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# 'Backlash' and the coupling between electron transport and proton translocation in bacterial chromatophores

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**At the onset of continuous illumination of chromatophores from *Rhodopseudomonas capsulata* there is a burst of $H^+$-disappearance from the external medium, lasting about 100 ms, which coincides with the period of membrane potential ($\Delta\psi$) development. Three 'backlash' factors contribute to the burst. (1) A period of rapid electron transport through the photosynthetic reaction centre and early donors and acceptors, with accompanying charge translocation, preceeds the steady state in cyclic electron transport whose rate is determined by the slower reaction in the cytochrome $b/c_1$ complex. Inhibition of the $b/c_1$ complex with antimycin A reduces by about 50%, the rapid phase of $H^+$-disappearance and $\Delta\psi$ generation. (2) During the early turnovers of electron transport, before $\Delta\psi$ has reached a maximum, the rate of electron transport is relatively unrestricted by thermodynamic back-pressure from $\Delta\psi$ and proceeds rapidly. The rate of electron transport decreases as $\Delta\psi$ rises. The rate of electron transport at high $\Delta\psi$ in steady-state is shown to have a sensitive dependence on the magnitude of $\Delta\psi$. (3) As $\Delta\psi$ rises at the onset of illumination, the rate of passive ion flux across the chromatophore membranes increases. Because of the non-ohmic conductance of the membrane, the rate at which $\Delta\psi$ is dissipated increases disproportionately as $\Delta\psi$ develops. Consequently, the effect of the dissipative reactions becomes more pronounced as the burst progresses. Valinomycin and $K^+$ lead to an enhanced rate of light-induced $H^+$ disappearance during the period in which the development of $\Delta\psi$ is depressed. The stimulation of the apparent $H^+$ uptake is a consequence of both an increase in the rate of electron transport and a decrease in the rate of $\Delta\psi$-driven $H^+$ efflux. These results are consistent with energy coupling hypotheses in which electron transport is coupled to the translocation of protons between the bulk aqueous phases. In particular, it is shown that electron transport is kinetically tightly coupled to bulk phase proton translocation and $\Delta\psi$ generation, and that $\Delta\psi$ formation restricts electron transport and the appearance of protons in the bulk phase.**

## Introduction

The successful determination of the coupling ratio between proton translocation and electron transport in mitochondrial, bacterial and thylakoid membranes by direct measurement requires that the protons translocated "during a brief burst of activity from rest" do not "immediately pass back across the membrane in the closed circuit that operates in the steady state" [1]. Mitchell described this effect, by analogy with mechanical coupling devices, as 'backlash' [1]. It has been customary to increase natural backlash by performing measurements of the coupling or $H^+/e^-$ ratio in the presence of counter ions, such as

Abbreviation: Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]glycine.

$SCN^-$ or $K^+$ in valinomycin-treated membranes. This report is concerned with both the natural or intrinsic backlash and the increased backlash due to electrophoretic ions. Because the electric capacitance of biological membranes is low, in the absence of compensating ions the translocation of only a few protons is expected to lead to the development of a large membrane potential ($\Delta\psi$) and the rapid establishment of the steady state. The build-up of a large $\Delta\psi$ after only a few turn-overs of the electron-transport chain will lead to (a) a rapid return of $H^+$ across the coupling membrane and (b) a decrease in the rate of electron transport due to the 'back-pressure' of $\Delta\psi$. However, in experiments with intact mitochondria and bacteria in the absence of added compensating ions some results have been reported which are said to be at variance with the simple predictions of the chemiosmotic hypothesis [2–5]. It has been suggested that these results call for major revision of the chemiosmotic hypothesis; for example, that protons are not translocated between bulk aqueous phases on either side of the membrane, but are conducted by specific proteins between the electron-transport complexes and the ATP synthase [6,7]. Photosynthetic membranes present the opportunity to study the pre-steady-state events in electron-transport-driven proton translocation. (a) Energy can be supplied in the form of light so that mixing artefacts do not interfere with observations by spectroscopy (compare oxygen pulse experiments in mitochondria and chemosynthetic bacteria). (b) Measurements can be made on a rapid time scale. (c) Electrochromic absorbance changes in the photosynthetic pigments can be used to record $\Delta\psi$ and ionic currents across the membrane [8]. In this work we have used chromatophores from photosynthetic bacteria to study backlash effects at the onset of illumination.

Inward proton translocation upon illuminating a chromatophore suspension takes place in a burst lasting for about 100 ms followed by a much slower rate of uptake [9]. These measurements were made with pH indicators which, by several criteria [10,11], appeared to report from the external medium in which the chromatophores were suspended. In this report we shall examine the possibility that three backlash factors can, in principle, contribute to the burst. (1) Backlash in the electron-transport chain: due to the fact that electron transport through the photosynthetic reaction centre is much faster than through the cytochrome $b/c_1$ complex, there will be a burst of electron flow through the reaction centre before the steady-state cycle is reached. (2) The rate of cyclic electron transport may become progressively inhibited by thermodynamic back pressure from the developing $\Delta\psi$ (cf., respiratory control). (3) The rate of return of $H^+$ outwards across the chromatophore membrane will increase as $\Delta\psi$ develops. This effect may be pronounced because of the non-ohmic nature of the chromatophore membrane [12]. In principle, a fourth factor might contribute to the burst of $H^+$-uptake at the onset of illumination: the $H^+/e^-$ ratio might change during this period. Some authors [13] have suggested that the coupling ratio might decrease as the magnitude of the proton-motive force ($\Delta p$) increases, i.e., the electron-transport-driven proton pump might 'slip' as $\Delta p$ is increased. Recently, however, we provided positive evidence against the existence of $\Delta p$-dependent slip during photosynthetic electron flow in chromatophores [14].

