Miller, D. L., and Weissbach, H. (1974), *Methods Enzymol. 30,* 219–232.
Möller, W. (1974), in Ribosomes, Nomura, M., Tissieres, A., and Lengyel, P. Ed., Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, pp 711–731.
Nakamura, S., Arai, K. I., Takahashi, K., and Kaziro, Y. (1975), *Biochem. Biophys. Res. Commun. 66,* 1069–1077.
Nishizuka, Y., Lipmann, F., and Lucas-Lenard, J. (1968), *Methods Enzymol. 12B,* 708–721.
Pacaud, M., and Richaud, C. (1975), *J. Biol. Chem. 250,* 7771–7779.
Pacaud, M., and Uriel, J. (1971), *Eur. J. Biochem. 23,* 435–442.
Platt, T., Files, J. G., and Weber, K. (1973), *J. Biol. Chem. 248,* 110–121.
Rohrbach, M. S., Dempsey, M. E., and Bodley, J. W. (1974), *J. Biol. Chem. 249,* 5094–5101.
Rosenbusch, J. P. (1974), *J. Biol. Chem. 249,* 8019–8029.
Scatchard, G. (1949), *Ann. N.Y. Acad. Sci. 51,* 660–672.
Skar, D. C., Rohrbach, M. S., and Bodley, J. W. (1975), *Biochemistry 14,* 3922–3926.
Suter, P., and Rosenbusch, J. P. (1975), *Eur. J. Biochem. 54,* 293–299.
Suter, P., and Rosenbusch, J. P. (1976), *J. Biol. Chem. 251,* (in press).
Takacs, B. J., and Rosenbusch, J. P. (1975), *J. Biol. Chem. 250,* 2339–2350.
Tanford, C. (1968), *Adv. Protein Chem. 23,* 121–282.
Travers, A. A. (1973), *Nature (London) 244,* 15–18.
Travers, A. A., Kamen, R. I., and Schleif, R. F. (1970), *Nature (London) 228,* 748–751.
Wade, M., Laursen, R. A., and Miller, D. L. (1975), *FEBS Lett. 53,* 37–39.
Weiner, A. M., Platt, T., and Weber, K. (1972), *J. Biol. Chem. 247* 3242–3251.
Wetlaufer, D. B. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 697–701.

A Simple, Quantitative Approach to the Coupling of Photophosphorylation to Electron Flow in Terms of Proton Fluxes†

Richard E. McCarty* and Archie R. Portis, Jr.‡

ABSTRACT: A simple relationship between observed phosphorylation efficiencies (*P/e* ratios) and internal proton concentration in spinach chloroplast thylakoids has been derived. *P/e* ratios, varied by either changing the light intensity or by adding the energy transfer inhibitor, 4′-deoxyphlorizin, were found to change with internal proton concentration in accordance with this relationship. A quantitative prediction of the effect of uncouplers on the *P/e* ratio can probably also be made. By extrapolation of plots of observed *P/e* ratios against internal proton concentration divided by the overall rate of electron flow, a maximum intrinsic *P/e* of about 0.66 is obtained. Assuming that two protons appear inside thylakoids per electron transferred, a *P/e* ratio of 0.66 suggests that three internal protons are consumed for each ATP formed. Internal protons may be considered to be substrates for the phosphorylation reaction. Hill plots of phosphorylation rate vs. internal proton concentration also indicate that three protons are consumed for each ATP synthesized. Thus, the H^+ concentration gradient behaves quantitatively, as well as qualitatively, as if it is the connecting link between electron flow and phosphorylation in illuminated thylakoids.

Strong evidence supporting at least the basic postulates of the chemiosmotic interpretation (Mitchell, 1966) of the coupling of phosphorylation to light-driven electron flow in chloroplast thylakoids has been presented. Illuminated thylakoids catalyze a light-dependent, uncoupler-sensitive H^+ uptake (Neumann and Jagendorf, 1964), resulting in a pH differential of 3 units or more across thylakoids membranes (Rottenberg et al., 1972; Schuldiner et al., 1972; Portis and McCarty, 1973, 1974). Since pH differentials formed artificially across thylakoid membranes in the dark can serve as the driving force for ATP synthesis (Jagendorf and Uribe, 1966), it is logical to conclude that the pH differential generated by light-induced electron flow serves this purpose also.

