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THE RELATION BETWEEN MEMBRANE IONIC CURRENT AND ATP SYNTHESIS IN CHROMATOPHORES FROM *RHODOPSEUDOMONAS CAPSULATA*

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(1) Under conditions in which membrane potential ($\Delta\psi$) was the sole contributor to the proton-motive force, the steady-state rate of ATP synthesis in chromatophores increased disproportionately when $\Delta\psi$ was increased: the rate had an approximately sixth-power dependence on $\Delta\psi$. (2) Simultaneous measurements showed that the dissipative ionic current (J_{DIS}) across the chromatophore membrane had a related dependence on $\Delta\psi$, i.e., the membrane conductance increased markedly as $\Delta\psi$ increased. (3) For comparable $\Delta\psi$ values, J_{DIS} was greater in phosphorylating than in non-phosphorylating chromatophores. For comparable actinic light intensities, $\Delta\psi$ was smaller in phosphorylating than in non-phosphorylating chromatophores. (4) At either low pH or in the presence of venturicidin, oligomycin or dicyclohexylcarbodiimide to inhibit ATP synthesis, J_{DIS} was substantially depressed, particularly at high $\Delta\psi$. Even under these conditions the membrane conductance was dependent on $\Delta\psi$. (5) Also in intact cells, J_{DIS} was depressed in the presence of venturicidin. Points 1–5 are interpreted in terms of a $\Delta\psi$ -driven H^+ flux through the F_0 channel of the ATPase synthase. The high-power dependence of the F_0 conductance on $\Delta\psi$ determines the dependence of the rate of ATP synthesis on $\Delta\psi$. The $\Delta\psi$ -dependent conductance of F_0 dominates the electrical properties of the membrane. In chromatophores the ionic current accompanying ATP synthesis was more than 50% of the total membrane ionic current at maximal $\Delta\psi$. (6) The rate of cyclic electron transport was calculated from J_{DIS}. This led to an estimate of 0.77 ± 0.22 for the ATP/$2e^-$ ratio and of 3.5 ± 1.3 for the H^+/ATP ratio. (7) Severe inhibition of the electron-transport rate by decreasing the light intensity led to an almost proportionate decrease in the rate of ATP synthesis. The chromatophores were able to maintain proportionality by confining electron-transport phosphorylation to a narrow range of $\Delta\psi$. This is a consequence of the remarkable conductance properties of the membrane.

1. Introduction

The high-energy intermediate in electron-transport phosphorylation in mitochondria, chloroplasts and bacteria may be the protonic potential difference (Δp) across the coupling membrane. According to the chemiosmotic hypothesis, Δp is generated by electron transport-driven H^+ translocation and is utilised by a proton flux through the ATP synthase [1]. This model is in general consistent with many different kinds of experimental data (see Ref. 2 for review) and is given further support by the results presented below: we show that a component of the ionic current across the membrane of bacterial chromatophores coincides with the process of ATP synthesis. More specifically, we have investigated the form of the relationship between the membrane ionic current, the

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Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; Mes, 4-morpholineethanesulphonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine; BChl, bacteriochlorophyll.

rate of ATP synthesis and the magnitude of Δp. The electrochromic absorption changes of the endogenous carotenoid pigments in chromatophore membranes provide a convenient probe for the measurement of both ionic current and Δp [3–5]. The steady-state approach that we use complements earlier experiments with pulsed light [6,7]. It has the advantage that higher values of Δp are attained and it is more comparable with experiments carried out in mitochondria and chemosynthetic bacteria.

The magnitude of Δp has been found to depend non-linearly on the rate of electron transport in mitochondra [8–10], chloroplasts [11] and bacteria [12,13]. In general, strong inhibition of electron transport leads to only a small decline in Δp. At least in photosynthetic bacteria this can be wholly explained [5,13] by Nicholls' suggestion [8] that the membrane is non-ohmic – the ionic conductance of the membrane increases with increasing Δp. In this report we show that the ionic current vs. Δp curves indicate a similar behaviour in chromatophores prepared from the bacteria.

In chromatophores [14] and in other membranes [15–19] the rate of ATP synthesis has been found to depend non-linearly on Δp – it increases disproportionately as Δp is increased. We confirm this and demonstrate a striking resemblance between the ATP synthesis vs. Δp curve and the ionic current vs. Δp curve. The close parallel between the ionic current and the ATP synthase activity is supported by experiments with specific inhibitors of the F_0 component of the enzyme. A calibration procedure for the ionic current enables us to calculate limiting values of the $P/2e^-$ and H^+/ATP ratios and provides an internal check on the use of this method for examining the role of H^+ currents in electron-transport phosphorylation. The important conclusion from a physiological standpoint is that inhibition of electron transport leads to an almost proportionate decrease in the rate of ATP synthesis in a range where Δp falls hardly at all. This arises from the dominating influence of the gated ATP synthase upon the conductance properties of the membrane.

2. Methods

Rhodopseudomonas capsulata strain N22 was grown under photosynthetic conditions as previously described [20]. The harvested cells were washed in 10% sucrose, 50 mM K_2SO_4, 8 mM $MgCl_2$, 50 mM Tricine, pH 7.4, and broken in the same medium supplemented with a few crystals of DNAase in a French press at 11 000 lb/inch^2. The chromatophore fraction, sedimenting between $30\,000 \times 30$ $g \cdot$ min and $76\,000 \times 180$ $g \cdot$ min was resuspended in 10% sucrose, 100 mM KCl, 8 mM $MgCl_2$, stored at 4°C and used within 5 days of preparation. Bacteriochlorophyll was assayed in acetone/methanol extracts by the method of Clayton [21].

Absorbance changes were measured in a cross-beam spectrophotometer in a 1×1 cm cuvette. The actinic light, from a 150 W quartz-halogen lamp passed through 4 cm of water and two layers of Wratten 88A gelatin filter, was directed into the cuvette from below. The light was turned on and off with a leaf shutter (opening and closing time less than 6 ms) and was attenuated with grey filters calibrated at 800 nm. The maximum light intensity incident upon the cuvette face was approx. 2.3×10^5 erg $\cdot$ cm$^{-2} \cdot$ s^{-1} calibrated against a Hewlett Packard thermopile with a silicon photodiode.

