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Inhibition of Photosynthetic Electron Transport by DDT and DDE

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Abstract

The effect of DDT and DDE (a metabolite of DDT) on chloroplast electron transport was investigated. Photosynthetic electron transport in isolated spinach and barley chloroplasts as well as chloroplasts isolated from macroscopic green algae, *Codium fragile* and *Chaetomorpha aerea*, was inhibited by both compounds. Photoreduction and photophosphorylation measured in the presence of ferricyanide showed 50% inhibition at 2×10^{-5} M DDT and DDE. P/2*e* ratios were 1·2–1·5, and remained constant in the presence of both inhibitors. The addition of uncouplers such as ammonium ion and carbonyl cyanide, *m*-chlorophenylhydrazone did not overcome the inhibition of the chlorinated hydrocarbons. Inhibition of phenazine methosulfate-catalyzed cyclic photophosphorylation by DDT and DDE was observed at low light intensities but was not seen at $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} and above. In the presence of DDT, a slow rise in measuring beam fluorescence was observed. The actinic beam fluorescence was slightly less than that in the control. Inhibition by DDT and DDE appears to be similar to that of DCMU. Brief sonication of the chloroplasts increases the sensitivity to DDT. The lack of penetration of DDT to terrestrial plant chloroplasts may be the reason why these are protected from this insecticide.

Introduction

Little is known about the toxicity of DDT* to plants. Most literature deals with uptake, accumulation and effects in animals. DDT-resistant and DDT-susceptible varieties have been recognized in barley,[1] although the mechanism of resistance is unknown. DDT in cultures of marine phytoplankton have been shown to reduce photosynthetic CO_2 fixation.[2,3] DDT also reduced activity of the Hill reaction in susceptible barley chloroplasts, as measured by reduction of 2,6-dichlorophenolindophenol.[4]

In animals DDT exerts its effects on various systems, and many of these are associated with membranes. Two adenosinetriphosphatases associated with the $20{,}000 \times g$ fraction from rat brain preparations were inhibited by DDT and DDE.[5] One of these, the Na^+ K^+ Mg^{2+} adenosinetriphosphatase, was 1000 times more sensitive to DDT than to DDE, 50% inhibition occurring at 3×10^{-7} M for DDT. A concentration of 5×10^{-4} M DDT in the surrounding medium created a marked reduction in the sodium

* Abbreviations: DDT, 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane (99+%); DDE, 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (99%); PMS, phenazine methosulfate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; Tricine, N-tris (hydroxy methyl) methyl glycine; CCCP-carbonyl cyanide, *m*-chlorophenylhydrazone.

and potassium flux during the falling phase of the action potential across isolated lobster giant axons.[6] In the presence of DDT the activity of rat liver microsomal enzymes that hydroxylate steroid hormones was stimulated.[7] Oxidative phosphorylation in subcellular fractions of houseflies was inhibited by DDT in concentrations ranging from 5×10^{-6} M to 5×10^{-5} M.[8]

The inhibition of photosynthetic $^{14}CO_2$ uptake in marine phytoplankton by DDT and the effects of DDT on the Hill reaction in susceptible barley suggest that the energy metabolism of the chloroplast lamellar membranes might be involved. Studies in animal systems indicate that cellular membranes are target sites of DDT effects. These considerations prompted the present investigation using spinach and barley chloroplasts and chloroplasts isolated from the intertidal marine green algae, *Codium fragile* and *Chaetomorpha aerea*. The effects of both DDT and DDE on photoreduction and photophosphorylation were studied, using $Fe(CN)_6^{3-}$ and PMS. Evidence will be presented as to the mode of inhibition by DDT and DDE in photosynthetic electron transport and phosphorylation.

Methods

Chloroplast Isolation

Spinach chloroplasts were isolated from young, field-grown spinach (Early Hybrid 11 and Early Hybrid 424, Dessert Seed Co., El Centro, Calif.), using the methods of Gee *et al.*[9] with the substitution of Tricine (Sigma Chemical) for the Tris buffer. Barley plants used (Arivat variety) were 6 days old grown indoors under hydroponic conditions. Conditions for isolating chloroplasts from them were the same as for spinach, except Tricine buffer was pH 8·4. The homogenate was centrifuged as $250 \times g$ for 90 sec to remove cell debris and the chloroplasts were obtained by centrifugation of the resulting homogenate at $750 \times g$ for 90 sec.[10] Chloroplasts from *Codium* and *Chaetomorpha* were isolated in a number of buffer systems. The most active preparation was obtained by grinding freshly harvested algae using the same techniques as for spinach. When required, sonic disruption of spinach chloroplasts was carried out using a sonicator (Branson Inst., Stamford, Conn.) equipped with a rosette cell. The sonicator was operated for either 2 or 10 sec at 20 kc and 100 W. Chloroplast suspensions were cooled in ice during these short exposures to sonication. Chlorophyll concentrations were measured as described by Arnon.[11] The chloroplast suspension was diluted with 0·05 M Tricine of pH 7·8 (8·4 for barley), 10 mM KCl and 2·5 mM $MgCl_2$ to give about 25–30 μg chlorophyll per 200 μl. At this point the chloroplasts were tested for their ability to couple electron transport to phosphorylation, using the methods of Izawa and Good.[12] For the spinach, only those chloroplast preparations which showed a two-fold increase of $Fe(CN)_6^{3-}$ reduction under phosphorylating conditions were used.