Qualitatively, the pH differential (ΔpH) was found to have properties consistent with its being the connecting link between electron flow and ATP synthesis. For example, phosphorylation and uncouplers (Portis and McCarty, 1974; Pick and Avron, 1973) diminish the magnitude of ΔpH. However, quantitative estimates of ΔpH under different conditions were required to provide critical tests of the chemiosmotic hypothesis Spinach chloroplast thylakoids appeared to be a good experimental subject for these investigations. Thylakoids catalyze rapid electron flow and phosphorylation and have very low ATPase activity. Rates of electron flow and phosphorylation may be very easily altered simply by changing the incident light intensity. More significantly, the H^+ electrochemical gradient may be more readily determined in thylakoids than in other phosphorylating organelles. Illuminated spinach chloroplast thylakoids suspended in media containing high Cl^- concentrations appear to have only a slight electrical potential across them at the steady state (Rottenberg et al., 1972; Schröder et al., 1971). In contrast, the pH differential, which

† From the Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853. *Received June 23, 1976* This work was supported by a grant from the National Science Foundation (GB-44233X).

‡ Predoctoral trainee of the National Institutes of Health (GM 00824).

can be reliably estimated from the distribution of [^{14}C]hexylamine cation, is large under these conditions.

Previously, we showed (Portis and McCarty, 1974) that phosphorylation follows an apparent third-order dependence on the internal H^+ concentration ($[H^+]_{in}$), suggesting that three internal H^+'s are consumed per ATP synthesized Moreover, it was predicted (Portis and McCarty, 1976) that the reciprocal of the phosphorylation efficiency should be proportional to $1/[H^+]_{in}^2$ as long as the permeability of thylakoids to H^+ is unchanged. Although this proportionality was verified experimentally (Portis and McCarty, 1976) a more straightforward treatment of the data was desired. Here, we report that using a very simple chemiosmotic model it is possible to derive a relationship between the phosphorylation efficiency and $[H^+]_{in}$. The data fit this expression rather well, suggesting that the pH differential satisfies quantitative as well as qualitative tests of its suitability as the link between electron flow and ATP synthesis.

Materials and Methods

Spinach chloroplast thylakoids (McCarty and Racker, 1967) were suspended in 0.3 M mannitol, 0.02 M Tricine[1]-NaOH (pH 8.0), and 0.01 M NaCl. ΔpH, phosphorylation, and electron flow were assayed as previously reported (Portis and McCarty, 1976). All 0.1-ml reactions mixtures contained: 20 or 40 mM Tricine-NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM $K_3Fe(CN)_6$, and 0.025 mM hexylamine. For the assay of ΔpH, 0.03 μCi of [^{14}C]hexylamine and 0.1 μCi of [^{3}H]sorbitol were also added. When phosphorylating conditions were used, 3 mM ADP and 3 mM P_i were also present. Illuminations with about 6×10^5 ergs cm^{-2} s^{-1} of white light (unless otherwise noted) were carried out at room temperature for 30–120 s within the microcentrifuge. Light intensity was varied through the use of neutral density filters from Eastman Kodak. Dichlorophenyl-1,1-dimethylurea (DCMU) was purchased from K & K Laboratories and was recrystallized three times from ethanol-water mixtures.

Results and Discussion

Rumberg and Siggel (1969) predicted that the rate of nonphosphorylating (basal) electron flow should be proportional to $[H^+]_{in}$ as long as the permeability of thylakoids to H^+ is not altered. This prediction was recently found to hold true when either light intensity (Portis et al., 1975) or DCMU (Portis and McCarty, 1976) was used to vary $[H^+]_{in}$ and electron flow and ADP or ATP was present. Thus, the rate of basal electron flow may be expressed in terms of H^+ as $R_b = k_{H^+}[H^+]_{in}$, where R_b is the rate of basal electron flow and k_{H^+} is the proportionality constant which is related to the permeability constant of thylakoids for H^+. This proportionality also holds true in the presence of gramicidin D (Portis and McCarty, 1976) and allows the determination of the rate constant for H^+ efflux in terms of μeq h^{-1} (mg of chlorophyll)$^{-1}$ μM^{-1} $[H^+]_{in}$. Since the rate of basal electron flow is a linear function of $[H^+]_{in}$ when these parameters were varied by changing the light intensity or DCMU concentration, the permeability constant of thylakoid membranes to H^+ must be independent of light intensity or DCMU concentration. In confirmation of this conclusion, the *apparent* first-order rate constant for H^+ efflux in the dark from thylakoids illuminated to the steady state was not affected by light intensity or by DCMU. For example, at pH 7.8 and 20 °C, the half-time for H^+ efflux in the dark from chloroplasts illuminated in the presence of 0.1 mM methylviologen, 2 mM Tricine-NaOH, 50 mM NaCl, 0.1 mM ATP, and 5 mM $MgCl_2$ was 5.3 ± 0.5 s over a light intensity range of 4.5×10^5 to 4.5×10^4 ergs cm^{-2} s^{-1}. DCMU at 0.5 μM, which reduced the extent of H^+ uptake by nearly 50%, also had no effect on the apparent rate of H^+ efflux.

