microsomes are able to conjugate a larger variety of aglycones than the immobilized rat liver UDPGT suggests that not all isozymes were covalently bound or solubilized.

The lack of stability of UDPGTs seems to be the main problem in purification of these membrane-bound enzymes, which may be explained by the loss of protection offered by the lipid bilayer. Immobilization of partially or fully purified rat liver UDPGT enzymes to Sepharose prolongs their stability activities as seen with rabbit liver [4]. Therefore, the immobilization of rat liver UDPGT potentially provides a versatile method for studying drug metabolism *in vitro* and for synthesizing UDPGA-conjugated metabolites, and offers an alternative to the rabbit preparation.

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REFERENCES

1. Mulder GJ, Coughtrie MWH and Burchell B, Glucuronidation. In: *Conjugation Reactions in Drug Metabolism* (Ed. Mulder GJ), pp. 51–105. Taylor & Francis, New York, 1991.
2. Falany CN and Tephly TR, Separation, purification and characterization of three isoenzymes of UDP-glucuronsyltransferase from rat liver microsomes. *Arch Biochem Biophys* **227**: 248–258, 1983.
3. Kirkpatrick RB, Falany CN and Tephly TR, Glucuronidation of bile acids by rat liver 3-OH Androgen UDP-glucuronsyltransferase. *J Biol Chem* **259**: 6176–6180, 1984.
4. Parikh I, MacGlashan DW and Fenselau C, Immobilized glucuronosyltransferase for the synthesis of conjugates. *J. Med Chem* **19**: 296–299, 1976.
5. Fenselau C, Pallante SL and Parikh I, Solid-phase synthesis of drug glucuronides by immobilized glucuronsyltransferase. *J Med Chem* **19**: 679–683, 1976.
6. Lehman JP, Ferrin I, Fenselau C and Yost GS, Simultaneous immobilization of cytochrome P450 and glucuronsyltransferase for synthesis of drug metabolites. *Drug Metab Dispos* **9**: 15–18, 1981.
7. Pallante SL, Lisek CA, Dulik DM and Fenselau C, Glutathione conjugates. Immobilized enzyme synthesis and characterization by fast atom bombardment mass spectrometry. *Drug Metab Dispos* **14**: 313–318, 1986.
8. Dulik DM and Fenselau C, Species-dependent glucuronidation of drugs by immobilized rabbit, rhesus monkey, and human UDP-glucuronosyltransferases. *Drug Metab Dispos* **15**: 473–477, 1987.
9. El Mouelhi M, Ruelius HW, Fenselau C and Dulik DM, Species-dependent enantioselective glucuronidation of three 2-arylpropionic acids. *Drug Metab Dispos* **15**: 767–772, 1987.
10. Burchell B and Weatherhill P, 4-Nitrophenol UDP-glucuronsyltransferase (rat liver). *Methods Enzymol* **77**: 161–177, 1981.
11. Mulder GJ and van Doorn ABD, A rapid NAD^+-linked assay for microsomal uridine diphosphate glucuronosyltransferase of rat liver and some observations on substrate specificity of the enzyme. *Biochem J* **151**: 131–140, 1975.
12. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J. Biol Chem* **193**: 265–275, 1951.
13. Cleland WW, The statistical analysis of enzyme kinetic data. In: *Advances in Enzymology* (Ed. Nord FF), pp. 1–32. Interscience, New York, 1967.
14. MacKenzie PI, Lang MA and Owens IS, Effect of different detergent systems on the molecular size of glucuronosyltransferase and other microsomal drug-metabolizing enzymes. *Membrane Biochem* **5**: 193–207, 1984.

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Effects of chronic ethanol feeding on rat liver mitochondrial energy metabolism

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Abstract—Chronic alcohol consumption is known to decrease hepatic mitochondrial respiration rate. It was shown here that the proton leak through the mitochondrial inner membrane was unaffected by chronic ethanol treatment. This indicates that changes in proton leak are not responsible for the alterations in respiration found in mitochondria isolated from ethanol-treated rats. Therefore, the lowered coupled respiration rate is solely due to a decrease in the activity of the electron transport chain. However, this alteration was only evident in coupled respiration (i.e. state 4) and was not apparent in uncoupled respiration. Thus, chronic ethanol treatment decreases the activity of the mitochondrial electron transport chain components which have control over coupled, but not uncoupled, respiration. Mitochondrial energy metabolism is regulated by thyroid hormone status. It was shown that the chronic alcohol treatment did not affect the circulating levels of thyroxine. Furthermore, the activity of mitochondrial α-glycerophosphate dehydrogenase, which is strongly affected by thyroid hormones, was unaltered by alcohol treatment. Thus, the effects of ethanol treatment on mitochondria occur independently of changes in circulating thyroid hormone levels.