Membrane potential ($\Delta\psi$) was measured from the electrochromic absorption change of the endogenous carotenoid pigments. The absorption change depends linearly on $\Delta\psi$ so that the membrane potential axes of the figures, in units of carotenoid absorption change, are directly proportional to $\Delta\psi$ (Ref. 4; and, for a review, see Ref. 22). Experiments in which only electrochromic changes were recorded, were carried out in medium A containing 0.5 mM NADH, 0.4 mM sodium succinate, 100 mM KCl, 8 mM $MgCl_2$, 50 mM Tricine, 10% sucrose, final pH 7.6, and chromatophores at a final BChl concentration of 10 μM. Anaerobiosis was attained by allowing the chromatophores to take up residual oxygen from the argon-sparged medium by respiration during a 30 min pre-incubation period in a darkened, completely filled and stoppered cuvette. Anaerobic conditions were chosen so as to provide a stable, reproducible redox environment. The carotenoid band shift was calibrated by the method described earlier [4,23]. The chromatophores were washed once in a K^+-free medium containing 10% sucrose, 50 mM NaCl, 50 mM Tricine, 8 mM $MgCl_2$, pH

7.6. The calibration was carried out in this medium under aerobic conditions in the dark. The suspension was treated with 0.2 μg/ml valinomycin and incubated for 10 min to permit ionic equilibration across the membrane. The carotenoid shift generated by the addition of KCl, corrected for the dilution artifact, was then recorded. The slope of the linear relation between the carotenoid absorption change and the logarithm of the added KCl was taken to be 58 mV at 20°C. The calibration procedure carried out on just one occasion gave a value for light-induced membrane potential similar to those found in earlier work [4,23] (300 mV under non-phosphorylating conditions) and this was used as a normalising standard for all the values quoted in this report.

The membrane ionic current is proportional to the rate of change of electrochromic absorption as first discussed by Junge and Schmid [3]. The proportionality constant for converting $\Delta A/s$ to nmol ion equiv./μmol BChl per s was calculated on the basis [5] that saturating single-turnover flash activation of antimycin-treated chromatophores leads to the electrogenic transfer of a single electron and the binding of a single proton in each photosynthetic reaction centre [24]. From five chromatophore preparations, the ratio of the maximum absorbance change produced by continuous illumination to the absorbance change produced by a short flash in the presence of 2.5 μM antimycin A was 5.4 S.D. ± 0.6. In the same preparations the mean ratio of bulk BChl/reaction centre was 107 S.D. ± 20 estimated from the absorption change at 540 nm [25] produced by a train of closely spaced flashes in chromatophores poised at high redox potential with potassium ferricyanide.

When simultaneous measurements of electrochromism and ATP synthesis were required, medium A was supplemented with 100 μM cresol red and the Tricine was omitted. The pH of the medium was adjusted with dilute KOH by comparison with the optical absorption of a similar suspension, buffered to pH 7.6. Anaerobic conditions were achieved as described above. At the end of the experiment the stopper was removed and the cresol red response was calibrated by adding small quantities of standard HCl. The addition of 2 μg/ml rotenone prior to this stage was used to eliminate pH drift due to NADH oxidase activity. Separate glass-electrode experiments showed that 100 μM cresol red had no measurable effect on the rate of ATP synthesis. Note that, in the combined experiments, the carotenoid band shift was measured at a single wavelength (503 nm, a peak in the difference spectrum) but in the absence of ATP determinations, both of the measuring beams were used to record the carotenoid shift (503–487 nm).

Intact cell suspensions of *Rps. capsulata* were prepared as described in Ref. 20 and the experiments, with 10 μM BChl, were carried out in argon-sparged fresh growth medium after a 30 min dark incubation in a filled, stoppered cuvette to ensure complete anaerobiosis.

3. Results

3.1. The dependence of the rate of ATP synthesis and the membrane ionic current upon membrane potential

Fig. 1 shows the simultaneous recording of membrane potential and ATP synthesis during a short period of continuous illumination in an anaerobic suspension of chromatophores from *Rps. capsulata*. The rate of ATP synthesis was measured from the colour change at 572 nm of added cresol red, due to the uptake of H^+ accompanying the

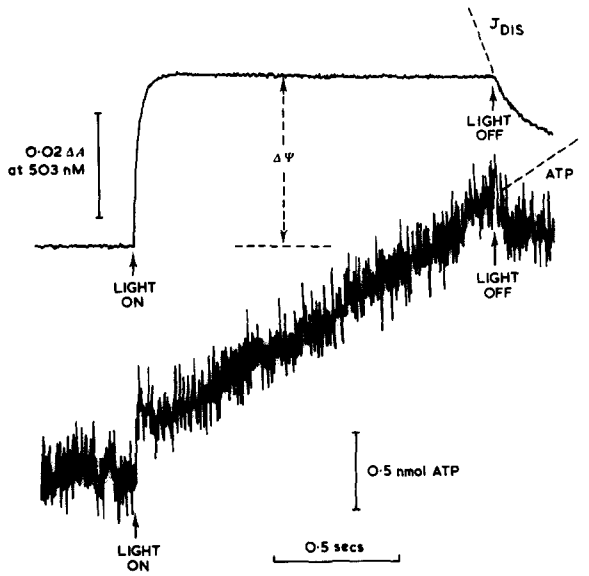

Fig. 1. Simultaneous measurement of $\Delta\psi$, J_{DIS} and the rate of ATP synthesis during steady-state illumination of chromatophores from *Rps. capsulata*. See Section 2 for details. The medium was supplemented with 1.0 mM ADP and 1.0 mM phosphate (K^+ salt, pH 7.6).

phosphorylation reaction [26]. The initial transients in the 572 nm absorption change were due to background changes in the absorption of the chromatophore pigments and can be ignored. The steady-state rate of the 572 nm absorption change, established after 100 ms of illumination, was entirely suppressed when the medium was supplemented with Tricine buffer at pH 7.6 and it was strictly dependent on the presence of added ADP. This steady-state rate of change is therefore due to the scalar proton-uptake reaction accompanying ATP synthesis. We assumed a value of 0.92 H^+/ATP from Ref. 26. The carotenoid absorption change reached an approximately constant value within 100 ms and this, we assumed, was a measure of the steady-state magnitude of $\Delta\psi$. The dissipative ionic current (J_{DIS}) associated with this $\Delta\psi$ was estimated from the initial rate of decay of the carotenoid absorption change upon rapidly darkening the suspension. This is explained in more detail in Ref. 5.

The dissipative ionic current determined by this procedure must be predominantly a proton flux. Even in the unlikely situation that another ion in the system, more permeable than the proton, very rapidly reaches electrochemical equilibrium during illumination, then upon darkening, net flux of this ion can only follow the H^+ flux. This argument breaks down if the chromatophore membranes possess an electrogenic, light-driven pump for ions other than H^+ (for which there is no evidence). It has been shown experimentally that the rate of re-release of H^+ from chromatophores after short flashes coincides with the rate of decay of $\Delta\psi$ measured from the carotenoid absorption change [27]. The experiment in Fig. 1 was carried out in the presence of 1.3 μM nigericin to ensure that the ΔpH component of Δp was eliminated. However, separate experiments showed that this reagent had little effect on the results; presumably, ΔpH makes an insignificant contribution on this fairly rapid time scale.

