Table 1. *Half-times of oxidation of the slow component at 590nm*

A suspension (1 ml) of *Nitrobacter* whole cells (anaerobic) (20 mg dry wt./ml) was mixed with 1 ml of aerated medium (100 mM-KCl, 10 mM-Tris-HCl, pH 7.4, 30°C) in an apparatus modified from the design of Strittmaster (1964). Carbonyl cyanide phenylhydrazone and dichlorophenylmethylurea were preincubated with the cells for 2 min.

<table>
<thead>
<tr>
<th>590–574 nm</th>
<th>Carbonyl cyanide phenylhydrazone</th>
<th>Dichlorophenylmethylurea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additions</td>
<td>None</td>
<td>(200 μM)</td>
</tr>
<tr>
<td>$T_{1/2}$ (s)</td>
<td>5.5</td>
<td>15.0</td>
</tr>
</tbody>
</table>

There is a rapid oxidation, but also a much slower oxidation, of which the initial half-time and kinetics are dependent on the ‘energized’ state. Initial half-times of between 3 s and 15 s have been recorded under different conditions (Table 1). The slow 590 nm component is identified with the high-potential 590 nm cytochromes and the fast component with the low-potential 590 nm cytochrome. These findings are in agreement with those of Kiesow (1967) and Cobley & Chappell (1974), who have shown that NO$_2^-$ oxidation is an energy-dependent process, and those of W. J. Ingledew & J. B. Chappell (unpublished work), who have shown, by using the inhibitor dichlorophenylmethylurea, that NO$_2^-$ oxidation occurs on a branch chain from the NADH to O$_2$ chain and that this side chain contains a nitrite-binding cytochrome 590.

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**Respiration-Driven Proton Translocation in *Hydrogenomonas eutropha* H-16**

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The facultative autotroph *Hydrogenomonas eutropha* H-16 was grown as described by Bongers (1967). Uncouplers such as carbonyl cyanide phenylhydrazone and 2,4-dinitrophenol stimulate the respiration of whole cells in air-saturated medium. After the cells were incubated anaerobically in excess of 2 h until any pH drift was either negligible or absent, air-saturated medium was added resulting in acidification of the cell suspension. That carbonyl cyanide phenylhydrazone catalyses acid–base titrations and diminishes the acidification of the medium (Figs. 1a and b) indicates the change in pH is due to proton translocation. There exists an optimum O$_2$ concentration to obtain the highest H$^+$/O
Bacteria (3.6 mg cell dry wt./ml) were incubated under anaerobic conditions at 30°C in 150 mM-KCl–2 mM-Tris–HCl in the initial pH range 6.9–7.0. O₂ (25 ng-atoms) was added as air-saturated medium at the arrows. Carbonyl cyanide phenylhydrazone (25 μM) was in (b), potassium thiocyanate (50 mM) in (c), carbonyl cyanide (25 μM) and potassium thiocyanate (50 mM) in (d). The quantity of H⁺ ions equivalent to the pH changes are indicated for the 4 ml of medium. A decrease in the pH of the suspension is upwards.

Fig. 1. Time-course of respiration-driven ΔpH

Bacteria (3.6 mg cell dry wt./ml) were incubated under anaerobic conditions at 30°C in 150 mM-KCl–2 mM-Tris–HCl in the initial pH range 6.9–7.0. O₂ (25 ng-atoms) was added as air-saturated medium at the arrows. Carbonyl cyanide phenylhydrazone (25 μM) was in (b), potassium thiocyanate (50 mM) in (c), carbonyl cyanide (25 μM) and potassium thiocyanate (50 mM) in (d). The quantity of H⁺ ions equivalent to the pH changes are indicated for the 4 ml of medium. A decrease in the pH of the suspension is upwards.

ratios whereas excess of O₂ causing diminished H⁺/O values are perhaps due to the build up of the proton electrochemical potential. When the incubation takes place in the presence of the ionophore valinomycin and K⁺ ions or in the presence of the lipid soluble anion thiocyanate, the proton pulse is enhanced. With optimum O₂ and thiocyanate concentrations, the mean of the extrapolated H⁺/O values of ten experiments is 7.88 ± 0.32. Values of 8 have been reported in one other organism, Micrococcus denitrificans (Scholes & Mitchell, 1970). Either valinomycin and K⁺ ions in antiport or thiocyanate in symport with proton translocation would nullify the electrical potential caused by proton translocation.

In the presence of valinomycin and K⁺ ions (Fig. 2b), the effect of carbonyl cyanide phenylhydrazone is potentiated, whereas in the presence of thiocyanate and carbonyl cyanide phenylhydrazone approximately the same extent of the proton pulse is obtained as with thiocyanate alone (Figs. 1c and 1d). A suggested hypothesis is that the positively charged valinomycin–K⁺ ion complex facilitates the movement of the uncoupler anion across the lipid portion of the membrane through charge interaction. Conversely, the increased negative-charge density in the membrane owing to the accumulation of thiocyanate might retard the movement of the anionic form of carbonyl cyanide phenylhydrazone (Eisenman et al., 1968; Liberman & Topaly, 1968). It is significant that uncoupler and thiocyanate does not diminish the magnitude of the proton pulse, but increases the decay rate (Fig. 1d). If uncoupler hydrolyses the high-energy intermediate X-I which drives the proton pump, as is sometimes postulated, a diminished pulse would be expected.

It is suggested that electrogenic proton translocation is caused directly by respiration and that these results are consistent with either an X-I-driven proton pump or with a
Fig. 2. *Time-course of respiration-driven ΔpH*

Bacteria (5.0 mg cell dry wt./ml) were incubated under anaerobic conditions at 30°C in 150 mM-KCl–2 mM-Tris–HCl in the initial pH range 6.5–6.6. O₂ (24 ng-atoms) was added as air-saturated medium at the arrows. Valinomycin (2.5 mg/g cell dry wt.) was in (a), valinomycin (2.5 mg/g cell dry wt.) and 2.5 μM-carbonyl cyanide phenylhydrazone in (b), 2.5 μM-carbonyl cyanide phenylhydrazone in (d). The quantity of H⁺ ions equivalent to the pH changes are indicated for the 4 ml of medium. A decrease in the pH of the suspension is upwards.

vectorial movement of protons linked directly to the oxidoreductions of the electron transport chain as proposed by Mitchell (1966). These results are inconsistent with the hypothesis that the primary movement in respiration-driven proton translocation is an X~I-driven cation pump with proton movement linked secondarily either by an electro-neutral exchange or by an electrophoretic migration of protons, since in this case uncoupler would be obliged to act directly on X~I and would diminish the magnitude of the respiratory pulse with or without thiocyanate.

We thank Mrs. L. Clark for skilled technical assistance.


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**The Effect of Cations on Membrane Surface Properties of Rat Liver Mitochondria as Determined by Microelectrophoresis**

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Microelectrophoresis has proved to be extremely useful in the determination of the nature of the surface properties of larger biological structures such as erythrocytes, bacteria and viruses (see Mehrishi, 1972). Some investigations of mitochondria have