Chronic ethanol feeding of rats for 3–4 weeks causes a general depression of mitochondrial energy metabolism (for a review, see Ref. 1). This includes a decrease in state 3 and coupled (i.e. state 4) respiration rates and a decrease in the rate, but not the efficiency, of ATP synthesis [1]. Both of these effects correlate with a decrease in the amount or activity of a number of electron transport chain and ATP synthase components [1]. There are also changes in mitochondrial lipids on chronic ethanol treatment [2] which, as electron transport and ATP synthesis activities are affected by mitochondrial lipid composition [3, 4], may cause some of the changes seen on ethanol treatment. The control of respiration in isolated mitochondria is distributed over a number of steps [5–7]; therefore, the decreases in respiration following chronic ethanol treatment may be due to changes in several factors.

The proton leak through the mitochondrial inner membrane has significant control over mitochondrial respiration [8–10] and this leak is altered by changes in mitochondrial lipid and protein composition [9, 4, 11]. In the present work, the effect of chronic ethanol feeding on the proton leak through the mitochondrial inner membrane has been investigated. There was no difference in the proton leak through the mitochondrial inner membrane and therefore the decrease in coupled respiration rate following chronic ethanol treatment is solely due to decreased activity of the electron transport chain. However, this alteration was only evident in coupled respiration and was not apparent in uncoupled respiration. In addition, the changes in respiration rate were shown to occur independently of alterations in levels of circulating thyroid hormone, which have a range of effects on mitochondrial metabolism [5].

Materials and Methods

Treatment of experimental animals. Two groups of male Wistar rats were fed either alcohol or isocaloric-control diets [12]. The alcohol group were fed a liquid diet supplemented with 5% (w/v) ethanol and the isocaloric group were pair-fed the same volume of liquid diet as was consumed by the ethanol group but with dextrin-maltose (1:1, w/w) isocalorically substituted for ethanol. Rats were weaned onto the ethanol diet over 5 days by gradually increasing the alcohol concentration from 0 to 5% (w/v). Matched alcohol and isocalorically pair-fed rats were used for experiments after 4–5 weeks on the liquid diets, by which time "fatty liver" was evident in the alcohol-treated rats.

Isolation of mitochondria. One rat from each group was killed each day and parallel experiments were carried out on the two separate mitochondrial preparations. The rats were killed by stunning followed by cervical dislocation and liver mitochondria were prepared by homogenization followed by differential centrifugation in ice-cold medium containing 250 mM sucrose, 5 mM Tris and 1 mM EGTA adjusted to pH 7.4 (HCl) at 25° [13]. The mitochondrial protein content was determined by the biuret method using bovine serum albumin as a standard [14].

Measurement of respiration rate and mitochondrial membrane potential ($\Delta\psi$).* The respiration rate was measured using an oxygen electrode (Rank Brothers, Bottisham, U.K.) contained in the base of a thermostatted and rapidly stirred 3-mL perspex chamber. Respiration rate was calculated assuming an oxygen concentration of 475 nmol O/mL in the experimental medium at 25° [15]. The mitochondrial $\Delta\psi$ was determined from the distribution of the lipophilic cation methyltriphenyl phosphonium (TPMP) across the mitochondrial inner membrane [16]. The matrix concentration of TPMP was calculated using mitochondrial matrix volumes and TPMP binding correction factors measured in separate experiments outlined below. The distribution of TPMP between the mitochondrial matrix and the external medium was measured using a TPMP-sensitive electrode [17]. The TPMP electrode was inserted through the perspex lid of the oxygen electrode chamber giving an airtight seal. This enabled the simultaneous measurement of the membrane potential and respiration rate of a mitochondrial suspension. Experiments to measure respiration rate and $\Delta\psi$ simultaneously were carried out in the presence of the ionophore nigericin and 120 mM KCl. This clamped the pH gradient (ΔpH) across the mitochondrial inner membrane close to zero [18, 19]. The ΔpH component of the protonmotive force (Δp) was therefore negligible and $\Delta\psi$ was the sole component of Δp.