In view of the difficulties encountered in the explanation of results with intact bacterial cells [2–5], our objective has been to discover whether the $H^+$ burst in chromatophores can be explained by chemiosmotic principles and to assess which of the above factors might be responsible for the burst. The comparison of $H^+$ uptake and $\Delta\psi$ formation at the onset of illumination is made in more detail than in earlier reports [10,14,15]. The specific intention is to elaborate in relatively simple membrane vesicles, the principles which must also apply in more complex intact organisms. Our results are also considered briefly from the standpoint of localised proton coupling hypotheses [6,7].

## Methods

The anaerobic, phototrophic growth of *Rps. capsulata* strain N22 and the preparation of chromatophores have been described [12]. The preparation medium was 10% sucrose/50 mM $K_2SO_4$/8 mM $MgCl_2$/50 mM Tricine-KOH (pH 7.4) and the final resuspension medium was similar, but lacked the tricine. The chromatophores were stored at 4°C and used within 5 days of

preparation. Two basic media were used for experiments, either 10% sucrose/100 mM KCl/8 mM $MgCl_2$/50 mM Tricine (pH 7.6) or for cresol red measurements of $H^+$ uptake, the same but lacking tricine. In the experiments using valinomycin at defined, low potassium concentrations, the chromatophores were prepared in a medium in which $Na^+$ replaced $K^+$.

Absorbance changes were recorded on a laboratory-constructed dual-wavelength spectrophotometer equipped with an electronic shutter on the excitation beam as described earlier [12]. The recordings were averaged in a microcomputer at $8.3 \cdot 10^{-3}$ Hz for the number of sweeps described in the figure legends. Experiments with single illumination periods show that the absorbance changes relaxed completely during 2 min of darkness.

## Results and Discussion

*The contribution of antimycin-insensitive electron transport to the burst of proton translocation at the onset of a period of illumination*

In Fig. 1 the kinetics of the light-induced external pH change in a chromatophore suspension are compared with the kinetics of the $\Delta\psi$-indicating carotenoid band shift. It is known that after short flash excitation, the kinetics of $H^+$ uptake and electric-field generation can be distinguished, and this has led to the identification of discrete electrogenic and proton-binding electron-transport reactions [16,17]. In the present experiments on a slower time scale involving multiple turnovers, these processes are not resolved, and the similarity between the kinetics of the cresol red change and the carotenoid band shift during the burst are consistent with the thesis that the transfer of bulk phase protons from the external medium is associated with membrane potential development. Following the burst, after about 100 ms, a much slower change in external pH is evident, which is not reflected in the $\Delta\psi$ kinetics. On a time scale of seconds the carotenoid band shift is known partially to decay during illumination and co-inside with the slow phase of $H^+$ disappearance measured with a glass electrode [15,18]. These observations lead to the view that the slow external pH change following the burst (Fig. 1) is due to the slow electrophoretic transport of of counter ions and the beginning of a partial replacement of $\Delta\psi$ by $\Delta$pH.

The effects of antimycin on the burst of $H^+$ translocation and $\Delta\psi$ development are also shown in Fig. 1. Antimycin is a specific and high affinity inhibitor of the cytochrome $b/c_1$ complex in chromatophores [19]. The concentration used in Fig. 1 was in excess of that required for maximum inhibition, so that this experiment reveals the contribution to the burst of the external pH change from the proton-binding reactions which accompany electron transport to and from the photosynthetic reaction centre on the high-potential and low-potential side of the antimycin block. In the conditions of the experiment, this represented 49% of the total $H^+$ burst. Similarly, the antimycin-insensitive burst of $\Delta\psi$ development (47% of the change in the absence of antimycin) is a measure of the extent of charge separation in the photosynthetic reaction centre during electron transport from the donors on the high-potential side of the block to the acceptors on the low-potential side and a small contribution from an electrogenic component in the cytochrome $b/c_1$ complex [17].

These results illustrate how, under the severe rate limitation introduced by antimycin, a pronounced burst in charge translocation can be observed. It represents a 'backlash' effect in the electron transport reactions during the approach to steady state: at the onset of illumination, electron transport associated with components adjacent to the photosynthetic reaction centre is much more rapid than electron transport through the inhibited cytochrome $b/c_1$ complex, but in the steady state all reactions in the cycle must have the same slow rate as that governed by the antimycin block. Backlash in the electron-transport chain is the adjustment of individual reactions to this unique, overall rate. Antimycin is believed to inhibit at a point in the electron-transport chain which is close to the intrinsic rate-limiting step [19]. In this case the antimycin-insensitive components of $H^+$ translocation and $\Delta\psi$ generation (each about 50%) should represent upper limits of the contribution from electron transport backlash to the burst in the absence of inhibitor.

If the antimycin block of electron transport were complete then, following the burst of $H^+$