This set of experiments was repeated at a series of reduced actinic light intensities on the same chromatophore sample. The rate of ATP synthesis and J_{DIS} were plotted as a function of $\Delta\psi$ in Fig. 2 (A and B). A broadly similar relation between the rate of ATP synthesis and Δp to that shown in Fig. 2A has been found in chloroplasts [15–18] and chromatophores [14] on earlier occasions using

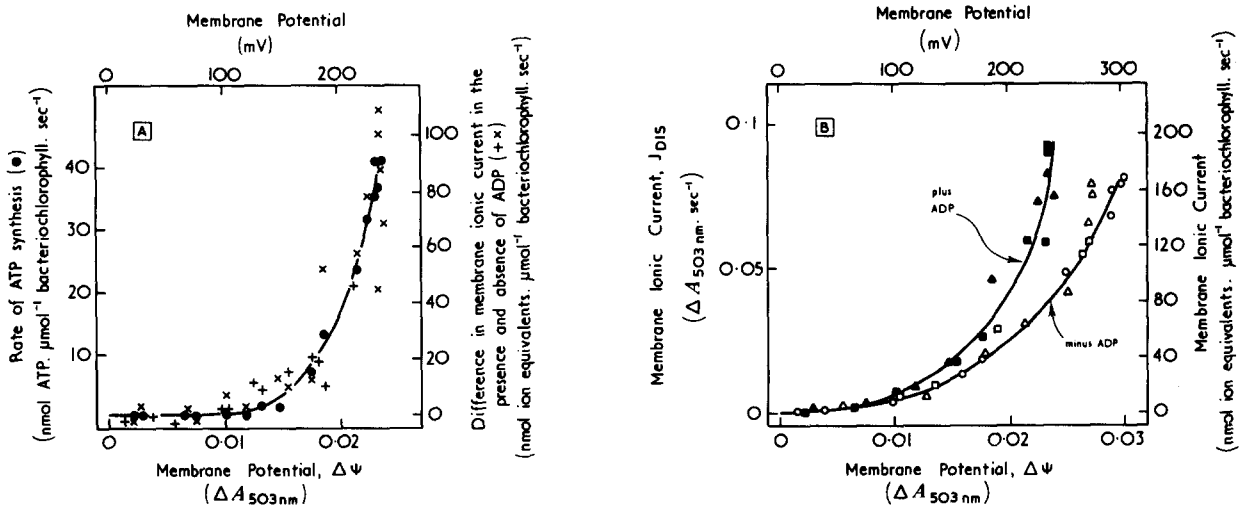

Fig. 2. The dependence of the rate of ATP synthesis and of J_{DIS} on the value of $\Delta\psi$ in chromatophores. The data were collected from experiments similar to those shown in Fig. 1 but at a series of reduced light intensities. (●———●) Dependence of the rate of ATP synthesis on $\Delta\psi$; (▲———▲ and ■———■) dependence of J_{DIS} on $\Delta\psi$ in the presence of ADP and phosphate (the symbol sets indicate two different experiments); (○———○, △———△ and □———□) dependence of J_{DIS} on $\Delta\psi$ in the presence of phosphate and absence of ADP (three sets of experiments);(+———+ and ×———×) difference between the membrane ionic current in the presence and absence of ADP (the data points are the subtracted values between the points ■ and ▲ and the line fitted through the points ○, △ and □). The absolute rate of ATP synthesis, J_{DIS} and $\Delta\psi$ were calculated as described in the text.

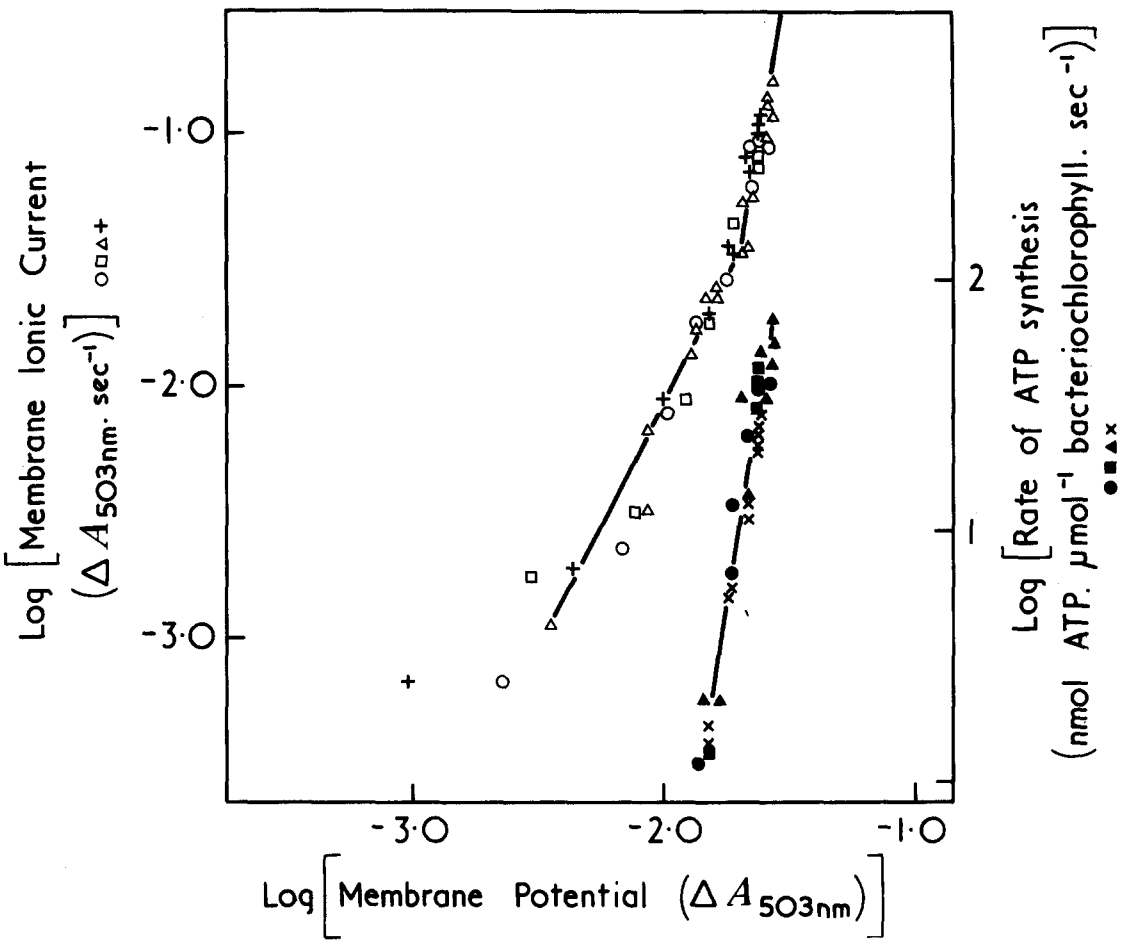

Fig. 3. The logarithmic relationships between the rate of ATP synthesis and J_{DIS} on the value of $\Delta\psi$ in chromatophores. (▲, ■, ●, X) ATP synthesis rates; (△, □, ○, +) J_{DIS} values. The data were taken from Fig. 2 and from two other sets of experiments performed on different chromatophore preparations. The error on the data points of the J_{DIS} vs. $\Delta\psi$ profile at low $\Delta\psi$ values was too severe to define the precise relation. The line (slope = 2) is merely intended to indicate a divergence from the higher-power dependence at high values of $\Delta\psi$.

a variety of measuring techniques. At low and even at moderate values of $\Delta\psi$ the rate of ATP synthesis was very low but the rate increased disproportionately as $\Delta\psi$ was increased. A logarithmic plot of the data from four experiments on different chromatophore preparations (Fig. 3) shows that rate of ATP synthesis had an approximately sixth-power dependence upon the value of $\Delta\psi$.

