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Proton-coupled Energy Transduction by Biological Membranes

Principles, Pathways and Praxis

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A brief outline of certain features of the chemiosmotic hypothesis of the mechanism of free-energy transfer between the reactions of electron transport and adenosine triphosphate synthesis catalysed by biological membranes is given. Pulses of electron transport induced by the addition of small quantities of oxygen to suspensions of the bacterium *Paracoccus denitrificans* lead to vectorial H^+ movements into the aqueous phase external to the organisms, where they may be detected with a glass pH electrode. The stoichiometry of the number of protons translocated into the bulk phase external to the organisms, per oxygen atom reduced, is essentially unchanged when the amount of oxygen reduced is varied, in a manner inconsistent with the predictions of the chemiosmotic-coupling hypothesis. These and other observations lead to the view that the energy-coupling proton-transfer processes utilised in reactions such as electron-transport phosphorylation are confined to the membrane phase. Mechanisms which most easily account for this are discussed.

It is now well established that localised and specific proton-transfer processes play an important role in enzymatic catalysis.[1-8] In addition it has become clear that free-energy transfer in biological membrane systems is often effected by means of a current of " energised " protons. The classical example of this is electron-transport phosphorylation, in which the passage of pairs of electrons down a thermochemical gradient (*e.g.* from reduced nicotinamide adenine dinucleotide, NADH, to dioxygen; $\Delta G^{\circ\prime} = -210$ kJ mol^{-1}) is coupled to the otherwise endergonic synthesis of adenosine triophosphate, ATP, from adenosine diphosphate, ADP, and inorganic phosphate ($\Delta G^{\circ\prime} = +31$ kJ mol^{-1}).[9] The two sets of reactions are catalysed by spatially separate enzyme complexes embedded in a contiguous, fluid mosaic [10] lipoprotein " coupling " membrane of molecular thickness, and the problem with which we are concerned is " how is the free energy released by electron transport coupled to the synthesis of ATP?".

Current thinking [11] contends that the activity of certain components of the electron-transport chain is more or less tightly coupled to an initial proton translocation across the coupling membrane (fig. 1), and that the " energised " protons so translocated (" pumped ") pass to the ATPase enzymes, so as to provide the free energy to drive the synthesis of ATP. Controversy attaches both to mechanisms by which the protons are pumped by the electron-transport chain and used by the ATPase [11] and to the pathway taken by the " energised " coupling protons in electron-transport phosphorylation. In the present article we confine our questioning to the latter problem.

Recognising the high mobility of protons in aqueous media [12-16] Mitchell,[17-21] in his chemiosmotic hypothesis, proposed that the unit of energy coupling was the entire volume that the coupling membrane surrounded, so that the coupling protons

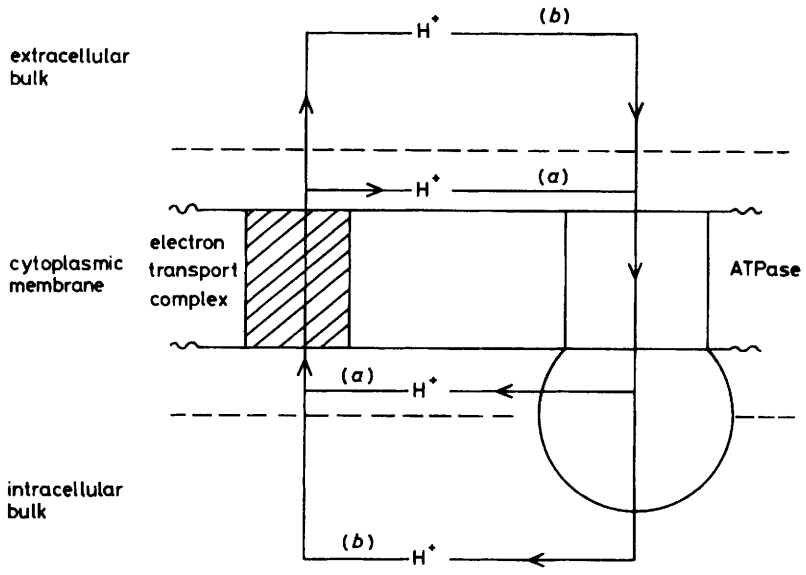

FIG. 1.—Proton-coupled reactions in electron transport phosphorylation. The figure gives a diagrammatic representation of a bacteria! cytoplasmic membrane separating the intracellular and extracellular spaces, and containing lipoprotein electron transport and ATPase complexes. It is supposed that the general ion and proton permeability of the coupling membrane is low, and that electron transport and ATP synthesis are coupled to vectorial proton translocation. In the present case we wish to distinguish direct and localised pathways of proton transfer (*a*) from proton movements in the bulk aqueous phase external to the bacteria (*b*). We use the symbol H^+ to denote protons of unspecified degrees of hydration.

