Effect of 1:1:1-Trichloro-2:2-di-(*p*-chlorophenyl)ethane (DDT) and Related Compounds on the Adenosine Triphosphate-Phosphate Exchange Catalysed by a Particulate Fraction from the Mosquito

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Many attempts have been made to relate the toxic action of 1:1:1-trichloro-2:2-di-(*p*-chlorophenyl)-ethane (DDT) to an interference with specific enzymes. DDT at a relatively high concentration (mM) was reported to inhibit cytochrome oxidase from both mammals and flies (Sacktor, 1949; Johnston, 1951; Morrison & Brown, 1954). A much lower concentration of DDT (50 μ M) was found to suppress oxidative phosphorylation in a subcellular fraction from the adult housefly (Sacklin, Terrier & Remmert, 1955) and from the mosquito (Gonda, Traub & Avi-Dor, 1957). The incorporation of 32 P into ATP catalysed by insect respiratory particles proved also to be sensitive to DDT (Avi-Dor & Gonda, 1959). In work described in this paper an attempt was made to relate the inhibition of the adenosine triphosphate 32 P exchange by various DDT analogues to their solubility in the respiratory particles and to their toxicity for insect larvae.

EXPERIMENTAL

Materials. DDT and its analogues were either purified commercial products or synthesized. DDT (I), m.p. 108°, commercial product; 1:1-dichloro-2:2-di-(*p*-chlorophenyl)-ethane (II), m.p. 109–110° (Mosher, Cannon, Conroy, Van Strien & Spalding, 1946); 1:1:1-trichloro-2:2-di-(*p*-methoxyphenyl)-ethane (III), m.p. 88°, commercial product; 1:1-dichloro-2:2-di-(*p*-chlorophenyl)ethylene (IV), m.p. 88–89°, prepared by dehydrochlorination of DDT by ethanolic KOH (Grumitt, Buck & Jenkins, 1945); di-(*p*-chlorophenyl)-acetic acid (V), m.p. 164–166° (Grumitt, Buck & Egan, 1955); 1:1:1:2-tetrachloro-2:2-di-(*p*-chlorophenyl)ethane (VI), m.p. 92–93° (Grumitt, Buck & Jenkins, 1945); 1:1-di-(*p*-chlorophenyl)ethanol (VII), m.p. 69–70° (Grumitt, Buck & Becker, 1945); 1:1:1-trichloro-2:2-diphenylethane (VIII), m.p. 64–65° (Baeyer, 1872); 1:1-di-(*p*-chlorophenyl)-2:2:2-trifluoroethanol (IX) (Kaluszyner, Reuter & Bergmann, 1955); 1:1-di-(*p*-chlorophenyl)-2:2:2-trichloroethyl acetate (X), m.p. 128–129° (Bergmann & Kaluszyner, 1958). The other chemicals used, the composition of the buffers and the colony of *Aedes aegypti* L. employed were as described by Avi-Dor & Gonda (1959).

Methods. The procedure for the isolation of respiratory particles from mosquito, the method of assay of the exchange between adenosine triphosphate (ATP) and 32 P catalysed by the respiratory particles and other analytical methods used have been described by Avi-Dor & Gonda

(1959). The composition of the assay medium, unless indicated otherwise, was as follows: 2-amino-2-hydroxymethyl-propan-1:3-diol (tris) buffer, pH 7.4 (10 mM), sodium phosphate buffer, pH 7.4 (2.7 mM), 0.2 ml. of a stock solution of 32 P which contained approx. 1.5 μ Ci/ml. (giving 1.5×10^6 counts/min./ml. in a thin mica-window Geiger tube), sucrose (0.25 M), ATP (7 mM), human serum albumin (1%, w/v), ethylenediaminetetra-acetic acid (EDTA; 1 mM), respiratory particles from mosquito (equivalent to approx. 4 mg. dry wt./ml. of assay medium). The mixture was made up to 1.5 ml. with water. Time of incubation was 10 min.; temp. 28°. DDT and its analogues were added in propanol solution. The final concentration of propanol in the reaction mixture was 1% (w/v). The same amount of propanol was added to the controls.

