase.

The four enzymic complexes of the respiratory chain were partially or completely separated. Of these complexes cytochrome oxidase exhibits the largest microwave Hall mobility.

The maximum hydration content of loosely bound water for freeze-dried preparations of cytochrome oxidase is 5% by weight; 60% of this hydration content is driven off by microwave power. Since the effective ac resistivity of the samples of cytochrome oxidase did not appreciably vary with changes in hydration content, the true resistivity of cytochrome oxidase has a value of the order 5×10^3 ohm cm and possibly much lower.

The electron transport pathway (as measured by Hall signal) of cytochrome oxidase is irreversibly damaged by prolonged exposure to microwave irradiation at 9·2 GHz. This is accompanied by the complete loss of capacity to oxidise ferrocytochrome *c*. Such changes do not occur with HBHM or with the other respiratory complexes.

There appears to be a direct relationship between observed Hall signals and the capacity of cytochrome oxidase to oxidize ferrocytochrome *c*. There is a "background" signal which is not directly related to electron transport but which is dependent on the conformation of the cytochrome oxidase.

The observed electronic parameters of cytochrome oxidase do not depend appreciably on its redox state.

Acid denaturation of cytochrome oxidase drastically reduces the Hall signal, to include almost complete removal of the "background" signal. It also more than doubles ac resistivity.

An electron tunnelling model is outlined.

Introduction

The mechanisms of charge transfer in the respiratory chain are not fully understood. The fact that charge transfer is greatly inhibited and

† Present address: School of Engineering Science, University College of North Wales, Bangor, Caerns.

possibly completely stopped at liquid nitrogen temperatures has been interpreted in favour of transport of electrons via mobile carriers (e.g. coenzyme Q, cytochrome *c*) rather than mechanisms based on resonance energy transfer or long range electron transport through conduction bands.[1,2]

The observed temperature variation does not, in fact, preclude the possibility that charge transfer occurs via conduction bands within individual molecular complexes of the cytochrome system. Transfer between complexes could involve a temperature activated tunnelling or hopping mechanism between the individual conduction band systems. Potential energy barriers could also exist within a particular cytochrome complex, the barrier shape and hence temperature variation of charge transfer being dependent on the molecular conformation.

In previous work[3] it was shown that freeze dried preparations of rat liver mitochondria gave an N-type Hall signal, much greater than that obtained from bovine serum albumin at the same resistivity values. This signal was significantly reduced by cyanide, but not by rotenone or Antimycin A.

Materials and Methods

The microwave Hall mobility measurements described here have been obtained using a microwave system based on that described by Trukhan.[4] A detailed account of the pertinent theory and experimental procedure has been given elsewhere.[5]

Preparations of heavy beef heart mitochondria (HBHM) were obtained as described by Smith.[6] The enzymic activities of the electron transport chain were partially or completely resolved to give Complex I + III (NADH-cyt. *c* reductase[7]), Complex II (succinate-coenzyme Q reductase[8]) and Complex IV (cytochrome oxidase[9]).

NADH-cytochrome *c* reductase was assayed as described by Halefi and Rieske,[7] in the absence of added phospholipid. Cytochrome oxidase was assayed as described by Wharton and Tzagoloff.[10] Succinate-cyt. *c* reductase (Complex II + III) activity was assayed as described by King.[11] Succinate-coenzyme Q reductase was not assayed.

No contamination of cytochrome oxidase by NADH-cyt. *c* reductase or succinate cyt. *c* reductase could be demonstrated. The NADH-cyt. *c* reductase was contaminated to less than 5% with cytochrome oxidase and less than 0·01% with succinate-cyt. *c* reductase.

Protein was determined by the method of Lowry, Rosebrough, Farr and Randall.[12]

Preparations of cytochrome oxidase were treated as follows: to 1 ml samples were added (a) 1 ml of aqueous KCN (final conc. 10^{-4} M); 0·2 ml of hydrogen peroxide (10 vols); sodium dithionite (1 mg); 1 ml of ferrocytochrome *c* (1%, produced as described by Wharton and

Tzagoloff;[10] 1 ml of ferrocytochrome *c* followed by 1 ml of KCN (final conc. 10^{-4} M) or sodium azide (final conc. 10^{-4} M).

