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ANTIMYCIN A AS AN UNCOUPLER AND ELECTRON TRANSPORT INHIBITOR IN PHOTOREACTIONS OF CHLOROPLASTS

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SUMMARY

1. The effect of antimycin A on photoreactions of isolated chloroplasts in the μM range was shown to depend strongly on chloroplast concentration, pH of reaction and duration of preincubation.

2. Antimycin A was shown to be an uncoupler of photophosphorylation and an inhibitor of electron transport. The latter activity sets in at lower concentrations, but both activities overlap to a considerable extent.

3. The inhibition of electron transport by antimycin A is potentiated by high pH and by the presence of uncouplers.

4. The uncoupling activity of antimycin A was manifested by inhibiting ATP synthesis (noncyclic and cyclic), stimulation of electron flow and stimulation of the dark decay of the proton pump.

5. The two effects of antimycin A can be separated by the addition of serum albumin, which presumably removes part of the inhibitor from the reaction mixture.

INTRODUCTION

Antimycin A is a well-known inhibitor of electron transport in oxidative phosphorylation whose site of inhibition is generally considered to be located between cytochrome *b* and *c* (ref. 1). Low concentrations of this compound were shown to inhibit endogenous cyclic phosphorylation in bacterial chromatophores[2]. The effect of antimycin A on isolated chloroplasts, however, is not clear. WHATLEY *et al.*[3] found no effect on phosphorylation supported by FMN or vitamin K in the μM concentration range. But later it was shown by BALTSCHEFFSKY[4] that antimycin A in slightly higher concentrations did inhibit cyclic phosphorylation. BAMBERGER *et al.*[5] reported that antimycin A, at 0.1 mM, inhibits ATP formation coupled to $NADP^+$ reduction, without inhibiting the latter.

TAGAWA *et al.*[6] have shown that 10 μM antimycin A inhibited strongly ferredoxin cyclic phosphorylation, claiming that this inhibition is specific for the above-mentioned reaction. Several reactions in whole algae, which are supposedly dependent on cyclic endogenous phosphorylation, were also shown to be inhibited by this

Abbreviations: PMS, phenazine methosulfate; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

compound[7–9]. On the other hand, IZAWA *et al.*[10] have recently found, that, at low light intensity, any type of phosphorylation is inhibited. The latter authors envisage antimycin A as an inhibitor of electron transport which does not affect the basal electron flow. This interpretation was adopted by HIND AND OLSON[11], who found that antimycin A inhibited the photoreduction of cytochrome *f* but did not inhibit the photoreduction or photooxidation of cytochrome b_6.

We reinvestigated the effect of antimycin A on photoreactions of isolated chloroplasts and found it to inhibit electron flow in the μM range, whereas at somewhat higher concentration (in the 10 μM range), it exhibits uncoupling activity as well. Both effects strongly depend upon reaction conditions.

MATERIALS AND METHODS

Chloroplasts were prepared from lettuce (*Lactuce sativa* var. romaine). 30 g of leaves were blended in a 220-V Waring Blendor for 20 sec at 120 V, in 100 ml of medium containing 0.4 M sucrose, 0.01 M NaCl, 0.01 M Tris, 0.05 M ascorbate and 0.1 % human serum albumin. The pH of this mixture was brought to 8.0. The homogenate was filtered through gauze and was centrifuged at low speed. The chloroplast pellet was then collected by centrifuging for 7 min at 1500 × *g*, suspended in 5 mM Tris–0.01 % human serum albumin (pH 8.0), centrifuged again for 7 min at 12000 × *g* and resuspended finally in 0.4 M sucrose, 0.01 M Tris (pH 8.0), 0.01 M NaCl, 0.7 % serum albumin, at a chlorophyll concentration of approx. 0.5 mg/ml. The beneficial effect of serum albumin was reported recently in detail[12]. In several experiments (stated in the text), tris(hydroxymethyl)methylglycine (Tricine) buffer was used instead of Tris.

Chlorophyll was determined according to the procedure of ARNON[13]. Ferricyanide reduction was measured either by loss of absorption at 420 nm of the deproteinized solution, by measuring the appearance of ferrocyanide[14] or by continuous recording of the decrease in absorbance with a Cary 15 spectrophotometer as previously described[12]. Occasionally it was also tested by following O_2 evolution with an oxygen electrode. $NADP^+$ photoreduction was followed by measuring increase of absorbance at 340 nm. Ferredoxin was prepared and purified from mangold leaves[15]. Phosphorylation was measured by following ^{32}P incorporation into ATP according to AVRON[16]. Light-induced proton uptake was measured by a radiometer pH electrode connected to a recorder. The pH changes were calibrated by addition of a standard acid to the reaction mixture.