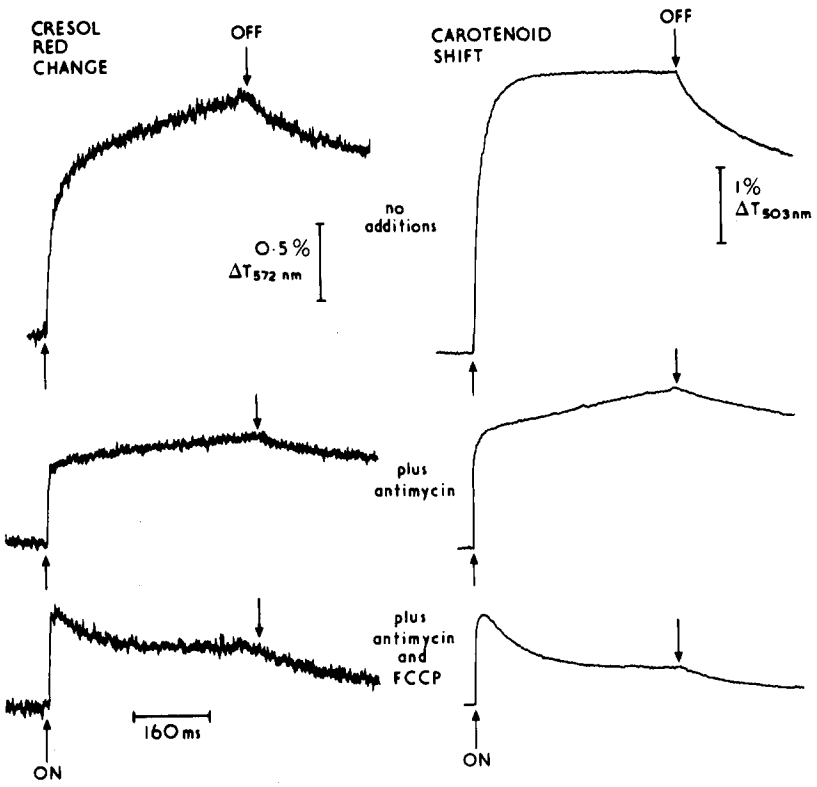


Fig. 1. The kinetics of $H^+$ disappearance and $\Delta\psi$ formation in chromatophores at the onset of illumination. The bacteriochlorophyll concentration of the chromatophore suspension was 10 $\mu$M. The experiments were carried out in unbuffered medium containing 10% sucrose/100 mM KCl in the presence of 100 $\mu$M cresol red at pH 7.6 (see Methods). The traces are an average of 16 recordings. The antimycin was present at 2.4 $\mu$M and the FCCP at 2 $\mu$M, where shown. In control experiments in the presence of buffer, light-induced absorbance changes at the wavelength used for the cresol red recordings (572 nm) were negligible.

uptake and $\Delta\psi$ generation, it would be expected (Fig. 1C and D) that $H^+$ would be re-released from the lumen of the chromatophores and $\Delta\psi$ would slowly collapse due to the intrinsic ionic conductance of the membrane. Since this does not occur, and since $H^+$ uptake and $\Delta\psi$ formation continued very slowly to increase, it is reasoned that the dissipative ionic current in these conditions must be less than the rate of proton translocation driven by the 'leak' round the antimycin block. This supposition is strengthened by the observation (ref. 12, see also below) that at low $\Delta\psi$ the membrane conductance is extremely small and by the result (Fig. 1E and F) that further treatment with the protonophore, FCCP, does lead to the $H^+$ re-release and $\Delta\psi$ collapse, while the actinic light remains on. This experiment provides evidence that the $H^+$ uptake reactions associated with antimycin-insensitive electron transport are vectorial and not scalar.

Short flash experiments have shown that $1H^+$ and $2H^+$, respectively, are bound to the outside of the chromatophore membrane per electron transported in the presence and absence of antimycin A [1,16]. In the present series of experiments, the change in $pH_0$ after 100 ms in the absence of antimycin yielded an average of 1.9 protons per reaction centre (P-870). This is less than the approx. five charges per reaction centre which are needed to reach the steady state, calculated on the basis of carotenoid shift measurements, assuming that saturating short flashes in the presence of antimycin A lead to a single charge separation per reaction centre [20]. We offer two explanations for this discrepancy. (1) Some cytochrome $c_2$ may have been lost during chromatophore preparation.

It is known that cytochrome-*c*-depleted chromatophores have a reduced $H^+/e^-$ stoichiometry, whereas $\Delta\psi$ generation through the reaction centre is less sensitive to the loss of cytochrome *c* [16]. (2) A fraction of the chromatophore membranes may not have become topologically inverted during preparation (e.g., see Ref. 12). With respect to the cresol red signal, proton efflux in those membranes retaining right-side-out polarity would effectively subtract from proton uptake in the inside-out vesicles. The electrochromic signal, however, would not discriminate between the two populations.

The observation that only approx. five net charges per reaction centre are required to raise $\Delta\psi$ to its maximum value (approx. 300 mV by electrochromic determinations [12]) confirms earlier estimates of the low capacitance of the chromatophore membrane [22]. Even though the $H^+/e^-$ coupling ratio is only 2.0, rather more than 2.5 turnovers of the electron-transport chains will be required to achieve the maximum, because $H^+$ efflux takes place coincidently with the uptake reactions (see below).

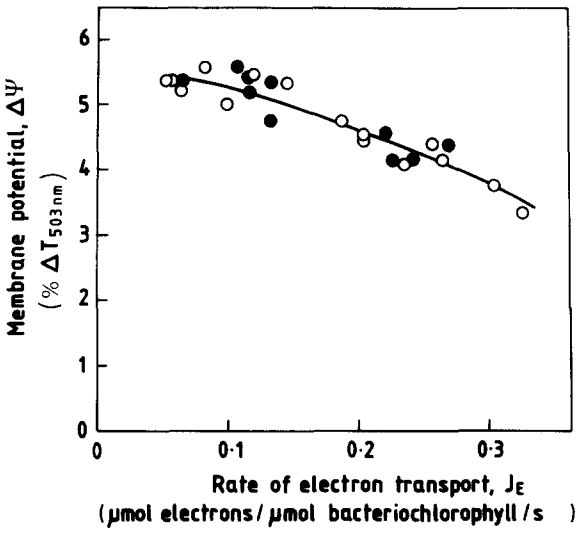


Fig. 2. The dependence of electron-transport rate on the value of $\Delta\psi$. The rate of electron transport was measured from the initial rate of P-870 reduction estimated at 542 nm (●) and from the value of $J_{dis}$ from carotenoid absorbance changes (○) using the assumptions described in Ref. 9. The lowest rates of electron transport were those recorded in the absence of FCCP. Electron-transport rates were increased by adding FCCP (up to 1.2 μM for the highest rate measured). At high rates of electron transport the method is limited by the accuracy by which initial rates can be measured.