The dependence of J_{DIS} on $\Delta\psi$ also showed a pronounced upward curvature with increasing $\Delta\psi$ (Fig. 2B), i.e., the ionic conductance of the membrane was dependent on membrane potential (the membrane was non-ohmic). The logarithmic data from the compiled experiments (Fig. 3) reveal a correlation between J_{DIS} and the rate of ATP synthesis at high $\Delta\psi$ whereas at low $\Delta\psi$ there is evidence of another, unrelated component of ionic current.

The omission of ADP from the sample led to an increased value of $\Delta\psi$ and a marked reduction in J_{DIS} (Fig. 2B). At $\Delta\psi = 240$ mV (the maximum $\Delta\psi$ reached during ATP synthesis), the ionic current was about 50% of that achieved under phosphorylating conditions. The difference between the two sets of data (J_{DIS} in the presence and absence of ADP taken from Fig. 2B) is replotted with the ATP synthesis data in Fig. 2A. There is clearly a

close correlation and this is discussed below.

Even in the absence of ADP the ionic conductance of the membrane was steeply dependent on $\Delta\psi$. Separate experiments with cresol red-indicated pH changes showed that this was not due to the phosphorylation of endogenous ADP. Indeed, it is unlikely that substrate concentrations of ADP would survive the chromatophore preparation procedure. Experiments with F_0 inhibitors (subsection 3.3) show that the $\Delta\psi$ -dependent change in the ionic conductance in the absence of ADP nevertheless originates from the behavior of the ATP synthase.

The phosphorylation potential, ΔG_p, was not controlled in the experiments described in Fig. 2. During the 1–2 s illumination period required for the measurement of the rate of photophosphorylation, the ATP concentration increased by not more than 10^{-6} M even at the highest light intensities. At the end of a similar experiment to that from which Fig. 2 was constructed, the ADP concentration, measured enzymically with pyruvate kinase and lactate dehydrogenase, has not decreased significantly from the starting concentration of 10^{-3} M. In another series of experiments (not shown) it was found that the presence of 5 μM P^1,P^5-bis(5'-adenosyl) pentaphosphate to inhibit adenylate kinase had no effect on the kind of profile shown in Fig. 2B. Finally, the profile was not significantly affected by the presence of a glucose/hexokinase trap.

3.2. The dependence of the rate of ATP synthesis on the electron-transport rate

In the steady state ($\Delta\psi$ = constant), the rate at which $\Delta\psi$ is generated by photosynthetic electron transport must be equal to the rate at which it is dissipated ($=J_{DIS}$). There is evidence in chromatophores [28] for two electrogenic reactions with an associated uptake of two protons for a single turnover of cyclic electron transport ($H^+/e^- = 2$). Hence, the rate of electron transport is given by $J_{DIS}/2$. Because the electron-transport chain is cyclic, its operating rate is otherwise very difficult to measure (see Ref. 29). We shall assume that H^+/e^- has a constant value of 2 even though some workers [10] have argued that the H^+/e^- stoicheiometry is variable and depends on Δp: we have recently shown [13] that at least for the

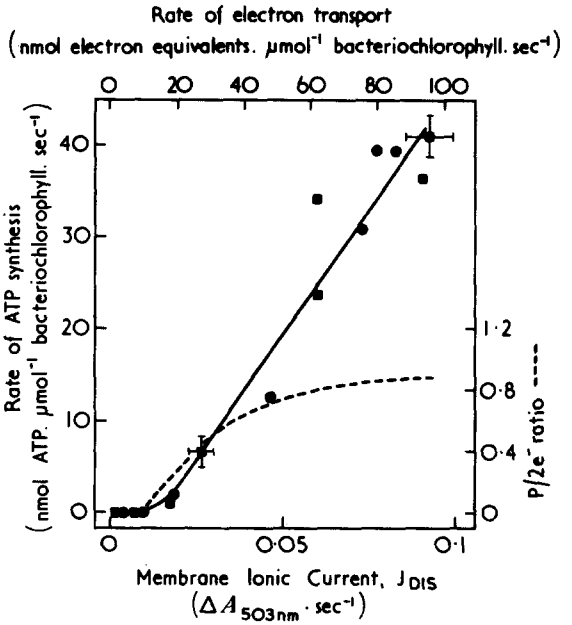

Fig. 4. The dependence of the rate of ATP synthesis on the rate of electron transport in chromatophores. Taken from the data from which Fig. 2 was constructed. (●——● and ■——■) Results of two sets of experiments on the same chromatophore preparation. The data were first plotted as the rate of ATP synthesis vs. J_{DIS} (bottom axis). The rate of electron transport (top axis) was then calculated from J_{DIS} as explained in the text. (------) ATP/2e$^-$ ratio calculated from the line through the data points. The error bars give the standard deviation for six measurements on two chromatophore samples.

respiratory chain in photosynthetic bacteria, the experimental results upon which the argument for variable stoichiometry is based are better explained by the non-ohmic conductance properties of the membrane.

The relationship between the rate of ATP synthesis and J_{DIS} is shown in Fig. 4. From the argument given above, this is equivalent to the dependence of the rate of ATP synthesis on the rate of electron transport. The top axis of this figure is calibrated in electron-transport rate units using the assumptions discussed above and in Section 2. Two conclusions emerge from this data. The first is that under optimal conditions (high electron-transport rate, high $\Delta\psi$), the ATP/2e$^-$ ratio in these chromatophores was about 0.9. The second conclusion (dashed line in Fig. 4) is that the ATP/2e$^-$ ratio was relatively insensitive to changes in the rate of electron transport, i.e., the

TABLE I
VARIATION OF P/2e⁻ RATIO IN DIFFERENT CHROMATOPHORE PREPARATIONS

Preparation No.	1	1	2	3	4	5	6	7	7
Rate ATP synthesis (nmol ATP/μmol BChl per s)	48	43	42	27	48	28	70	40	39
Rate electron transport (nmol e^-/μmol BChl per s)	157	121	96	78	88	130	128	106	122
P/2e⁻	0.61	0.71	0.88	0.69	1.09	0.43	1.09	0.75	0.64

coupling between electron transport and ATP synthesis was maintained even when the energy input to the chromatophores was considerably reduced. At low rates of electron transport the ATP/2e⁻ ratio declined more sharply. The maximum ATP/2e⁻ ratio (viz., that measured at high light intensities) varied slightly for different chromatophore preparations. Table I summarises data taken from nine different experiments from seven chromatophore preparations. The mean value was 0.77 S.D. ± 0.22.

Another conclusion from the steady-state assumption is that because J_{DIS} (and therefore the electron-transport rate) was approx. 10% greater in phosphorylating conditions than in the absence of ADP (Fig. 2), our chromatophores showed 'photosynthetic control' with an index of about 1.1. The mean value of the photosynthetic control index in seven different experiments was 1.20 ± 0.06.