Antimycin A was purchased either from Sigma or from Calbiochem. It was dissolved with ethanol to a concentration of 4 mM. The maximal final concentration of ethanol in the reaction mixture was 1 %. All other reagents were of analytical grade.

RESULTS

The effect of antimycin A on $NADP^+$ photoreduction and its coupled ATP formation is presented in Fig. 1. Both processes are inhibited by this compound; however, with increasing concentrations of antimycin A, ATP formation is inhibited more than electron transport, and P/e_2 (see ref. 17 for notation) is correspondingly reduced.

From the results of Fig. 2A it can be seen that the inhibition of $NADP^+$ photoreduction by antimycin A increases markedly when the concentration of chlorophyll in the reaction mixture is decreased. The differential effect of antimycin A on electron transport and on ATP formation may be seen in Fig. 2B. At the lower concentration

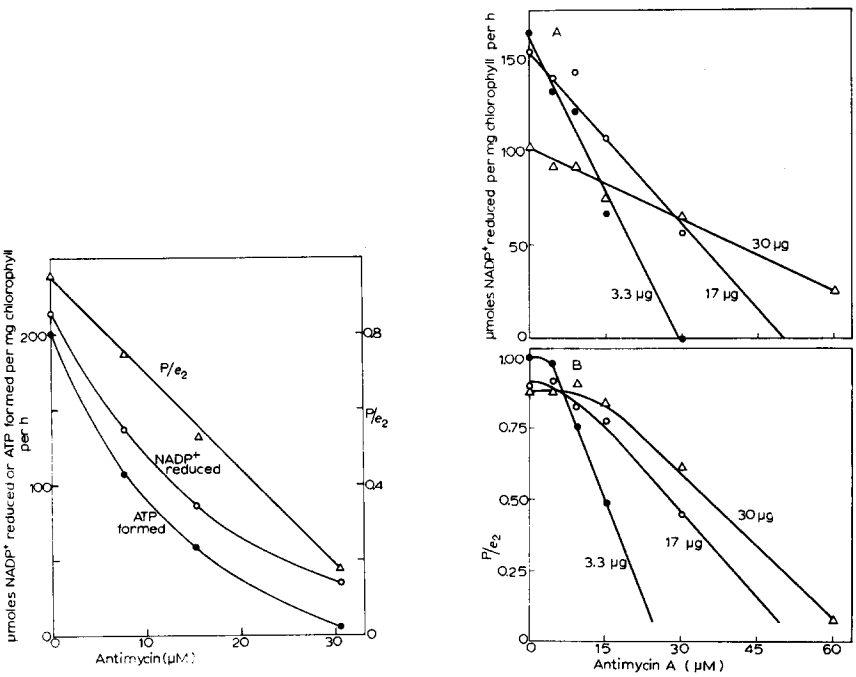


Fig. 1. Effect of antimycin A on $NADP^+$ photoreduction and its coupled ATP formation. The reaction mixture contained in 3.0 ml the following in µmoles: Tris (pH 8.0), 50; NaCl, 70; P_i, 10; ADP, 3; $NADP^+$, 0.75; and ferredoxin, 0.015. It also contained chloroplasts equivalent to 21 µg chlorophyll. Antimycin A was added prior to illumination at concentrations as specified. The reaction mixture was illuminated for 2 min by a 150-W bulb shielded by a water bath providing 3500 ft candles at the level of the test tubes.

Fig. 2. Effect of antimycin A on $NADP^+$ photoreduction and ATP formation at various chloroplast concentrations. Experimental conditions as in Fig. 1, except that the concentrations of chloroplasts were varied as indicated.

range (7–10 µM) of antimycin A added, the P/e_2 is not affected but electron transport is inhibited (compare with 2A), whereas at somewhat higher concentrations of antimycin A, the P/e_2 is progressively lowered. The decrease of P/e_2 becomes also more severe upon lowering the chlorophyll concentration. We suggest that antimycin A is an electron transport inhibitor and an uncoupler, with both activities overlapping to a considerable extent, but the latter sets in at a slightly higher concentration.

