

## **Microwave Hall Mobility Measurements on Rat Liver Mitochondria and Spinach Chloroplasts**

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### *Abstract*

The effect of known respiratory inhibitors on the charge carrier Hall mobility of rat liver mitochondria has been investigated using a microwave technique. Potassium cyanide and rotenone were found to reduce the Hall mobility, but no effect was observed for antimycin-A. The marked effect of potassium cyanide and the low mobility value obtained for the lipid extract of the mitochondria, suggests that electronic conduction through the electron transport chain is being observed. Volume-corrected values of between 50 and 80 cm<sup>2</sup>/V sec are found for the electron Hall mobility.

Measurements on spinach chloroplasts give P-type Hall mobility values of 0.5 and 0.8 cm<sup>2</sup>/V sec.

### *Introduction*

Our present extensive knowledge of biological oxidation and photosynthesis is based on classical biochemical methods of enzyme isolation and identification, supplemented more recently by electron microscopic studies of the intact organized membrane systems. In the respiratory chains in the mitochondria of animals, plants and protozoa, or in the protoplast membranes of certain bacteria, biological oxidation of substrates involves electron transport between the substrates and molecular oxygen, via dehydrogenase proteins, coenzymes, cytochromes and cytochrome oxidase. Photophosphorylation in chloroplasts involves a cyclic electron flow terminated by photo-sensitive pigments, involving an electron-transport chain whose components are not completely known. Knowledge of the physical conduction mechanisms of such biological electron-transport chains in the intact cellular membranes may obviously be approached from the viewpoint of solid-state physics. Here we describe preliminary measurements on rat liver mitochondria and spinach chloroplasts using a microwave technique which allows us to identify the sign and to measure the mobility of the majority charge carriers in these biological particles.

### *Measurement Technique*

Electrical conductivity and Hall effect measurements of charge carrier mobility are normally used in the studies of conduction processes in semiconductors. Unfortunately, conventional d.c. and a.c. electrical measurements on polycrystalline biological materials

are complicated by electrode effects and heterogeneities associated with intercrystalline boundaries and intracrystalline defects arising from denaturation and sample preparation. In particular, low-frequency Hall effect measurements on inhomogeneous materials can give erroneous values for the magnitude and sign of the charge carrier mobilities. Trukhan has shown<sup>1,2</sup> that these difficulties are overcome by taking measurements at frequencies of the order 10 GHz.

The dielectric and Hall mobility measurements given here have been obtained using a microwave system based on that described by Trukhan.<sup>3</sup> The sample, in the form of a lightly compressed thin disc, is placed in the centre of a cylindrical bimodal cavity<sup>4</sup> operating in the TE<sub>11</sub> mode. The unloaded resonant frequency and  $Q$ -factor of the cavity are 9.23 GHz and 9200, respectively, and the complex permittivity ( $\epsilon' - j\epsilon''$ ) of the specimen is given by the perturbations of these values resulting from introduction of the specimen into the cavity. A modification of the equations of von Aulock and Rowen<sup>5</sup> gives the resonant frequency shift  $\Delta f/f_0$  and  $Q$ -factor change  $\Delta(1/Q)$  as

$$2(\Delta f/f_0) - j\Delta(1/Q) = \{(\epsilon' - 1) - j\epsilon''\} 2tR/L$$

where  $t$  is the specimen thickness,  $L$  the cavity length and  $R$  is a geometry factor. For our cavity of diameter 2.90 cm, the value of  $R$  for a 5-mm diameter disc is 0.063. The effective a.c. conductivity of the specimen is given by

$$\sigma = \sigma_0 + 2\pi f\epsilon_0 \epsilon''$$

where  $\sigma_0$  is the d.c. conductivity and  $f$  is the measurement frequency.

The Hall mobility values are obtained from measurement of the transmitted power through the bimodal cavity resulting from the Faraday-type rotation of the plane of polarization of the incident power as it passes through the specimen under the influence of a steady magnetic field. The theory of the microwave Hall effect for biological materials is complicated by the presence of displacement current arising from loosely bound water. It can be shown<sup>6</sup> that inclusion of the relevant Hall fields into Maxwell's equations gives the Faraday-type rotation as

$$\theta = \frac{1}{2}(\mu/\epsilon)^{1/2} \{\sigma^2 R_c - w^2 \epsilon^2 R_D\} Bt$$

where  $B$  is the magnetic field strength and  $R_c$  and  $R_D$  are the conduction current and displacement current Hall coefficients, respectively. It can be seen that the effect of a displacement current Hall effect is to reduce the effective Faraday rotation produced by free charge carriers.  $R_c$  is inversely proportional to the density of free charge carriers, and by analogy  $R_D$  will be inversely proportional to the number of dipoles associated with the displacement current (see ref. 7). We have estimated<sup>8</sup> the electron carrier density for conduction in the BPA/strongly bound H<sub>2</sub>O system to be of the order 10<sup>9</sup> cm<sup>-3</sup>. If this can be taken as a representative charge carrier density for biological materials in general, then even for the lowest conceivable hydration state, the number of dipoles associated with loosely bound water will ensure that  $R_c \gg R_D$  and that the free carrier dominates the Faraday rotation. The contribution (if any) of Faraday rotations associated with free radicals and bound donor or acceptor sites can be determined by taking measurements with the specimen placed at various positions between the node and anti-node of the microwave electric field.