Photoreduction

The photoreduction of $Fe(CN)_6^{3-}$ was measured by following the absorbancy change at 420 nm using techniques described by Izawa and Good.[12] This reaction was carried out in a total volume of 3·0 ml in a pyrex cuvette. The reaction mixture contained: Tricine, 125 μmoles, pH 7·8; $MgCl_2$, 6·7 μmoles; KCl, 25 μmoles; $K_3Fe(CN)_6$, 4 μmoles; ADP (Sigma Chemical), 1 μmole; K_2HPO_4, 10 μmoles; DDT (*p,p'* isomer,

Aldrich Chemical) as indicated, DDE (*p,p'* isomer, Aldrich Chemical) as indicated, and chlorophyll 30 μg. DDT and DDE were added as ethanol solutions (25 μl), and ethanol controls were also run. The reaction components were first placed together in 1·5 × 10·0 cm test tubes. Because of the hydrophobic nature of these chlorinated hydrocarbons, these compounds were added to the tubes after the addition of the chloroplasts, which in turn followed the buffer and ADP. The tubes were gently and briefly vortexed after addition of the DDT or DDE, and after the addition of all components. Contents were then transferred to pyrex cuvettes. The temperature was 22°C with illumination provided by Sylvania Sun Gun Model 1 at an intensity of $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1}. A filter consisting of a dilute solution of copper sulfate was placed in front of these cuvettes. Energy was measured with a radiometer (Yellow Springs Instr., Yellow Springs, Ohio). In light-intensity experiments the energy was as indicated. When added, the final concentration of $(NH_4)_2SO_4$ was 8×10^{-5} M and that of CCCP 3×10^{-7} M. The concentrations of uncouplers used produced maximum uncoupling in isolated spinach chloroplasts.

Photophosphorylation

Photophosphorylation was measured in 1·5 × 10·0 cm test tubes using the temperature and light conditions of photoreduction. Non-cyclic photophosphorylation was measured after transfer to cuvettes using $Fe(CN)_6^{3-}$ and following photoreduction simultaneously. Cyclic photophosphorylation was measured using PMS as the cofactor. The reaction contained in 3·0 ml: Tricine, 125 μmoles, pH 7·8; KCl, 25 μmoles; $MgCl_2$, 6·7 μmoles; ADP, 1 μmole; $K_2H^{32}PO_4$, 10 μmoles containing 30 μCi of ^{32}Pi; and either 4 μmoles of $K_3Fe(CN)_6$ or 0·1 μmole of PMS (Sigma Chemical). The reactions were run for 5 min at intensity of $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} and killed with 0·5 ml of 20% trichloroacetic acid. The esterified phosphate was determined as described by Avron.[13] Light intensity when varied was as indicated.

Fluorescence Measurements

The instrument for measuring fluorescence yield is as described by Yamashita and Butler.[14] Two Corning filters (No. 9830, and No. 2030) were used which allowed light of wavelengths greater than 680 nm to pass. The energy of the measuring beam at 650 nm was 100 erg cm^{-2} sec^{-1} and that of the actinic light at 650 nm was 10^4 erg cm^{-2} sec^{-1}. Spinach chloroplasts were prepared as described previously. The reaction volume contained: 125 μmoles Tricine pH 7·8; 6·7 μmoles $MgCl_2$, 25 μmoles KCl and chloroplasts containing 25 μg chlorophyll in 3·0 ml. Twenty-five microliters of ethanol was added as a control and had only a small effect on fluorescence. DDT at a final concentration of 10^{-4} M, was added as 25 μl of an ethanol solution to fresh chloroplasts. DCMU was added to a final concentration of 10^{-6} M.

Results

The inhibition by DDT and DDE of non-cyclic photophosphorylation and electron transport in spinach chloroplasts using $Fe(CN)_6^{3-}$ as an electron acceptor, is shown in Table I. It can be seen that both reactions are affected to about the same degree by both compounds. A comparison of control reactions run with and without ethanol

indicates that 25 μl ethanol added to a 3·0 ml reaction mixture inhibits photoelectron transport by about 10% with slight uncoupling. The inhibition by DDT and DDE occurs without significant change in the P/2*e* ratio, but when DDT reaches a concentration of 10^{-4} M this drops by 50%. This uncoupling is not observed with DDE. Comparative tests performed with acetone indicate a more significant effect on photo-