The data shown in Figs. 2–4 were obtained by progressive reduction in the actinic light intensity. The light intensity dependence of J_{DIS}, $\Delta\psi$ and the

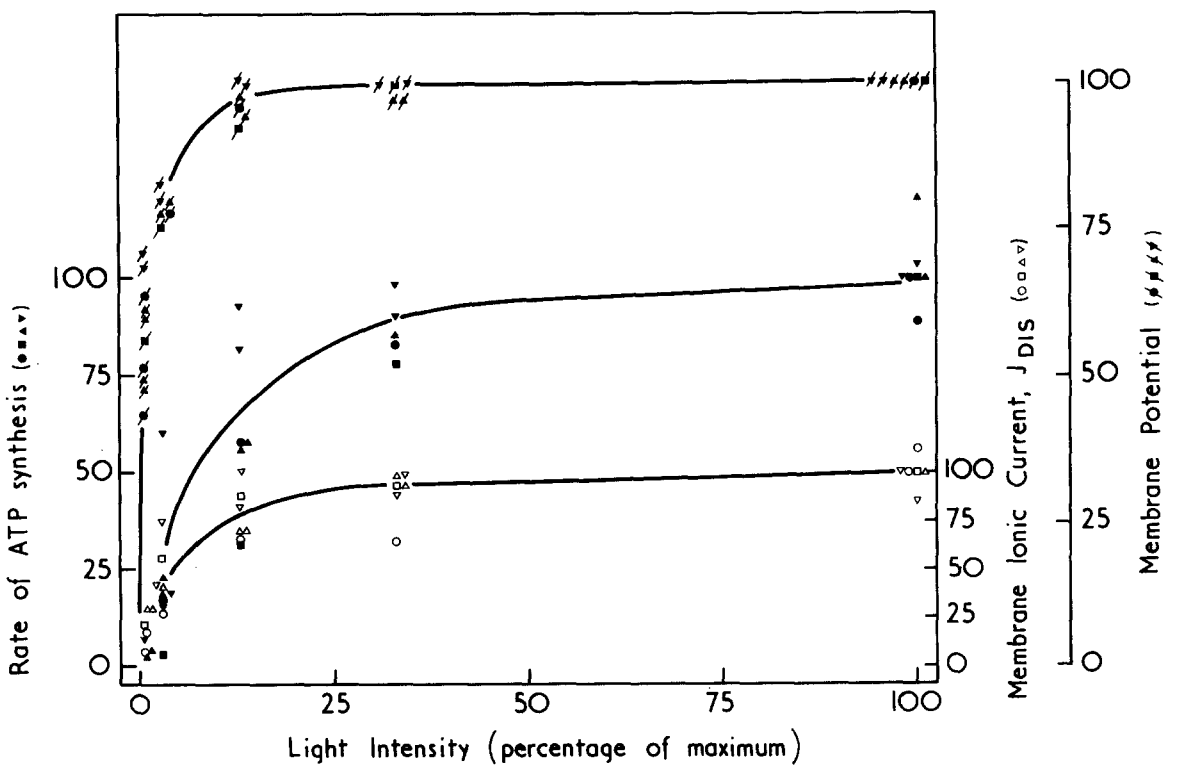

Fig. 5. The light intensity dependence of the rate of ATP synthesis, J_{DIS} and $\Delta\psi$ in chromatophores. Conditions as described in Figs. 1 and 2. The results of four separate experiments are shown. (●, ■, ▲, ▼) Rate of ATP synthesis; (○, □, △, ▽) J_{DIS}; (◖, ◧, ◭, ◥) $\Delta\psi$. All values are expressed as a percentage of the values reached at maximum light intensity.

rate of ATP synthesis are shown in Fig. 5. It is evident that the highest light intensities were close to saturation for each of these three parameters. Because of the proportionality between the electron-transport rate and J_{DIS} (see above) we can conclude that electron transport was saturated at maximum light intensity. In contrast, the data of Fig. 2 suggest that the $\Delta\psi$ reached at the maximum light intensity was not sufficient to saturate the ATP-synthesising capacity of the chromatophores.

3.3. The effect of ATP synthase inhibitors on the dependence of the membrane ionic current on membrane potential

Inhibitors of the ATP synthase (F_0F_1-ATPase) had a pronounced effect on the relation between J_{DIS} and $\Delta\psi$. Of the three inhibitors used, venturicidin was the most potent and was studied in detail. At a concentration of 0.1 μg/ml, and maximum light intensity, venturicidin markedly reduced the ionic current and slightly increased $\Delta\psi$. This was less pronounced at low light intensities so the overall effect was to decrease the curvature in the J_{DIS} vs. $\Delta\psi$ relation (Fig. 6). Again invoking the assumed proportionality between J_{DIS} and the electron-transport rate, it follows from Fig. 6 that (at maximum light intensity) venturicidin reduced the electron-transport rate by about 70%.

Oligomycin and DCCD had very similar effects on the J_{DIS} vs. $\Delta\psi$ relation (Fig. 7) but it is interesting that none of the three inhibitors reduced the relation to a linear dependence even at concentrations which were more than enough to abolish ATP synthesis. For example, in separate experiments (not shown) we found that 0.03 μg/ml