were in energetic equilibrium with the electrochemical potential of protons in the bulk aqueous phases that the coupling membrane served to separate. In this way he could describe their free energy in terms of macroscopic thermodynamics as a measurable " proton-motive force," Δp, given by the equation:

$$\Delta p = \Delta\tilde{\mu}_{H}{}^{+}/F = \Delta\psi - 2.303\, RT\, \Delta pH/F \qquad (1)$$

where $\Delta\tilde{\mu}_{H}{}^{+}$ is the electrochemical potential difference of protons between the 2 bulk phases that the membrane separates, and $\Delta\psi$ and ΔpH are, respectively, the electrical transmembrane potential and pH gradient between these phases.[22,23] R,T and F have their usual thermodynamic meanings. According to this hypothesis, the proton-motive force equilibrates reversibly with the reaction catalysed by the membrane ATPase in the steady state.

However, much experimental evidence [24-26] indicates that the energy-coupling process in electron-transport phosphorylation is much more localised than this, and following the original proposals of Williams,[27-30] a number of workers have adopted the view that the " energised " coupling protons are retained on the surfaces of the coupling membrane,[25,31-33] so that they are out of equilibrium with the bulk phase electrochemical proton potentials even in the steady state.

Since one of the apparently most persuasive pieces of evidence for the chemi-osmotic interpretation of processes such as electron-transport phosphorylation is the observation of electron-transport-linked proton ejection into the bulk aqueous phase external to suspensions of respiratory bacteria,[34] we decided to reinvestigate this process in the bacterium *Paracoccus denitrificans*. It is concluded that those H^+ that are ejected into the bulk aqueous phase during electron transport are not kinetically

competent to drive otherwise endergonic reactions such as ATP synthesis under any circumstance studied.

EXPERIMENTAL

P. denitrificans (NCIB 8944) was grown and maintained as described.[35] Cells from mid-log phase cultures were washed 3 times and resuspended, at *ca.* 3 (mg dry weight) cm^{-3}, in a 6 cm^3 reaction medium in a thermostatted vessel at 30 °C containing 150 mmol dm^{-3} KCl + 0.25 mmol dm^{-3} glycylglycine (pH 6.5). Carbonic anhydrase was added to a concentration of 80 $\mu g\ cm^{-3}$. Vigorous stirring was effected [35] and potentiometric measurements were carried out [36] by means previously described. H^+ movements in the aqueous phase external to the organisms were calibrated with anaerobic HCl and KOH, and oxygen pulses were delivered as air-saturated saline, in the manner described by Scholes and Mitchell.[34] The endogenous steady-state respiration rate of the cell was *ca.* 75 (ng-atom O) min^{-1} (mg dry weight)$^{-1}$ as measured using a Clark-type oxygen electrode.[37] In our hands the relationship 1 (mg dry weight of cells) = 5.59×10^8 organisms = 2.0 mm^3 internal volume was obtained [*cf.* ref. (25) and (38)]. Chemicals and biochemicals were obtained as previously.[35]

RESULTS

Scholes and Mitchell [34] demonstrated that the addition of pulses of O_2, as air-saturated 150 mmol dm^{-3} KCl (235 μmol dm^{-3} O_2 at 30 °C), to weakly buffered, anoxic suspensions of *Micrococcus* (now *Paracoccus*) *denitrificans* elicited the vectorial ejection of protons into the bulk aqueous phase external to the organisms, where they could be detected with a sensitive glass electrode (see fig. 1). We have repeated such experiments, with the resulting traces shown in fig. 3. Fig. 3 shows that in the absence of compounds such as SCN^- (see fig. 2) the rate of H^+ ejection is rather slow ($t_{\frac{1}{2}} \approx 5$ s) compared with the calculated time of O_2 reduction (<1 s, see Experimental), and the apparent stoichiometry of H^+ translocated per O atom reduced, the $\rightarrow H^+/O$ ratio, is rather small, *ca.* 2.5, compared with the expected value [34,39-42] of *ca.* 8. When the experiment is repeated [fig. 3(*b*)] in the presence of 100 mmol dm^{-3} KSCN, however, the ejection of the protons to, and their decay from, the bulk aqueous phase are much more rapid, and the $\rightarrow H^+/O$ ratio observed is increased approximately threefold to a value of *ca.* 7.5 [*cf.* ref. (34) and (39)-(42)]. When carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone, FCCP, is present [fig. 3(*c*) and (*d*)] essentially no H^+ movements are observable, indicating presumably that all protons previously seen to be ejected had been translocated back across the membrane (fig. 2), and were not just the products of scalar chemical reactions, since it is believed [43,44] that, *inter alia*,[45] one role of FCCP lies in accelerating greatly the passage of H^+ back across the coupling membrane (fig. 2). These data are in substantial quantitative agreement with those obtained by Scholes and Mitchell.[34]