Measurement of mitochondrial volume. The mitochondrial volume was determined using [^{14}C]sucrose as an extra-mitochondrial marker and ^3H_2O to determine the total pellet volume [18, 20]. The matrix volume was calculated as ^3H_2O space − [^{14}C]sucrose space.

Malonate titration of $\Delta\psi$ and respiration rate. Mitochondria (6 mg of mitochondrial protein) were suspended in 3 mL of medium [120 mM KCl, 5 mM KH_2PO_4, 2 mM $MgCl_2$, 10 mM Hepes, 1 mM EGTA, 13.3 μM rotenone and nigericin (0.2 μg/mL); pH 7.2 (KOH) at 25°] in an airtight, rapidly stirred 3-mL chamber thermostatted at 25°. The magnetic stirrer was switched on and TPMP (5 × 5 μL of 0.6 mM; Br^- salt) was then added to calibrate the TPMP electrode giving a final concentration of 5 μM TPMP. Five minutes after switching on the stirrer 5 mM succinate (K^+ salt) was added to initiate respiration and establish a $\Delta\psi$. This was followed by a series of small, sequential additions of malonate (K^+ salt) at 2-min intervals; the amounts of malonate added varied from titration to titration. At the end of the incubation excess carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP; 0.33 μM) was added to allow the TPMP electrode trace to return to its baseline. From the experimental traces the respiration rate and $\Delta\psi$ were determined for each new steady state brought about by the addition of malonate. The steady state distribution of TPMP between the mitochondria and the external medium was calculated taking into account the slight drift of the electrode trace.

Measurement of serum [T_4] and mitochondrial α-glycerophosphate dehydrogenase activity. Blood samples were taken on killing the rats, placed on ice and the serum was separated from the blood clot by centrifugation (10 min × 2000 *g*) in a bench-top centrifuge. The serum was assayed for total [T_4] by a standard radioimmunoassay procedure designed for human serum. The activity of mitochondrial α-glycerophosphate dehydrogenase (EC 1.1.99.5) was measured by the method of Lee and Lardy [21].

Determination of the TPMP binding correction. The degree of binding of intra-mitochondrial TPMP was determined for mitochondria from rats on the two diets by the method of Brown and Brand [22]. Mitochondria (2 mg protein) were incubated in 1-mL plastic minifuge tubes in medium (250 mM sucrose, 5 mM Hepes, 1 mM EGTA, 5 mM succinate pH 7.2 with TMA^+OH^- at 25°) containing the following additions: 5 μM $TPMP^+Br^-$, 0.2 μM valinomycin, oligomycin (2 μg/mL), ^{86}RbCl (2.5 nCi/mL) and [^{3}H]TPMP (50 nCi/mL). KCl (0–10 mM) was added to the tubes, each KCl concentration in triplicate, and the final volume of each incubation was 1 mL. The tubes were incubated for 3 min at 25° after which the mitochondria were pelletted by centrifugation in a bench top centrifuge

* Abbreviations: FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; $\Delta\psi$, electrical potential across the mitochondrial inner membrane; ΔpH, pH gradient across the mitochondrial inner membrane; Δp, protonmotive force across the mitochondrial inner membrane; TMA, tetramethyl ammonium; TPMP, methyltriphenylphosphonium; T_4, L-thyroxine.

Table 1. Properties of liver mitochondria from rats on ethanol or isocaloric diets

Diet	α-Glycerophosphate dehydrogenase (ΔA_{500}/min.mg protein)	Serum [T_4] (nmol/L)	Respiration rate Coupled (nmol O/min.mg protein)	Uncoupled	TPMP binding correction	Matrix volume (μL/mg protein)
Isocaloric	0.15 ± 0.08	79.3 ± 7	26.3 ± 1.5	131.6 ± 9.3	0.39 ± 0.02	0.97 ± 0.04
Ethanol	0.19 ± 0.04	71.0 ± 4	17.5 ± 0.7	140.3 ± 5.6	0.35 ± 0.02	1.03 ± 0.05

The α-glycerophosphate dehydrogenase activity, total serum [T_4] and mitochondrial volumes were determined as outlined in Materials and Methods and the data shown here are the means ± SEM for experiments on five, six and three different mitochondrial preparations, respectively.