*Rat Liver Mitochondria*

Rat liver mitochondria were prepared for us by Miss Elizabeth Shephard using the technique previously described by herself and Professor G. Hübscher.<sup>9</sup> After the first prepared samples were found to exhibit a moderately large Faraday-type rotation, two more batches were prepared so that the effect of known mitochondrial respiratory inhibitors could be investigated. The inhibitors used were antimycin-A, potassium cyanide and rotenone. The samples were incubated with an inhibitor addition at 37°C for 33 min and subsequently freeze dried. A control sample without any inhibitor addition was given the same incubation and freeze-drying treatment.

The permittivity, resistivity and Hall mobility values obtained are presented in Table I, together with the protein concentration for each batch and the inhibitor concentration. Rat liver mitochondria contains about 38% lipid mostly in the form of phospholipid. The results obtained for lipid extract from rat liver mitochondria are also given in Table I. The Hall mobility values were obtained for an applied d.c. magnetic field of 1.21 T. The samples were in the form of lightly compressed 5-mm diameter discs and the cavity was flushed with dry nitrogen during the measurements. To aid comparisons of results, they have been given as far as possible for roughly equal a.c. resistivity values.

TABLE I. Permittivity, resistivity and Hall mobility values obtained for rat liver mitochondria, at 9.16 GHz and 23°C, showing the effect of inhibitor additions. The Hall mobility results are not volume corrected

	$\epsilon'$	$\epsilon''$	$\rho$ (ohm cm)	Hall mobility (cm <sup>2</sup> /V sec)
Batch I: 16 mg protein per millilitre				
Control	2.15	$6.98 \times 10^{-2}$	$2.8 \times 10^3$	4.8 N-type
0.25 $\mu$ g/mg protein, antimycin-A	2.23	$7.02 \times 10^{-2}$	$2.8 \times 10^3$	4.3 N-type
0.125 $\mu$ g/mg protein, rotenone	2.15	$7.36 \times 10^{-2}$	$2.7 \times 10^3$	4.9 N-type
Potassium cyanide ( $1 \times 10^{-4}$ M)	2.95	$7.61 \times 10^{-2}$	$2.6 \times 10^3$	1.5 N-type
Batch II: 12 mg protein per millilitre				
Control	2.45	$7.0 \times 10^{-2}$	$2.8 \times 10^3$	7.6 N-type
0.50 $\mu$ g/mg protein, antimycin-A	1.92	$5.48 \times 10^{-2}$	$3.6 \times 10^3$	8.4 N-type
0.25 $\mu$ g/mg protein, rotenone	3.25	$8.34 \times 10^{-2}$	$2.4 \times 10^3$	4.1 N-type
Potassium cyanide ( $1 \times 10^{-4}$ M)	3.17	$7.03 \times 10^{-2}$	$2.8 \times 10^3$	3.2 N-type
Lipid extract	2.22	$8.55 \times 10^{-3}$	$2.3 \times 10^4$	0.96 N-type
Cytochrome-c (horse heart)	4.32	$2.70 \times 10^{-1}$	$7.3 \times 10^2$	<0.5 N-type

When first placed in the microwave cavity, the samples exhibited an effective a.c. resistivity of the order  $1.0 \times 10^3$  ohm cm, which slowly increased over a period of 5 h to a final steady value of between  $3.0$ – $5.5 \times 10^3$  ohm cm, depending on the sample. This process was reversible and was almost certainly associated with the dehydration action of the dry nitrogen and microwave power. The total resistivity change was much smaller than that observed for bovine plasma albumin<sup>8</sup> and the mobility values obtained for the mitochondrion were at least six times greater than those obtained for BPA at similar resistivity values, as is shown in Fig. 1. It is a feature of the interpretation of the Faraday effect that an over-estimated value for the true effective conductivity

of the specimen produces an underestimated value for the Hall mobility.<sup>3</sup> Such an overestimation of the conductivity can arise if large dielectric losses, such as that associated with loosely bound water, are present. This is the underlying factor behind the increase of apparent mobility with dehydration of the specimens and the scatter of complex permittivity values exhibited by the various mitochondrial samples. The variation of the relative complex permittivity components for BPA with hydration is shown in Fig. 2. The zero hydration level was obtained by weighing the sample after it had been baked in an oven at 110°C for 17 h. Accurate determinations for  $\epsilon''$  for hydration levels below 1% were difficult, but  $\epsilon''$  values below  $10^{-3}$  were obtained yielding effective resistivity values of  $10^5$  ohm cm and greater.