A sample of cytochrome oxidase was denatured by titration with 6 N-HCl to pH 1 in the presence of Triton X-100 (final conc. 0·5%).

Preparations were freeze-dried for 18 hr prior to making discs for the Hall signal measurements.

Results

The results presented in Table I show that samples of HBHM give Hall signals which, as in preparations of mitochondria from rat liver,[3] are significantly affected by cyanide, but not by antimycin-A and rotenone. The Hall mobility is proportional to the square root of the intensity of the Hall signal.[4]

TABLE I. Initial permittivity, resistivity and Hall mobility measurements on freeze-dried samples of HBHM

	ϵ'	Resistivity (ohm cm)	Hall Mobility* ($cm^2/V.sec$)
HBHM	2·27	$4·15 \times 10^3$	12·2
HBHM + potassium cyanide	2·32	$2·84 \times 10^3$	8·15
HBHM + antimycin-A	2·24	$4·60 \times 10^3$	11·2
HBHM + rotenone	2·39	$5·80 \times 10^3$	11·7

* Calculated on basis that disc is 100% protein (see text).

All the Hall effect results are N-type and the applied field was 1·21 tesla. 1 ml of a preparation of HBHM or 1 ml of a preparation of HBHM treated with aqueous KCN (final conc. 10^{-4} M), ethanolic antimycin-A (0·125 μg/mg protein) or ethanolic rotenone (0·25 μg/mg protein) were freeze dried. Samples of these preparations were used for the measurements.

The Hall mobility values are expressed on the basis that all the sample placed in the bimodal cavity of the Hall apparatus is protein. This approach is used since previous work has shown that mitochondrial lipid gives a small Hall signal.[3]

The marked effect of cyanide in reducing the observed Hall signal suggests that the signal obtained from untreated HBHM may originate from the cytochrome oxidase part of the respiratory chain, since the site of action of cyanide is with the cyt.-a_3 moiety of cyt. oxidase[13] and the associated copper.[14]

To test this hypothesis, the various respiratory complexes were completely or partially purified from HBHM. Hall signal measurements were made on samples of the complexes. Typical results are given in Table II.

The greatest Hall mobility is given by cytochrome oxidase. Smaller Hall signals were given by NAD-cyt. *c* reductase and succinate-coenzyme Q reductase.

TABLE II. Initial permittivity, resistivity and Hall mobility measurements on freeze-dried samples of respiratory complexes

	ϵ'	Resistivity (ohm cm)	Hall mobility* ($cm^2/V.sec$)
HBHM	2·27	$4·15 \times 10^3$	12·2
Complex I + III (NADH-cyto. *c* reductase)	2·46	$2·54 \times 10^3$	4·32
Complex II (Succinate-coenzyme Q reductase)	2·38	$5·13 \times 10^3$	7·5
Complex IV (cytochrome oxidase)	2·56	$3·82 \times 10^3$	26·4

* Calculated on basis that disc is 100% protein.
All the Hall effect results were N-type and the applied field was 1·21 tesla. Complexes prepared as given in *Materials and Methods.*

Apart from exhibiting the largest Hall signal, cytochrome oxidase differed from HBHM and the other respiratory complexes in that the observed Hall signal decayed appreciably with time. The Hall signal given by cytochrome oxidase decayed from 26·4 to 15·75 $cm^2/V.sec$ in approx. 24 hr whereas the signal from HBHM decayed from 12·2 to 11·5 $cm^2/V.sec$ in 6 hr and appeared to reach a constant value.

The decay of observed Hall signal given by cytochrome oxidase is shown in Fig. 1.

Not only did the Hall signal from the cytochrome oxidase preparation decay with time, but the overall shape of the Hall signal changed in that the normal Hall signal shape around 0·2–0·5 tesla, where paramagnetic absorption effects are expected to be observed, decreased in magnitude and extent with time. This is also shown in Fig. 1.

The decay in Hall signal from cytochrome oxidase was paralleled by a decrease in the ability of cytochrome oxidase (when reconstituted in an aqueous medium) to oxidize ferrocytochrome *c*. This result, on which a preliminary note has been published,[15] is for convenience again presented in Fig. 2.