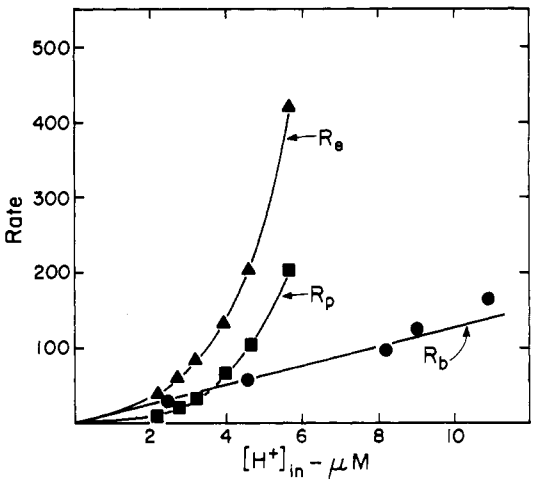

FIGURE 1: Relationships between phosphorylation, electron flow, and internal H^+ concentration. Phosphorylation, associated electron flow, and ΔpH were determined as a function of light intensity (3×10^3 to 6×10^5 ergs cm^{-2} s^{-1}). Basal electron flow was estimated in the absence of ADP and P_i, but in the presence of 0.1 mM ATP. R_e stands for the rate of electron flow under phosphorylating conditions, R_b the rate of basal electron flow, and R_p for the rate of phosphorylation. Rates are expressed as μmol h^{-1} (mg of chlorophyll)$^{-1}$.

Under phosphorylating conditions, the rate of electron flow is no longer proportional to $[H^+]_{in}$ when light intensity is used to vary these parameters. As the light intensity increases, the rate of electron flow increases much more sharply than $[H^+]_{in}$. As previously suggested (Portis and McCarty, 1976), this relationship between the rate of electron flow and $[H^+]_{in}$ under phosphorylating conditions is likely to be a result of the fact that 3 internal H^+ are consumed per ATP formed. The overall rate of electron flow may thus be expressed as

$$R_e = R_b + R_{ep}$$

where R_e is the overall rate of electron flow, R_b, the basal rate, and R_{ep}, the rate due to phosphorylation. The rate of electron flow which is specifically a consequence of the phosphorylation-dependent H^+ efflux is simply equal to the H^+/P ratio times the rate of phosphorylation, or

$$R_{ep} = R_p(H^+/P)$$

where R_p is the observed rate of ATP synthesis, and H^+/P is the stoichiometry of the phosphorylation reaction with respect to H^+ (the ratio of number of H^+ ions to the number ATP's). The R_b and R_{ep} may thus be determined as a function of $[H^+]_{in}$. An experiment in which light intensity was used to vary R_b, R_e, and $[H^+]_{in}$ is illustrated in Figure 1. As expected, R_e is much more sharply dependent on $[H^+]_{in}$ than R_b. As the light intensity is decreased, the contribution of R_b to the overall rate of electron flow increases. That is to say, at low light intensities a higher proportion of the electron flow is a consequence of the H^+ leak which is not coupled to phosphorylation. Thus, one would predict that the P/e_2 ratio would decrease with decreasing light intensity. A light intensity lag in photophos-

[1] Abbreviations used: Tricine, *N*-tris(hydroxymethyl)methylglycine; DCMU, dichlorophenyl-1,1-dimethylurea; P_i, inorganic phosphate; ADP and ATP, adenosine-5′-di- and triphosphates, respectively; ATPase, adenosine triphosphatase.