The inhibition of electron transport increases markedly with increase in pH of the reaction mixture (Table I). The decrease in P/e_2 on the other hand, does not show such a dependence. The larger inhibition of $NADP^+$ reduction at high pH might be due to a faster permeation of the inhibitor to its site of action, since by preincubating the reaction mixture at pH 8.0, there is a marked inhibition of $NADP^+$ reduction

TABLE I

EFFECT OF ANTIMYCIN A ON NADP+ REDUCTION AND P/e_2 AS A FUNCTION OF pH

Tris–P_i (each at 17 mM) was titrated to the specified pH. Antimycin A, where indicated, was added at 15 μM, 5 min before the onset of the reaction. The reaction mixture contained chloroplasts equivalent to 18 μg chlorophyll. Otherwise, experimental conditions as in Fig. 1.

pH	*NADP+ reduction*			P/e_2	
	μmoles/mg chlorophyll per h		*% of control*	*−antimycin*	*+antimycin*
	−antimycin	*+antimycin*			
6.5	28	33	118	0.98	0.14
7.0	89	78	87	1.00	0.26
7.5	143	102	71	1.10	0.65
8.0	133	26	20	1.08	0.28
8.5	62	4	6	0.93	0.01
9.0	36	0	0		

TABLE II

EFFECT OF pH DURING PREINCUBATION AND DURING THE REACTION PROPER, ON INHIBITION OF NADP+ REDUCTION BY ANTIMYCIN A

The reaction mixture contained in a total volume of 3.0 ml the following in μmoles: $NADP^+$, 0.5; NaCl, 70; Tris, 50; P_i, 10; and antimycin A where indicated, 0.0225. It also contained a saturating amount of ferredoxin and chloroplasts equivalent to 18 μg chlorophyll. The preincubation was performed for 5 min. Otherwise experimental conditions as in Fig. 1.

pH of preincubation	*pH of experiment*	*NADP+ photoreduction (μmoles NADP+ reduced per mg chlorophyll per h)*	
		−antimycin	*+antimycin (7.5 μM)*
8	8	147	51
8	7	59	36
7	7	57	57
7	8	135	120

even at pH 7.0, whereas without such a preincubation there is none (Table II). The inhibition by antimycin A is a time-dependent process. It increases with increasing time of preincubation of the chloroplasts with it (Fig. 3).

Antimycin A inhibited electron transport more severely in the presence of uncouplers or after treatment with EDTA (Table III).

The effect of antimycin A on ferricyanide reduction and on the accompanying ATP formation is described in Fig. 4. At pH 8.5, both in de presence and absence of phosphorylating reagents, ferricyanide reduction is inhibited. At pH 7.5 antimycin A behaves as a typical uncoupler, inhibiting ATP formation while stimulating electron transport.

The pH optimum of "uncoupled" electron flow is known to shift toward a more acidic pH (ref. 17). This is clearly the case with antimycin A as well (Fig. 5). The crossover point for the basal electron flow curve and the "uncoupled one" in the presence of 30 μM antimycin A is at pH 7.8.

The effect of antimycin A on cyclic ATP formation is shown in Fig. 6. 50%

inhibition with phenazine methosulfate (PMS) is obtained at 4 μM antimycin A. ATP formation with 2,6-dichlorophenolindophenol (DCIP)–ascorbate is also inhibited while electron transport from $DCIPH_2$ to $NADP^+$ is slightly stimulated. The rate of ATP formation and the inhibition by antimycin A do not depend on the presence of $NADP^+$ (data not shown), indicating that even in the presence of the acceptor, the electron flow is of the cyclic type.