According to the interpretation of these data given by Scholes and Mitchell,[34] it is proposed that in the absence of compounds such as SCN^- a large transmembrane potential [eqn (1)] is set up by the translocation of a small fraction of the pumped H^+. It is assumed that this electrical potential causes the H^+ to pass back across the membrane electrophoretically from the outer bulk aqueous phase before it can be observed, since the half-response time of our glass electrode and recording system is *ca.* 0.5 s. Transmembrane electrophoretic cotransport, with the pumped H^+, of SCN^- ions (which, unlike Cl^-, cross the cytoplasmic membrane of this organism fairly rapidly)[46] would act to disspate this bulk-to-bulk phase membrane potential and thus

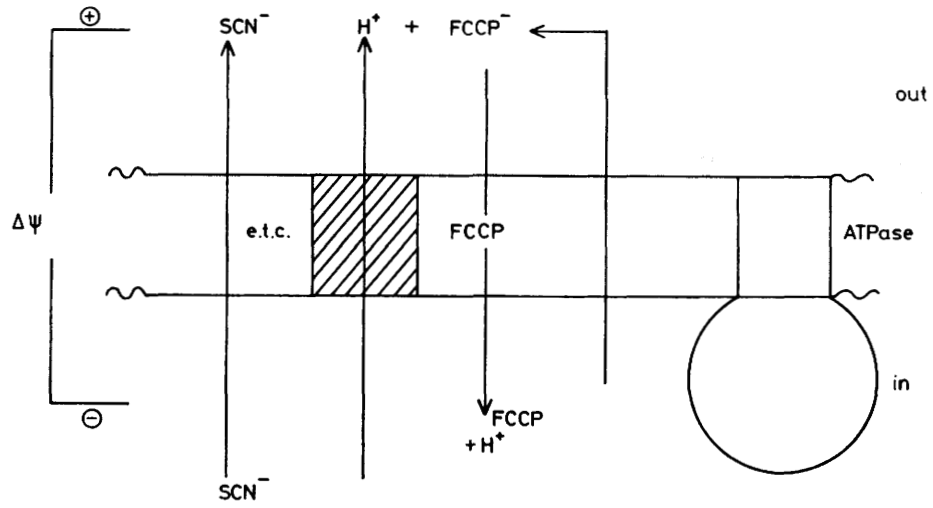

FIG. 2.—Conventional explanation of the role of compounds such as SCN^- and FCCP in affecting the apparent stoichiometry of respiration-driven proton translocation. It is supposed that transmembrane H^+ translocation driven by the electron-transport complex (e.t.c.) sets up a large membrane potential, $\Delta\psi$, between the two bulk phases that the membrane separates. Electrophoretic movement of SCN^- in response to the $\Delta\psi$ allows more H^+ to be pumped into the bulk. FCCP is a lipophilic weak acid which can cross the membrane in both neutral and anionic forms, thus catalysing the electrogenic passage of pumped H^+ back across the coupling membrane.

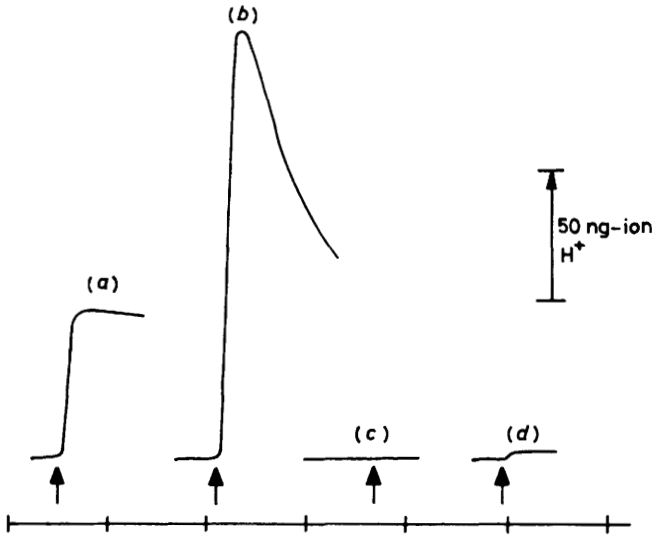

FIG. 3.—Respiration-driven H^+ translocation in *P. denitrificans*. Respiration-driven H^+ translocation was measured as described in the Experimental section. All reaction media contained, in a final volume of 6 cm^3 at 30 °C, 150 mmol dm^{-3} KCl, 0.25 mmol dm^{-3} glycylglycine (pH 6.5), 480 μg carbonic anhydrase and 1.05×10^{10} cells. In addition, traces (*b*) and (*d*) contained 100 mmol dm^{-3} KSCN. In traces (*c*) and (*d*) 2 μmol dm^{-3} FCCP was also present. At the arrows, 50 mm^3 air-saturated KCl (23.5 ng-atom O) was added to the closed reaction vessel. At no time did the pH change exceed 0.05 pH units.

allow measurement, by extrapolation to the half-life of O_2 reduction, of the true stoichiometry of H^+ translocation, in this case *ca.* 7.5. The amount of oxygen added in each case (fig. 3) was the same, and it is assumed (but see Discussion) that large amounts of ATP are not produced when SCN^- is absent.

