Evaluation of Electron Transport as the Basis of Adenosine Triphosphate Synthesis after Acid–Base Transition by Spinach Chloroplasts*

C. D. Miles† and A. T. Jagendorf

ABSTRACT: Adenosine triphosphate synthesis in chloroplasts driven by a pH gradient, whether formed by an acid–base transition in the dark or by a light–dark treatment, has been assumed not to involve electron transport. Recent data show that added redox dyes are affected by such pH transitions, suggesting that some electron transport may result from a pH transition and raising the possibility that adenosine triphosphate formation is driven by this sort of electron flow. We have studied the effect of electron transport inhibitors, and of conditions affecting the redox state of the electron transport chain, on acid–base adenosine triphosphate synthesis in order to evalutate this possibility. Chemical inhibitors, removal of chloride, and addition of reducing reagents whether under aerobic or anaerobic conditions all had profound effects on electron transport as measured by means of a modified Mehler reaction, but had no effect on acid–base adenosine triphosphate synthesis. Prior exposure to oxidizing conditions also had no effect on this adenosine biphosphate synthesis. We conclude that electron transport through the known electron transport chain including the coupling site (s) between photosystems I and II cannot be important for adenosine triphosphate synthesis driven by a pH gradient.

Following a brief preillumination period (Hind and Jagendorf, 1963) or after a transition from acid to base (Jagendorf and Uribe, 1966) spinach chloroplast thylakoid discs are able to synthesize some ATP in the dark. To a large extent it was presumed that these treatments are not able to invoke a sufficient volume of dark electron transport to account for the amount of ATP that is formed. On the basis of this assumption, first an unknown high-energy intermediate (Hind and and Jagendorf, 1963), and later a hydrogen ion electrochemical activity gradient (Jagendorf and Hind, 1963) was postulated to be the stored form of energy which supports phosphorylation in these experiments.

The assumption of the absence of electron transport following an acid–base transition was severely challenged recently by Lynn (1968) who was able to observe the reduction of a small amount of added cytochrome *c*, and apparent oxidation of bound neutral red, following an acid–base transition. These changes of the dyes were ascribed to the effect of varying the pH on the oxidation–reduction potential of internal components and of the added dyes. It has always been likely that endogenous chloroplast electron carriers might change their redox potentials due to alterations in pH, and indeed the recent data of Cramer and Butler (1969) showed that the unknown carrier "Q" (identified by its ability to quench the fluorescence of chlorophyll) has a redox potential which becomes more oxidized as the pH is lowered.

In view of these data serious consideration has to be given to the possibility suggested by Lynn (1968), that phosphorylation when driven by a proton concentration difference is really the result of electron flow through as yet unidentified carriers. However, the data presented so far have not been sufficient to show whether (a) net electron flow occurs through entirely internal chloroplast components in the absence of added neutral red or cytochrome *c*, (b) whether any such flow does generate some ATP, or (c) whether a sufficient volume of electron flow results to account for the total amount of ATP that can be formed.

In order to test this concept further we have applied a variety of inhibitors of electron transport to chloroplasts; demonstrated their effect on observed redox reactions and compared it with their effect on the phosphorylation after an acid–base transition. Attempts have also been made to "clog" the electron transport chain by an excess of internal reducing power, or to drain off electrons by prior oxidizing conditions.

Methods

Chloroplast fragments were prepared from the leaves of market spinach as indicated previously (Miles and Jagendorf, 1969) and resuspended in 10 mM NaCl. Chloroplast preparations were depleted of chloride by washing either with 10 mM KNO_3, or else with 0.4 M sucrose–30 mM $MgSO_4$ at pH 7.4 (Hind *et al.*, 1969) at 10,000*g* for 10 min. Chlorophyll was determined by the method of Arnon (1949). Acid–base-induced phosphorylation reactions were carried out following a procedure very similar to that of Jagendorf and Uribe (1966). Esterfied [^{32}P]P_i was measured using the procedure of Avron (1960). Light intensities used in these experiments were measured with YSI-Kettering Model 65 radiometer.