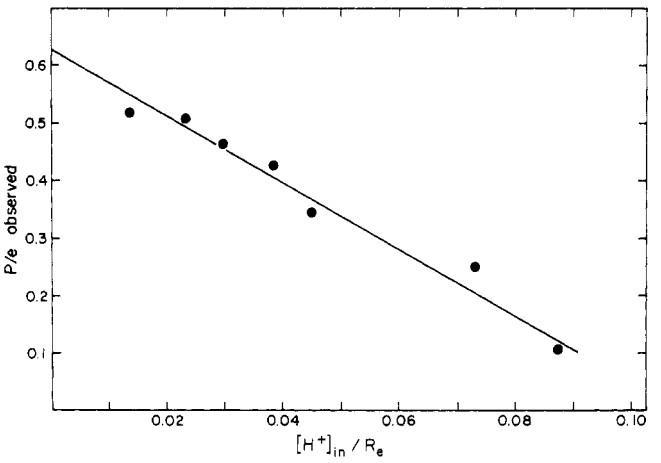

FIGURE 2: Relationship between the observed phosphorylation efficiency (P/e) and $[H^+]_{in}/R_e$. The data given in Figure 1 were used except for the data for the lowest light intensity (R_e, 13.5; $[H^+]_{in}$, 1.2 μM) which was omitted from Figure 1 for the sake of clarity. R_e stands for the overall rate of electron flow under phosphorylation conditions. The line was drawn by least-squares linear regression analysis. The linear correlation is 0.98.

phorylation has been observed (Sakurai et al., 1965). Moreover, uncouplers, which increase k_{H^+} and, therefore, R_b, make the light intensity lag more pronounced (Saha et al., 1970).

The behavior of the observed phosphorylation efficiency with respect to $[H^+]_{in}$ can be predicted from a simple relationship. As pointed out previously, the overall rate of electron flow is the sum of that due to the passive H^+ leak and that due to the outward H^+ pumping due to phosphorylation. This relationship may be expressed in terms of H^+ fluxes:

$$(H^+/e)R_e = k_{H^+}[H^+]_{in} + (H^+/P)R_p \qquad (1)$$

where H^+/e is the proton to electron ratio or the number of H^+ ions which appear inside thylakoids per electron transferred through the chain. Dividing through by (H^+/P) and R_e, the following expression is obtained:

$$\frac{(H^+/e)}{(H^+/P)} = \frac{k_{H^+}[H^+]_{in}}{R_e(H^+/P)} + \frac{R_p}{R_e} \qquad (2)$$

Since the quantity $(H^+/e)/(H^+/P)$ is equivalent to the maximum P/e $[(P/e)_{max}]$ and since R_p/R_e is the observed P/e ratio $[(P/e)_{obsd}]$, the above equation becomes:

$$(P/e)_{obsd} = (P/e)_{max} - \frac{k_{H^+}[H^+]_{in}}{R_e(H^+/P)} \qquad (3)$$

Thus, the observed phosphorylation efficiency should be a linear function of $k_{H^+}[H^+]_{in}/R_e$ or, in other words, of the ratio of basal electron flow to the overall electron flow. As k_{H^+} approaches zero, the observed P/e ratio approaches the maximum, theoretical value. Moreover, since k_{H^+} is defined in terms of electron flow, the H^+/e ratio is a part of this constant. Thus, as the rate of basal electron flow approaches the rate of overall electron flow, the term $(H^+/e)R_b/R_e(H^+/P)$ becomes $(H^+/e)/(H^+/P)$ or $(P/e)_{max}$ and the observed P/e ratio would be zero.

Plots of $(P/e)_{obsd}$ vs. $[H^+]_{in}/R_e$ should be linear if k_{H^+} is constant. The intercept at the ordinate would give $(P/e)_{max}$ and the slope, $k_{H^+}/(H^+/P)$. Figure 2 shows the results of an experiment in which phosphorylation, electron flow, and $[H^+]_{in}$ were varied with light intensity. An excellent fit of the data to the derived relationship was found. The extrapolated $(P/e)_{max}$ is 0.63 ± 0.03, indicating a P/e_2 ratio of 1.26 ± 0.06. Quite similar results were obtained when $[H^+]_{in}$ and the rates of phosphorylation and electron flow were varied by DCMU concentration. Moreover, the intercept on the abscissa is $(H^+/e)/k_{H^+}$. The k_{H^+} determined from this intercept had a value quite similar to that estimated from the slopes of a plot of R_e vs. $[H^+]_{in}$.

