by ATPase may serve to link the activity of the two enzymes (see ref. 10). Whatever the mechanism of coupling of this glycolytic enzyme to ATPase its ultimate result is that energy production from glycolysis is varied appropriately to the energetic demands of active cation transport in the erythrocyte.

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Some biochemical and X-ray diffraction studies of mitochondrial outer membrane

During the preparation of mitochondrial outer membrane for X-ray diffraction studies, it was observed that mild sonication stripped off a large proportion of the marker enzymes monoamine oxidase[1] and rotenone-insensitive NADH:cytochrome *c* oxidoreductase[2]. The diffraction experiments and parallel electron microscopy studies indicated that the thickness of the outer membrane in a hydrated state is of the order of 115 Å and that there is a small change (detectable at the dried state) in membrane dimension associated with removal of protein by sonication.

Preparation of outer membranes. A homogenate of liver tissue from 6–8-week-old rats was prepared in 0.88 M sucrose. (All sucrose solutions were neutralized with sodium bicarbonate.) It was felt that the hypertonic medium would serve to maintain an intact inner bag during subfractionation of the mitochondria. Tissue (1 g per 9 ml of sucrose) was homogenized at 4° by two strokes with a pestle-type homogenizer (clearance 0.29 mm) at 500 rev./min. The homogenate was strained and centrifuged

at 1000 × *g* for 10 min and the supernatant spun again at 9000 × *g* for 10 min. In order to remove any light fluffy material, the surfaces of the resulting mitochondrial pellets were washed twice with a few ml of 0.88 M sucrose. Three mitochondrial washes were then carried out by resuspension of the pellets in one half the original volume of 0.88 M sucrose and subsequent centrifugation at 9000 × *g* for 10 min. In each case, the pellets were resuspended by one stroke of homogenization. Microsomal and lysosomal contamination in the final mitochondrial pellet, as measured by glucose-6-phosphatase and arylsulphatase, respectively, was of the order of 5%.

The mitochondria were subfractionated by two methods. A fractionation by water lysis was essentially similar to the method of CAPLAN AND GREENAWALT[3]. The mitochondria were dispersed by pipette in deionized water (9 ml per initial 10 g of tissue) neutralized with sodium bicarbonate and immediately centrifuged at 80 000 × *g* for 30 min. The procedure was repeated and the final pellets suspended in 0.3 M sucrose. An alternative subfractionation involved a mild sonication in which the mitochondrial pellets were suspended in 0.88 M sucrose (2 ml per initial 5 g of tissue) by one stroke of homogenization, and sonicated at 0°, 1.3 A, in aliquots of 4.5 ml for 7 sec.

Subfractions of both preparations were isolated by centrifugation at 100 000 × *g* for 2 h through a discontinuous sucrose gradient in a 3 × 20 ml swing-out rotor. The gradient consisted of 4.5 ml each of payload (sonicated or lysed mitochondria), 1.37 M sucrose and 1.60 M sucrose. After centrifugation, layers were present at both interfaces and there was a pellet at the bottom of the tube. Since relatively large quantities of the upper layer were required for X-ray diffraction studies, in some experiments 6 ml of payload were layered on 12 ml of 1.37 M sucrose. Electron micrographs of thin sections through a pellet prepared from this upper fraction indicated that it was a clean preparation of membranes.

Enzyme assays. The subfractions from the gradient were sedimented by centrifugation and resuspended in deionized water neutralized with sodium bicarbonate. Residual particulate matter was removed from the soluble fraction (which was taken to be the fraction above the first interface of the gradient) by centrifugation at 157 000 × *g* for 1 h. Glucose-6-phosphatase and arylsulphatase activities were measured according to the methods of HÜBSCHER AND WEST[4] and ROY[5], respectively. Succinate dehydrogenase was measured by the reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride[6]. Monoamine oxidase and NADH: cytochrome *c* oxidoreductase were assayed by monitoring the reduction of benzylamine at 250 mμ (ref. 7) and cytochrome *c* (in the presence of 1 mM KCN and 30 μM rotenone) at 550 mμ (ref. 2), respectively. Glutamate dehydrogenase was measured basically according to the method of STRECKER[8] with the modification that the initial reaction mixture was 1 mM in EDTA, 3 mM in $MgCl_2$ and 2 mM in NAD.