*Contribution to the burst of $H^+$ disappearance from uncontrolled rates of electron transport*

The chemiosmotic hypothesis does not provide quantitative predictions for the mechanism of respiratory (and photosynthetic) control, but it explains the phenomenon as the result of a balance between the driving force of the redox reactions and the back pressure of $\Delta\psi$ upon electron transport [1]. The relationship between the electron transport rate and $\Delta\psi$ in the steady state has been explored in mitochondria [23] and in thylakoid membranes [24]. Fig. 2 shows data for photosynthetic electron transport during steady-state illumination in chromatophores. Electron-transport rates were measured from both the absorbance changes of P-870 (at 542 nm) and from ionic current determinations assuming a constant $H^+/e^-$ ratio of 2.0 [9]. $\Delta\psi$ was lowered progressively by titrating with FCCP. Within the range that can be measured, the electron-transport rate had a sensitive dependence on $\Delta\psi$: small decreases in $\Delta\psi$ led to large changes in the rate of electron flow. There is therefore a case for supposing, at the onset of illumination, before $\Delta\psi$ reaches its maximum value, that the rate of cyclic electron transport might be faster than in the steady state, and this might contribute to the burst of $H^+$ uptake. The actual rate of cyclic electron transport during the pre-steady state is not easily measured. The redox state of P-870 gives an indication of the way in which the rate of cyclic electron transport varies with time (Fig. 3). It can be seen that the rate of electron transport continually decreases during the rise of $\Delta\psi$ and that the electron-transport rate does not reach a constant value until the development of $\Delta\psi$ is complete. This is consistent with the view that electron transport is increasingly restricted by the rising $\Delta\psi$. Fig. 3 also shows that FCCP caused an increase in the rate of P-870 re-reduction by cyclic electron transport within 5 ms of switching on the actinic light, which suggests that $\Delta\psi$ already exerts some measure of control on electron transport during this period: long before the chromatophore membrane is charged to its full potential.

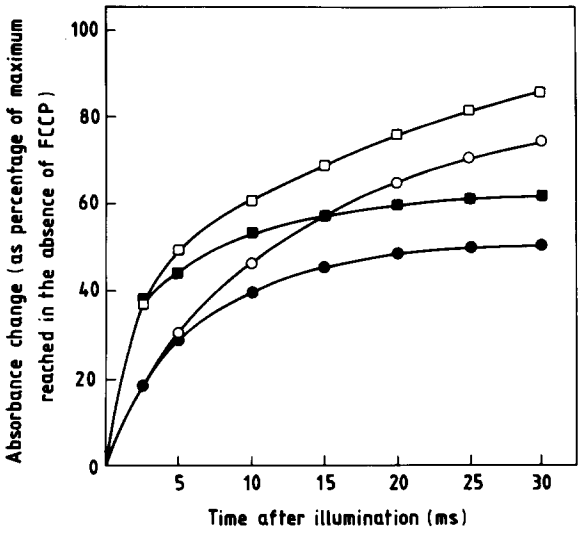


Fig. 3. The effect of FCCP on light-induced redox changes of P-870 and the carotenoid band shift at the onset of continuous illumination. P-870 (squares) measured at 542 nm and the carotenoid shift (circles) at 503 nm. The data were normalised to 100% as shown on the figure. The bacteriochlorophyll concentration was 10 $\mu$M. Open symbols, control, no FCCP. Closed symbols, plus 2 $\mu$M FCCP.

*Consequences of the non-ohmic conductance properties of the chromatophore membrane upon the nature of the burst of $H^+$ disappearance*

$H^+$ uptake by chromatophores upon illumination may be coupled directly to electron transport (see above), whereas $H^+$ efflux is a 'passive' process driven by $\Delta p$ ($\Delta\psi$ when $\Delta pH = 0$). So far, we have only discussed the effect of the $H^+$-uptake reactions in shaping the burst. The nature of the $H^+$ efflux reactions will also be influential. The experiment described in Fig. 4 was designed to examine the effect of $H^+$ efflux upon the burst kinetics and also to demonstrate the important consequence of the non-ohmic nature of the chromatophore membrane. The data are taken from electrochromic recordings. The photosynthetic light was extinguished at different times during the progress of the burst. At the instant before switching off the light the rate of change of $\Delta\psi$ (described as d $\Delta\psi/dt$ in Fig. 4A) is proportional to the total current flowing across the membrane (current $= C \cdot$ d $\Delta\psi/dt$, where $C$, the membrane capacitance, is constant [12,25]). The total membrane current at any instant is the arithmetic sum of the generative current ($J_{gen}$) and the dissipative current ($J_{dis}$) due to $H^+$ and other ions. In our steady-state analysis [12] we have pointed out that the initial rate of decay of $\Delta\psi$ upon darkening the suspension is a direct measure of $J_{dis}$. To a first approximation (but see below) the same reasoning applies in the experiment shown in Fig. 4A: d $\Delta\psi/dt$ at the instant before switching off the light is a measure of the total current ($J_{gen} + J_{dis}$) and at the instant after switching off the light, a measure of $J_{dis}$. Consequently, $J_{gen}$ can be calculated by subtraction. In Fig. 4B, the parameters $J_{gen}$, $J_{dis}$ and d $\Delta\psi/dt$ are plotted as a function of time. We have adopted the convention that the inward flux of positive charge is positive. The change in d $\Delta\psi/dt$ with time gives a description of the shape of the burst. It declines steeply at the beginning of illumination and then rapidly flattens out to zero