Electron transport in chloroplasts was measured by following oxygen uptake in the presence of the autooxidizable dye, methyl viologen. The procedure was similar to that of Izawa *et al.* (1966). Oxygen uptake was determined with a Yellow Springs 4044 Clark-type electrode which was protected

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† Present address: Department of Botany, University of Missouri, Columbia, Missouri 65201.

TABLE I: Effect of Electron Transport Inhibitors on Photophosphorylation and Acid–Base ATP Formation in Chloroplasts.[a]

Inhibitor	Concn for 20% Inhibition of Acid–Base ATP Synthesis (μM)	Concn for 50% Inhibition of Photophosphorylation (μM)	Reference
DCMU	100	0.03	(Izawa *et al.*, 1966)
CMU	100	3.00	(Jagendorf and Margulies, 1960)
HOQNO	10	0.60	(Avron, 1961)
BDHB	50	50.00	(Avron and Shavit, 1965)
Antimycin A	500	15.00	(Izawa and Good, 1968)
Simazine	10	0.40	(Bishop, 1962)
o-Phenanthroline	100	30.00	(Yamashita and Horio, 1968)

[a] The procedure for the acid–base reaction was as follows: (250 μg of chlorophyll) chloroplasts were injected into 10 mM succinate (pH 3.8) containing the inhibitors. After 20 sec the contents of the acid stage was injected into a second tube containing (in μmoles); tricine–NaOH, 100; ADP, 0.2; P_i, 2.0; $MgCl_2$, 5.0; and 5×10^5 cpm of [^{32}P]P_i; and water to 0.9-ml total volume. The pH after acid stage addition was 8.4. The phosphorylation reaction was stopped after 15 sec by injecting 0.2 ml of 20% trichloroacetic acid. The reactions were performed at 4° in the dark. Inhibitor concentrations are shown for the final volume of 1.8 ml, in the acid–base experiments. The inhibitor concentrations for photophosphorylation are taken from the literature; references are indicated in parentheses.

TABLE II: Effects of Electron Transport Inhibitors on Acid–Base-Induced ATP Synthesis.[a]

No.	Reaction Conditions (μM)	nmoles of ATP/mg of Chlorophyll	% of Control
1	Standard acid–base reaction, no inhibitor	97	
2	No shift in pH, always 8.5, no inhibitor	7	8
3	DCMU (1.4) in acid stage	91	94
4	DCMU (1.4) in base stage	84	87
5	BDHB (50) in acid stage	74	76
6	BDHB (50) in base stage	77	80
7	Standard acid–base reaction, no inhibitor	75	
8	Antimycin A (10) for 1 min[b]	84	111
9	Antimycin A (10) for 10 min[b]	91	120
10	Antimycin A (10) for 30 min[b]	109	144
11	Antimycin A (10) for 80 min[b]	117	155
12	Standard acid–base reaction, no inhibitor	81	
13	DCMU[c] (1)	89	110
14	Antimycin A[c] (10)	96	119
15	HOQNO[c] (10)	96	119
16	DCMU (1) and antimycin A[c] (10)	97	119
17	DCMU (1) and HOQNO[c] (10)	97	120
18	HOQNO (10) and antimycin A[c] (10)	96	119
19	DCMU (1) and HOQNO (10) and antimycin A[c] (10)	84	104

[a] Acid–base reaction as in Table I. [b] Incubated with chloroplasts for time indicated, before reaction. [c] Incubated with chloroplasts for 30 min before reaction.

from direct light. The reaction cell was illuminated with 713-nm light provided by Baird-Atomic narrow band-width interference filter and a Sylvania Sungun. The light intensity used in most experiments was 4×10^5 ergs/cm^2 per sec. The reaction mixture was maintained at a constant temperature by a circulating water bath and the temperature of the reaction mixture was always monitored with a Tri-R Instruments Model TGB electronic thermometer. Temperature of the reaction mixture and oxygen uptake were recorded simultaneously on a Mosley 7100 B strip chart recorder, for at least 2 min.