This result strongly suggests that there exists a close relationship between the observed Hall signal and the capacity of cytochrome oxidase to oxidize ferrocytochrome *c*, and that the prolonged exposure to microwave radiation (60 mW) produces deleterious effects on the cytochrome oxidase complex. A large residual Hall signal is given by the cytochrome oxidase preparation when extrapolated to zero enzymic activity. This may indicate charge transfer pathways not connected with electron transport in the respiratory chain.

In the microwave cavity, the samples lose weight. A 3% weight loss occurs after 4 hr in the cavity while a specimen left in the cavity for

24 hr (in a vacuum of 0·133 N/m²) shows a loss of weight of 4·93%. The loss of weight is interpreted to be a loss of water from the sample.

The observed reduction in the Hall signal of cytochrome oxidase

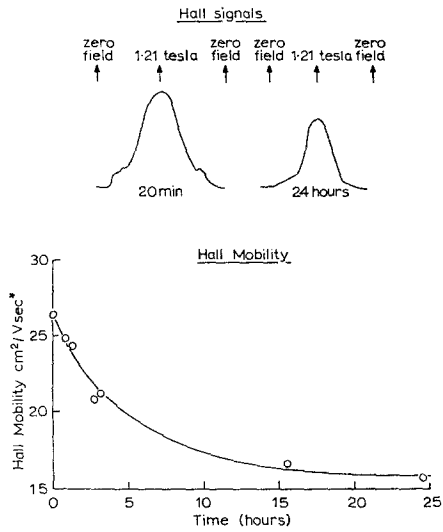

Figure 1. Variation of Hall signal, and Hall mobility of cytochrome oxidase with time spent in the bimodal cavity. * Hall mobility corrected to 100% protein.

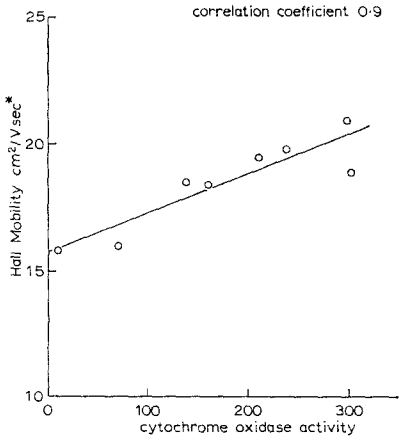

Figure 2. Variation of Hall mobility with cytochrome oxidase activity (from ref. 15). Cytochrome oxidase was assayed as indicated in ref. 10. * Hall mobility corrected to 100% protein.

with time in the bimodal cavity could possibly result from the oxidation of cytochrome oxidase (although this effect must be minimal since the bimodal cavity is flushed with N_2 during the course of each experiment) or the denaturation of the sample by microwave power.

To examine these possibilities, experiments were performed with fully reduced and oxidized cytochrome oxidase. The effect of supplying the electron transport chain with excess free electrons by adding ferrocytochrome *c* to cytochrome oxidase was also tested, together with the effects of cyanide and azide in the presence of these excess free electrons (Table III).

The addition of a drop of H_2O_2 to 1 ml of the cytochrome oxidase preparation caused an immediate precipitation of the cytochrome oxidase in a similar manner to the acid denaturation of cytochrome oxidase. Hence, the change in Hall signal probably reflects denaturation rather than oxidation of cytochrome oxidase.

Both cyanide and azide, known inhibitors of cytochrome oxidase, drastically reduce the Hall signal compared with an untreated cytochrome oxidase preparation. The addition of ferrocytochrome *c* as a free electron source did not appreciably alter the observed electronic parameters of cytochrome oxidase or the way in which the Hall signals decayed with time spent in the microwave cavity, although the Hall signals did exhibit larger paramagnetic absorption effects in the field region around 0·2–0·5 tesla than those for the untreated cytochrome oxidase preparation. Addition of cyanide or azide to the ferrocytochrome *c* reduced preparations produce the same fall in Hall signal as in the absence of a free electron source. Addition of dithionite to reduce the cytochrome oxidase did not change the observed electronic parameters or make an appreciable difference in the way the Hall signals decayed with time spent in the microwave cavity.