The foregoing explanation (fig. 2) of the role of compounds such as SCN^- was challenged by the work of Archbold *et al.*,[47] of Conover and Azzone [48] and of Gould and Cramer.[49] The latter authors, who carried out experiments with *Escherichia coli* in the absence of SCN^-, showed that when the cell/O_2 ratio was made very high, *i.e.* when the calculated membrane potential was energetically insignificant (see later), the measured $\rightarrow H^+/O$ ratio remained much lower than its limiting stoichiometric value, obtained in the presence of SCN^-, of *ca.* 4. Further, when a second O_2 pulse was added immediately following the first one the stoichiometry of H^+ ejection caused by the second pulse was the same as that caused by the first. This result would not be expected, according to the conventional view, since the membrane potential should be so large after the first pulse that no H^+ at all should be seen to enter the outer bulk aqueous phase in response to the second O_2 pulse. We have therefore carried out experiments of a similar nature, simply by varying the size of a single O_2 pulse, in *P. denitrificans*.

Fig. 4 is a plot of the number of H^+ translocated into the bulk phase external to

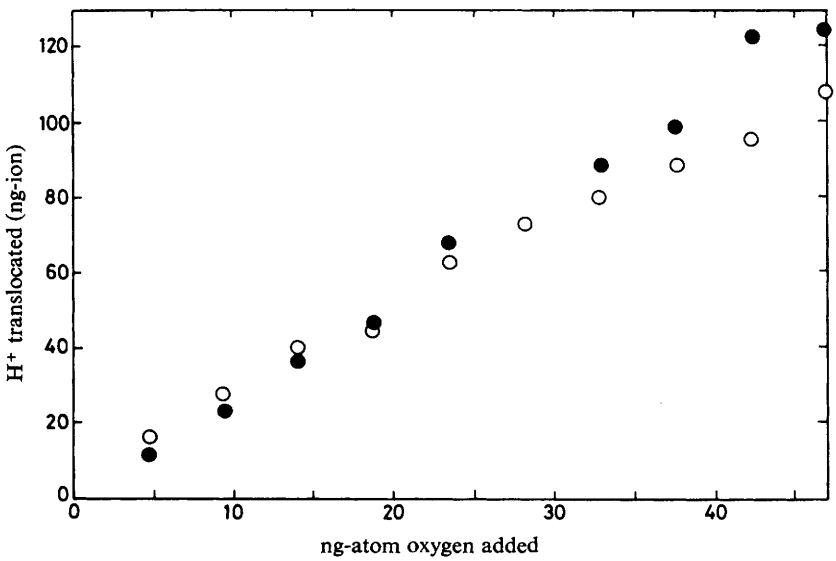

FIG. 4.—Effect of the size of the oxygen pulse on respiration-driven H^+ translocation in *P. dentrificans*. Respiration-linked H^+ movements were measured as described in the legend to fig. 3, trace (*a*), except that either 1.09×10^{10} (○) or 3.26×10^{10} (●) cells were present. The size of the oxygen pulse was varied as indicated.

the organisms as a function of the size of the oxygen pulse, obtained under the same conditions as the trace in fig. 3(*a*). It should be stressed that, throughout the range of O_2 pulses used, $t_{\frac{1}{2}}$ for H^+ ejection was similar to that observed, ± 1 s, in fig. 3(*a*). This slow $t_{\frac{1}{2}}$ cannot be attributed to a protonmotive reversal of the reaction catalysed by the membrane ATPase since this reaction is extremely slow in *P. denitrificans*.[50,51] It is clear that the build-up of a membrane potential, which should in principle have stopped the increase in the number of protons translocated at an added oxygen

concentration below even that in fig. 3(*a*) (see above), has no significant effect upon the stoichiometry of H^+ ejection into the bulk aqueous phase at any cell/O_2 ratio examined (fig. 5).