Anaerobic conditions were provided by conducting the reactions in Warburg vessels under an argon atmosphere. Oxygen was removed from the solution by addition of glucose and glucose oxidase before chloroplasts were mixed with acid, etc. The reaction mixtures were preincubated for 5 min during which time the vessel was flushed with argon. According to measurements with the Clark electrode, all (over 99%) of the oxygen was removed by this procedure.

DCMU[1] and CMU were supplied by the DuPont Company and HOQNO was the gift of Dr. J. W. Lightbown, National Institute for Medical Research, Mill Hill, London.

Results

Electron Transport Inhibitors. Several known inhibitors of electron transport in chloroplasts, including CMU, DCMU, BDHB, HOQNO, Simazine, and antimycin A were tested for their effect on acid–base-induced phosphorylation. Most inhibitors have very little effect; in the case of CMU and *o*-phenanthroline even a 20% inhibition was never achieved. Table I compares the concentration needed for 20% inhibition of acid–base phosphorylation, with that needed for 50% in-

[1] Abbreviations used are: DCMU, 3-(3,4-dichlorophenol)-1,1-dimethylurea; CMU, 3-(*p*-chlorophenol)-1,1-dimethylurea; HOQNO, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide; BDHB, *n*-butyl 3,5-diiodo-4-hydroxybenzoate; Tricine, tris(hydroxymethyl)methylglycine; DCPIP, 2,6-dichlorophenolindophenol; Simazine, 2-chloro-4,6-bis(ethylamino)-*s*-triazine.

TABLE III: Inhibition of Electron Transport in Spinach Chloroplasts.[a]

Inhibitor	Concn (μM)	Oxygen Uptake[b]	Inhibition (%)
Experiment 1			
Simazine	10	36	92
CMU	10	50	89
BDHB	10	86	82
o-Phenanthroline	30	217	53
Experiment 2			
DCMU	2	31	95
HOQNO	10	202	68

[a] Reaction mixture contained in millimolar concentrations; tricine, 50; $MgCl_2$, 2.5; KH_2PO_4, 1.0; ADP, 0.1; methyl viologen 0.1; NaN_3, 0.5; and 1.0 mg of chlorophyll in a total volume of 5 ml at pH 8.0. The reaction was illuminated with 713-nm light at 4×10^5 ergs/cm^2 — 1 sec. [b] *n* equiv of electrons/mg of chlorophyll × min. Control values were 470 in expt 1, and 630 in expt 2.

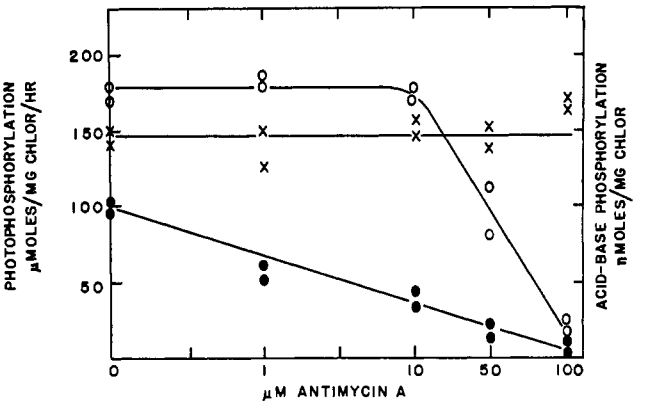

FIGURE 1: Effect of antimycin A on photophosphorylation with 0.1 mM methyl viologen (○); with 0.4 mM $K_3Fe(CN)_6$ (●); or on acid–base-induced phosphorylation (×). The photophosphorylation mixture contained 50 mM tricine-Na (pH 8.0), 5.0 mM $MgCl_2$, 2.0 mM phosphate, 5×10^5 cpm of [^{32}P]P_i, 2 μM ADP, and 250 μg of chlorophyll. The reaction was illuminated for 2 min at a red light (Corning 2418 red filter) intensity of 4×10^5 ergs cm^{-2} sec^{-1} at 23°. Acid–base phosphorylation reaction as in Table I.