X-ray diffraction. For these studies the membrane preparation was washed 5 times by successive resuspension in 15 ml of 5 mM NAD, pH 7.4 and centrifugation at 100 000 × *g* for 10 min (final packing for 15 min). A sample of the packed membranes was then mounted in a controlled humidity chamber on the diffraction camera as previously described[9,10]. Serial diffraction patterns (20-min exposures) were recorded whilst the membrane preparation was allowed to dry slowly at 92% relative humidity over a period of 10–12 h.

Specimens for electron microscopy. Specimens were fixed in buffered 1% osmium tetroxide at intermediate stages of drying and embedded in araldite.

The upper layer from the gradient consisted of membranous vesicles with less than 5% microsomal contamination (glucose-6-phosphatase). Measurement of glutamate dehydrogenase in this fraction indicated that matrix protein was absent. Contamination by mitochondrial inner membrane, on the basis of succinate dehydrogenase activity was 4% (ratio of recovery of inner membrane to outer membrane marker with respect to payload). The vesicular fraction obtained by both methods of subfractionation possessed monoamine oxidase and NADH:cytochrome *c* oxidoreductase activities (Table I). This, together with the low level of succinate dehydrogenase activity, indicated that this subfraction consisted predominantly of mitochondrial outer membrane. In the preparation by water lysis, the specific activities of monoamine oxidase and the cytochrome *c* reductase were about 3 times greater than those of payload material (Table I); measurement of glutamate dehydrogenase activity indicated that only about 10% of matrix protein remained in this payload material. (These enrichments in marker enzymatic activity are comparable to those reported by others[1,2] for preparations of outer membrane where sonication was not employed.) There was, however, little or no increased specific activity of monoamine oxidase or the cytochrome *c* reductase in sonicated membranes (Table I). This difference was accounted for by the presence of a large proportion of these enzyme activities in the soluble fraction of the preparation by sonication and no detectable activities in the equivalent fraction of the other preparation (Table I). The activities were recoverable in both types of preparation. Sonication for 7 sec of the outer membrane fraction prepared by water lysis caused solubilization of both enzymes, but the extent was dependent upon the concentration of membrane suspension treated. The relatively mild sonication required to strip monoamine oxidase

TABLE I

MONOAMINE OXIDASE AND ROTENONE-INSENSITIVE NADH:CYTOCHROME *c* REDUCTASE ACTIVITIES IN SUBFRACTIONS OF RAT-LIVER MITOCHONDRIA

N = not detectable. A and B were separate experiments.

Method of fractionation	*Expt.*	*Sonication*			*Water lysis*		
		Payload	*Outer membrane*	*Soluble fraction*	*Payload*	*Outer membrane*	*Soluble fraction*
Monoamine oxidase activity as % of payload	A	—	1.7	15.9	—	27	N
	B	—	4	26	—	30	N
Specific activity of monoamine oxidase (mμmoles of benzaldehyde per min per mg of protein)	A	25	36	—	21.7	53.6	—
	B	27.8	29.4	—	22	71	—
NADH:cytochrome *c* reductase activity as % of payload	A	—	3.8	43.5	—	25	N
	B	—	3.1	36	—	46.4	N
Specific activity of NADH:cytochrome *c* reductase (mμmoles of reduced cytochrome *c* per min per mg of protein)	A	128	133	—	173	460	—
	B	69	66.5	—	64	247	—

TABLE II

X-RAY DIFFRACTION SPACINGS FOR MITOCHONDRIAL OUTER MEMBRANE FROM RAT LIVER

The membranes were isolated from water-lysed mitochondria. A and B were separate experiments.

	Expt.	*Progressive stages of dehydration*						
		I: Three orders of lamellar repeat			*II*		*III: fully dried*	
Spacings in Å	A	118.5	59.2	39.5	93.5	48.9	90.5	39.1
	B	115	57.5	37.9	93.6	49.3	90.8	41.0

and the cytochrome *c* reductase off the mitochondrial outer membrane suggests that these enzymes are quite loosely bound.