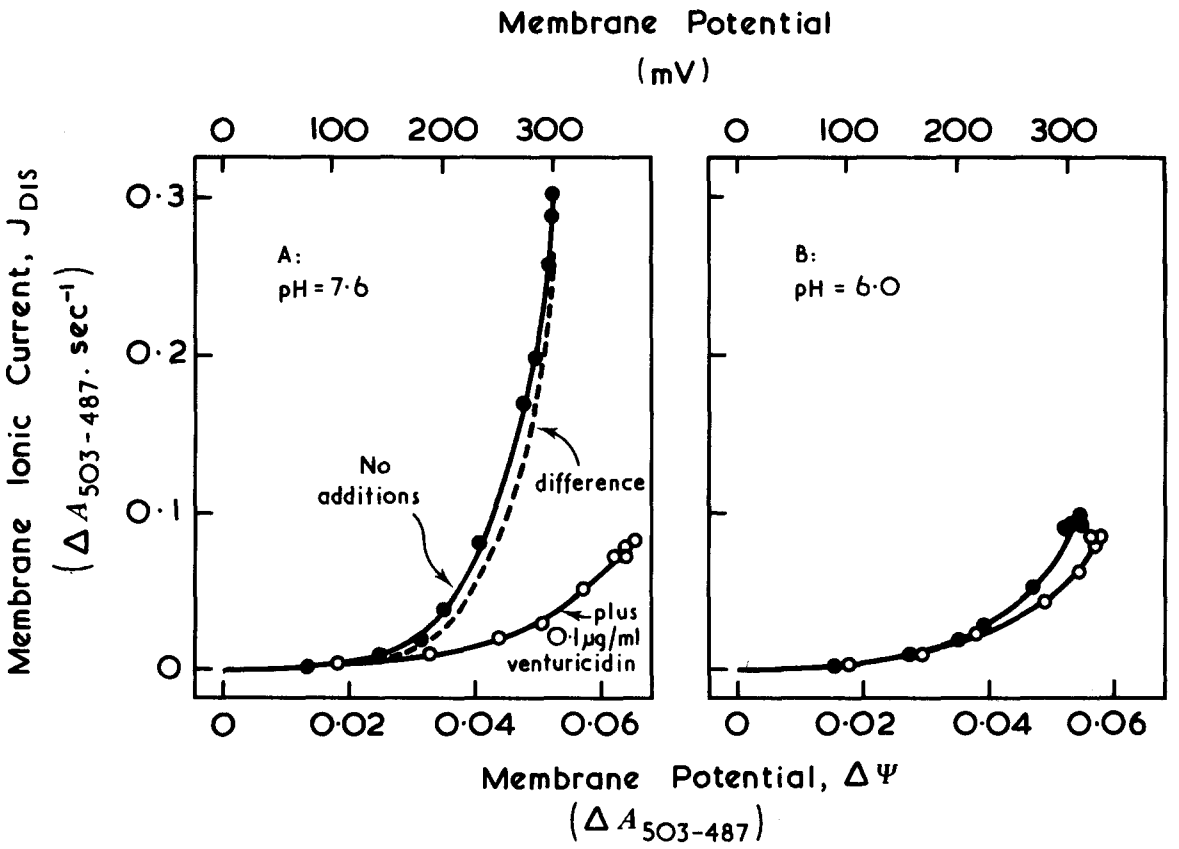

Fig. 6. The effect of venturicidin on the dependence of J_{DIS} on $\Delta\psi$ in chromatophores. (A) At pH 7.6 (using buffered medium, as described in Section 2; (B) at pH 6.0 (using a buffered medium in which Tricine was replaced with 50 mM Mes). (●———●) No addition; (○———○) plus 0.1 μg/ml venturicidin (the inhibitor was added to the cuvette before the 30 min pre-incubation period; see Section 2; (— — —) difference in J_{DIS} ± venturicidin.

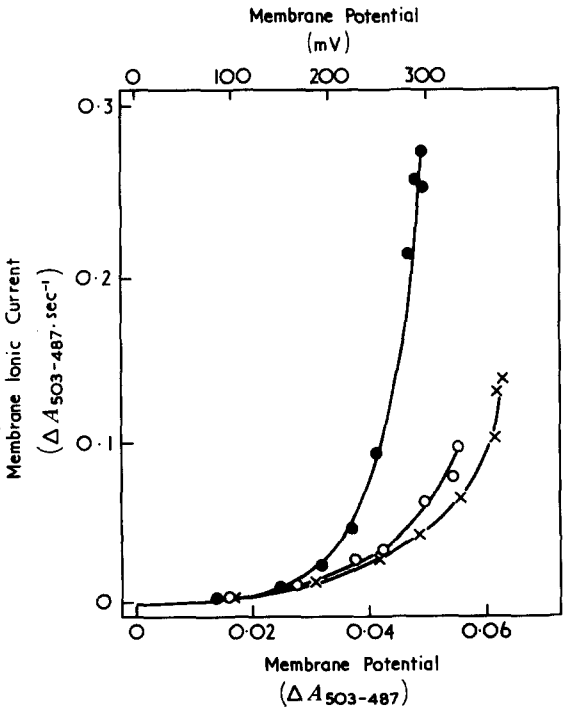

Fig. 7. The effect of oligomycin and DCCD on the dependence of J_{DIS} on $\Delta\psi$ in chromatophores. Conditions as described in Section 2. (●———●) No additions; (×———×) plus 1 μg/ml oligomycin (the inhibitor was added to the cuvette before the 30 min pre-incubation period; see Section 2); (○———○) plus DCCD. We adopted the procedure used by Casadio and co-workers [45] for this inhibitor: 0.2 ml chromatophore suspension containing 0.18 μmol BChl was mixed with 0.2 ml glycerol and incubated at room temperature in the dark for 1 h with 250 μM DCCD. The chromatophores were then stored at −20°C until required (a few hours). In separate experiments (not shown) it was found that this concentration of DCCD completely inhibited ATP synthesis. Control experiments showed that glycerol treatment and −20°C storage had no significant effect on the J_{DIS} vs. $\Delta\psi$ curve or on the ATP-synthesis rate.

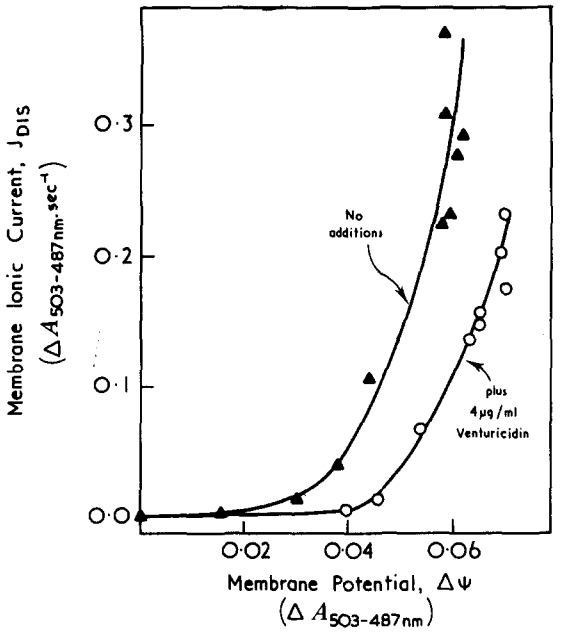

Fig. 8. The effect of venturicidin on the dependence of J_{DIS} on $\Delta\psi$ in intact cells of *Rps. capsulata*. Experiments were carried out as described in Section 2 and Ref. 5. (▲———▲) Experiments in fresh growth medium; (○———○) plus 4 μg/ml venturicidin.

venturicidin (i.e., less than was used in Fig. 6) led to about 90% inhibition of the rate of ATP synthesis. Even 1.0 μg/ml venturicidin had no further effect on the $J_{DIS}/\Delta\psi$ relation than the 0.1 μg/ml shown in Fig. 6.

Venturicidin completely blocked the enhancement of J_{DIS} by the addition of ADP in the presence of phosphate (cf. Fig. 2B), providing evidence that this increase in the ionic current is a direct consequence of ATP synthesis. Further evidence is given in Fig. 6B. At pH 6.0, the rate of light-induced ATP synthesis is extremely low. Under these conditions, the relation between J_{DIS} and $\Delta\psi$ in the absence of inhibitor resembled that seen at pH 7.6 in the presence of the ATP synthase inhibitor (cf. Fig. 6A and B). At pH 6.0 neither the stimulation of J_{DIS} with ADP (not shown) nor the marked depression of J_{DIS} with venturicidin was evident.

We have shown on earlier occasions [20] that even in intact cells of *Rps. capsulata*, venturicidin but not oligomycin is capable of specifically inhibiting the ATP synthase. In Fig. 8 the effect of venturicidin on the ionic current vs. $\Delta\psi$ curve of intact cells is shown. The higher concentration of venturicidin that was required in the cells compared with the chromatophores probably reflects a partial permeability barrier due to the outer membrane of the bacteria. Nevertheless, a marked decrease in the membrane ionic current could be observed.