Previously we used a different method to treat light intensity data (Portis and McCarty, 1976). This treatment involved plots of the reciprocal of the observed phosphorylation efficiency vs. $1/[H^+]_{in}^2$ and is similar to the treatment presented here: eq 1 may be rearranged to

$$R_e/R_p = \frac{k_{H^+}[H^+]_{in}}{(H^+/e)R_p} + \frac{(H^+/P)}{(H^+/e)}$$

which is equivalent to

$$(e/P)_{obsd} = \frac{k_{H^+}[H^+]_{in}}{(H^+/e)R_p} + (e/P)_{max}$$

Since $R_p = k_p[H^+]_{in}^3$, where k_p is the overall catalytic rate constant for phosphorylation, the above equation may be written

$$(e/P)_{obsd} = \frac{k_{H^+}}{(H^+/e)k_p}\frac{1}{[H^+]_{in}^2} + (e/P)_{max}$$

Thus, the reciprocal of the observed phosphorylation efficiency should be proportional to the reciprocal of the square of $[H^+]_{in}$. However, this method for treating the data is cumbersome and can not be used to predict the effects of limiting ADP or P_i concentrations or of energy-transfer inhibitors on the observed P/e ratio since it has to be assumed that the overall catalytic rate constant for phosphorylation was unaffected by the procedure used to vary the rates of phosphorylation and electron flow.

However, the simple approach given here does not have this limitation. Thus, we may predict what should happen to $(P/e)_{obsd}$ when the phosphorylation reaction is specifically inhibited by an energy-transfer inhibitor. Provided that ADP (or ATP) is present, energy-transfer inhibitors, such as Dio-9 (McCarty et al., 1965) or phlorizin and related compounds (Winget et al., 1967), have essentially no effect on the rate of basal electron flow. Thus, it is unlikely that these reagents affect k_{H^+}. By inhibiting phosphorylation, energy-transfer inhibitors increase $[H^+]_{in}$ (Portis and McCarty, 1976). It is evident, therefore, that the rate of basal electron flow must be increased by an energy-transfer inhibitor as phosphorylation is inhibited since $R_b = k_{H^+}[H^+]_{in}$. P/e_2 ratios approaching a value of 2 were calculated by Izawa and Good (1968) who subtracted the basal rate of electron flow measured under nonphosphorylating conditions from the overall of electron flow to derive the rate of electron flow due to phosphorylation. This is undoubtedly an overcorrection since phosphorylation decreases $[H^+]_{in}$ by threefold or more.

As with decreasing light intensity, increasing concentrations of an energy-transfer inhibitor will result in a higher proportion of the overall electron flow being a consequence of the passive H^+ leak and an apparent uncoupling, reflected by a decreasing $(P/e)_{obsd}$, would be expected. Since phosphorylation is fully sensitive to energy-transfer inhibitors, whereas nonphosphorylating electron flow is insensitive, a full uncoupling may be seen. Figure 3 shows a plot of $(P/e)_{obsd}$ vs. $[H^+]_{in}/R_e$ with data obtained with 4′-deoxyphlorizin, an energy-transfer inhibitor. Once again, a good fit of the data to eq 3 was found. The $(P/e)_{max}$ was 0.65 ± 0.02 in agreement with that obtained in the light intensity experiments.