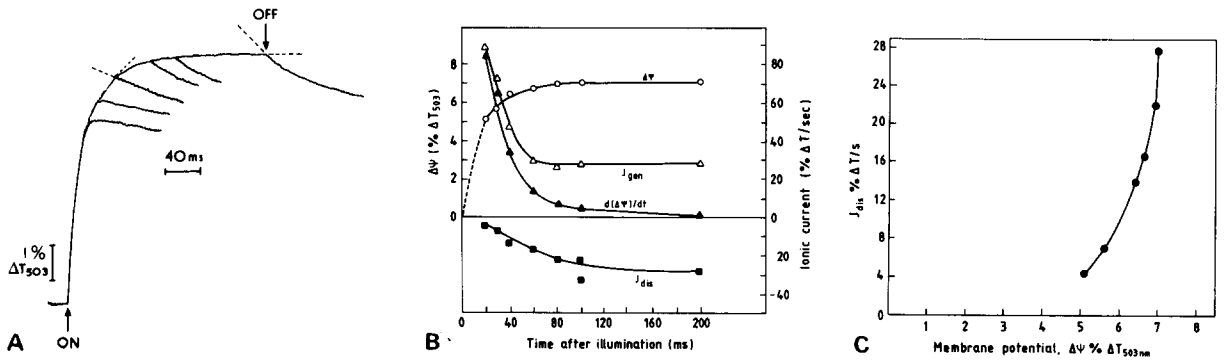


Fig. 4. The competing effects of generation and dissipation on the rise of membrane potential at the onset of illumination. (A) The results of a series of experiments in which the light was extinguished after different periods of illumination. The dashed lines show calculated rates of $J_{dis}$ and d $\Delta\psi/dt$ for two experiments as described in the text. (B) Data taken from (A) and as described in the text. (C) Data from (B) replotted to show the dependence of $J_{dis}$ on $\Delta\psi$.

in the steady state. The burst kinetics in the earliest period were dominated by $J_{gen}$: the dissipative ionic current was extremely small. With increasing time, however, the curves for $J_{gen}$ and $d(\Delta\psi)/dt$ diverged as the contribution from $J_{dis}$ became more pronounced. The conductance of the chromatophore membrane is known to increase with $\Delta\psi$ [12]. In agreement with this, $J_{dis}$ increased (became more negative) disproportionately with $\Delta\psi$ during the progress of the reaction. For example $J_{dis}$ increased by a factor of 5.6 from the first to the last measurement in Fig. 4, even though $\Delta\psi$ increased only by a factor of 1.4 during this period. The effect was to make the burst more abrupt and to hasten the establishment of the steady state in which the net current across the membrane was zero ($d\Delta\psi/dt = 0$). Obversely, the non-ohmic character of the chromatophore membrane can be demonstrated from these data as in Fig. 4c. The plot of $J_{dis}$ vs. $\Delta\psi$ is complementary to that obtained in steady state by varying actinic light intensity [12], and gave similar results.

A problem which cautions against too detailed an analysis by this procedure is evident in Fig. 4A in the recordings at very short illumination periods. When the light was extinguished the membrane potential continued to rise slightly for a few milliseconds. This is clearly analogous to the rise of $\Delta\psi$ in the millisecond time scale after single-turnover (microsecond) flash excitation [20]. This phenomenon arises in pre-steady-state conditions before all the component reactions in the cyclic electron-transport chain have reached an equal rate and interferes with accurate determination of $J_{dis}$.

When electron transport is in a steady-state, $J_{gen} = nJ_E$, where $J_E$ is the electron-transport rate and $n$ the $H^+/e^-$ coupling ratio. However, $J_E$ can not be indiscriminately calculated from the $J_{gen}$ data of Fig. 4, because as explained above, at the onset of illumination, component reactions in the cyclic electron-transport chain do not occur at equal rates. Using the antimycin-insensitive kinetics of Fig. 1 as an upper limit, the electron-transport backlash will be complete in less than 40 ms. In Fig. 4 $J_E$, calculated from $J_{gen}/2$, from 40 ms to the end of the burst, decreased by 45%, while $\Delta\psi$ rose by only 13%, confirming the sensitive dependence of $J_E$ on $\Delta\psi$ found in Fig. 2.

In principle, provided $\Delta$pH remains insignificant, a similar analysis to that in Fig. 4 can be performed with a combination of the cresol red and electrochromic data to yield the dependence of proton translocation upon $\Delta\psi$. Qualitatively, the results lead to the same conclusions about the shaping of the burst of $H^+$ uptake by the increasing inhibition of electron transport and by the increasing conductance of the membrane for protons as $\Delta\psi$ rises (not shown). The rates of proton translocation (from the cresol red data) and of charge translocation (from the electrochemic data) can be calibrated by the same procedure as the extents, as discussed above and in more detail in Ref. [12]. The two sets of data from simultaneous experiments are compared in Fig. 5 for the initial rate of decay after varing periods of illumination, plotted as a function of $\Delta\psi$. Throughout the entire range of $\Delta\psi$, the initial rate of outward proton translocation upon darkening was lower than the rate of total charge displacement. As noted recently there appears to be variability between these relative rates in different chromatophore preparations [14]. We formerly ascribed the discrepancy between the total current and the proton current to inaccuracies arising from the poor signal-to-noise ratio in the cresol red data. However, even with improved data it has emerged that the total