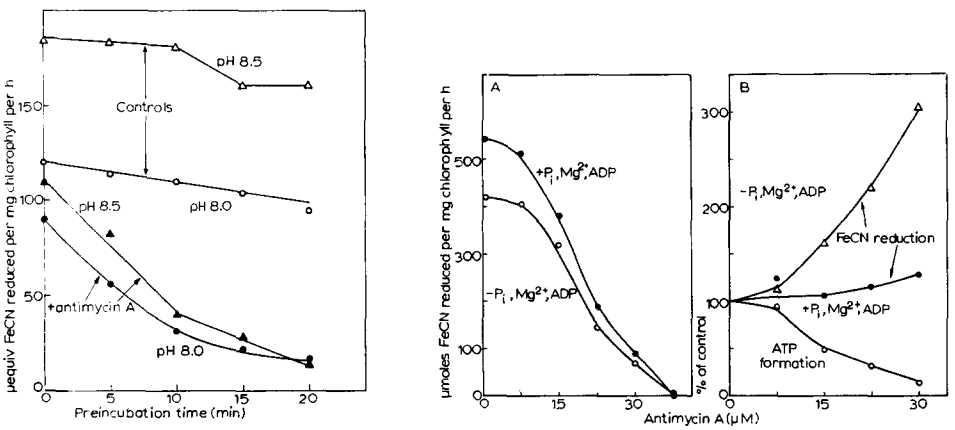


Fig. 3. Time course of inhibition by antimycin A. The reaction mixture contained in 3.0 ml the following in μmoles: Tricine (pH 8.5), 50, or Tris (pH 8.0), 50; NaCl, 70; $NADP^+$, 0.75; and ferredoxin, 0.015. It also contained chloroplasts equivalent to 20 μg chlorophyll and antimycin A at 22 μM where indicated, added at different time intervals before the onset of reaction. Otherwise experimental conditions as in Fig. 1.

Fig. 4. Effect of antimycin A on ferricyanide reduction and ATP formation. A. The reaction mixture contained in 3.0 ml the following in μmoles: Tricine (pH 8.5), 50; NaCl, 70; ferricyanide, 1.8; and when indicated, also $MgCl_2$, 5; ADP, 3; and P_i, 10. In addition it contained chloroplasts equivalent to 36 μg chlorophyll. Antimycin A was added at concentrations as indicated, 5 min before the onset of the reaction. Ferricyanide was added immediately before turning the light on. Illumination was by white light (providing 3000 ft candles at the level of the vessel). Electron flow was measured by following O_2 evolution with a Clark oxygen electrode attached to a Gilson oxygraph. The rate of the reaction was calculated from the slope of the recorded traces. B. The reaction mixture was the same as in A, except that the Tricine buffer added was at pH 7.5 and when ATP formation was measured, ^{32}P was also included. The test tubes were illuminated for 3 min with white light (providing 3000 ft candles at the level of the test tubes). Ferricyanide reduction was measured by loss of absorption at 420 nm. The activity of the controls, in μmoles/mg chlorophyll per h, were as following: reduction of ferricyanide, 100; reduction of ferricyanide in the presence of phosphorylating reagents, 150; formation of ATP, 75.

Antimycin A was reported to be highly inhibitory to cyclic electron flow of ferredoxin[6]. We observed indeed a somewhat higher susceptibility of this reaction of antimycin A, obtaining 50 % inhibition by 5 μM in the presence of a high concentration of chloroplasts (70 μg chlorophyll per ml). Part of this higher sensitivity is due to the presence of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) in the reaction mixture.

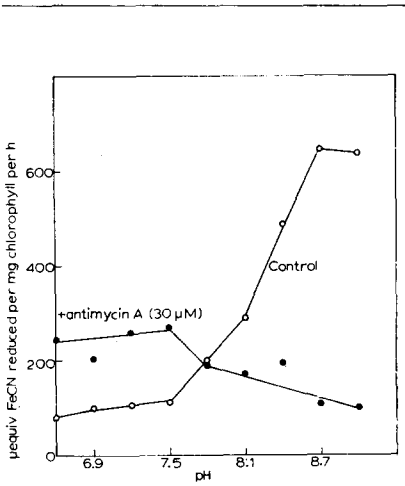
The effect of antimycin A on the light-induced proton uptake is described in Table IV. The extent and the initial rate of formation were inhibited by antimycin A, whereas the dark decay is stimulated. This stimulation of the dark decay can also

TABLE III

EFFECT OF ANTIMYCIN A ON "UNCOUPLED" ELECTRON FLOW

Expt. 1: The reaction mixture contained in 3 ml the following in μmoles: Tris (pH 8.0), 50; NaCl, 70; and ferricyanide, 1.8. It also contained chloroplasts equivalent to 20 μg chlorophyll. Ferricyanide reduction was assessed by measuring O_2 evolution with a Clark oxygen electrode attached to a Gilson oxygraph. The rates of the reaction of the controls and after addition of antimycin A (15 μM) were calculated from the slopes of the recorded traces. *Expt. 2*: Chloroplasts isolated as usual were incubated at a concentration of 30 μg/ml in 0.75 mM EDTA (pH 8) for 20 min at 0°. They were centrifuged for 10 min at 17000 × *g*, resuspended in distilled water, centrifuged, resuspended again in distilled water and centrifuged. They were finally resuspended as described in MATERIALS AND METHODS. The controls were initially incubated in 0.75 mM Tricine (pH 8.0) and otherwise were treated as described above. The reaction mixture was as in Expt. 1. Ferricyanide reduction was measured by recording continuously the decrease in absorbance at 420 nm with a Cary spectrophotometer.