If we treat the organisms as 1 μm diameter spherical-shell capacitors, with a membrane capacitance of 1 μF cm^{-2},[52] it is possible to calculate the maximum bulk-phase

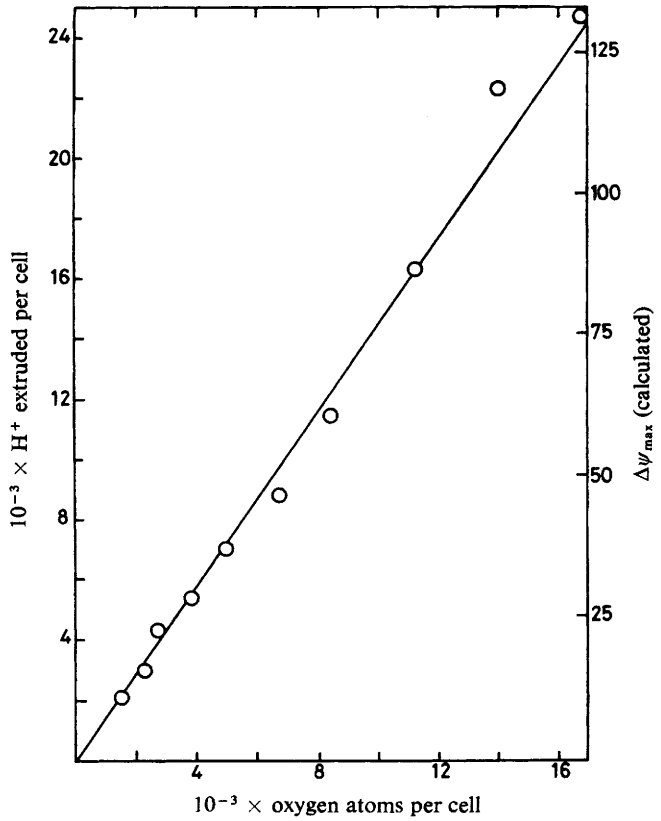

FIG. 5.—Lack of effect of the size of the oxygen pulse on the stoichiometry of respiration-driven H^+ translocation in *P. denitrificans*. Measurements of respiration-driven H^+ translocation were carried out as described in the legend to fig. 4, except that *ca.* 5×10^{11} cells were present. The number of cells and the size of the oxygen pulse were varied to give the oxygen/cell ratios indicated. The maximum possible membrane potential that could have been built up, $\Delta\psi_{max}$, was calculated as described in the text.

transmembrane potential, $\Delta\psi_{max}$, built up by electrically uncompensated H^+ translocation from the formula [49]

$$\Delta\psi_{max} = en/C \qquad (2)$$

where n is the number of protons translocated across the membrane capacitance of a single bacterial cell of total capacitance C, and e is the elementary electrical charge. By varying the size of the O_2 pulse and/or the number of cells, $\Delta\psi_{max}$ will also be varied, and may be made arbitrarily small, according to eqn (2), since, given the constancy of e and of C, $\Delta\psi_{max}$ depends only on the number of oxygen atoms (and hence H^+ translocated) per bacterial cell. As $\Delta\psi_{max}$ tends to zero the orthodox view [34] would have it that there is nothing to stop the ejection of H^+ equal to the true limiting stoichio-

metry, as seen when SCN^- is present (fig. 3), into the bulk phase external to the organisms.

Fig. 5 shows a plot of the H^+ translocated as a function of the oxygen/cell ratio added during the pulse, in which the $\Delta\psi_{max}$, calculated using eqn (2) with a value of C of 3×10^{-14} F,[49] was as low as 12 mV. It may be observed (fig. 5) that there is no significant increase in the $\rightarrow H^+/O$ ratio as the O_2/cell ratio is decreased to very low levels. Whilst the difference in the absolute values of the $\rightarrow H^+/O$ ratios observed in the experiments of fig. 4 and 5 is due to variations between batches of cells, this variation makes no difference to the present analysis and interpretation, which is independent of the absolute values measured. Care was taken to ensure that both the cell and O_2 concentrations were varied in experiments such as those shown in fig. 5, so as to ensure that imperfect mixing did not constitute a potential artefact in these measurements.[53] Essentially similar data to those shown in fig. 4 and 5 were obtained when all K^+ salts were substituted by the corresponding sodium or choline salts (data not shown), indicating that electrically compensating cation movements into the cells were not the cause of the observable H^+ movements.

As an alternative approach to assessing the role of compounds such as SCN^- in stimulating the apparent $\rightarrow H^+/O$ ratio, we chose to study, for reasons intimated,[25,54] the effect of the more lipophilic, membrane-permeable tetraphenylborate (TPB^-) anion on respiration-driven proton translocation. At the concentrations used, this compound had no effect upon the steady-state respiration rate of these organisms (data not shown).