For the determination of the median lethal dose (LD₅₀) of DDT analogues, 20 larvae of *Aedes aegypti* L. (fourth-instar) were exposed to varying dilutions of the respective compound. The assay was carried out in Petri dishes of 9 cm. diameter at 28°; time of incubation was 24 hr.

For the determination of the partition of the DDT analogues between the aqueous phase of the reaction medium and between the suspended respiratory particles, the respiratory particles were equilibrated for 10 min. at 28° with the assay medium to which a known amount of the analogue in propanol solution has been added. Then the particles were separated by centrifuging at 8000 *g* for 7 min. at 0°, drained, transferred with the aid of 2 ml. of ethanol (96%, v/v) to a 10 ml. beaker and dried under an infrared lamp. The dried precipitate was dissolved by gentle warming in 3 ml. of a mixture of fuming nitric acid (1 vol.) and conc. sulphuric acid (1 vol.). The assay of the DDT analogues was performed by the method of Schechter, Soloway, Hayes & Haller (1945), in a sample of the solution so obtained. In many experiments the amount of DDT left in the supernatant was also determined. The practically complete recovery indicated that no metabolism of the compounds tested took place during the time of the experiment.

RESULTS

Factors affecting the partition of DDT and its analogues between the aqueous phase and the suspended respiratory particles

Effect of the concentration of serum albumin in the assay medium. The ATP- 32 P exchange catalysed by insect particles is dependent on the addition of serum albumin to the assay medium (Avi-Dor & Gonda, 1959). Albumin had also a marked effect on

the stability of the DDT suspensions. When a reaction mixture containing $0.1 \mu\text{mole}$ of DDT/ml. was exposed to the centrifugal force ($8000 g$ for 7 min.) which was used to separate the suspended respiratory particles, more than $0.09 \mu\text{mole}$ of DDT/ml. sedimented in the absence of serum albumin. This sedimentation was avoided only when the concentration of the albumin was at least 1% (w/v) (Fig. 1). Serum albumin also reduced the amount of DDT which was absorbed by the respiratory particles and, as a corollary, the extent of inhibition of the exchange reaction (Fig. 2). In our experiments, the standard assay medium contained 1% (w/v) of serum albumin. This level of albumin sufficed for maintaining the exchange reaction, for avoiding sedimentation of DDT and its analogues during centrifuging and yet allowed the absorption of these compounds by the particles.

Effect of the concentration of DDT, its analogues and of respiratory particles. Externally added substances with specific affinities toward lipids are accumulated by subcellular particles of high lipid content. This applies also to DDT and its analogues,

which have hydrophobic character and high lipid solubilities. In Fig. 3 the amount of DDT and its analogues absorbed by a constant amount of particles is plotted against the concentration of these compounds in the medium. [A 0.1 mM -solution of DDT or of its analogues is completely transparent in the presence of 1% (w/v) serum albumin. Since, however, it is not known whether these compounds form true solutions under such condition, 'concentration' denotes the amount of the compound (in μmoles) added in a propanol solution per l. of medium.] The partition of DDT and related compounds between the medium and the respiratory particles varied within wide limits. From a 0.1 mM -solution, 80% of DDT, but only 5% of 2:2-di-(*p*-chlorophenyl)acetic acid (V), was removed by the particles. Furthermore, the accumulation of DDT and its analogues depended on the ratio of the total amount of the compounds to the total amount of the respiratory particles in the

Fig. 1. Effect of the concentration of serum albumin on the sedimentation of DDT. From the standard assay medium ATP and ^{32}P were omitted, and the concentration of the serum albumin was varied as indicated. DDT was added ($0.1 \mu\text{mole/ml.}$ of assay medium); total volume 15 ml. After centrifuging at $8000 g$ for 7 min. at 0° the amount of DDT sedimented was determined.