In the X-ray diffraction experiments, discrete low-angle diffraction bands began to appear after 3–5 h when the amount of water remaining in the preparation was of the order of 20–30% with respect to final dried weight. The first pattern showed three orders of diffraction of a lamellar repeat measuring about 115 Å (Stage 1, Table II) for both membrane preparations. Subsequently, the lamellar spacing decreased and a sharp reflection developed at about 49 Å which appeared unrelated to the lamellar spacing (Stage II, Table II). Patterns from fully dried specimens of membranes (Stage III, Table II) prepared by water lysis, showed two apparently unrelated bands, one at 40 Å and another at about 90 Å. At this fully dried stage, the sonicated membranes also gave rise to a 40-Å band, but the second band appeared to be closer to 80 Å. A direct comparison of equivalent patterns confirmed that there was a difference probably of the order of 5–10 Å. Previous studies[9,10] would suggest that at this stage the 90-Å bands represent a residual lipoprotein membrane whereas the 40-Å band probably arises from a separated lipid phase.

Electron micrographs of the condensed membrane system have indicated that the mitochondrial membranes come together to form lamellar arrays in which the repeating layer includes only one membrane thickness. The unit membrane or trilamellar features of the membrane structure do not achieve close contact and the layer thickness is of the order of 100 Å.

The sequences of X-ray diffraction patterns showed strong similarities to those previously obtained during the dehydration of isolated myelin and erythrocyte ghosts[10]. It can therefore be suggested that the initial X-ray diffraction spacing of 115 Å represents the thickness of the mitochondrial outer membrane in a state which retains the water of hydration essential to its structural integrity. A loss of protein from the sonicated membrane as indicated by a measured decrease in the protein to phospholipid ratio and by reduction of monoamine oxidase and NADH: cytochrome *c* oxidoreductase activities may be related to the small decrease in layer thickness in this preparation, detected at the dried stage.

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Voltage-controllable negative differential resistance in *Nitella translucens*

An investigation into the current–voltage characteristics of the plasma membrane of *Nitella translucens* has revealed a voltage-controllable negative differential

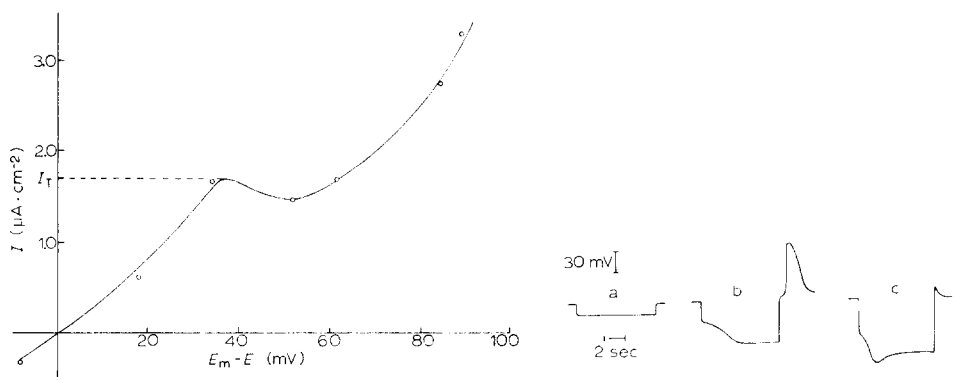

Fig. 1. Part of the steady-state current–voltage curve for *N. translucens* for hyperpolarising currents. The applied current (I) shifts the membrane potential from its normal resting value, E_m, to a new value E. The average value of E_m was close to −80 mV, the cells being bathed in 1.0 mM NaCl, 0.1 mM KCl, 1.0 mM $CaCl_2$. A regenerative transition occurs under constant-current conditions whenever the applied current amplitude exceeds the threshold current (I_T). The curve was plotted from data obtained from a series of potential responses to applied rectangular current pulses some of which are shown in Fig. 2.

Fig. 2. Some membrane potential responses to applied rectangular current pulses. In (a) the applied pulse is sub-threshold and the response is capacitative with the membrane in its low-resistance state. In (b) the applied pulse is only slightly larger than threshold and about 1 sec after the beginning of the pulse the membrane commences to switch from the low-to the high-resistance state; following the break of the applied pulse an inductive potential transient occurs which triggers an action potential. Finally in (c) the applied pulse is well above threshold; in this case both the switching from low to high membrane resistance and the termination of the applied pulse are followed by an inductive membrane potential transient.