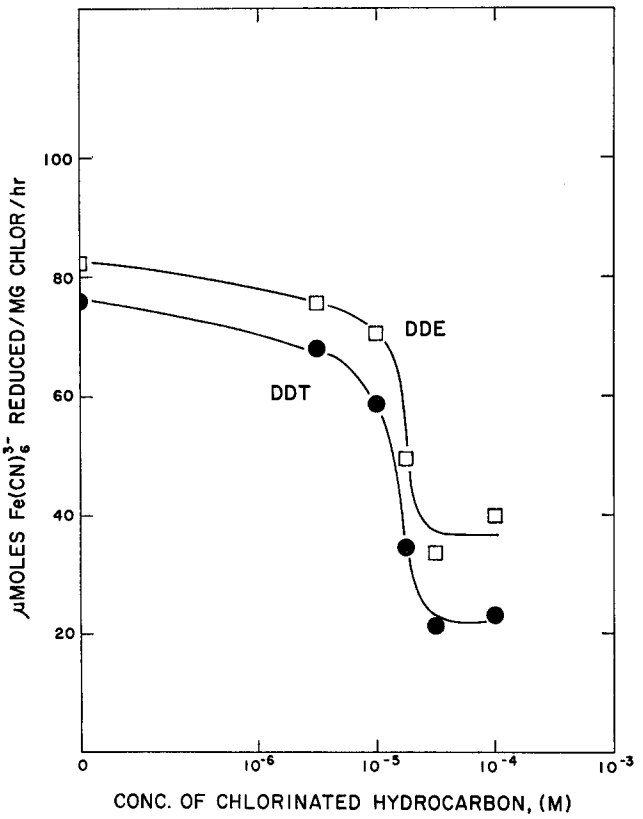

Figure 1. Inhibition of $Fe(CN)_6^{3-}$ photoreduction in *Chaetomorpha aerea* chloroplasts by DDT and DDE. To a total volume of 3·0 ml the following were added: chloroplasts having 70 μg chlorophyll; Tricine, 125 μmoles, pH 7·8; $MgCl_2$, 6·7 μmoles; KCl, 25 μmoles; $K_3Fe(CN)_6$, 4 μmoles; ADP, 1 μmole; K_2HPO_4, 10 μmoles; and DDT or DDE as indicated. The reactions were run at 22°C with $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} for 5·0 minutes. DDT and DDE were added in 25 μl ethanol. Ethanol (25 μl) was added to the mixture indicated as containing O chlorinated hydrocarbon.

reduction and coupling. For this reason, ethanol was used as the solvent in all experiments.

The $Fe(CN)_6^{3-}$ Hill reaction in the two marine algae, *Codium fragile* and *Chaetomorpha aerea*, and in barley, was also inhibited to the same extent by DDT and DDE. The curves of inhibition for *Chaetomorpha* are shown in Fig. 1 and for barley in Fig. 2. The concentration at which 50% inhibition occurs is about 2×10^{-5} M for both compounds and is the same for spinach.

The simultaneous inhibition of phosphorylation and electron transport prompted further experiments to determine the sites of action. Ammonia and CCCP are known to uncouple the phosphorylation reactions from electron transport in chloroplasts.[15] It can be observed in Fig. 3 that in the presence of 8×10^{-5} M $(NH_4)_2SO_4$, DDT and DDE still inhibit electron transport, and to the same extent as when this compound is absent. This indicates that DDT and DDE act at a point prior to the site of uncoupling by ammonia. DDT and DDE inhibition curves made in the presence of CCCP also show the same inhibition of the Hill reaction as in the absence of the uncoupler. With both $(NH_4)_2SO_4$ and CCCP, the concentration of the chlorinated hydrocarbon showing 50% inhibition was not changed.

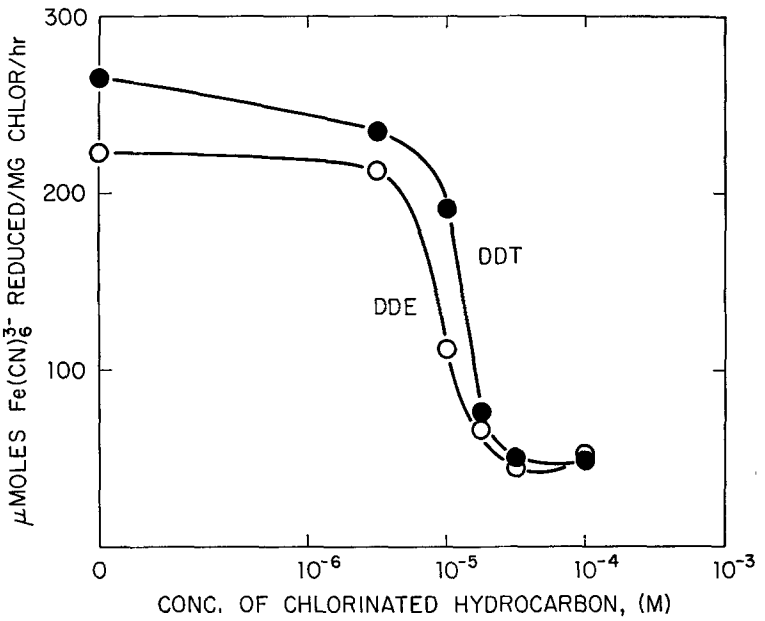

Figure 2. Inhibition of $Fe(CN)_6^{3-}$ photoreduction in barley chloroplasts by DDT and DDE. Experimental conditions are the same as those outlined in Fig. 1, except for the presence of 21 μg chlorophyll.