Expt. No.	*Addition*	*Ferricyanide reduction (μequiv reduced per mg chlorophyll per h)*	
		—antimycin	*+antimycin*
1	None	475	368
	NH_4Cl, 3 mM	648	216
	Atebrine, 20 μM	520	160
	Gramicidine, 10 μM	648	152
2	Control	500	350
	EDTA-treated chloroplasts	735	338



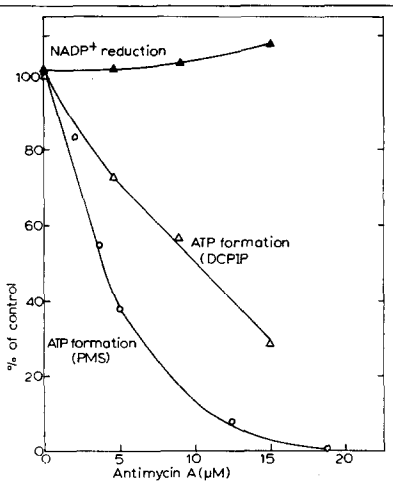


Fig. 5. Effect of antimycin A on ferricyanide reduction as a function of pH. The reaction mixture contained in 1.0 ml the following in μmoles: buffer *N*,*N*-bis(2-hydroxyethyl)glycine(bicine)–maleate, 17 (of each), at the specified pH; NaCl, 20; and ferricyanide, 0.6. It also contained chloroplasts equivalent to 6.4 μg chlorophyll. Antimycin A where indicated was added, 5 min before the onset of the reaction at 30 μM. The reaction mixture was illuminated for 2 min. Photoreduction was assessed by measuring appearance of ferrocyanide.

Fig. 6. Effect of antimycin A on cyclic phosphorylation. The reaction mixture for PMS phosphorylation contained in 3 ml the following in μmoles: Tris (pH 8.0), 50; NaCl, 70; $MgCl_2$, 5; P_i, 10; ADP, 3; and PMS, 0.09. It contained also ^{32}P and chloroplasts equivalent to 19 μg chlorophyll. Antimycin A was added at a concentration as specified. It was preincubated with the reaction mixture for 5 min. Illumination for 2 min was provided by white light (3500 ft candles). The reaction mixture for DCIP phosphorylation was the same as above, except that instead of PMS it contained the following in μmoles: $NADP^+$, 0.75; ferredoxin, 0.015; DCIP, 0.75; ascorbate, 15; and DCMU, 0.003. Illumination as above. The control activities in μmoles/mg chlorophyll per h were as following: for PMS phosphorylation, 595; for ascorbate–DCIP phosphorylation, 65; for $NADP^+$ reduction, 70.

TABLE IV

EFFECT OF ANTIMYCIN A ON THE PROTON PUMP

The reaction mixture contained in 2.0 ml: 0.03 μmole PMS, 100 μmoles NaCl and chloroplasts equivalent to 29 μg chlorophyll. The initial pH of the reaction mixture was adjusted to 6.1. A wide band of red light was provided by a 500-W projector, which was filtered through a Corning 2-60 filter. The pH extent of the control was about 0.25 pH unit. Measurements were made as described in MATERIALS AND METHODS. Antimycin A was added 5 min before illumination, except in Column 4, where it was added immediately after turning off the light.

Addition	*Extent (μequiv H^+ per mg chlorophyll)*	*Initial rate of formation (ΔpH/2 sec)*	*$t_{1/2}$ of decay (sec)*	*$t_{1/2}$ of decay (sec)*
None	1.55	0.100	6.0	5.5
Antimycin A, 11 μM	1.28	0.095	4.0	4.5
Antimycin A, 22 μM	0.43	0.055	1.5	4.0
Antimycin A, 33 μM	0.10	0.020	1.7	3.5

TABLE V

RESTORATION OF P/e_2 BY SERUM ALBUMIN

The reaction mixture contained in a total volume of 3 ml the following in μmoles: Tricine, 50 (pH 8.5); NaCl, 70; $MgCl_2$, 5; ADP, 3; P_i, 9; and ferricyanide, 1.8. It also contained chloroplasts equivalent to 18 μg chlorophyll. The reaction mixture was preincubated for 5 min in darkness at room temperature with or without 15 μM antimycin A. Human serum albumin was added at the end of the pre-incubation prior to illumination. Illumination time, 2 min. Ferricyanide reduction was assayed by measuring the decrease in absorbance of the deproteinized solution.