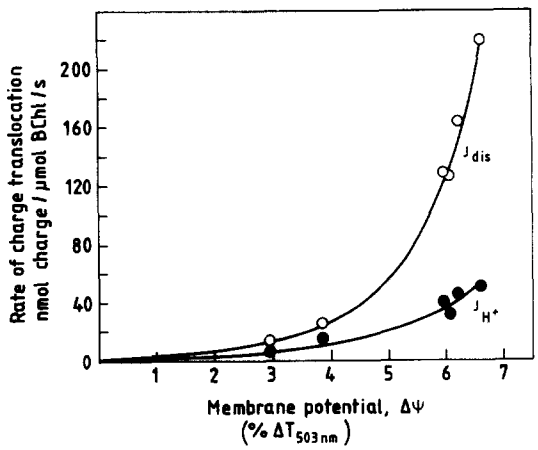


Fig. 5. Comparison between $J_{dis}$ and $J_{H^+}$. ○, Data from a series of experiments as described in Fig. 4C. Absolute values of $J_{dis}$ were calculated as in Ref. 14. ●, Data from a parallel series of experiments but monitoring the cresol red signal. The calibrated initial rate of $H^+$ reappearance on darkening was plotted against $\Delta\psi$ from the carotenoid shift determinations.

membrane ionic current exceeds the proton current consistently by between 2- and 4-fold, depending on chromatophore preparation. We offer two possible explanations. (1) The conductance of other ions in the system exceeds that of the proton conductance. (2) The chromatophore preparation contains a population of non-inverted membrane vesicles (varying between 20% and 37% of the total togive the 2- and 4-fold difference).

*Modification of the burst of proton translocation and $\Delta\psi$-development by valinomycin and $K^+$*

It is generally assumed that the stimulatory effect of valinomycin upon primary proton translocators is a result of the dissipation of $\Delta\psi$ through electrophoretic $K^+$ flux. A justification for this assumption is shown in Fig. 6. The chromatophore suspension was pre-equilibrated with a moderately high concentration of valinomycin and a relatively low concentration of $K^+$. These conditions were chosen to ensure that electrophoretic efflux from

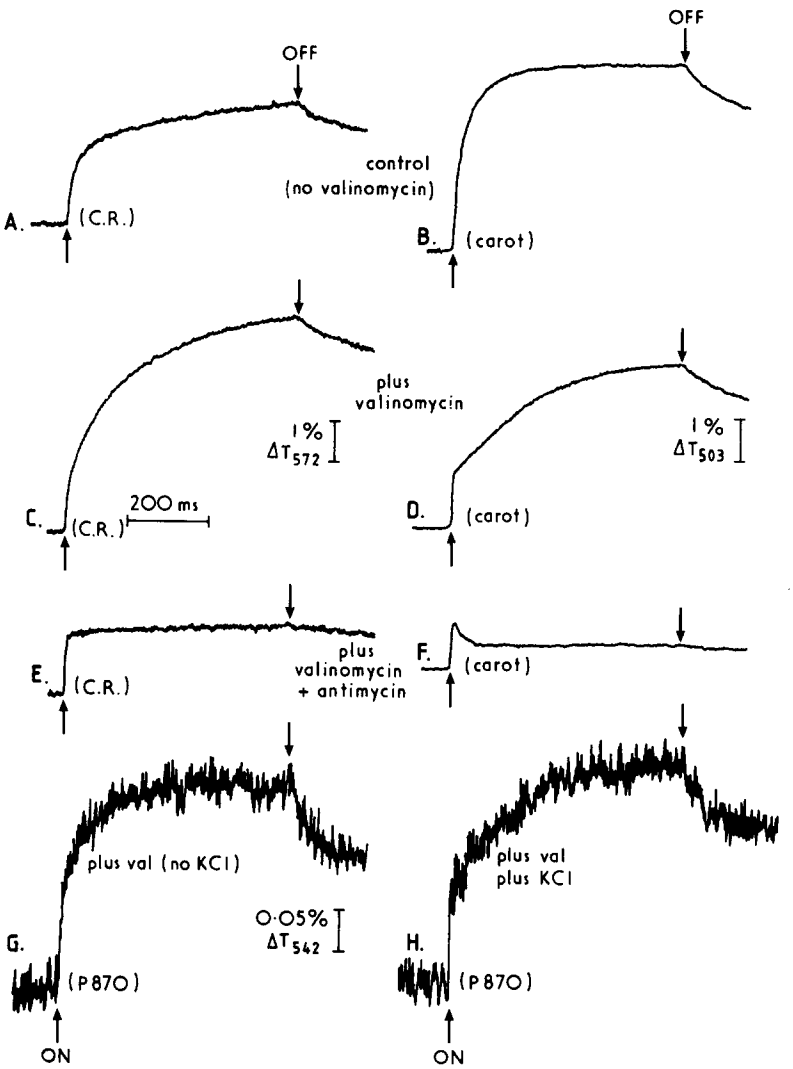


Fig. 6. The effect of valinomycin at low $K^+$ concentration on the burst of $H^+$-disappearance, $\Delta\psi$ formation and P-870 oxidation at the onset of illumination. Conditions as in Fig. 1. The $K^+$ concentration was 5 mM and $Na^+$ 95 mM, except in trace G where $K^+$ was omitted. Traces A (cresol red (C.R.)), B (carotenoid shift) and G (P-870) were controls. Valinomycin was present in traces C, D, E, F, G and H at 0.4 $\mu$g/ml, and antimycin was present in traces E and F at 2.4 $\mu$M.

the chromatophore lumen would be rapid, but would last for only a short time. From the electrochromic data in Fig. 6D, it can be seen that the burst of $\Delta\psi$ formation, due to rapid charge translocation through the reaction centre and which is insensitive to antimycin (see above), is too fast to be affected by the electrophoretic $K^+$ flux (Fig. 6E and F). Following the initial rapid charge displacement, the rate of development of $\Delta\psi$ was depressed, presumably due to $K^+$ efflux (Fig. 6D). However, in the limiting $K^+$ concentration of the experiment, potassium ion electrochemical potential equilibrium was quickly reached and $\Delta\psi$ subsequently rose to more than 90% of its uninhibited value. In accordance with this reasoning, at higher concentrations of $K^+$ the lag in the development of $\Delta\psi$, after the rapid phase was increased and the steady-state magnitude of $\Delta\psi$ was decreased (data not shown). The central point of this experiment is that the period during which stimulation of the light-induced pH change was observed, exactly co-incided with the period during which the development of $\Delta\psi$ was delayed (compare Fig. 6C and D).