Fig. 6 shows the effect of low concentrations of sodium tetraphenylborate on the apparent $\rightarrow H^+/O$ ratio in *P. denitrificans*. There is initially an essentially linear in-

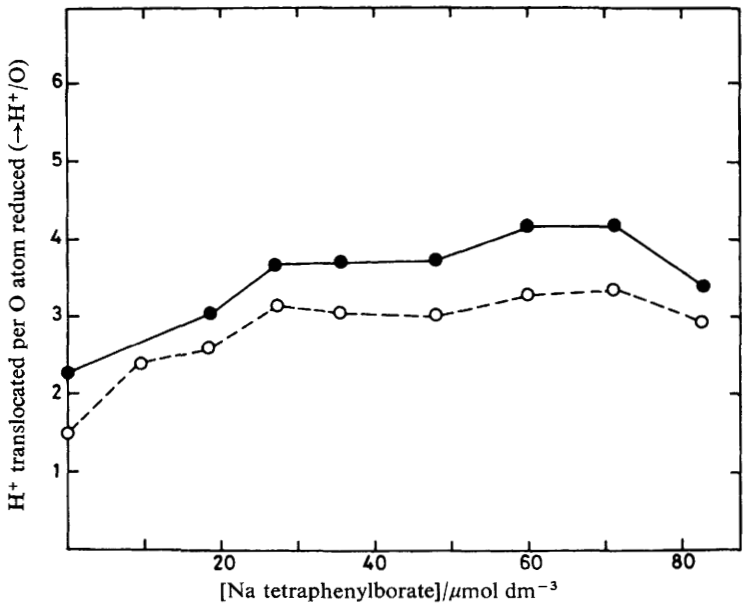

FIG. 6.—Effect of sodium tetraphenylborate on the apparent stoichiometry of respiration-driven H^+ translocation in *P. denitrificans*. Measurements were carried out as described in the Experimental section, except that all potassium salts were replaced by the corresponding sodium salts. Sodium tetraphenylborate was added to the concentration indicated. The number of cells present was 1.29×10^{10} and the amount of oxygen added was either 14.1 ng-atom (●) or 42.3 ng-atom (○). All respiration-linked pH changes were abolished by 5 μmol dm^{-3} FCCP.

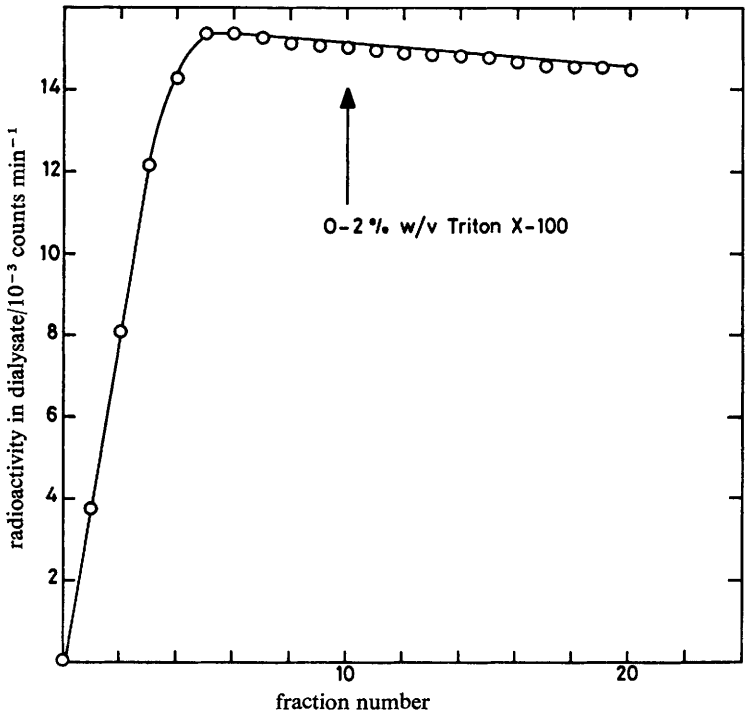

FIG. 7.—Lack of a Donnan potential (positive inside) across the cytoplasmic membrane of *P. denitrificans*. Cells were harvested and resuspended at 12.3 (mg dry weight) cm^{-3} in 0.1 mol dm^{-3} tris acetate, pH 7.3. 1 cm^3 of this suspension was placed in the upper chamber of a flow dialysis cell as described.[55] At time zero, 20 μmol dm^{-3} $KS^{14}CN$ (60 mCi $mmol^{-1}$) was added to the upper chamber and the flow started. A decrease in radioactivity corresponds to uptake by the cells. At the point indicated, the detergent Triton X-100 was added to a final concentration of 0.2% w/v to disrupt the cytoplasmic membrane. No release of KSCN is observed, indicating that no concentrative uptake of SCN^- had taken place. Identical results were obtained in the absence of cells.