Chow-fed rats had total serum [T_4] of 76.3 ± 4.0 nmol/L (N = 10). The binding correction for TPMP was determined as described in Materials and Methods. To determine respiration rates mitochondria (6 mg protein) were suspended in 3 mL of medium [120 mM KCl, 5 mM KH_2PO_4, 2 mM $MgCl_2$, 10 mM Hepes, 1 mM EGTA, 13.3 μM rotenone; pH 7.2 (KOH) at 25°] in an oxygen electrode chamber thermostatted at 25°. Five minutes later succinate (5 mM; K^+ salt) was added and the coupled respiration rate was determined. Three minutes after the addition of succinate excess FCCP was added and the uncoupled respiration rate was determined.

Data are the means ± SEM for experiments on four different mitochondrial preparations. The coupled, but not the uncoupled, respiration rates for mitochondria from ethanol and isocaloric fed rats were significantly different by Student's *t*-test ($P < 0.01$).

(2 min at 10,000 *g*) and the supernatant and pellets were separated and counted in a scintillation counter using appropriate quench and crossover corrections. The $^{86}Rb^+$ accumulation ratio was plotted against that for TPMP and the slopes of these plots, determined from linear regression, were taken as the TPMP binding correction factor.

Materials. [^{14}C]Sucrose, ^{86}RbCl and ^3H_2O were from Amersham International (Amersham, U.K.). The scintillant "Ecolite™ (+)" was from ICN Biomedicals Ltd. All other chemicals and enzymes were from the Sigma Chemical Co. or BDH (both Poole, U.K.).

Results and Discussion

Many of the metabolic effects of chronic alcohol treatment are due to elevated cytosolic NADH concentrations [23] which would be decreased by induction of mitochondrial α-glycerophosphate dehydrogenase. The possibility that mitochondrial α-glycerophosphate dehydrogenase is induced to protect against elevated NADH levels was tested by measuring the activity of this enzyme in liver mitochondria from alcohol-fed rats. As can be seen in Table 1, there was no significant change in the activity of mitochondrial α-glycerophosphate dehydrogenase following chronic alcohol treatment. In addition, because the activity of this enzyme alters considerably in response to changes in circulating thyroid hormones [21], these data imply that there is no significant change in circulating thyroid hormones in chronic alcohol treatment. This was confirmed by measurements of total serum [T_4] which are shown in Table 1. Therefore, the effects of chronic ethanol feeding on mitochondrial metabolism are not mediated by alterations in circulating thyroid hormones which have many effects on mitochondrial energy metabolism [5].

The control of respiration in isolated mitochondria is reasonably well understood [5–7]. Respiration in coupled mitochondria (i.e. state 4) is controlled by the proton leak through the mitochondrial inner membrane and, to a lesser extent, by the respiratory chain and substrate transporters [24]. Respiration in uncoupled mitochondria is controlled by the respiratory chain and the substrate transporters [5–7]. Therefore, changes in either of these rates as a result of chronic alcohol treatment may indicate the mitochondrial sites affected by ethanol treatment. In Table 1, it can be seen that chronic alcohol treatment has little effect on uncoupled respiration but decreases coupled respiration. The finding that the respiration rate of coupled mitochondria is lower after chronic ethanol feeding is in agreement with many other reports [1]. This suggests that chronic alcohol treatment has either decreased the proton leak through the mitochondrial inner membrane or has decreased the activity of the components of the mitochondrial electron transport chain or substrate transporters which have control over coupled respiration [8–11]. To distinguish between these possibilities, the proton leak through the mitochondrial inner membrane was determined as a function of its driving force, the protonmotive force. This entails simultaneous measurement of respiration rate and protonmotive force [8]. Nigericin and 120 mM KCl eliminated the pH gradient [18, 19] and therefore $\Delta\psi$ was equivalent to the protonmotive force. The distribution of TPMP was used to measure $\Delta\psi$, which requires TPMP binding corrections and matrix volumes for mitochondria from the two groups of rats. The TPMP binding corrections wee determined and in Table 1 the values of the binding corrections and mitochondrial matrix volumes are given. It can be seen that the matrix volumes are identical for the two groups of mitochondria and that the fraction of matrix TPMP bound to the mitochondrial inner membrane is not significantly different in mitochondria from the ethanol-treated rats compared with those from the control group. These correction factors and matrix volumes were used in all subsequent calculations of $\Delta\psi$.