TABLE III. Initial permittivity, resistivity and Hall mobility measurements for cytochrome oxidase preparations

	ϵ'	Resistivity (ohm cm)	Hall mobility* ($cm^2/V.sec$)
1. Cytochrome oxidase	2·56	$3·82 \times 10^3$	26·4
2. Cytochrome oxidase + cyanide	1·99	$2·18 \times 10^3$	9·92
3. Cytochrome oxidase + H_2O_2	2·49	$3·21 \times 10^3$	4·94
4. Cytochrome oxidase + dithionite	2·12	$4·91 \times 10^3$	16·92
5. Cytochrome oxidase + ferrocytochrome *c*	2·61	$3·10 \times 10^3$	13·4
6. Cytochrome oxidase + ferrocytochrome *c* + cyanide	2·44	$3·19 \times 10^3$	5·74
7. Cytochrome oxidase + ferrocytochrome *c* + azide	2·29	$4·13 \times 10^3$	8·33
8. Acid denatured cytochrome oxidase	2·02	$1·07 \times 10^4$	4·08

* Calculated on basis that disc is 100% protein.

Details of treatments applied to samples of cytochrome oxidase are given in Materials section.

Acid denaturation of cytochrome oxidase causes a drastic alteration in electronic properties. Besides the reduction in Hall signal, the resistivity of the sample changed significantly. Although a drastic fall in Hall signal occurred on H_2O_2 treatment, no change in resistivity was observed. This indicates a major difference in the action of the two sets of treatments.

Discussion

The observed reduction of the Hall signals with time for cytochrome oxidase was attributed to irreversible denaturation rather than oxidizing effects. Supporting evidence for such a conclusion comes from the observation of Muijers *et al.*[16] that, whereas at least three, possibly four different conformations of oxidized cytochrome oxidase can be distinguished depending upon the presence of molecular oxygen, a denaturation of a small amount of this enzyme during oxygenation might have a more profound effect on the observed oxidation rate than conformational charges induced by oxygenation. Also, conformation changes of cytochrome *c* upon oxidation have apparently no influence on the oxidation rate constants.[17]

From measurements on whole mitochondria from rat liver, it was concluded that the observed Hall signals corresponded to a true microscopic Hall mobility of 50–80 $cm^2/V.sec$, measurements on cytochrome oxidase prepared from HBHM gave Hall mobility values of the order 20 $cm^2/V.sec$ when calculated on a protein content basis. The fact that addition of cyanide and also azide, reduced the observed Hall effect for cytochrome oxidase strongly suggests that the observed signals are associated with electron transport in cyt. a_3 and/or the associated copper atoms. Since the molecular weight of cytochrome oxidase is probably some four times greater than that of cyt. a_3, the true microscopic Hall mobility for cyt. a_3 can be estimated to be of the order of 80 $cm^2/V.sec$.

Dickerson *et al.*[18] have recently reported their results for the 2·8 Å structure determination of horse ferricytochrome *c*. One of the reasons for obtaining this structure was to aid the understanding of how electron transfer occurs into or out of cyt. *c*. The essential feature of the cyt. *c* structure is that the haem is tightly enveloped in hydrophobic groups, with two "channels" filled with hydrophobic side chains leading to the right and left from the haem to the surface of the molecule. It was observed that the aromatic groups have a remarkable tendency to occur in approximately parallel pairs and these authors speculated on the transfer of electrons via the overlap of aromatic π-electron bonds. Another possible electron pathway could involve residues 92 to 104 which form an α-helix. Three of the residues forming the α-helix (Leucine 94 and 98, Iso-leucine 95) have side chains which pack

against the haem, whilst the two lysine[99, 100] residues lie at the edge of the right "channel", which contains two parallel aromatic groups. It would be of interest to see if theoretical calculations applied to this combination of α-helix and overlapping π-electron bonds could indicate the existence of a well defined energy band for rapid electron transfer. If electron transport was confined to a similar pathway in cyt. a_3 (i.e. of the order 15% of the total molecule) then the true microscopic Hall mobility for cyt. a_3 would be one of the order 500 $cm^2/V.sec$.