crease in the $\rightarrow H^+/O$ ratio as the TPB^- concentration is increased, over at least a three-fold change in O_2-added per cell. If the mechanism by which this was occurring was by simple outward electrophoresis of intracellular TBP^- (as in the conventional explanation, fig. 2), the " extra " H^+ observed should be accompanied by a similar number of TBP^- ions. However, assuming that there is no pre-existing Donnan potential across the bacterial cytoplasmic membrane (see fig. 7), we may calculate, from the known intracellular volume of the cells (see Experimental), the maximum number of TPB^- ions that *could* move from the intracellular bulk phase to the extracellular bulk phase in response to electrogenic H^+ pumping. Under the conditions used (fig. 6) this is equal to 0.46 ng-ion for each added 10 μmol dm^{-3} TPB^-. The " extra " H^+ observed under these conditions is, especially with the larger O_2 pulse, greatly (>20 times) in excess of this, as the TPB^- concentration is raised from zero to 50 μmol dm^{-3}.

Had there been a pre-existing Donnan potential (positive inside) across the bacterial cytoplasmic membrane, the concentration of free TPB^- inside the bacteria prior to the O_2 pulse would have been greater than that outside by a factor given by the Nernst equation:

$$\Delta\psi_{\text{Donnan}} = 2.303 \frac{RT}{F} \log_{10} \frac{[\text{TPB}^-]_{\text{in}}}{[\text{TPB}^-]_{\text{out}}}. \quad (3)$$

However, the experiment displayed in fig. 7 shows that, since no concentrative uptake of the permeant SCN^- ion is observed in these cells, no such Donnan equilibrium exists across the cytoplasmic membrane of *P. denitrificans*, the lower limit of detection under these experimental circumstances being *ca.* 20 mV.[55] Thus, since bulk-phase intracellular TPB^- molecules should have been very much depleted at very low $\rightarrow H^+/O$ ratios under the conditions described if they were passing electrophoretically from one bulk phase to another in response to the primary H^+ movements, the independence on O_2 concentration of the TPB^- stimulation of the apparent $\rightarrow H^+/O$ ratio, in addition to the evidence described, would seem to negate the view that the sole effect of ions such as SCN^- and TPB^- on the appearance of the true number of H^+ in the bulk phase during $\rightarrow H^+/O$ measurements lies in their ability to collapse a bulk-to-bulk phase membrane potential.

DISCUSSION

The chief question to which we wish to address ourselves in the present submission concerns the pathway taken by the protons pumped across the bacterial cytoplasmic membrane in response to a pulse of respiratory activity. In confirmation of the original findings of Scholes and Mitchell [34] it was observed (fig. 3) that the addition of a small pulse of dioxygen, as air-saturated KCl, to a suspension of anoxic *P. denitrificans* elicited the vectorial ejection of protons into the bulk aqueous phase external to the organisms. Both the rate and extent of H^+ ejection were markedly stimulated by the addition of 100 mmol dm^{-3} KSCN, such that the stoichiometry of protons translocated per oxygen atom reduced, the $\rightarrow H^+/O$ ratio, attained under these conditions a value approaching its accepted [34,39-42] limiting stoichiometry. Various authors [*e.g.* ref. (42), (56) and (57)] have discussed possible reasons why even this value may be an understimate of the " true " stoichiometry, but for our present purposes we wish to know what happened to the " missing " protons when this experiment was performed in the *absence* of KSCN.

It is worth mentioning at the outset that, owing to the relatively low electrical capacitance of the coupling membrane when compared with the differential buffering capacitance of the system, for every electrically uncompensated H^+ moved across the membrane into the external aqueous phase there will be a much greater increase in the transmembrane potential than in the pH gradient.[18] Thus, in the experiment depicted in fig. 3(*a*) in the absence of KSCN, a large transmembrane potential will be set up, and this may, for instance, be rapidly used for ATP synthesis. If this membrane potential is thus dissipated, the residual pH gradient will be thermodynamically too small to drive further ATP synthesis, and thus the ejected protons will remain for a relatively long time in the external aqueous phase. A similar pattern would also be seen [58] if a leak of protons back across the coupling membrane that is not coupled to ATP synthesis is also highly non-ohmic, so that at high values of the protonmotive force the decay rate is particularly rapid [*cf. e.g.* ref. (59)]. However, this type of phenomenon fails to explain the slow half-life of ejection of H^+ into the bulk phase [fig. 3(*a*)], since, if this type of phenomenon alone were acting to lower the apparent $\rightarrow H^+/O$ ratio in the absence of KSCN, the apparent rate of H^+ ejection should be the same when KSCN is present. Under conditions in which the calculated value of $\Delta\psi$ is very small, the extrapolated $\rightarrow H^+/O$ ratio should be increased to its true stoichiometric value of *ca.* 8, a phenomenon which is not observed. In any event, it has been demonstrated with phospholipid vesicles,[36] including those containing the proton pump bacteriorhodopsin,[60] that the rate of decay of a pH gradient across the phospholipid membrane is directly proportional to the magnitude of the pH gradient, even

when this is initially caused by artificial means to exceed 4 pH units.[36] Thus a non-ohmic *leak* of protons back across the coupling membrane seems an unlikely explanation of the present data.