The dependence of the proton leak on $\Delta\psi$ was determined by simultaneous measurement of respiration rate and $\Delta\psi$ during an inhibitor titration of coupled respiration [8]. Such a plot indicates the dependence of proton leak through the mitochondrial inner membrane on $\Delta\psi$. This is because multiplying the respiration rate by an appropriate stoichiometry of proton pumping by the electron transport chain transforms the plot into one of proton efflux as a function of $\Delta\psi$. In a steady state the proton efflux must equal the proton leak therefore the plot is effectively one of the proton leak as a function of $\Delta\psi$. This assumes no slip in the proton pumps [8, 9]. If this assumption is not made the plot is then one of $\Delta\psi$ against the respiration rate balancing the proton leak. It is still a description, albeit less quantitative, of the proton leak as a function of $\Delta\psi$. This approach eliminates any secondary changes in

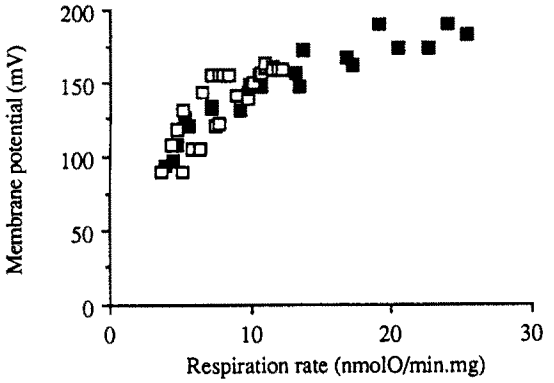

Fig. 1. Malonate titration of mitochondrial $\Delta\psi$ and respiration rate. Mitochondrial $\Delta\psi$ and respiration rate were measured simultaneously as described in Materials and Methods on mitochondria isolated from rats fed isocaloric (■) or ethanol (□) diets. This experiment shows data from several titrations carried out on one day. This was repeated on two further occasions with the same superimposition of plots of $\Delta\psi$ against respiration rate for mitochondria from rats fed isocaloric or ethanol diets, as shown here.

leak due to changes in $\Delta\psi$. In Fig. 1 it can be seen that the plots of $\Delta\psi$ against respiration during an inhibitor titration for mitochondria from rats on the two diets are superimposable and that there is no difference in proton leak through the mitochondrial inner membrane on chronic ethanol feeding. Therefore, the decrease in coupled respiration is caused by a decrease in the activity of the electron transport chain or substrate transporters; however, this decrease in activity is not evident in uncoupled mitochondria. This indicates that chronic ethanol treatment affects segments of the electron transport chain or substrate transporters which have control over respiration in coupled mitochondria but has essentially no effect on the activity of those mitochondrial components which control respiration in uncoupled mitochondria [5].

In conclusion, it has been demonstrated that the effects of chronic ethanol feeding on the energy metabolism of liver mitochondria are not mediated by changes in thyroid hormones. The proton leak through the mitochondrial inner membrane is unaffected by chronic ethanol feeding. Therefore, the decrease in coupled respiration is not due to a decrease in the proton leak but is caused by a decrease in the activity of the mitochondrial electron transport chain components which have control over coupled respiration. This decrease in activity of the electron transport chain was not evident in uncoupled mitochondria; therefore, the mitochondrial sites affected by ethanol treatment have control over coupled, but not uncoupled, respiration.