Electron transport in thylakoids with water as the electron

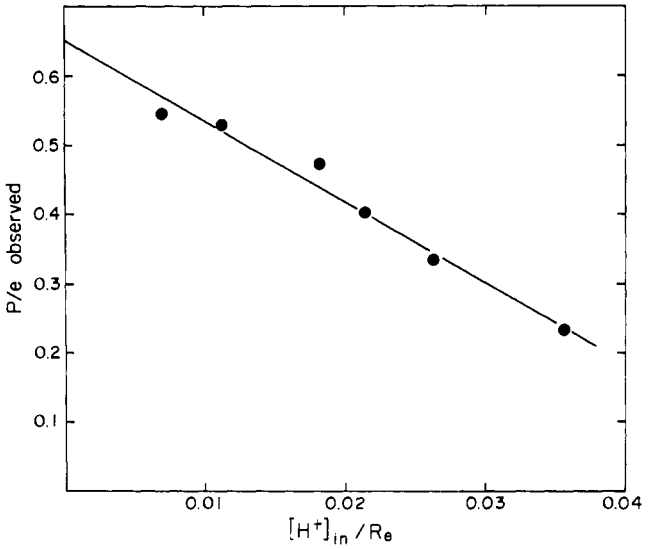

FIGURE 3: Relationship between the observed phosphorylation efficiency (P/e) and $[H^+]_{in}/R_e$. Phosphorylation, electron flow (R_e), and ΔpH were determined as a function of 4-deoxyphlorizin concentration. The line was drawn according to least-squares analysis of the data. The linear correlation is 0.98. R_e in the absence of 4′-deoxyphlorizin was 651 μmol of ferricyanide reduced h^{-1} (mg of chlorophyll)$^{-1}$, whereas, in the presence of 0.85 mM 4′-deoxyphlorizin, R_e was 248.

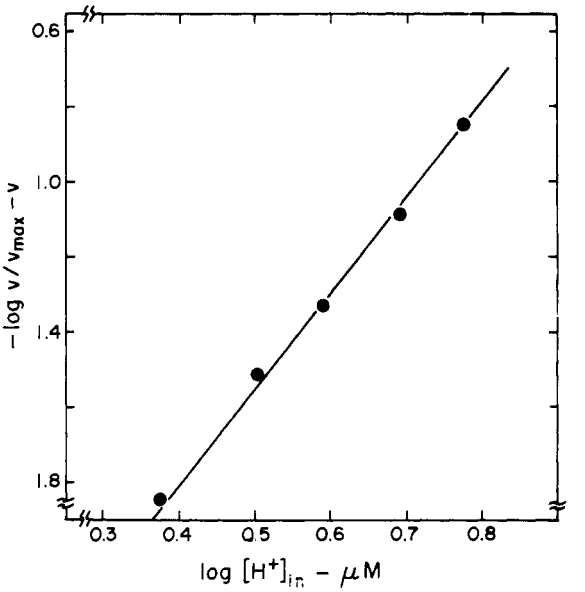

FIGURE 4: A Hill plot relating phosphorylation rate and internal H^+ concentration. Phosphorylation and ΔpH were varied using concentrations of DCMU varying from 0 to 2 μM. v_{max} was assumed to be 1200 μmol h^{-1} (mg of chlorophyll)$^{-1}$, but the slope was unaffected by the magnitude selected for v_{max}.

donor and ferricyanide as the electron acceptor most likely generates two H^+ inside thylakoids per electron transferred (an H^+/e ratio of 2). As a consequence of electron flow through the plastoquinone region of the chain, one H^+ per electron is translocated across thylakoid membranes (Junge and Ausländer, 1974). The photooxidation of water, which is thought to take place toward the inside of thylakoid membranes, generates another internal H^+ per electron transferred (Junge and Ausländer, 1974; Fowler and Kok, 1974). Since $(P/e)_{max} = (H^+/e)/(H^+P)$, the H^+/P ratio may be calculated from $(P/e)_{max}$ given that the H^+/e is 2. The calculated $(P/e)_{max}$ of 0.65 is clearly consistent with an H^+/P ratio of 3.

We previously tentatively suggested (Portis and McCarty, 1974, 1976) an H^+/P ratio of 3 on the basis of the observation that phosphorylation follows an apparent third-order dependence on $[H^+]_{in}$. To reach this conclusion, we assumed that $[H^+]_{in}$ was the rate-limiting factor in phosphorylation so that log (rate of phosphorylation) should be proportional to n log $[H^+]_{in}$, where n is the H^+/P ratio. With saturating ADP and P_i concentrations, the data relating phosphorylation rate (v) to internal H^+ concentration fit the Hill equation (Hill, 1910):

$$\log \frac{v}{v_{max} - v} = n \log [H^+]_{in} - \log k'$$

where v is the observed rate of phosphorylation, v_{max}, the maximum rate, and k', a complex constant. In the experiment shown in Figure 4, phosphorylation rates and ΔpH were measured as a function of DCMU concentration and the data were plotted as log $(v/(v_{max} - v))$ vs. log $[H^+]_{in}$. The data fit the Hill equation rather well and the slope is 2.9. This result could indicate that 3 H^+ are required for each ATP formed in a highly cooperative manner.