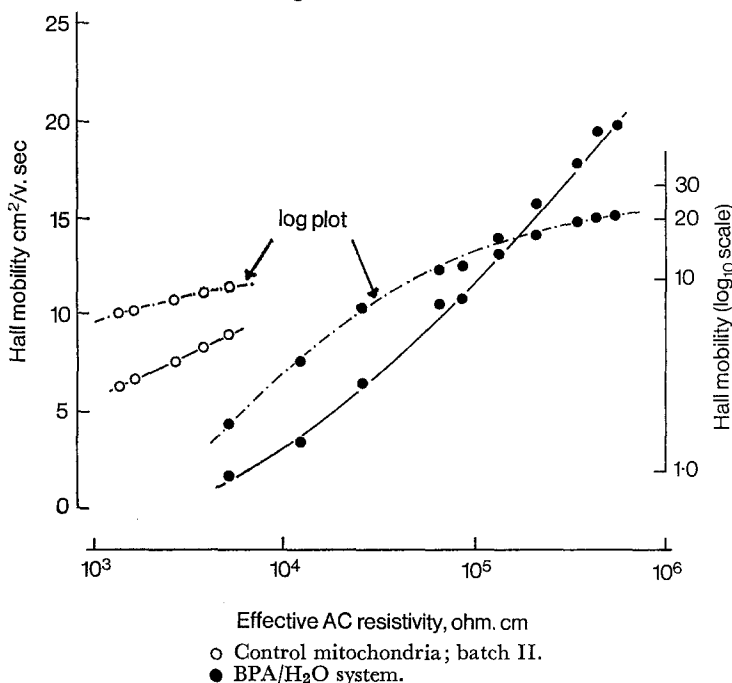


Figure 1. Variation of Hall mobility with effective a.c. resistivity during process of dehydration. The Mitochondria results are not volume corrected.

From Table I the low mobility value obtained for the lipid extract implies that the higher mobility values obtained for the whole mitochondria were associated with electron conduction in the electron transport chain or the associated structural proteins. The effect of potassium cyanide in reducing the observed mobility compared with the control sample strongly suggests that the electron transport chain was being studied. In batch II the concentrations of antimycin-A and rotenone were double that of batch I and rotenone was seen to reduce the mobility at this increased concentration. The concentrations of inhibitor used here were greater than that usually employed in respiratory inhibition studies, so that the insensitivity of antimycin-A could imply that the electrons have a lower mobility in the region specifically effected by this inhibitor than in other sections of the electron-transport chain. Future measurements on individual complexes of the transport chain will possibly clarify this point.

The logarithmic plot given in Fig. 1 appears to extrapolate to a limiting value of

about  $16 \text{ cm}^2/\text{V sec}$  for the Hall mobility of mitochondria batch II. The limiting Hall mobility for batch I extrapolates to a value of about  $10 \text{ cm}^2/\text{V sec}$ . Since the unit carrying the electron transport chain can be purified some five-fold relative to the mitochondrion,<sup>10</sup> then any structure presumed to be associated with electron transport should not exceed more than 20% of the total dry weight of the mitochondrion. Fernandez-Moran and his colleagues<sup>10</sup> have isolated an elementary particle they consider contains the complete electron transport chain. There are from  $10^4$  to  $10^5$  of these particles present in a single mitochondrion, accounting for 10–15% of the total mitochondrial volume. To a first approximation the Faraday rotation is proportional to the Hall mobility multiplied by the volume of the conducting particles (see ref. 3, equations 4 and 6). Assuming the

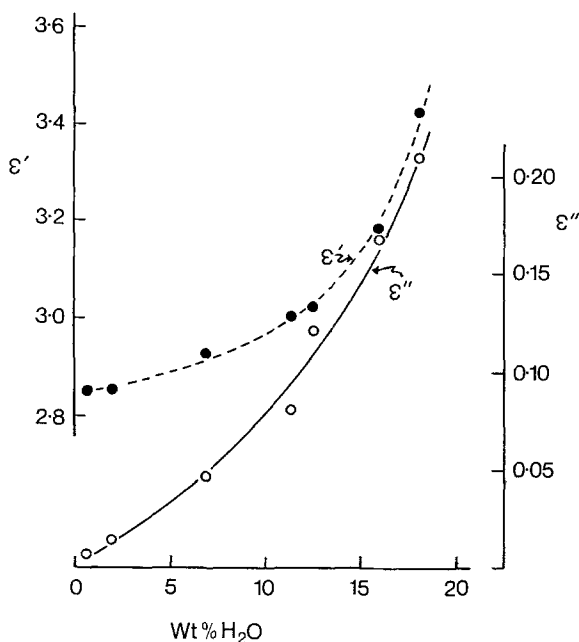


Figure 2. Variation of the complex permittivity components with hydration for Bovine Plasma Albumin.

electron transfer regions occupy less than 20% of the mitochondrion, then a value of between  $50\text{--}80 \text{ cm}^2/\text{V sec}$  for the true Hall mobility of electrons in the electron transport chain of rat liver mitochondria would not seem an over-estimation.