According to arguments developed above, stimulation of the light-induced pH change at the low $\Delta\psi$ maintained by valinomycin and $K^+$ might be a result of either an accelerated rate of electron-transport-driven inward proton translocation or it might be due to a decreased rate of $\Delta p$-driven $H^+$ efflux. In practice, both factors appear to contribute. Fig. 6 includes the result of an experiment in which the light-induced change in the redox state of P-870 was monitored in the presence of valinomycin and low $K^+$. The slow rise in the light-induced oxidation of P-870 (compare with the control, Fig. 6G) suggests that the rate of re-reduction starts off rapidly and decelerates during the period in which the electrochemical potential gradient of $K^+$ across the membrane approaches zero.

The flash-train experiment in Fig. 7 illustrates the effect of both stimulated electron transport and decreased rate of $H^+$ efflux in the presence of valinomycin and $K^+$. The chromatophores were illuminated with saturating short flashes (4 $\mu$s, half-peak width) at low frequency (1.0 Hz). A similar experimental design was employed by Cogdell and Crofts [26] to show that proton translocation measured during continuous illumination could be resolved into a series of $H^+$ binding reactions after each flash in a train. In our experiments we operate at low $K^+$ concentrations to ensure that the period of rapid electrophoretic $K^+$ efflux is confined to the early flashes in the train. The main figure (compare C and D with A and B) shows that the stimulatory effect of valinomycin/$K^+$ on $H^+$ disappearance at the start of the flash train coincides with the period in which $\Delta\psi$ was rapidly dissipated after the individual flashes (see the insets to Fig. 7B and D showing events on a more rapid scale). After approx. 7–8 flashes in the presence of valinomycin, the average value of $\Delta\psi$ began to rise due to a decreased rate of decay in between flashes (see the inset to Fig. 7B and D after 60 flashes), presumably as a result of depleted internal $K^+$. Over the same period, the stimulatory effect of valinomycin on $H^+$ disappearance was lost. The insets to Fig. 7A and C at the start of flash illumination show why $H^+$ disappearance is more pronounced in the presence of valinomycin and $K^+$. First, whereas the total amount of $H^+$ binding per flash decreases over the first five flashes in the absence of valinomycin, it remains constant in the presence of ionophore (see also Ref. 26). This suggests that the transport of electrons through the cytochrome $b/c_1$ complex in the dark time between flashes is incomplete in the absence and complete in the presence of valinomycin. The same conclusion is reached from an inspection of the extent of the carotenoid band shift after the flashes. Second, the ionophore reduces the rate of $H^+$ efflux in between flashes, and so enhances the net proton disappearance. As the flash number increases, both of these effects become less evident. The inset of the cresol red change following the 60th flash shows little difference in the presence and absence of valinomycin.

Kell and Hitchens [27] carried out an experiment similar to that performed in Fig. 7, using a sensitive glass electrode to record the extent of $H^+$ disappearance after a fixed number of short flashes. They deduced, in contradiction with our conclusions, that a delocalised $\Delta\psi$ arising from proton translocation does not lead to feed-back inhibition on electron transport. However, the better time resolution of the experiments in Fig. 7 shows that

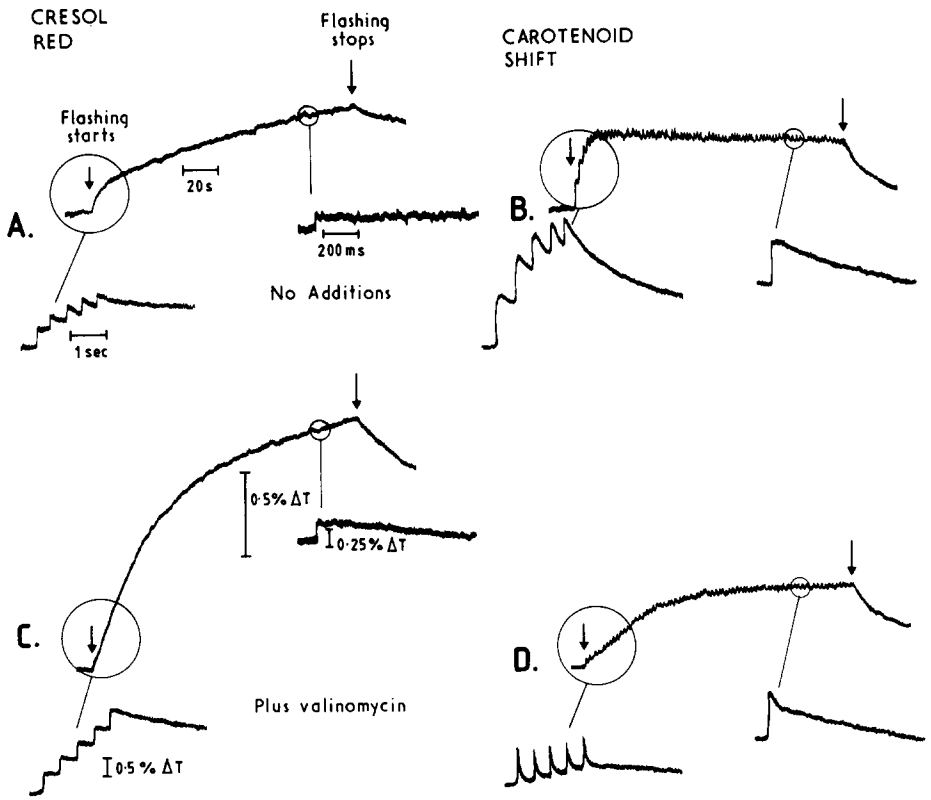


Fig. 7. The stimulatory effect of valinomycin on the burst of $H^+$ disappearance during low-frequency flash excitation. Conditions as Fig. 6 except that illumination was by 4 $\mu$s flashes fired at 1.0 Hz. Traces A (cresol red) and B (carotenoid shift) were controls in the absence of valinomycin. Valinomycin was present in traces C and D at 0.4 $\mu$g/ml. The insets show critical regions of the traces, but recorded on a more rapid time scale and averaged over flash times fired after a dark adaptation time of 60 s.