In its nonlogarithmic form, the Hill equation in this case would be:

$$v/v_{max} = \frac{[H^+]_{in}{}^n}{k' + [H^+]_{in}{}^n}$$

and it would be expected that plots of v vs. $[H^+]_{in}$ would be sigmoidal. However, $[H^+]_{in}$ never becomes sufficiently high to allow v to approach v_{max} and, thus, only the initial part of the curve, which approximates an exponential, is seen. A v_{max} for phosphorylation with ferricyanide may be calculated. In fully uncoupled chloroplasts, the maximum rate of ferricyanide reduction approaches 1800 μeq h^{-1} (mg of chlorophyll)$^{-1}$ (Stiehl and Witt, 1969; Portis and McCarty, 1976) which is equivalent to 3600 μeq of H^+ h^{-1} (mg of chlorophyll)$^{-1}$ for an H^+/e ratio of 2. Assuming that all of these H^+ could be utilized by the ATPase for phosphorylation, the maximum phosphorylation rate would be 1200 μeq h^{-1} (mg of chlorophyll)$^{-1}$ which is considerably higher than the highest observed rate of phosphorylation.

Plots of log (phosphorylation rate) against ΔpH were also found to linear when pyocyanine was used as the mediator of electron flow (Portis and McCarty, 1974). This result suggests that $[H^+]_{in}$ is the rate-limiting factor for phosphorylation even at phosphorylation rates in excess of 1000 μmol h^{-1} (mg of chlorophyll)$^{-1}$. Although it is not possible to calculate a v_{max} for cyclic phosphorylation since the rate of electron flow has not been measured, the v_{max} for cyclic phosphorylation is likely to be much higher than that for noncyclic phosphorylation. Thus, a rate of 1000 could still be far from the v_{max} for cyclic phosphorylation.

The relationship (eq 3) between $(P/e)_{obsd}$ and $[H^+]_{in}$ should also hold true in the presence of an uncoupler. Since, however, uncouplers increase H^+ permeability of thylakoids, k_{H^+} would vary with uncoupler concentrations. This constant would, therefore, have to be determined at each uncoupler concentration. This may be done by estimating $[H^+]_{in}$ and R_e at several light intensities and plotting $[H^+]_{in}$ as a function of R_e since the slopes of the lines are a measure of k_{H^+}. It would be a laborious task to determine k_{H^+} as well as the rates of phosphorylation, electron flow, and the magnitude of ΔpH at several different uncoupler concentrations. However, for a given concentration of gramicidin D, the P/e ratio observed

was found to correspond closely with that calculated from eq 3.

Thus a simple chemiosmotic model provides a rational framework for quantitative as well as qualitative aspects of the coupling of phosphorylation to electron flow. This model accounts for the decrease in the *P/e* ratios at low light intensity or at high concentrations of energy-transfer inhibitors, and probably by uncouplers as well. In partially uncoupled thylakoids, the enhancement of the rate of electron flow may be mostly lost even though phosphorylation may still occur at appreciable rates. This phenomenon, which has been difficult to explain, is called "loose coupling". Loose coupling may also be explained in terms of the model presented here. As a result of partial uncoupling, the basal electron flow would be a much higher percentage of the overall rate of electron flow. Yet, $[H^+]_{in}$ may not be sufficiently reduced to abolish phosphorylation.