It can be readily shown that the magnitude (S) of the oscillatory path described by the electrons under the influence of the microwave electric field is given by

$$S = \mu \hat{E}/2\pi f$$

where μ is the electron conductivity mobility, $\hat{E}$ the maximum electric field strength value in the sample and f is the microwave frequency. For our cavity and Klystron power source and assuming a specimen relative permittivity value of 2·5, then the value for $\hat{E}$ is of the order 11 V/cm. For a true microscopic Hall mobility of 500 $cm^2/V.sec$ (and assuming a 1:1 relationship between the Hall and conductivity mobilities), then for a frequence of 9 GHz, the electron pathway is of the order 9·7 Å, not an unreasonable value.

If the complete electron pathway within the cyt. a_3 molecule does involve a combination of α-helix and overlapping π-electron bonds, then studies of the variation of the Hall mobility with conformation changes should be of interest. Temperature variation measurements may reveal the activation energy required for the electron to traverse the "gap" between the π-bonds and the α-helix.

A Semiconductor Model for the Mitochondria

In order to construct a working hypothesis, we shall take the biological macromolecule as a potential energy box containing a full valence band of electrons and an empty conduction band separated by an energy gap $\Delta\epsilon$.[19, 20] An electron in the conduction band is regarded as tunnelling through the potential energy barrier between one macromolecule and the next, to give the observed conductivity of films and crystals of proteins and other organic semiconductors, see Fig. 3. This model has been advanced on general physical grounds over many years to account qualitatively for a large volume of experimental data, and for details reference should be made to a review.[21] In the absence of information on the state of hydration of proteins in the mitochondrial membrane, we assume they are held in their lipoidal environment in an essentially dry state. We know that a very large number of proteins, including cyt. c in the very dry state have $\Delta\epsilon \simeq 2{\cdot}6$ eV and we accept the idea of energy bands, width about 0·2 eV, based on mobile π electrons in the

$C = O \ldots HN$ hydrogen bond system,[22] realizing that modern quantum calculations give $\Delta\epsilon$ values of around 5 eV[23] which, however, may be brought down to the experimental value if polarization terms be included.[24] It is possible in favourable circumstances that these hydrogen-bonds combine with the mobile π electrons of phenyl side chains to give a specially favourable pathway, as we have just considered for cyt. *c*. The above interpretation regards the dry protein as an essentially intrinsic semiconductor with a very high room temperature resistivity of around 10^{17} ohm cm. By contrast, cytochrome oxidase shows a higher conductivity, with $\Delta\epsilon = 2{\cdot}0$ eV at >50°C and

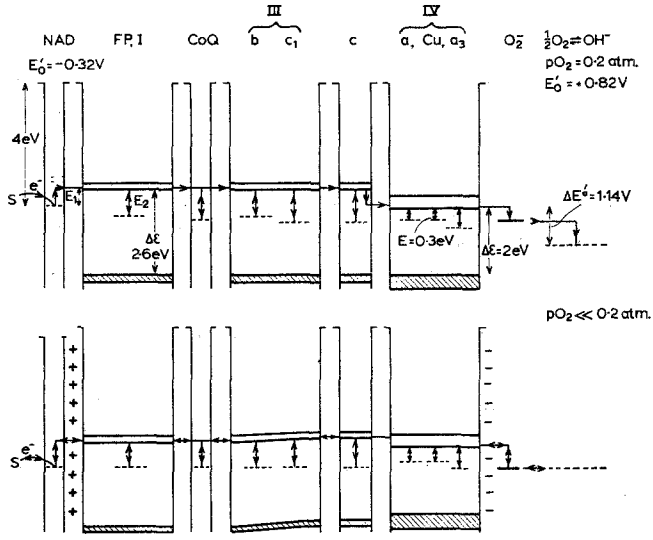

Figure 3. An electron potential energy box model for electron transport through the protein complexes of the mitochondrion. NAD nicotine adenine dinucleotide; FP I Flavoprotein Complex I; CoQ Coenzyme Q; III *b* c_1, Complex III, cytochromes *b* and c_1; C cytochrome *c*; IV *a*, Cu, a_3, Complex IV, cytochrome *a*, copper, cytochrome a_3. The zero of energy is the electron at infinity and the model is explained in the text.