The effect of DDT and DDE at $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} on cyclic photophosphorylation using PMS and on non-cyclic photophosphorylation using $K_3Fe(CN)_6$ in the same chloroplast preparation is shown in Fig. 4. No inhibition of PMS-catalyzed photophosphorylation by these chlorinated hydrocarbons was observed even at concentrations when the non-cyclic reaction shows 78% inhibition. A 1-h incubation of chloroplasts (kept in ice) with DDT added either in 25 μl acetone or ethanol gives the same results as in Fig. 4. The results presented here indicate that a site of inhibition is near Photosystem II.

Figure 5 presents the results of the effects of addition of DDT and DDE on cyclic photophosphorylation in barley chloroplasts at a light intensity of $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1}. Only a high concentrations of DDT does slight inhibition occur. This might be due to uncoupling, as seen at 10^{-4} M DDT in Table I. The slight stimulation by DDE

is a repeatable phenomenon but is within 10% of the control values. As can be seen in Table I, DDE does not show uncoupling effects at 10^{-4} M.

Table II demonstrates a striking relationship between light intensity and the sensitivity of cyclic phosphorylation to DDT. This effect is observed with both spinach and barley. At $0{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} cyclic photophosphorylation is inhibited 63% in barley and 40% in spinach chloroplasts by 5×10^{-5} M DDT. Increasing the light intensity to 1×10^5 erg cm^{-2} sec^{-1}, DDT inhibition is decreased to 50% in barley and 35% in

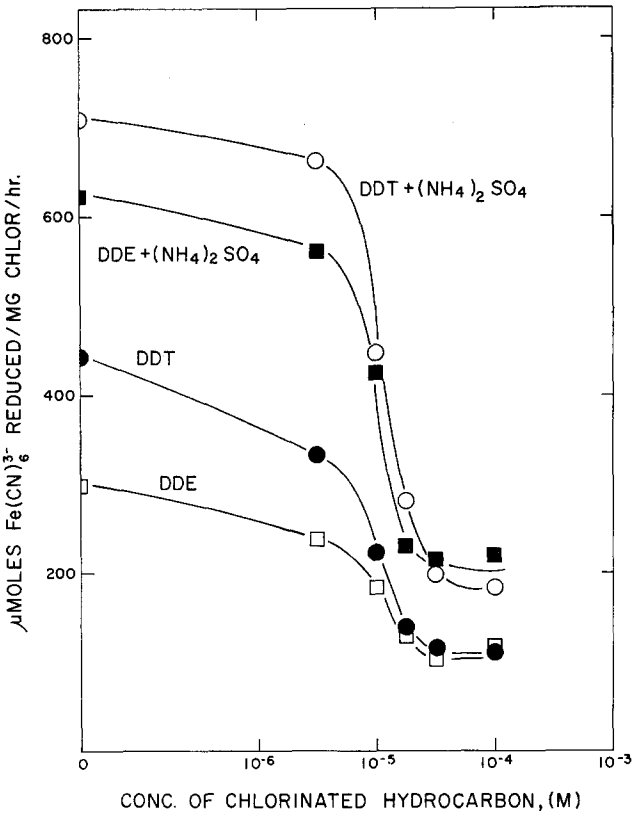

Figure 3. The effect of DDT and DDE on coupled and uncoupled spinach chloroplasts. The reaction conditions are the same as outlined in Fig. 1, except for the presence of 8×10^{-5} M $(NH_4)_2SO_4$ and 28 μg chlorophyll.

spinach. At $2{\cdot}0 \times 10^5$ erg cm^{-2} sec^{-1} DDT inhibition in barley was down to 14%. The inhibition of cyclic photophosphorylation in both barley and spinach was overcome at a light intensity of $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1}. The absence of inhibition was observed to intensities of $9{\cdot}0 \times 10^5$ erg cm^{-2} sec^{-1}. Experiments using DDE at light intensities of 0·5 and $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} show inhibition at the lower light intensity for both barley and spinach, which was also overcome at $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1}. Non-cyclic photophosphorylation was not affected by changing the light intensity.

Figure 6 shows the results of fluorescence yield studies. In the presence of 10^{-4} M DDT a slow rise in the fluorescence yield obtained from the 100 erg cm^{-2} sec^{-1} measuring

beam was observed. The fluorescence yield from the actinic beam is lowered, as in the case of 10^{-6} M DCMU. Fluorescence yield measurements using DDE give similar traces. These results indicate that the chlorinated hydrocarbons act at a site close to Photosystem II, perhaps at the DCMU site.