4. Discussion

4.1. Factors affecting the membrane ionic current in chromatophores

The relation between the membrane ionic current, measured by a direct procedure, and the rate of ATP synthesis, both expressed as a function of $\Delta\psi$, is shown for the first time in Fig. 2. Clearly the disproportionate increase in the phosphorylation rate as $\Delta\psi$ was increased was matched by a related change in the membrane ionic current. Together with the finding that treatment with the F_0 inhibitors, venturicidin, oligomycin and DCCD decreased the membrane ionic current (Figs. 6 and 7), this lends support to the chemiosmotic view that a proton current through the ATP synthase is responsible for ATP synthesis.

We will assume that the dissipative ionic current across the chromatophore membrane is made up of contributions from ionic fluxes through the ATP synthase, ion-transport systems and through ionic leaks. For any value of $\Delta\psi$ these contributions are additive (i.e., in the steady state the sum of all the current elements across the membrane is zero) so that in principle, the curve of dissipative ionic current vs. $\Delta\psi$ in Fig. 2B can be resolved into its components. In a qualitative way, a comparison of the curves for J_{DIS} (in the presence of ADP, Fig. 2B) and the rate of ATP synthesis (Fig. 2A) especially in their logarithmic forms (Fig. 3) shows that at high $\Delta\psi$ the dominating ionic current corresponds to ATP synthesis, whereas at low $\Delta\psi$ another component with an apparently lower-power dependence on $\Delta\psi$ can be resolved. In practice, an analysis of this kind may be complicated by secondary interactions, e.g., by a possible regulatory influence of electron-transport carriers on the ATP synthase [14,30]. Nevertheless, the following minimal conclusions may be drawn.

4.1a. The basal membrane current. After treatment of the chromatophores with either venturicidin, oligomycin or DCCD or in experiments at low pH, ionic current through the F_0 channels of the membrane should have been eliminated. The dependence of the residual ionic current on $\Delta\psi$ under these conditions was considerably flattened although it was still non-linear: the membrane conductance varied by 20-fold across the measured potential range, i.e., the membrane was still non-ohmic. This residual conductance may include contributions from specific ion-transporting systems in the chromatophore membrane. It is interesting in this context that the rate of alanine transport in a related species of photosynthetic bacteria has been shown to be non-linearly dependent on the magnitude of Δp [31]. On the other hand, the measured conductance values in these circumstances are so low (ranging from about 1 to 20 $mS \cdot m^{-2}$ by the calculation used in Ref. 5) that they are indistinguishable from values measured for artificial bilayer membranes [23] and may therefore reflect the ionic leakage of the chromatophore membrane. Even artificial, protein-free, lipid membranes generally show a voltage-dependent increase in ionic conductance (see discussion and references in Ref. 32). In some circumstances, particularly at the low $\Delta\psi$ values generated by short flashes, and under the dominating influence of ionophores, an assumption of a constant membrane conductance does provide a good fit for experimental data [33].

4.1b. The ionic current through F_0 channels. The difference between the ionic current vs. $\Delta\psi$ profile in the presence and absence of venturicidin (shown as the dashed line in Fig. 6) reveals the contribution to J_{DIS} of the F_0 channels of the ATPase. This is clearly the dominant ionic conductance pathway in the chromatophore membranes and is very largely responsible for their pronounced non-ohmic behaviour (the conductance changed by more than two orders of magnitude between $\Delta\psi = 50$ and 300 mV). At high values of $\Delta\psi$, about 90% of the total membrane ionic current proceeded by way of F_0. The contribution decreased as $\Delta\psi$ was lowered and was about 50% of the total current at $\Delta\psi = 150$ mV. This is roughly consistent with an effect of venturicidin of about 10–20% on the decay of the carotenoid band shift and a low ATP yield after single-turnover flashes observed in our earlier work [7,27]. Under those transient state conditions, $\Delta\psi$ reached a value of about 1/5 that achieved during the full steady-state illumination in the present series of experiments.

Although it is possible to identify the fraction of the ionic current passing through F_0 it is not possible from the present data to judge what proportion of that F_0 current is associated with ATP synthesis. Even under non-phosphorylating condi-

tions, i.e., in the absence of added ADP, the F_0 inhibitors substantially lowered the membrane ionic current. Clearly, therefore, some ionic current proceeded through the F_0 without accompanying ATP synthesis. There are two limiting cases to consider: either this ionic current proceeds through F_0 sites which are deficient in F_1 (or in which F_1 is damaged during chromatophore preparation), or else intact F_0F_1 ATP synthase can, in these circumstances, conduct protons even in the absence of net ATP synthesis (i.e., the ATP synthase can 'slip' protons).

In the event of the first alternative (a tacit assumption in the analysis of Gräber and Witt [17]), then the ionic current accompanying ATP synthesis can be obtained by subtraction of the values measured in the presence and absence of ADP (symbols + and x in Fig. 2A). The correlation with the rate of ATP synthesis is quite good. At the maximal value of $\Delta\psi$ achieved in these experiments under phosphorylating conditions, it would follow that approx. 50% of the total membrane ionic current was directly responsible for ATP synthesis, about 45% proceeded through naked F_0 sites (by comparing Figs. 2B and 6A) and about 5% was basal ionic current. Using this assumption for nine experiments on different chromatophore preparations, the average value for the proportion of the total proton current directly responsible for ATP synthesis was 67% S.D. $\pm$ 11%. On the other hand, if intact F_0F_1-ATP synthase can pass a 'decoupled' proton current, for which in thylakoids there is some indirect evidence [34], then the contribution of the phosphorylating ionic current cannot be unambiguously determined. It would represent a greater share of the total current than is estimated above.

The situation is equally ambiguous in intact cells (Fig. 8). We can rule out the likelihood of damaged F_1 units and perhaps the existence of F_0 channels devoid of F_1 but unfortunately the efficacy of the F_0 inhibitors is lower in intact cells. Some of the residual ionic current in the presence of venturicidin may therefore be attributed to uninhibited F_0 channels. The difference between the ionic current profiles in the presence and absence of venturicidin (70% of the total current at high $\Delta\psi$) therefore gives a minimal estimate of the proportion of the ionic current proceeding through F_0.

4.2. The ATP/2e$^-$ and the H$^+$/ATP ratio in chromatophores

Recently, we discussed the difficulties encountered in the measurement of electron-transport rates in the cyclic system of photosynthetic bacteria [29]. A repetitive-flash procedure was developed in an attempt to measure electron-transport rates and ATP/2e$^-$ ratios. In chromatophores from *Rps. capsulata* Ala pho$^+$ we calculated [29] an ATP/2e$^-$ ratio of 1.1. From the present experiments using a totally different set of assumptions, we estimate that the maximum value of the ATP/2e$^-$ ratio in *Rps. capsulata* N22 chromatophores (i.e., in the steady state at high light intensities) was 0.77 $\pm$ 0.22. The existence of phosphate-acceptor control of the rate of electron transport in this organism was also confirmed (see Section 3).