$\Delta\epsilon = 0{\cdot}6$ eV ($E = 0{\cdot}3$ eV) below 50°C.[25] While the first value may be the intrinsic energy gap, the second value is almost certainly an impurity semiconduction based on the copper atoms in the molecule. To date, it is interesting that we have no d.c. dark conduction evidence for the haems acting as impurity levels, although we believe that infrared photoconduction we have detected in haemprotein systems may very well arise from excitation of trapped electrons on these prosthetic groups, so that dark conduction data may be forthcoming in future.[26]

For the present purpose, we shall neglect the succinate-Fraction II pathway to coenzyme Q and consider the electron transfer series below, the components following in physical juxtaposition the order of their

redox potentials, values from Mahler and Cordes.[27] The abbreviations have their usual significance.

		I			III		
	NAD	→ FPN	→ CoQ	→ (cyt. b)$_2$ ... cyt. c_1	→ cyt. c	→
$E^{0'}$ (V)	−0·32	0·00	0·10	0·12	0·21	0·23	

IV

→ (cyt. a)$_n$ Cu$_m$ (cyt. a_3)$_n$ Cu$_n$ → O_2

0·29 0·35 0·82

More recent[28] relevant values of $E^{0'}$ are b 0·038, $c + c_1$ 0·227, a 0·205 and a_3 0·365 V. Erecinska *et al.*[29] give $E^{0'} = 0{\cdot}24$ V for the 830 nm copper signal in intact pigeon heart mitochondria, which is less than the 0·35 V quoted. In Fig. 3, we have arranged single-electron potential energy boxes representative of the different molecules from left to right, with dotted lines denoting the $E^{0'}$ levels, which represent the standard free energy of the electron on the prosthetic group, regarded as an impurity level below the conduction band of the protein concerned. The redox potential level may be regarded on this picture as a Fermi level between the actual impurity level and the bottom of the conduction band. The energy zero for the diagram is the electron at infinity, and the NAD redox level is fixed with respect to this by regarding it as equivalent to an electron affinity for the solvated molecule of 4·0 eV (cf. crystalline adenine 5·3 eV[30]). A value of 3·5 eV is ascribed to the electron affinity i.e., the bottom of the conduction band for the proteins, with the exception of cytochrome oxidase with its lower $\Delta\epsilon$ (2·0 as against 2·6 eV), where the valence band levels are matched. The band width for cytochrome oxidase is put at 0·4 eV, wider than the other proteins, on mobility grounds (see later). These small electron-transfer units may be regarded as fixed to the inner matrix side of the mitochondrial membrane, each unit held rigidly, apart from the mobile units coenzyme Q and cyt. c.[31] The linear dimension of the potential box is drawn to bear a rough relationship to the molecular weight of the molecule or molecular aggregate concerned. Each transfer unit will occupy about 50,000 Å^2 of membrane surface[32] and this gives about 3×10^4 transfer units on the cristae membrane of one liver cell mitochondrion, total area 16μ^2.[33] A later more detailed view[32] doubles the chain back across the *thickness* of the membrane, with cyt. a_3 on the matrix side leading to c on the "outer" or c side and back to the flavoprotein on the matrix side. This model is concerned with proton transfer across the membrane and is probably the best for further consideration from our viewpoint. However, at present we omit any attempt to link our model with the established models of proton transfer and phosphorylation[34] and concentrate on the electron path.

We suggest that the electrons travel mainly via the conduction bands, the redox levels acting as trapping states, as indicated by the arrows. An electron from substrate S is transferred to nicotine adenine dinucleotide (NAD) and hence via an activation energy of E_1 to the conduction band of the flavoprotein oxido-reductase, Complex I. Whether the measured activation energy of 14 Kcal mole^{-1} (0·6 eV)[35] refers to injection from substrate E_1, or excitation from the redox level E_2, is left open. The electron then descends down the redox level gradient, probably via conduction bands as shown, although tunnelling between impurity levels may need consideration (depending on the distance between neighbouring redox groups). Microwave Hall mobility values of 50 cm^2/Vs for the mitochondrion[36] and 25 cm^2/Vs for cytochrome oxidase (this paper) both reduceable by inhibitor concentration of KCN, identify cytochrome oxidase, complex IV as the particle of maximum electron mobility. This is emphasized by the correlation between the oxidase activity of cytochrome oxidase and its microwave Hall electron mobility.[14] For this reason we have ascribed it bands of 0·4 eV width, wider than the other proteins. However, even so, at the cyt. *c*–cytochrome oxidase interface it appears necessary for phonon emission to accompany electron tunnelling between conduction bands, a process known in solid state physics as inelastic phonon-assisted tunnelling.[37] This interface is one of the three mitochondrial sites for undergoing phosphorylation and it may be speculated that the emitted phonon (an energy quantum of lattice vibration) would provide the energy trigger for this process (cf. considerations by Cope and Straub[25]). It has been suggested that dark conduction in cytochrome oxidase and its enzymic activation energy for substrates, are equal to a Fermi level depth of 0·3 eV (energy level depth of 0·6 eV) below the conduction band.[38] There is very good evidence that a possibly similar reaction, the cyt. *c*-bacterio chlorophyll photoactivated reaction occurs by tunnelling with zero or very low activation energy below 50°K.[39] The O_2^- radical is a logical chemisorbed intermediate and this is not ruled out by biochemical evidence.[40] We have placed the O_2^- energy level at about 4·5 eV, by comparison with its energy level adsorbed on inorganic oxides like ZnO.[41] The final state, of course, is OH^-, the total drop in standard redox potential from NAD to OH^- being 1·14 V.