Albumin added (mg/ml)	*Control*			*+Antimycin A*		
	Ferricyanide reduction	*ATP formation*	*P/e_2*	*Ferricyanide reduction*	*ATP formation*	*P/e_2*
	(μmoles/mg chlorophyll per h)			*(μmoles/mg chlorophyll per h)*		
0	965	422	0.88	262	17	0.13
0.7	955	367	0.77	425	78	0.37
1.7	1000	382	0.76	455	110	0.48
2.3	970	413	0.85	410	130	0.64

be seen when the compound is added to the reaction mixture after the light is turned off (last column of Table IV).

Separation of the uncoupling effect from the inhibitory effect of antimycin A was achieved by adding serum albumin. As shown in Table V, addition of serum albumin after preincubation with antimycin A, increased electron flow by 56% and ATP formation by 670%. This separation might be due to a partial removal of antimycin A by serum albumin.

DISCUSSION

It has been shown in the present work that antimycin A has two activities in isolated chloroplasts: it inhibits electron transport and, in addition, exhibits uncoupling activity. However, at variance with other uncouplers which were shown

to inhibit electron transport at higher concentrations[17], antimycin A actually starts to inhibit electron flow (and therefore its coupled ATP formation) at lower concentrations than it acts as an uncoupler (Fig. 2). At higher concentrations both activities overlap to a considerable extent.

The inhibition of electron transport by antimycin A is strongly dependent on chlorophyll concentration, the duration of preincubation and pH of reaction. The decrease of inhibitory activity with increase in chlorophyll concentration indicates a strong partitioning of antimycin in favor of chloroplast material. Since inhibition increases with the duration of preincubation of antimycin with the chloroplasts (Fig. 3), it is possible that this compound has to permeate through a barrier prior to its action on electron flow. A lag in the inhibition by antimycin A and dependence on pH have been reported also for mitochondria electron transport[18], but, in the latter, the extent of inhibition decreased when the pH of the reaction mixture was raised.

The uncoupling activity of antimycin A is manifested by the following phenomena: (a) decrease in P/e_2 and inhibition of cyclic phosphorylation; (b) inhibition of the proton pump, most significantly stimulation of dark decay of the pH gradient (compare with the effect of other uncouplers on pH gradient (ref. 17)); (c) stimulation of electron transport.

Decrease in P/e_2 was shown with both $NADP^+$ and ferricyanide (Figs. 1, 2, 4B and Tables I and V). Antimycin A was shown to inhibit also cyclic ATP formation with DCIP–ascorbate and PMS (Fig. 6).

In view of the high susceptibility of ferredoxin cyclic photophosphorylation reported by TAGAWA *et al.*[6], it was of interest to study this reaction. We have found indeed that ferredoxin cyclic photophosphorylation is somewhat more sensitive to antimycin A than is PMS photophosphorylation. At 4.5 μM antimycin A, the former reaction was inhibited 63%, whereas the latter was only inhibited 31%. We have noted, however, that addition of DCMU, which is usually included in the reaction mixture[6] and which by itself has no effect, increases the inhibition caused by antimycin A. The higher sensitivity of cyclic ferredoxin photophosphorylation might be due to an involvement of an additional sensitive site in this reaction (perhaps cytochrome *b*) which is by-passed in the presence of other cofactors[6]. IZAWA *et al.*[10], on the other hand, have concluded that the higher inhibition takes place in any phosphorylation which is limited by the rate of electron transport and which they claim characterizes the ferredoxin cyclic phosphorylation.

Stimulation of electron flow can be shown only at a pH below 7.8 (Figs. 4B and 5, Table I), whereas at higher pH's the inhibitory effect of antimycin A is predominant (Figs. 3 and 5).

The dual effect of antimycin A and the dependence on chlorophyll concentration can explain some of the conflicting results reported previously. Thus, inhibition of ATP formation during $NADP^+$ reduction with no effect on the latter[5], could be due to the uncoupling effect of antimycin A and its activity as an electron flow inhibitor.

