Table 1 Proton uptake from the mitochondrial matrix

Condition	Respiratory rate (nmol $e^- s^{-1}$)	H^+/e^-
As Fig. 3	5.4 (±0.2 s.d.)	−1.87 (±0.11 s.d.); $n = 14$
+4.2 mM $MgCl_2$	16 (±0.9 s.d.)	−1.69 (±0.13 s.d.); $n = 9$

Experimental conditions were as described in the legend to Fig. 3. The data refer to experiments with eight different preparations of rat liver mitochondria. The H^+/e^- ratios were obtained from initial rates of proton uptake (Fig. 3*c*) and electron transfer (Fig. 3*b*), which were calibrated for each preparation of mitochondria as shown in Figs 2 and 3*a*, respectively. Electron transfer between succinate and ferricyanide was assumed to result in uptake of 1 H^+/e^- from the matrix phase (see Fig. 1 and the text).

Antimycin-insensitive reduction of ferricyanide by endogenous hydrogenated reductants can occur in some conditions[19,20] (Fig. 3, trace *a*). This causes scalar release of H^+ ions on the cytoplasmic side of the membrane, which should not be confused with true proton transport. During respiration with ferrocyanide (see Fig. 1) the rate of oxygen consumption (controlled separately with a Clark electrode; not shown, but see ref. 20) was found to match completely the net rate of generation of ferricyanide (Fig. 3, trace *b*), giving the electron transfer velocity. Without the pretreatment with ferricyanide there was in these conditions about 20% reduction in the net initial rate of production of ferricyanide relative to the consumption of O_2, due to reduction of formed ferricyanide by an endogenous reductant[19,20] (see also Fig. 3, trace *a*).

When the rate of absorption change of Neutral red was converted to ΔH^+ according to the calibration described above, it was found that the H^+/e^- ratio of proton uptake was close to 2.0 during oxidation of ferrocyanide (Table 1). This confirms that generation of protonmotive force by the cytochrome oxidase reaction is twice as effective as that of succinate–cytochrome *c* reductase per transferred electron, in full agreement with my original proposal[1] (Fig. 1). A threefold increase in the rate of oxidation of ferrocyanide brought about by addition of $MgCl_2$ had little effect on the measured H^+/e^- ratio (Table 1). Note that proton uptake and electron transfer were monitored from about 0.4 s onwards after addition of ferrocyanide (Fig. 3, traces *b*, *c*), that is, almost from the onset of e^- transport through cytochrome oxidase.

The possibility that changes in light scattering might significantly affect the measurements of Neutral red absorption was tested by using several different pairs of wavelengths, but this did not change the results. Selected pairs of wavelengths that gave absorption changes due to Neutral red and light scattering in the same or opposite direction gave the same results. The conditions chosen in Figs 2 and 3 are particularly insensitive to light scattering because measuring and reference wavelengths are closely spaced. Further controls showed that the absorption changes with ferrocyanide were blocked by cyanide, uncoupling agents[1] or nigericin, and were drastically diminished in other conditions in which the mitochondrial *p*H gradient is minimized, for example when inorganic phosphate and succinate were added in the absence of *N*-ethylmaleimide.

Although it seems clear from these data, and from earlier data of others[13–16], that the absorption changes of Neutral red measure changes in intramitochondrial H^+ activity, the main conclusion is independent of this notion. As the measured absorption changes are fully energy-dependent, the results show in any case that cytochrome oxidase is twice as effective an energy converter as succinate–cytochrome *c* reductase. This is inconsistent with the hypothesis of Mitchell[7] and Papa[8–10], in which the energy-conserving efficiency is the same for these segments of the respiratory chain. It is also not consistent with the hypothesis of Azzone[11] and Lehninger *et al.*[12], according to which the energy-conserving efficiency is three times higher for the oxidase reaction than it is for succinate–cytochrome *c* reductase.

Conclusive evidence of membrane transport requires the demonstration of both uptake and release of the transported species on the two sides of the membrane. Measurements of proton release on the cytoplasmic side of the membrane and of translocation of electrical charges have led to the model shown in Fig. 1. The present demonstration of uptake of 2 H^+/e^- from the matrix side of the membrane during cytochrome *c* oxidase activity completes this picture.

Whether Neutral red measures bulk matrix *p*H or more local H^+-linked changes on the matrix side of the membrane cannot be decided on the basis of the present results. It can nevertheless be concluded that cytochrome oxidase functions as a proton pump with the proton-translocating stoicheiometry suggested originally[1] (Fig. 1), and confirmed for cytochrome oxidase vesicles. The method presented here may also prove helpful in further assessment of proton translocation by enzymes other than cytochrome oxidase.

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Errata

'Insight' in the pigeon: antecedents and determinants of an intelligent performance

R. Epstein, C. E. Kirshnit, R. P. Lanza & L. C. Rubin
Nature **308,** 61–62 (1984)

IN the third sentence of the first paragraph on page 61, 'a classic problem with that Köhler confronted his chimpanzees' should read 'a classic problem with which . . .'.

VIP and noradrenaline act synergistically to increase cyclic AMP in cerebral cortex

Pierre J. Magistretti & Michel Schorderet
Nature **308,** 280–282 (1984)

ON page 281, the second and third paragraphs should read 'As shown in Fig. 1, the synergistic effect of 1 μM VIP and 10 μM NA was antagonized by the specific α-adrenergic antagonist phentolamine (100 μM) but not by (±)propranolol (100 μM), a specific β-adrenergic antagonist. Furthermore, as shown in Table 1, the specific α-adrenergic agonist phenylephrine . . .'.