The effects of sonication on the inhibition by DDT are shown in Fig. 7. A ten-fold decrease in the concentration of DDT required for 50% inhibition can be shown by

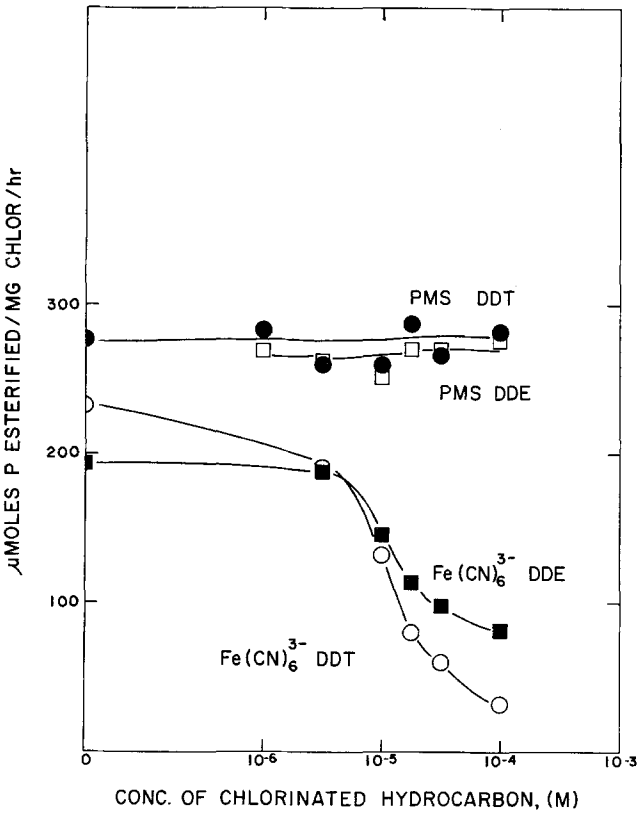

Figure 4. Effect of DDT and DDE on cyclic and non-cyclic photophosphorylation in spinach chloroplasts. The experimental conditions are the same as outlined in Table I, except that 0·1 μmole PMS replaced the $K_3Fe(CN)_6$ for the measurements of cyclic photophosphorylation.

sonication. Experiments with DDE showed the same increase in sensitivity. This effect can be shown using sonication periods as short as 2 sec, and increases slightly up to sonication times of 10 sec. Preincubation of the chloroplasts in the presence of DDT and DDE before addition of $K_3Fe(CN)_6$ and illumination also increased the inhibition. Preincubation of the chloroplasts for 20 min in the presence of the chlorinated hydrocarbons increased the inhibition two-fold. PMS-catalyzed cyclic photophosphorylation was not inhibited by the chlorinated hydrocarbons, at $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} when either a 20-min preincubation time was employed or a 10-sec sonication was used prior to the measurements. Cyclic photophosphorylation catalyzed by PMS shows similar

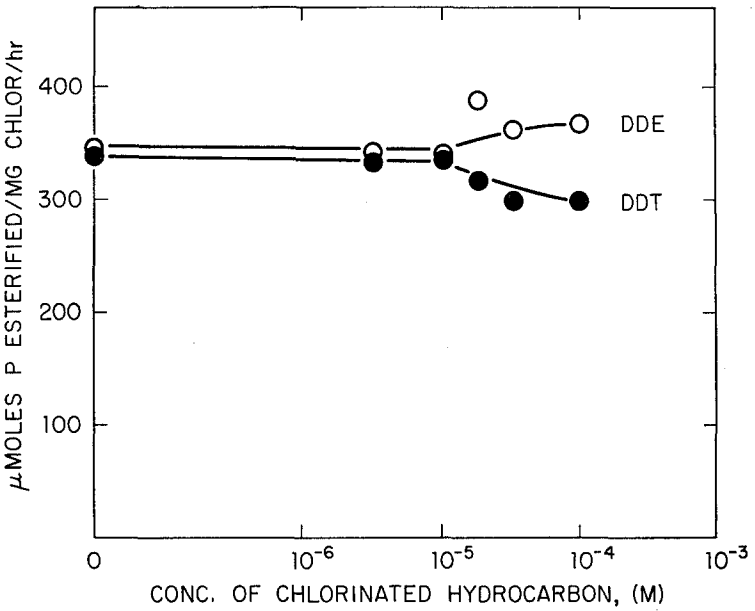

Figure 5. Effect of DDT and DDE on cyclic photophosphorylation in barley chloroplasts. To test tubes in a volume of 3·0 ml the following were added: Tricine, 125 μmoles, pH 8·4; KCl, 25 μmoles; $MgCl_2$, 6·7 μmoles; K_2HPO_4, 10 μmoles containing 30 μCi ^{32}P; ADP, 1·0 μmole; PMS, 0·1 μmole; and chloroplasts containing 32 μg chlorophyll. Other experimental conditions are as outlined in Table I.