The dc drift mobility of 2×10^{-2} cm^2/Vs[42] in mitochondria on this view corresponds to the tunnelling probability between protein molecules.[20] The microwave Hall mobility of 25–50 cm^2/Vs already mentioned, on the other hand, refers to the to-and-fro motion of the electron over about 10 Å at 9·2 GHz, along the path of highest mobility, identified in this and the preceding paper[15] as cytochrome oxidase.

If we de-energize the mitochondria by reduction of the oxygen

pressure, the equilibrium redox potential will rise (i.e. tend from positive to zero values) as shown in the bottom part of Fig. 3. In other words, as electrons pass down the chain, they will accumulate on the active centre of cytochrome oxidase, giving rise to a *change* in the electrostatic potential difference across the transfer unit and causing all the energy levels to rise. Where the redox levels throughout are equal we shall have equal numbers of electrons passing in both directions and an electrostatic potential of 1·14 V across the transfer unit. However, it might be more reasonable to allow for a change of 0·1 V in redox potential. Allowing an overall length per unit of 500 Å, so there will be a voltage drop of 2×10^4 V/cm, which should exert a marked piezoelectric strain (contraction) on a "soft" biopolymer system. The changes which result will depend on the geometrical relationship between transfer unit and membrane, but these could be quite large for certain geometries which might favour the "bimetallic strip" type of behaviour. Large conformational changes are, of course, observed in the membrane in its different states.[43]

We have made no attempt to speculate on the states of hydration of the proteins, although these can profoundly increase conductivity by introducing donor impurity levels on one view,[44] or changing the microscopic dielectric constant and contracting the energy gap between conduction and valence bands on another.[24] It is possible also that we have neglected a charge transfer role of coenzyme Q, which has been found to induce conductivity in cyt. *c* by a charge-transfer interaction (quinones act as electron acceptors with proteins[44]). In an early publication, Cardew and Eley[45] pointed out that a redox driving potential of 1 V could only drive a current through a dry protein fibre 1 micron long × 50 Å diameter (a primitive mitochondrial model) 10^{16} times smaller than the electron transfer rate due to respiration. Furthermore, the necessary activation energy of $\frac{1}{2}\Delta\epsilon$ i.e. 1·3 eV was much greater than the 0·48 eV usually observed for the overall process. Rosenberg and Postow[24] more recently reconciled the magnitudes by allowing for hydration of the protein. In the present model we are concerned essentially with electrons injected from the substrate over a barrier of 0·5 eV into the conduction band, so there should be no problem even with the dry protein, although we have not attempted as yet detailed calculations. The misleading concept in our past calculation was to base our calculations on electrons excited over $\Delta\epsilon = 2{\cdot}6$ eV from the valence band. We also note that Cope and Straub[25, 38] have reconciled the oxygen kinetics on the basis of a slow process of electron transfer from substrate through cytochrome oxidase.

In conclusion, we claim no more than that there is a logical case for considering membrane bound electron transfer enzymes from the viewpoint of organic semiconductors, and that the present model serves to reconcile the scanty evidence at present available. We

hope that it will serve to stimulate and guide further experimental work.

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