hibition of photophosphorylation in the light. It is apparent that CMU, DCMU, HOQNO, *o*-phenanthroline, Simazine, and antimycin A have hardly any effect on the acid bath phosphorylation even at concentrations orders of magnitude higher than those effective on photophosphorylation. Only BDHB has anything like comparable effects in the two systems; however, this compound seems to be an uncoupler in addition to an electron transport inhibitor (Gromet-Elhanen and Avron, 1965).

Table II illustrates the effect of different modes of addition of the inhibitors. The same inhibition was obtained if the compound was added in the base stage only, or in the acid stage (compare numbers 1–6). Preincubation of the chloroplasts together with the inhibitor for 30 min at 4° before the acid stage begins, actually increased the yield of ATP (Table II, 12–15) and the degree of stimulation increased with time (7–11).

In an effort to block all electron transport, phosphorylating or nonphosphorylating (Izawa and Good, 1968), combinations of inhibitors were used. Antimycin A, HOQNO, and DCMU were chosen as affecting three different sites in electron (Hind and Olson, 1966). Combinations of any two of these, or indeed all three added together, failed to cause detectable inhibition of ATP formation (Table II, 12–19).

In order to be certain that these compounds were effective inhibitors of the particular chloroplasts used, they were tested on the Mehler type of reaction involving net oxygen uptake in the presence of methyl viologen, with azide added to prevent the breakdown of hydrogen peroxide. As expected, severe inhibition of electron transport could be shown (Table III) at concentrations which have little or no effect on acid–base-induced phosphorylation. The inhibitor antimycin A may be particularly critical because it has been suggested to work specifically on the phosphorylating pathway of electron transport, and not on the nonphosphorylating pathway (Izawa and Good, 1968). As can be seen from Figure 1, strong inhibitions of photophosphorylation accompanying either cyclic (with pyocyanine) or noncyclic (with ferricyanide) electron transport can be demonstrated, at concentrations that have no effect on the acid–base-induced phosphorylation.

Table IV shows inhibition of electron transport either by partial chloride depletion (chloroplasts washed in KNO_3, 10 mM) which is supposed to inhibit on the water side of the system II (Hind *et al.*, 1969), or by DCMU which has been localized as acting on electron transport after the system II complex (Duysens and Sweers, 1963). Similar results were obtained using chloroplasts depleted of chloride by the procedure of Hind *et al.* (1969). Oxygen uptake was severely inhibited by DCMU addition, chloride deficiency, or the combination of these two; then restored and considerably stimulated by adding ascorbate-reduced DCPIP to bypass these two blocks. None of these conditions or reagents had any effect on acid–base ATP synthesis. If an electron transport pathway responsible for ATP synthesis is operating in the dark it would have to be a different one than that operating in the light.

Capacity and Redox State of the Electron Transport Chain. For electron transport to support phosphorylation in the dark, a sufficient number of mobile electrons and holes both must be present in the chain (see Discussion). Attempts were made to remove any mobile electrons from the chloroplast chain prior to their use in an acid–base ATP experiment. Although ferricyanide added to the acid stage does cause inhibition, so does ferrocyanide; these effects are currently being traced to an alteration in the terminal ATPase enzyme by a combination of acid pH and polyanions (G. Polya, unpublished data). As an alternative, we illuminated chloroplasts with 713-nm light to evoke system I activity only, in the presence of DCMU to block the use of water as an electron donor, and with either diquat or benzyl viologen present to facilitate transfer of any available electrons to oxygen. As seen in Table V, this oxidizing treatment did not cause any appreciable decrease in the amount of ATP that could be formed when the chloroplasts immediately afterward, were subject to an acid–base transition.