TABLE I. Inhibition of photoelectron transport and phosphorylation in spinach chloroplasts by DDT and DDE

	DDT			DDE		
Concentration of inhibitor	μmoles $Fe(CN)_6^{3-}$ reduced per milligram of chlorophyll per hour	μmoles P esterified per milligram of chlorophyll per hour	P/2*e*	μmoles $Fe(CN)_6^{3-}$ reduced per milligram of chlorophyll per hour	μmoles P esterified per milligram of chlorophyll per hour	P/2*e*
0	339	232	1·4	293	193	1·3
5×10^{-6} M	282	192	1·4	267	190	1·4
10^{-5} M	212	133	1·3	203	146	1·4
$2{\cdot}5 \times 10^{-5}$ M	133	80	1·2	137	114	1·6
5×10^{-5} M	106	61	1·2	125	98	1·5
10^{-4} M	108	33	0·6	119	82	1·4

To a pyrex cuvette in a total volume of 3·0 ml the following were added: Tricine, 125 μmoles, pH 7·8; KCl, 25 μmoles; $MgCl_2$, 6·7 μmoles; K_2HPO_4, 10 μmoles containing 30 μCi ^{32}P; ADP, 1·0 μmole; $K_3Fe(CN)_6$, 4·0 μmoles; and chlorophyll, 24·6 μg. The DDT and DDE were added in 25 μl ethanol to achieve the indicated final concentration. The same amount of ethanol was added to the mixtures indicated as containing 0 concentration of inhibitor. The reactions were run at 22°C with $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} for 5·0 minutes. The reactions were stopped by adding 0·5 ml of 20% trichloroacetic acid.

TABLE II. Effect of light intensity on the inhibition of cyclic and non-cyclic photoelectron transport

Light intensity	Barley				Spinach			
	μmoles P esterified per milligram of chlorophyll per hour		μmoles $Fe(CN)_6^{3-}$ reduced per milligram of chlorophyll per hour		μmoles P esterified per milligram of chlorophyll per hour		μmoles $Fe(CN)_6^{3-}$ reduced per milligram of chlorophyll per hour	
	Control	5×10^{-5} M DDT	Control	5×10^{-5} M DDT	Control	5×10^{-5} M DDT	Control	5×10^{-5} M DDT
0.5×10^5 erg cm^{-2} sec^{-1}	98	36	238	40	248	148	436	97
1.0×10^5 erg cm^{-2} sec^{-1}	203	103	238	48	692	455	558	124
2.5×10^5 erg cm^{-2} sec^{-1}	326	355	229	64	1000	946	479	116

To test tubes in a volume of 3·0 ml the following were added: Tricine, 125 μmoles, pH 7·8 for spinach, and 8·4 for barley; KCl, 25 μmoles; $MgCl_2$, 6·7 μmoles; K_2HPO_4, 10 μmoles containing 30 μCi ^{32}P; ADP, 1·0 μmole. When added, $Fe(CN)_6^{3-}$, 4·0 μmoles, and PMS, 0·1 μmole. The chlorophyll content of barley chloroplasts was 22 μg and that of spinach was 19 μg. DDT was added in 25 μl of ethanol to achieve the indicated final concentration. The same amount of ethanol was added to the control mixtures. The reactions were run at indicated light intensities for 5·0 min after transferring the mixed contents to pyrex cuvettes. The reactions were stopped by adding 0·5 ml of 20% trichloroacetic acid.

sensitivity to ethanol as noncyclic photophosphorylation using $Fe(CN)_6^{3-}$. When 25 μl ethanol is added to PMS-catalyzed chloroplasts with phosphorylation rates of 730 μmoles P esterified per milligram of chlorophyll per hour, inhibition of 10% was observed. The same volume of acetone inhibited the cyclic system 30%. When DDT was added little or no further inhibition was observed.