For every two electrons transported round the cyclic electron-transport chain, four protons are translocated inwards across the chromatophore membrane [28]. In the steady state only 67 $\pm$ 11% of the returning protons accompany ATP synthesis (a minimal estimate – see above). From the measured P/2e$^-$ ratio of 0.77 $\pm$ 0.22, it follows that the H$^+$/ATP ratio, i.e., the number of translocated protons accompanying each ATP synthesised, is 3.5 $\pm$ 1.3. This compares favourably with some values that have been determined in other systems (for a review see Ref. 2) but is larger than the values of approx. 1.7 and 2.25 $\pm$ 0.16 obtained in chromatophores after short flash excitation [35,36]. In those experiments, the quantity of charge translocation accompanying ATP synthesis was calculated from the relative rates of decay of the carotenoid shift in the presence and absence of ADP and may have been underestimated.

Fig. 4 shows that even a pronounced reduction of the rate of electron transport from its maximum value led to an almost proportionate decrease in the rate of ATP synthesis, i.e., the value of the ATP/2e$^-$ ratio declined only slightly under these conditions. In order to account for this within the terms of the chemiosmotic hypothesis, it must be reasoned that the proportion of the proton current accompanying ATP synthesis to the total membrane ionic current is fairly constant within this range. This is supported in fact by the data of Fig. 2: in the range of $\Delta\psi$ where high phosphorylation rates take place (Fig. 2A), the difference between the ionic current values in the presence and ab-

sence of ADP (i.e., the proton current accompanying ATP synthesis, assuming that there is no slip in the ATP synthase) is a constant ratio of the total membrane ionic current (Fig. 2B). Only at low values of $\Delta\psi$ does the 'non-phosphorylating' H^+ flux become significant and the ATP/$2e^-$ ratio drops steeply.

An explanation for this is provided by the coincidence of the two factors which are the main subject of this paper: (a) the rate of ATP synthesis increases as a high power dependence of $\Delta\psi$; (b) the ionic conductance properties of the membrane are dominated by the ATP synthase. The sequence of events can be described thus: when electron transport is inhibited, the tendency of $\Delta\psi$ to fall is largely offset by the $\Delta\psi$ -dependent decrease in the membrane proton conductance (determined predominantly by the properties of the ATP synthase). But, because the conductance of the ATP synthase is related (by the H^+/ATP ratio) to its catalytic activity, even a slight fall in the value of $\Delta\psi$ is sufficient to cause a significant drop in the rate of ATP synthesis. This exquisite control mechanism ensures a constant degree of coupling between electron transport and ATP synthesis and it enables the bacteria to maintain moderate values of $\Delta\psi$ at extremely low rates of electron transport.

4.3. The dependence of the steady-state rate of ATP synthesis on $\Delta\psi$

A broadly similar dependence of the rate of ATP synthesis on the value of Δp to that shown in Fig. 2A has been demonstrated previously in chloroplasts [15–18], chromatophores [14] and mitochondria [19]. The disproportionate increase in the rate of ATP synthesis as Δp is increased may be a general feature of electron-transport phosphorylation. It has been variously described as a threshold dependence, implying a discontinuity in the rate of ATP synthesis at a critical value of Δp (see Ref. 37), or it has been found to obey a sigmoidal dependence on $[H^+]_{in}$ [18] or a high-power dependence on either $[H^+]_{in}$ [15] or Δp [17]. From these descriptions, various conclusions about the H^+/P ratio and the mechanism and control of ATP synthesis have been drawn.

In experiments with photosynthetic vesicles in which the actinic light intensity is varied in order to change Δp, such as those presented in this article and in Refs. 14–17, a heterogeneity is introduced into the sample due to self-shading. Those vesicles proximal to the source of illumination operate at a higher Δp than those further away. Of course this will arise in any experiments performed at sub-saturating light intensities but the problem, which might lead to rather complex results, has not always been acknowledged. It may be severe in dense suspensions with a long actinic light path and is exacerbated (a) when the rates of ATP synthesis and the values of Δp are measured in different parts of the sample (e.g., when spectroscopic and quenching methods are combined), (b) when the determination of Δp depends on a logarithmic parameter (e.g., permeant ion distribution). The use of electron-transport inhibitors and uncouplers to lower Δp would seem to be a sensible alternative to the employment of reduced light intensities, although some workers believe that these procedures do not produce analogous effects [14]. In our experiments the problem was minimised by employing weak suspensions of chromatophores, by using a linear indicator of $\Delta\psi$ and by analysing both $\Delta\psi$ and the rate of ATP synthesis at the same position in the cuvette (with crossed measuring beams) along only approx. 3 mm of the actinic light path.

Our data from a number of experiments (Fig. 3) suggest that the rate of ATP synthesis has an approximately sixth-power dependence on $\Delta\psi$ (for $\Delta pH = 0$). The fact that the H^+ current through the ATP synthase even in the absence of ADP has a high-power dependence on $\Delta\psi$ (see Fig. 2B and above discussion) suggests that control of the proton conductance through the enzyme is not controlled by the catalytic reaction itself. The subunits of the ATP synthase are present in multiple copies. A possible explanation for the high-power dependence is that it may reflect co-operativity between the subunits. For example, if the proton-conducting activity (e.g., conformation or alignment) of individual subunits is influenced in a simple way by $\Delta\psi$ then the activity of the oligomeric enzyme would have a high-power dependence on $\Delta\psi$. It is interesting that there are some indications that the proteolipid subunit, which is known to be involved co-operatively in the transport of protons through the F_0, may be present as a hexamer (cf. Refs. 38 and 39).

Recently, a number of workers [40–43] have re-adopted an experimental approach first employed by Baum and colleagues [44] in which the rate of ATP synthesis is titrated with a combination of an electron-transport inhibitor and an ATP synthase inhibitor. The general result is that a partial block of ATP synthesis with one type of inhibitor does not affect the titre of the other inhibitor. It has been concluded from this [40] that the 'high-energy intermediate' generated by an electron-transport chain cannot delocalise amongst a number of ATP synthase complexes and this of course would rule out the possibility that Δp is the high-energy intermediate. We propose an alternative interpretation based on the kind of model described above. One other, simple constraint is required – we assume that, when activated by $\Delta\psi$ (or Δp), an individual ATP synthase complex will operate at its maximum rate, i.e., it will have zero-order kinetics with respect to Δp. Consequently, the rate of electron-transport phosphorylation is always limited (and in the same manner) by the proportion of the ATPase in the activated state. Where we disagree with other authors is that they assume that the position of the rate-limiting step can be shifted from the electron-transport proteins to the ATP synthase (and vice versa) depending on the inhibitor present [40]. We suggest that even when electron transport is strongly inhibited, rate limitation still arises from the restricted activation of the ATP synthase. Token support for this view is provided by a comparison of Figs. 2A and 5. The maximum light intensity used in our experiments was saturating (Fig. 5) and yet it appears from Fig. 2A that the rate of ATP synthesis had not reached its maximum value with respect to $\Delta\psi$. Although light saturation of the electron-transport reactions has not always been specified, a review of the data from other workers [14–16] shows that this may be a general effect: the available catalytic capacity of the ATP synthase always appeared to exceed that of electron transport. This arises from the fact that Δp not only drives the ATP synthase but also controls its activity.

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