The finding that the intrinsic P/e_2 ratio in thylakoids is likely to be 1.33 deserves special mention. First, if the ratio is the same in chloroplasts in situ, insufficient ATP would be formed by phosphorylation coupled to the reduction of $NADP^+$ to allow CO_2 fixation via the Calvin–Benson pathway. Egneus et al. (1975) proposed that coupled electron flow to oxygen could provide the needed extra ATP. Second, P/e_2 ratios of greater than 1.5 have been observed in carefully prepared intact chloroplasts lysed just prior to assay. P/e_2 ratios greater than 1.33 are clearly inconsistent with an H^+/e of 2 and an H^+/P of 3, estimated in thylakoids. However, Mitchell (1975) suggested that electron flow through the cytochrome *b*–plastoquinone region of the electron transport chain might result in the inward translocation of 2 H^+ per electron. It could be imagined that the ability of isolated chloroplasts to carry out Mitchell's quinone cycle is readily lost upon exposure of thylakoids to an artificial external medium. Thylakoids would, then, translocate H^+ only via a loop-type mechanism and only 1 H^+ per electron would be taken up.

Finally, our proposal that the H^+/P ratio in thylakoids is 3 is supported not only by the H^+/e and P/e ratios, but also by preliminary work, suggesting that 3 H^+ must be translocated down the pH differential to satisfy the maximum energy demands of photosynthetic phosphorylation. In mitochondria, the H^+/P ratio is likely to be 2 (Mitchell, 1976; Thayer and Hinkle, 1973). We find it somewhat disquieting that the H^+/P ratios for the chloroplast and mitochondrial ATPases may differ, especially in view of the close similarities between these two enzymes.

Acknowledgment

We thank Mr. James Davenport for his conceptual and experimental contributions.

References

Egneus, H., Heber, U., Mathieson, U., and Kirk, M. (1975), *Biochim. Biophys. Acta 408,* 252.
Fowler, C. F., and Kok, B. (1974), *Biochim. Biophys. Acta 351,* 299.
Hill, A. V. (1910), *J. Physiol. (London) 40,* 4.
Izawa, S., and Good, N. E. (1968), *Biochim. Biophys. Acta 162,* 380.
Jagendorf, A. T., and Uribe, E. (1966), *Proc. Natl. Acad. Sci. U.S.A. 65,* 170.
Junge, W., and Ausländer, W. (1974), *Biochim. Biophys. Acta 333,* 59.
McCarty, R. E., Guillory, R. J., and Racker, E. (1965), *J. Biol. Chem. 240,* 4822.
McCarty, R. E., and Racker, E. (1967), *J. Biol. Chem. 242,* 3435.
Mitchell, P. (1966), *Biol. Rev. Cambridge Philos. Soc. 41,* 445.
Mitchell, P. (1975), *FEBS Lett. 59,* 137.
Mitchell, P. (1976), *Biochem. Soc. Trans. 4,* 399.
Neumann, J., and Jagendorf, A. T. (1964), *Arch. Biochem. Biophys. 107,* 107.
Pick, U., and Avron, M. (1973), *FEBS Lett. 32,* 91.
Portis, A. R., Jr., Magnusson, R. P., and McCarty, R. E. (1975), *Biochem. Biophys. Res. Commun. 64,* 877.
Portis, A. R., Jr., and McCarty, R. E. (1973), *Arch. Biochem. Biophys. 156,* 621.
Portis, A. R., Jr., and McCarty, R. E. (1974), *J. Biol. Chem. 249,* 6250.
Portis, A. R., Jr., and McCarty, R. E. (1976), *J. Biol. Chem. 251,* 1610.
Reeves, S. G., and Hall, D. O. (1973), *Biochem. Biophys. Acta 314,* 68.
Rottenberg, H., Grunwald, T., and Avron, M. (1972), *Eur. J. Biochem. 25,* 64.
Rumberg, B., and Siggel, U. (1969), *Naturwissenshaften 56,* 130.
Sakurai, M., Nishimura, M., and Takamiya, A. (1965), *Plant Cell Physiol. 6,* 309.
Saha, S., Izawa, S., and Good, N. E. (1970), *Biochim. Biophys. Acta 223,* 158.
Schröder, H., Muhle, H., and Rumberg, B. (1971), *Proc. Int. Congr. Photosynth. Res., 2nd,* 919.
Schuldiner, S., Rottenberg, H., and Avron, M. (1972), *Eur. J. Biochem. 25,* 64.
Stiehl, H. H., and Witt, H. T. (1969), *Z. Naturforsch. B 24,* 1588.
Thayer, W. S., and Hinkle, P. C. (1973), *J. Biol. Chem. 248,* 5395.
Winget, G. D., Izawa, S., and Good, N. E. (1969), *Biochemistry 8,* 2067.