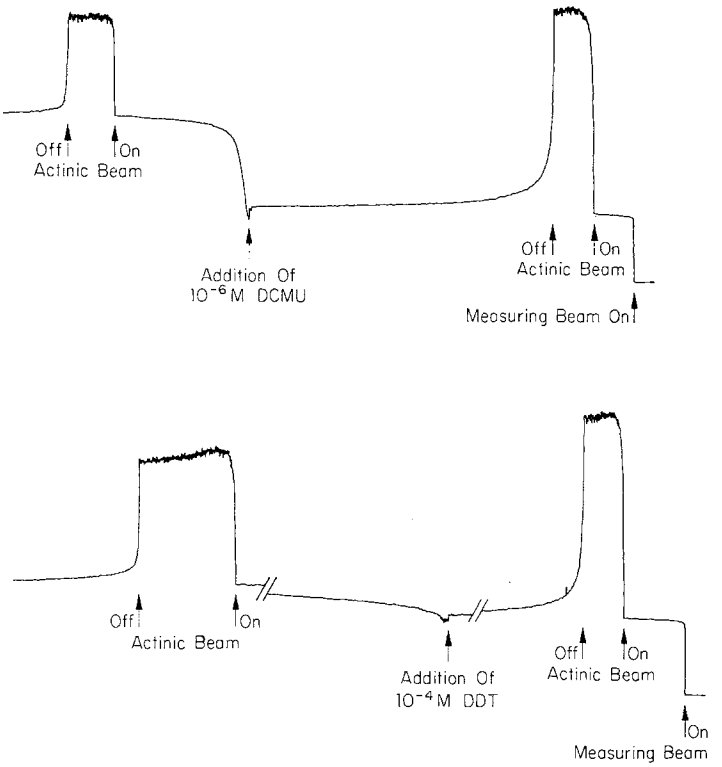

Figure 6. The upper fluorescence yield trace is that obtained in the absence and presence of DCMU. The lower trace represents a parallel experiment where DDT is used in place of DCMU in a fresh chloroplast preparation. The traces to the right are those obtained in the absence of inhibitor. The energy of the measuring beam at 650 nm was 100 erg cm^{-2} sec^{-1} and that of the actinic light at 650 nm was 10^4 erg cm^{-2} sec^{-1}. The reaction volume contained: Tricine, 125 μmoles, pH 7·8; $MgCl_2$, 6·7 μmoles; KCl, 25 μmoles; and chloroplasts containing 25 μg chlorophyll, in 3·0 ml. Twenty-five microliters of ethanol was added as a control. DDT at a final concentration of 10^{-4} M was added to fresh chloroplasts as 25 μl of an ethanol solution. DCMU was added to fresh chloroplasts. The instrument for measuring fluorescence yield is as described by Yamashita and Butler.[4]

Discussion

The above data indicate that the inhibition of $^{14}CO_2$ fixation in marine algae[2, 3] can be explained by the inhibition of electron transport. This inhibition occurs without change in the P/2*e* values in the non-cyclic system. Only at high concentrations of DDT can a drop in the P/2*e* ratio be observed. This change is not seen with DDE and may indicate lamellar membrane destruction at high concentrations of DDT.

The lack of change of P/2e ratios indicate that DDT is not an uncoupler. This fact, plus four other lines of evidence, suggests that the site of inhibition of DDT and DDE may be near the site of DCMU inhibition: (1) inhibition of photoreduction of $Fe(CN)_6^{3-}$; (2) inhibition of photoreduction of $Fe(CN)_6^{3-}$ in the presence of uncouplers; (3) the lack of inhibition of cyclic photophosphorylation; (4) the results of fluorescence studies.

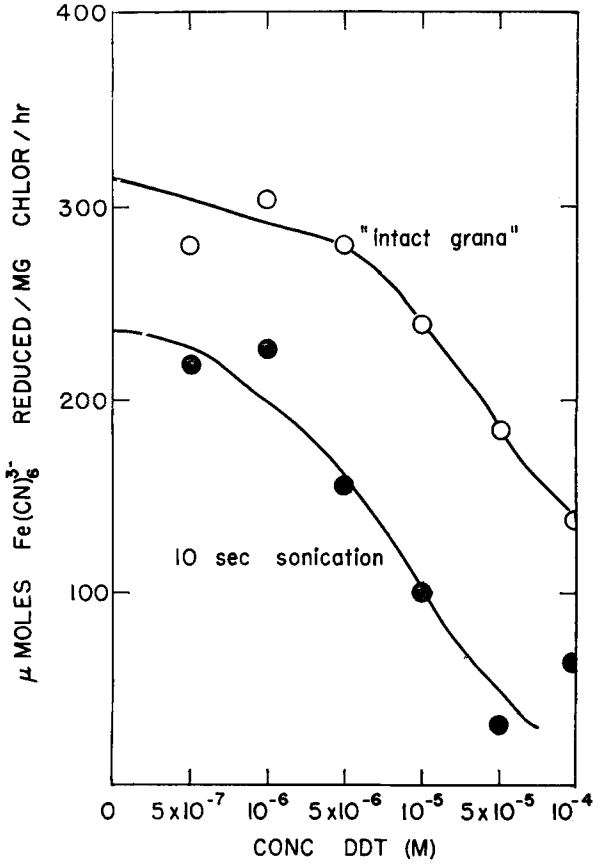

Figure 7. The effect of sonication on the inhibition by DDT in spinach chloroplasts. Conditions for the experiment are the same as outlined in Fig. 1, except the chlorophyll content was 29 μg. Sonication of chloroplasts in a rosette cell in ice was carried out using a Branson Instruments Sonifier.

The inhibition of the photoreduction of $Fe(CN)_6^{3-}$ was not affected by the addition of ammonia and CCCP, known uncouplers of photosynthetic phosphorylation.[15,16] These results indicate that the inhibition does not occur at a site in the energy conservation mechanism after the site of uncoupling. A difference in the effect of DDT and DDE on cyclic and noncyclic photophosphorylation was observed. The lack of inhibition by the chlorinated hydrocarbons on cyclic photophosphorylation at a light intensity of $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} and greater is similar to the effects seen with 10^{-6} M DCMU. The results with the cyclic system indicate that the site of inhibition of

DDT is not at the site of the "high-energy intermediate"[15,17] and is not in the part of electron flow associated with Photosystem I.