even at a frequency of 1 Hz and under anaerobic conditions, electron transport in the absence of valinomycin may not have been complete within the dark time between flashes. This interpretation is based on the observation that the extent of proton translocation and the extent of $\Delta\psi$ formation per flash decreased during the first few flashes. Consequently, in an experiment, where the flash frequency is raised from 3 to 5 Hz [27], there will be an expected increase in the extent of $H^+$ disappearance due to less $H^+$ efflux during the period between flashes, but also an expected decrease in $H^+$ disappearance due to the smaller quantity of reducing equivalents transported round the cycle during the dark time between flashes. Also in the presence of valinomycin and excess $K^+$ [27], we should expect a similar extent of $H^+$ disappearance whether the flashes are fired at 3 Hz or 5 Hz. In these circumstances electron transport will be complete in the dark time between flashes, and the rate of $H^+$ efflux from the chromatophores will be extremely slow. We conclude that these observations on $H^+$ disappearance in chromatophores [27] are not inconsistent with the chemiosmotic hypothesis.

## General discussion

An alternative to the chemiosmotic mechanism of energy coupling in oxidative and photophosphorylation is the possibility that protons, released in the oxidation/reduction reactions of the electron-transport chain can proceed directly to the catalytic site of the ATP synthase and drive ATP synthesis without leaving the membrane [6]. In more recent, and sophisticated models [7], the

proton circuit between an electron-transport complex and an ATP synthase complex is isolated from the bulk aqueous phases, except in two regions: a proton conductance connects the positive electrochemical potential side of the local circuit to one bulk aqueous phase and another proton conductance leads from the negative electrochemical potential side of the local circuit to the other bulk aqueous phase. If the value of these conductances were small compared with the proton conductance of the ATP synthase (or slip pathway) then the electron transport and ATP synthase 'super complexes' behave as discrete, isolated units; there would be no proton translocation and although a $\Delta\psi$ might conceivably develop as a result of a capacitative coupling of local charge displacements of the bulk phases, such a $\Delta\psi$ would not persist, but would discharge by way of transmembrane leaks. This model would be inconsistent with the results presented above. We do observe proton translocation which is tightly coupled to the development of a bulk phase membrane potential and this membrane potential appears both to drive the return of protons back across the coupling membrane and to feedback and inhibit the electron-transport rate. At the other extreme, if the proton conductances between the local circuit and the bulk phases are relatively large, the model [7] becomes indistinguishable from the chemiosmotic hypothesis and the discussion of the results given above need not be modified. The new model may be most useful when the resistive elements separating the local and bulk phase proton circuits are chosen with values intermediate between the above two extremes. In this case the proton circuit in electron-transport phosphorylation would be expected only partly to equilibrate with the bulk phases and energy coupling would have both localised and delocalised characteristics. The results presented above are not inconsistent with such a view: although only bulk phase protons, delocalised membrane potentials and transmembrane ionic currents are in evidence, the presence of local proton circuits within the membrane cannot be ruled out.

One of the reasons leading to the proposal of this model of energy coupling [7] was that the properties of respiration-linked $H^+$ efflux in bacteria and mitochondria in the absence of permeant ions were not easily reconciled with the simple predictions of the chemiosmotic hypothesis [2–5]. Such problems were not encountered in the experiments with chromatophores described in this report. Three conclusions are possible. Either (i) the resistive elements which prevent the proton currents from delocalising into the bulk phases in intact bacterial cells are disrupted during chromatophore preparation. Or (ii) the resistive elements are significant on the face of the cytoplasmic membrane which is exposed in intact cells, but not on the face which is exposed in chromatophores. Or (iii) experiments with intact cells are complicated in an unknown way by proton translocation accompanying other vectorial reactions across the cytoplasmic membrane and have led to an erroneous conclusion – in which case the justification for the revised model is weakened [32]. Chromatophores are single membrane structures of which the soluble, metabolic functions have been removed during preparation. They are capable of good rates of electron transport ATP synthesis, and their energy coupling properties remain stable for long periods in the conditions of our experiments. Despite their simplicity, three factors were shown (above) to influence the burst of proton translocation at the onset of light-driven electron transport: (i) the pre-steady-state kinetics of electron transport; (ii) the non-linear dependence of electron-transport rate on $\Delta\psi$; and (iii) the non-linear dependence of $H^+$ efflux on $\Delta\psi$. In intact bacterial cells another degree of complexity will arise from the secondary translocators for substrates and ions. A striking example of the ways in which secondary effects can modify the kinetics of pH changes in intact cells has been provided for the photosynthetic bacteria by Kobayashi and Nishimura [28–30]. In those experiments, not only the rate and extent of the light-induced pH changes detected by a glass electrode were influenced by the ionic composition of the medium, but also the polarity of the changes. Recent experiments [31] do reveal a rapid efflux of protons from intact cells of *Rps. capsulata* after short flashes. On a longer time scale during continuous illumination the kinetics of $H^+$ efflux are highly complex in intact cells [28–32] and at present prohibit the kind of analysis employed in this report.

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