It is known from the work of several groups with related systems [*e.g.* ref. (61)-(65)] that ATP synthesis may be driven by a transmembrane field alone, in the absence of observable bulk-phase proton movements. Controversy remains as to whether this is a thermodynamic or a kinetic phenomenon,[26] although it is worth noting that under such conditions ATP synthesis stops immediately upon cessation of electron transport.[66] Hanselmann [38] showed that, upon the initiation of respiration in *P. denitrificans*, ATP synthesis began following a variable lag of between 0 and 2 s. For technical reasons it was not possible for us to measure ATP synthesis on this timescale, nor, unfortunately, was an inhibitor of the ATP synthase enzyme itself found which was active in intact cells. Thus it would seem that some uncharacterised factors are operating to slow the expression of respiration-linked H^+ movements in the bulk phase for times that are extremely long compared with those expected from simple diffusion alone.[67] Various mechanisms [25,68] have been proposed to account for this, but as yet none has the benefit of extensive and rigorous experimental support.

We have proposed elsewhere [32,33] that, in addition to the proton *pumps* which are coupled to the activity of the electron transport chain and ATPase complexes, there exist in such coupling membranes proteinaceous devices whose role is to act as elements in specific networks (protoneural networks) for energised proton transfer along the membrane surfaces. It is envisaged [32,33] that the transmembrane field acts to change

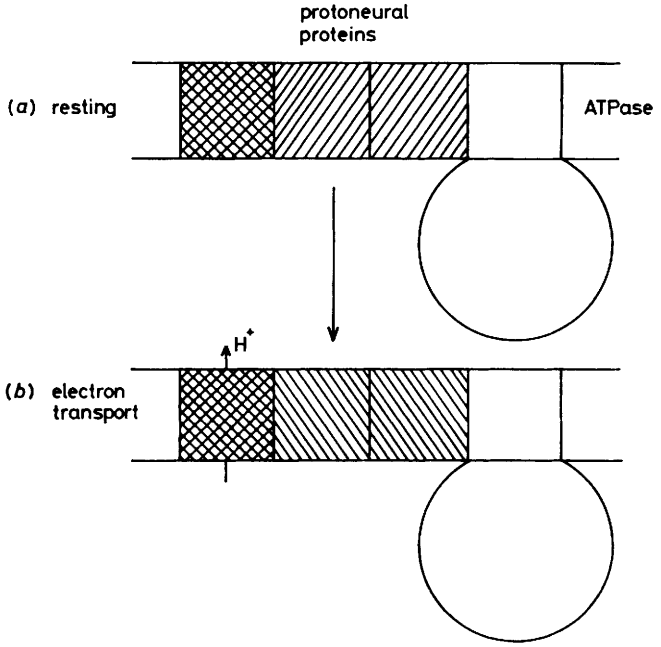

FIG. 8.—Proposed model of energy coupling in electron-transport phosphorylation. The diagram shows an energy coupling membrane containing an electron-transport complex(e.t.c.), an ATPase and protoneural proteins whose role is, upon initiation of electron transport (*b*), to change their conformation in a coherent fashion and effect passage of the pumped protons to the ATPase. In the resting state (*a*), in which no electron transport is taking place, the proton electrochemical potentials at the membrane surfaces are in equilibrium with those in the bulk. It is proposed that compounds such as SCN^- and TPB^- inhibit the conformational transitions of the protoneural proteins.

their conformation in a coherent fashion between proton-conducting and non-conducting states, so that only under the latter conditions do protons pumped across the coupling membrane come into equilibrium with those in the bulk aqueous phases that the coupling membrane separates. Such a minimal proposal would serve to explain all the data presented here, as well as many others in the literature [*e.g.* ref. (24)-(26), (32), (33) and (68)]. The role of TPB^- in increasing the apparent $\rightarrow H^+/O$ ratio, in addition to a transmembrane electrophoretic moment in response to electrogenic proton transport, is viewed as an inhibition of the transition of the protoneural networks between their non-conducting and conducting states (fig. 8).

In summary, electron transport in *P. denitrificans* is demonstrably coupled to H^+ translocation across the bacterial cytoplasmic membrane. However, under no conditions examined did those translocated protons which could be observed as changes in pH in the bulk aqueous phase external to the organisms appear to be kinetically competent to return across the membrane, and so effect free-energy transfer. It is suggested that there are controls over current flow between local devices in membranes which cannot be understood from studies of bulk-phase phenomena.

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