TABLE IV: Effect of Cl^- Deficiency and Electron Donor on Electron Transport and on Acid–Base-Induced ATP Synthesis.

Expt	Reaction Conditions[a]	Electron Transport, 35-sec Light		ATP/mg of Chlorophyll by Acid–Base Transition	
		n equiv/mg of Chlorophyll	Control (%)	nmoles	Control (%)
1	Plus Cl^-	122	100	167	100
	Plus Cl^-, DCMU	25	21	146	87
	Plus Cl^-, DCMU, $DCPIPH_2$	517	423	155	93
2	Minus Cl^-	64	53	153	92
	Minus Cl^-, DCMU	28	23	146	88
	Minus Cl^-, DCMU, $DCPIPH_2$	629	515	157	94

[a] All reactions contained 50 mM tricine, 2.5 mM $MgCl_2$, 1.0 mM KH_2PO_4, 0.1 mM ADP, 0.1 mM methyl viologen, 0.5 mM NaN_3, and chloroplasts containing 0.5 mg of chlorophyll in a total volume of 2.5 ml at pH 8.4. Where added, DCMU was 1.0 μM, DCPIP 0.1 mM, and ascorbate 3.0 mM. Plus chloride indicates chloroplasts prepared and washed in 10 mM NaCl; minus chloride means the chloroplasts were prepared and washed in 10 mM KNO_3. Illumination was with light at 713 nm using an interference filter, at 4×10^5 erg/cm^2 sec. Electron transport was measured as oxygen uptake; acid–base ATP formation was measured as indicated in Table I.

Conversely, some attempts were made to clog the electron transport chain with an excess of electrons. The combination of ascorbate and a relatively high concentration (0.1 mM) of DCPIP has been shown to provide electrons to the chloroplast chain before the phosphorylation site (Avron and Neumann, 1968). This combination had no effect on acid–base ATP synthesis (Table IV) under aerobic conditions. The experiment was repeated under anaerobic conditions. It was previously reported (Jagendorf and Uribe, 1966) that the lack of oxygen did not inhibit ATP synthesis by an acid–base transition. The data of Table VI indicate that there was no inhibition due to added ascorbate–$DCPIPH_2$ even when oxygen was not present. The lack of oxygen requirement was further checked by removing all oxygen from the solutions by a 5-min preincubation with glucose and glucose oxidase. These reactions were carried out in Warburg vessels with argon flushing. The removal of oxygen from the reaction mixture was checked with the Clark oxygen electrode; all (99%) was removed in 1.5 min when 6 mM glucose and 30 μg of glucose oxidase were added. When 3 mM glucose and 15 μg of enzyme were added all oxygen was removed in 3.5 min. ATP synthesis driven by an acid–base transition under this anaerobic condition is shown in Table VII. There was no increased inhibition when enzyme and substrate were added, over the amount of inhibition by glucose itself.

It coupled electron transport occurs in chloroplasts after an acid–base transition in the presence of DCMU, then it should certainly be affected one way or the other by simultaneous illumination. In the acid–base transition experiment DCMU is always present, hence light should serve to oxidize the chain by draining electrons through system I and out to oxygen. If a reservoir of internal electrons is the substrate for electron transport following the acid–base transition, this oxidizing effect of light might possibly enhance ATP yields.

TABLE V: Effects of Electron Acceptors and Light on Acid–Base ATP Synthesis.[a]

Reaction	nmoles of ATP/mg of Chlorophyll	Control (%)
Standard acid–base reaction	99	100
No pH shift	2	2
+ Benzyl viologen ($E_0' -0.327$)	100	100
+ Benzyl viologen, light for 30 sec	97	98
+ Diquat ($E_0' -0.350$)	110	111
+ Diquat, light for 30 sec	103	103

[a] Benzyl viologen and diquat were 0.5 mM, and 713-nm light was at 4×10^5 ergs/cm^2 sec. Acid–base reaction was as indicated in Table I. DCMU was present throughout at 1.0 μM.