Contrary to the report of IZAWA *et al.*[10], antimycin A does inhibit "basal" electron flow, namely electron flow in absence of phosphorylating reagents (Tables II, III, Figs. 3, 4A and 5). The insensitivity of basal electron transport to antimycin A reported by IZAWA *et al.*[10] may be explained by assuming that under their conditions (preparation of chloroplasts, buffer used, range of inhibitor concentrations *etc.*) the

two effects of antimycin A on electron flow: stimulation due to uncoupling and inhibition, cancelled each other. This could be so only if the crossover point between stimulation and inhibition is shifted in their preparation toward a higher pH. We also observed, however, a slight increase in the susceptibility of electron flow to the inhibitor upon addition of the phosphorylating reagents (Fig. 4A). It is possible that this higher sensitivity to antimycin A in the presence of phosphorylating reagents, uncouplers (Table III) or at high pH which has been assumed as causing uncoupling[19], may be due to changes in permeability, which increase the accessibility of antimycin A to the component sensitive to antimycin A. Drastic structural changes in chloroplast thylakoids caused by uncouplers have been reported by IZAWA AND GOOD[20].

The interference of antimycin A with ATP formation can be reversed and separated from its effect on electron transport by using serum albumin (Table V). This can be explained by assuming that the carrier of electron transport inhibited by antimycin A is located behind a permeability barrier, whereas the coupling site is exterior to it. It can, however, result from a difference in the affinity of the two sites toward the inhibitor. It is probable that albumin removes part of antimycin A.

While this work was in progress, it has been shown by HIND[21] that antimycin A stimulates $NADP^+$ reduction at pH 7.0 and reduces ATP/NADPH at pH 8.3, in complete agreement with the results reported herewith.

REFERENCES

1 E. RACKER, *Advan. Enzymol.*, 23 (1961) 326.
2 D. M. GELLER AND F. LIPMANN, *J. Biol. Chem.*, 235 (1960) 2478.
3 F. R. WHATLEY, M. B. ALLEN AND D. I. ARNON, *Biochim. Biophys. Acta*, 32 (1959) 32.
4 H. BALTSCHEFFSKY, *Acta Chem. Scand.*, 14 (1960) 264.
5 E. S. BAMBERGER, C. C. BLACK, C. A. FEWSON AND M. GIBBS, *Plant Physiol.*, 38 (1963) 483.
6 K. TAGAWA, H. Y. TSUJIMOTO AND D. I. ARNON, *Proc. Natl. Acad. Sci. U.S.*, 50 (1963) 544.
7 W. SIMONIS, in J. B. THOMAS AND J. C. GOEDHEER, *Currents in Photosynthesis*, Ad Donker, Rotterdam, 1966, p. 217.
8 W. TANNER, E. LOOS AND O. KANDLER, in J. B. THOMAS AND J. C. GOEDHEER, *Currents in Photosynthesis*, Ad Donker, Rotterdam, 1966, p. 243.
9 J. BARBER, *Nature*, 217 (1968) 876.
10 S. IZAWA, T. N. CONNOLLY, G. D. WINGET AND N. E. GOOD, *Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symp. Biol.*, 19 (1966) 169.
11 G. HIND AND J. M. OLSON, *Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symp. Biol.*, 19 (1966) 188.
12 M. FRIEDLANDER AND J. NEUMANN, *Plant Physiol.*, 43 (1968) 1249.
13 D. I. ARNON, *Plant Physiol.*, 24 (1949) 1.
14 M. AVRON AND N. SHAVIT, *Anal. Biochem.*, 6 (1963) 549.
15 N. NELSON AND J. NEUMANN, *Biochem. Biophys. Res. Commun.*, 30 (1968) 142.
16 M. AVRON, *Biochim. Biophys. Acta*, 40 (1960) 257.
17 N. GOOD, S. IZAWA AND G. HIND, in D. R. SANADI, *Current Topics in Bioenergetics*, Vol. 1, Academic Press, New York, p. 76.
18 R. W. ESTABROOK, *Biochim. Biophys. Acta*, 60 (1962) 236.
19 J. NEUMANN AND A. T. JAGENDORF, *Biochem. Biophys. Res. Commun.*, 16 (1964) 562.
20 S. IZAWA AND N. E. GOOD, *Plant Physiol.*, 41 (1966) 544.
21 G. HIND, *Photochem. Photobiol.*, 7 (1968) 369.