The rate of oxidation of pyruvate by isolated mitochondria occurs with  $Q_{\text{O}_2}$  values (microlitres of  $\text{O}_2$  per milligramme dry weight per hour) in the range 40–80 (see ref. 11). The median dry weight of rat liver mitochondria is  $1.1 \times 10^{-13} \text{ gm}$  (see ref. 11), so this respiratory quotient corresponds to an average electron flow of  $1.8 \times 10^5$  electrons per mitochondrion per second, equivalent to a total current of  $2.9 \times 10^{-14} \text{ A}$  per mitochondrion. The cristae of a single liver mitochondrion have a total surface area of about  $16 \times 10^{-8} \text{ cm}^2$  (see ref. 11), giving the current density per mitochondrion as  $0.18 \mu\text{A}/\text{cm}^2$ , assuming the current flow to be normal to the cristae. If 10% of the  $c.5 \times 10^4$  electron transport particles are transporting a pair of electrons at any one instant, this

will correspond on average to  $10^4$  electrons occupying a volume equal to about 15% of the total mitochondrial volume. The average overall spheroidal dimensions of a rat liver mitochondrion is  $3.3 \mu \times 1.0 \mu$  (see ref. 11), giving an effective electron density  $n$  of  $2.6 \times 10^{16} \text{ cm}^{-3}$ . For a current density of  $0.18 \mu\text{A}/\text{cm}^2$  this gives the mean electron velocity as  $4.4 \times 10^{-5} \text{ cm/sec}$  and the transit time through an 180-Å long charge transfer chain† as envisaged by Green *et al.*<sup>10</sup> as 41 m sec. Assuming a 1:1 relationship between the Hall mobility  $\mu_H$  and drift mobility  $\mu_D$ , the mean resistivity of the charge transport chain  $(nq\mu_D)^{-1}$  is calculated to be 3.8 ohm cm. Certain substituted benzoquinone charge-transfer complexes and the *o*-chloranil complex of tetrathiotetracene in particular,<sup>12</sup> have resistivity values of this order.

Measurements on samples of horse heart cytochrome-*c* [obtained from Seravac Laboratories (Pty) Ltd.] are complicated by electron spin resonances, but the effective Hall mobility is estimated to be N-type and lower than  $0.5 \text{ cm}^2/\text{V sec}$  (see Table I). It is of great interest that the mobilities observed for the electron transport system of the mitochondrion are some 100 times this value. This may suggest that the protein conformation of the various cytochromes within the mitochondria is particularly suitable for electron transport and that this ideal conformation is lost in the extracted soluble cytochrome-*c*. The recent work of T. F. Chuang *et al.*<sup>13</sup> may be particularly relevant here where the structure and catalytic capacity of the two hemoproteins, cytochromes *c* and *a*, are found to be phospholipid dependent.

### *Spinach Chloroplasts*

Two separate samples of spinach chloroplasts were prepared by Dr. M. J. Chappell in this Department. The results obtained are presented in Table II.

TABLE II. Permittivity, resistivity and Hall mobility values obtained for spinach chloroplasts at 9.16 GHz and 23°C

Sample	$\epsilon'$	$\epsilon''$	$\rho$ (ohm cm)	Hall mobility ( $\text{cm}^2/\text{V sec}$ )
I	3.5	$3.17 \times 10^{-2}$	$6.18 \times 10^3$	0.80 P-Type
II	3.47	$8.86 \times 10^{-2}$	$2.22 \times 10^3$	0.53 P-Type

The most obvious difference between these results and those obtained for the mitochondria is the change in sign and ten-fold decrease of the Hall mobility. Future measurements may indicate if in fact holes are the dominant charge carriers in the chloroplasts, or whether these results reflect the damaged state of the quantasomes, for example.

### *Conclusions*

We believe that future microwave measurements based on those described here will enable biological activity involving electron-transfer mechanisms to be investigated

† This model has been criticized, but suffice to indicate the order of the dimensions needed for our calculations. (e.g. Mitochondrial Structure and Compartmentation, eds E. Quagliariello *et al.*, Adriatica Editrice, Bari, 1967.)

at the electronic level and will provide an important measurement technique in the general field of organic and biological semiconduction studies.

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