TABLE VI: Effect of $DCPIPH_2$ on Acid–Base Phosphorylation with or without Oxygen.[a]

Phosphorylation Reaction	Atmosphere	nmoles of ATP/mg of Chlorophylll
No pH shift	Air	6
Acid–base shift	Air	107
Acid–base shift	N_2	107
Acid–base shift + 1 mM $DCPIPH_2$	Air	102
Acid–base shift + 1 mM $DCPIPH_2$	N_2	96

[a] Acid–base reaction were carried out as indicated in Table I. DCMU was 1 μM.

However, absolutely no effect of red light could be found on the acid–base ATP formation, even at intensities as high as 2.5×10^6 erg/cm^2 sec, applied during the base (phosphorylation) stage.

Discussion

For coupled electron transport to occur without the input of light energy there must be a pool of reduced carriers, a pool of oxidized acceptors, and an electron transport chain connecting the two in which at least one step is coupled to phosphorylation. In the case of a hypothetical system operating during and after the acid–base transition, the redox potentials of the two pools would have to behave like a "seesaw" with a change of direction when in acid and a reversion to the original in the base or phosphorylation stage. Mobile electrons would flow first one way in acid (in the direction normally uphill), then back to the normal position in the base stage, creating some ATP in the process.

A critical piece of evidence in favor or this model would be finding at least one inhibitor of electron transport, not having additional uncoupling powers, which blocks ATP synthesis following an acid–base transition. We have tested inhibitors known to act fairly specifically on electron transport, shown their marked effect on electron flow (Table III) and on photophosphorylation (Figure 1), and have seen that they are essentially without effect on acid–base phosphorylation (Tables I, II, IV). These data argue strongly against a requirement for electron transport in acid–base phosphorylation, unless by some chance the electron carrier sequence which is involved does not include the particular inhibitor-sensitive sites. We consider this to be rather unlikely, since inhibitors without any effect include HOQNO, antimycin A, and DCMU. Antimycin A has a locus of action on the phosphorylating electron transport chain (Izawa *et al.*, 1966), probably between cytochrome b_{559} and cytochrome *f* (Hind and Olson, 1966). DCMU inhibits the chain between the two light reactions, probably only one step removed from photoreaction II (*i.e.*, right after "Q") (Duysens and Sweers, 1963), and HOQNO inhibits cytochrome reactions both on the phosphorylating and on the nonphosphorylating pathway in chloroplasts (Izawa *et al.*, 1966). It is not likely that a significant length of the chain, containing a phosphorylation step in touch with two fairly large pools, would remain unaffected by the three reagents together.

Additional evidence is found in the recent important discovery by Anderson and McCarty (1969) that acid–base phosphorylation is possible in chloroplasts completely depleted of their plastocyanin.

These data represent critical evidence against the involvement of electron flow at the specific parts of the chain affected. They do not rule out electron transport completely, but rather restrict the areas in which it is possible to speculate that the "unidentified carriers" (Lynn, 1968) might occur.

Acid–base phosphorylation can occur under anaerobic conditions (this work, and Jagendorf and Uribe, 1966). Any "seesaw" electron transport must be between an ample pool of reducing equivalents and an equal pool of oxidizing equivalents (or "holes"), not oxygen. Measurements of pool sizes—*i.e.*, number of mobile electrons, number of available holes, or total redox equivalents available–are therefore able to provide critical data concerning this concept.