Fluorescence yield studies of chloroplasts in the presence of inhibitors can distinguish the site of inhibition as to before or after Q (Q being the fluorescence quencher described by Duysens[18]). The steady-state fluorescence obtained by the measuring beam will be decreased if the site of inhibition is before Q, and increased if the site of inhibition is after Q—such as the DCMU results obtained. The slow rise of fluorescence yield of the measuring beam in the presence of 10^{-4} M DDT indicates that the inhibition is similar to DCMU, where there is a concomitant small leak of electrons past Q (Dr. W. L. Butler, personal communication.) Further, cyclic photophosphorylation catalyzed by PMS is not inhibited by DDT or DDE at $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} or higher light intensities. At these light intensities DDT and DDE behave in a manner similar to that of a weak DCMU-type of inhibitor. The results of low light intensities can be best explained by the redox state of PMS.

The results reported here with both spinach and barley, and *Codium* and *Chaetomorpha*, are not consistant with those obtained from chloroplasts in DDT-susceptible barley by Lawler and Rogers[4,19] PMS-catalyzed photophosphorylation measured by them at high light intensity in the presence of ascorbate was inhibited by DDT but not DDE. It is not known whether Arivat barley, used in our experiments, is susceptible or resistant to DDT. The lack of inhibition of cyclic photophosphorylation shown above in spinach chloroplasts was demonstrated even when the chloroplasts were sonicated, which gave large increases in inhibition of the $Fe(CN)_6^{3-}$ Hill reaction by DDT and DDE. This result was not changed when the spinach chloroplasts were isolated and resuspended in medium containing 50 and 25 mM ascorbate, respectively. The cause of inhibition of cyclic photophosphorylation at high light intensity in DDT-susceptible barley reported by Lawler and Rogers is not known. Further, electron flow to ferricyanide was seen to be consistently inhibited by DDT and DDE in spinach, barley, *Codium* and *Chaetomorpha*. Chloroplast particles isolated from *Dunaliella tertiolecta*, a marine phytoplankter shown to be resistant to DDT through a wide range of concentrations,[3] demonstrated the same sensitivity.* Lawler and Rogers[19] reported both ferricyanide reduction and non-cyclic photophosphorylation were not affected by DDT in chloroplasts of DDT-susceptible barley, although photoreduction of 2,6-dichlorophenolindophenol was inhibited.

Experiments performed at light intensities less than $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1} showed an inhibition of cyclic photophosphorylation by DDT and DDE in spinach and barley chloroplasts. The inhibition of cyclic photophosphorylation decreased with increasing light intensities up to $2{\cdot}5 \times 10^5$ erg cm^{-2} sec^{-1}. The inhibition of ferricyanide reduction by DDT and DDE was not affected by change in light intensity. The inhibition at low light intensities by DDT of cyclic photophosphorylation can be explained in two ways: one, that DDT affects another site, or two, the state of reduction of PMS at low light intensity is of importance. In the presence of DCMU, PMS cyclic photophosphorylation is inhibited under oxidizing conditions.[20] There is a non-enzymatic photoreduction of PMS. PMS would be relatively more reduced under high light intesnities.[21] Under low light intensities in an aerobic system the PMS should be more oxidized. Increasing the light intensities would increase the amount of reduced PMS. Zweig and Avron[22]

* Gerald W. Bowes (in preparation).

have demonstrated that the ability of PMS to catalyze phosphorylation depends on its redox state. These experiments are in agreement with inhibition of PMS-catalyzed phosphorylation where the PMS is oxidized.[23]

The sonication experiments indicate that the main problem associated with the effect of DDT on chloroplast systems is its ability to reach the site of inhibition. Chloroplasts in the leaf are not subjected to treatments as drastic as sonication, yet leaves and marine and fresh water algae may be exposed to chlorinated hydrocarbons for long periods. Chloroplasts isolated from spinach when subjected to preincubation periods with DDT and DDE showed increases of sensitivity to these inhibitors. At 20 min of exposure to these compounds the amount of inhibitor required for 50% inhibition was one half that required originally. Experiments are now in progress to determine the site of inhibition and binding of DDT to chloroplast membranes.[24,25]

The chlorinated hydrocarbons studied are not known for their herbicidal effects on terrestial plants. Although DDT and DDE appear to act on electron transport associated with Photosystem II, their ability to inhibit is far lower than DCMU. This may be related to the higher degree of lipid solubility of the former compounds. The relatively dramatic effect of DDT and DDE on terrestrial plant chloroplasts shown above indicates the presence of a natural penetration barrier for DDT in the intact plant, the waxy cuticle of its leaves. Chloroplasts isolated from marine macroscopic green algae and a phytoplankton species show the same sensitivity. This indicates that the sensitivity of marine algae is governed by the ability of DDT to penetrate the cell.

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