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REFERENCES

1. Cunningham CC, Coleman WB and Spach PI, The effects of chronic ethanol consumption on hepatic mitochondrial energy metabolism. *Alcohol Alcoholism* **25**: 127–136, 1990.
2. Cunningham CC and Spach PI, The effect of chronic ethanol consumption on the lipids in liver mitochondria. *Ann NY Acad Sci* **492**: 181–192, 1987.
3. Daum G, Lipids of mitochondria. *Biochim Biophys Acta* **822**: 1–42, 1985.
4. Hoch FL, Lipids and thyroid hormones. *Prog Lipid Res* **27**: 199–270, 1988.
5. Brand MD and Murphy MP, Control of electron flux through the respiratory chain in mitochondria and cells. *Biol Rev* **62**: 141–193, 1987.
6. Tager JM, Wanders RJA, Groen AK, Kunz W, Bohnensack R, Kuster U, Letko G, Bohme G, Duszynski J and Wojtczak L, Control of mitochondrial respiration. *FEBS Lett* **151**: 1–9, 1983.
7. Moreno-Sanchez R and Torres-Marquez ME, Control of oxidative phosphorylation in mitochondria, cells and tissues. *Int J Biochem* **23**: 1163–1174, 1991.
8. Brand MD, The proton leak across the mitochondrial inner membrane. *Biochim Biophys Acta* **1018**: 128–133, 1990.
9. Murphy MP, Slip and leak in mitochondrial oxidative phosphorylation. *Biochim Biophys Acta* **977**: 123–141, 1990.
10. Brand MD, The contribution of the leak of protons across the mitochondrial inner membrane to standard metabolic rate. *J Theor Biol* **145**: 267–286, 1990.
11. Hafner RP, Nobes CD, McGown AD and Brand MD, Altered relationship between protonmotive force and respiration rate in non-phosphorylating mitochondria isolated from rats of different thyroid status. *Eur J Biochem* **178**: 511–518, 1988.
12. Lieber CS and DeCarli LM, The feeding of alcohol in liquid diets. *Alcohol Clin Exp Res* **10**: 550–553, 1986.
13. Chappell JB and Hansford RG, Preparation of mitochondria from animal tissues and yeasts. In: *Subcellular Components: Preparation and Fractionation*, 2nd Edn (Ed. Birnie GD), pp. 77–91. Butterworths, London, 1972.
14. Gornall AG, Bardawill CJ and David MM, Determination of serum proteins by means of the biuret reaction. *J Biol Chem* **177**: 751–766, 1949.
15. Reynafarje B, Costa LE and Lehninger AL, O_2 solubility in aqueous media determined by a kinetic method. *Anal Biochem* **145**: 406–418, 1985.
16. Azzone GF, Pietrobon D and Zoratti M, Determination of the proton electrochemical gradient across biological membranes. *Curr Topics Bioenergetics* **13**: 1–77, 1984.
17. Kamo N, Muratsugu M, Hongoh R and Kobatake Y, Membrane potential of mitochondria measured with an electrode sensitive to tetraphenyl phosphonium and relationship between proton electrochemical and phosphorylation potential in steady state. *J Membr Biol* **49**: 105–121, 1979.
18. Murphy MP and Brand MD, The control of electron flux through cytochrome oxidase. *Biochem J* **243**: 499–505, 1985.
19. Hafner RP and Brand MD, Effect of protonmotive force on the relative proton stoichiometries of the mitochondrial proton pumps. *Biochem J* **275**: 75–80, 1991.
20. Halestrap AP, The regulation of matrix volume of mammalian mitochondria *in vivo* and *in vitro* and its

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role in the control of mitochondrial metabolism. *Biochim Biophys Acta* **973**: 355–382, 1989.
21. Lee Y-P and Lardy HA, Influence of thyroid hormones on L-α-glycerophosphate dehydrogenases and other dehydrogenases in various organs of the rat. *J Biol Chem* **240**: 1427–1436, 1965.
22. Brown GC and Brand MD, Thermodynamic control of electron flux through mitochondrial cytochrome bc1 complex. *Biochem J* **225**: 399–405, 1985.
23. Kennedy NP and Tipton KF, Ethanol metabolism and alcoholic liver disease. *Essays Biochem* **25**: 137–195, 1990.
24. Brand MD, Hafner RP and Brown GC, Control of respiration in non-phosphorylating mitochondria is shared between the proton leak and the respiratory chain. *Biochem J* **255**: 535–539, 1988.