Using biochemical and biophysical techniques, pool sizes of chloroplast electron transfer components have been estimated in recent years. Aside from P700, the cytochromes, and plastocyanin, all of which are present on the order of one or two per 400 chlorophyll molecules, major pool sizes are indicated immediately after each photo act. After photoreaction II there exists a primary acceptor (Joliot's "E" or Duysens' "Q") (Joliot, 1961; Duysens and Sweers, 1963) present in small amount, together with a larger pool of acceptor molecules ("A" or "P," respectively). The combined abundance of both acceptor pools has been found to be about 1 per 35 chlorophylls (using maximum estimates), or 12 per 400 chlorophylls (Kok *et al.*, 1966). After photosystem I, a recently discovered entity, designated "CRS" for "cytochrome-reducing substance," is estimated to be present at the level of 1 for every 10 chlorophylls, or 40 per 400 chlorophylls (Fujita and Murano, 1966). The relation of these physiologically defined components to the extractable plastoquinine A is not clear; half or more of the plastoquinine may be in nonfunctional lipid droplets (Friend *et al.*, 1966) and the active component is likely to constitute the "A" or "P" pools noted above. In order to be overgenerous one might consider all of the plastoquinine A to be active (and encompassing the "A" pools, etc.), and therefore providing 1 equiv for every 10 chlorophylls, or another 40 molecules per 400 chlorophylls.

Thus the absolute maximum number of mobile electrons, and of available holes (assuming equal distribution between the major pools) would be 40–52 per 400 chlorophylls; and this is probably unrealistically high. The number of phosphorylating sites in the chloroplast chain is not known with certainty, but most estimates do not rise above two (Izawa and Good, 1968). Of these, at least one must be unused in the acid–base experiments, as indicated by the failure of antimycin A to inhibit (Figure 1). Thus the 40–52 mobile electrons would traverse at most one (unspecified) phosphorylation site, and at a normal ratio of two electrons per anhydro bond formed, would result in 20–26 ATP molecules synthetized per 400 chlorophylls. This contrasts with the formation of 40 molecules of ATP per 400 chlorophyll molecules as a rather low value, to 80–100 ATPs

TABLE VII: Effects of Anaerobic Conditions on Acid–Base-Induced ATP Synthesis.[a]

Phosphorylation Reaction	Addition	ATP Formed (nmoles)/ Chloro- (phyll mg)
No pH shift		8
Acid–base pH shift		139
Acid–base pH shift	6 mM glucose	51
Acid–base pH shift	30 μg of glucose oxidase	112
Acid–base pH shift	6 mM glucose + 30 μg of glucose oxidase	50

[a] Acid–base reactions were carried out as indicated in Table I. The reaction mixture was preincubated with the indicated addition for 5 min while the reaction vessel was flushed with argon. DCMU was at 1 μM.

per 400 chlorophylls as a maximum, in daily acid–base ATP experiments. On these stoichiometric grounds the use of electron transport as the transducer between a hydrogen ion gradient and ATP formation in the acid–base experiments can largely be ruled out.

Perhaps the most concrete evidence that we have obtained indicating the absence of a seesaw electron flow, is the absence of any effect on ATP formation of reagents or conditions which will effect the overall redox balance within the chain. In the seesaw model there must be a limited number of available mobile electrons and of available holes, both in the acid and in the base stages. Draining off some of these electrons *via* an oxidizing pool, or clogging the system with a glut of electrons from an external reductant, would surely be an easy way to inhibit the volume of electron transport that could occur and therefor the amount of ATP that would be formed. No such inhibition could be observed, whether system I light was used as oxidant (in the presence of DCMU, and of benzyl viologen to carry electrons to oxygen) prior to the phosphorylation (Table V), whether DCPIP reduced by ascorbate was used as reductant (Table VI) either in air or under nitrogen, or whether light was used to speed electron transport during phosphorylation.

Taken together, the theoretical arguments and our present experimental data argue strongly against Lynn's suggestion (1968) that electron transport intervenes between the acid–base induced pH gradient and the consequent phosphorylation. The observation that some electron transport is induced by the acid–base transition is of intense interest in its own right; but we feel at this point that the burden of proof must be on those who propose a major role for this electron transport in ATP formation.

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