Fig. 2. Effect of the concentration of serum albumin on the reabsorption of DDT by the respiratory particles and on the extent of inhibition of the ATP- ^{32}P exchange. ○, μmole of DDT absorbed/mg. dry wt. of particles; composition of the reaction mixture was as given in Fig. 1. ●, Extent of inhibition of ATP- ^{32}P exchange; standard reaction mixture (see Experimental section). Respiratory particles were added in both experiments in a concentration equivalent to 4 mg. dry wt./ml. of medium. The amount of DDT added was $0.1 \mu\text{mole/ml.}$ of assay medium. A correction was applied for the amount of the DDT which sedimented under conditions of the assay in the absence of respiratory particles.

assay medium. This can be seen from Figs. 4 and 5. In Fig. 4, the variable factor is the volume of the reaction mixture while the concentration of DDT and the total amount of respiratory particles in the system are kept constant. In Fig. 5, the absorption of the DDT analogues is presented as a function of the total amount of the particles.

Inhibition of the adenosine triphosphate ^{32}P exchange by DDT and related compounds

In Table 1 the inhibitory action of various analogues of DDT on the exchange between ATP and inorganic phosphate on the one hand and of their toxicity for larvae of *Aedes aegypti* L. on the other hand are compared. The concentration of the compounds that produced 50% inhibition was read off from titration curves as shown in Fig. 6. The corresponding values for the concentration of the analogues in the respiratory particles proper was obtained from experiments described in Fig. 3. It can be seen from the Table that the concentration of various compounds in the particles, which produced a 50% reduction in the rate of ATP- ^{32}P ex-

change, varied within fairly narrow limits (0.01–0.017 $\mu\text{mole/mg. dry wt.}$). The corresponding variations of the concentration in the aqueous phase were ninefold and of the LD_{50} for mosquito larvae more than 2000-fold.

DISCUSSION

Theories based either on physical and chemical properties of DDT (electromagnetic and steric configuration, lipid solubility, dehydrohalogenation etc.) or on its interaction with specific enzymes failed to explain satisfactorily its physiological activity (cf. Wigglesworth, 1955). Tomizawa (1957) observed that when radioactive phosphate was injected into the abdomen of insects which were treated with DDT, the greatest changes in the labelling of the phosphorylated intermediates occurred in the ATP + ADP fraction. However, it was not shown whether this action is confined only to highly toxic derivatives of DDT.

Fig. 3. Effect of the concentration of the insecticide in the aqueous phase on the amount absorbed by the particles. Composition of the assay medium and the conditions of the experiment were as given in Fig. 2. DDT analogues (see Table 1): ○, I; △, II; □, III; ●, IV; ▲, V; ■, VI; ■, X.

Fig. 4. Effect of variations in the total amount of DDT in the medium on the amount absorbed by the respiratory particles. Concentration of DDT in the medium was 0.1 mM. Respiratory particles were added in an amount corresponding to 4 mg./dry wt. The total amount of DDT in the system was changed by varying the volume of the medium. In other respects conditions of the reaction were as given in Fig. 1.

Fig. 5. Effect of the concentration of the respiratory particles on the amount of insecticide absorbed. Concentration of DDT and its analogues was 0.1 mM; serum albumin was 1% (w/v); other components were as in Fig. 1. Symbols for the analogues are as given in Fig. 3. Total volume was 15 ml.

Fig. 6. Effect of the concentration of DDT and its analogues on the extent of inhibition of ATP-³²P exchange. Standard reaction medium was used. DDT analogues: O, I; Δ , II; $\blacktriangle$, V.

Table 1. Correlation between the toxicity of DDT analogues, their partition between the aqueous phase and the respiratory particles and their inhibitory effect on adenosine triphosphate-³²P exchange

LD₅₀ values for the various insecticides towards mosquito larvae were assayed as described under Methods. Concentration of respiratory particles was 4 mg. dry wt./ml. The amount of insecticides accumulated by the particles and the ATP-³²P exchange was measured as described under Methods.

DDT analogues tested	LD ₅₀ (μmole/ml.)	Concn. of DDT analogues when ATP- ³² P exchange is inhibited by approx. 50 %	
		In aqueous phase (μmole/ml.)	In respiratory particles (μmole/mg. dry wt.)
1:1:1-Trichloro-2:2-di-(<i>p</i> -chlorophenyl)ethane (I)	0.0002	0.07	0.013
1:1-Dichloro-2:2-di-(<i>p</i> -chlorophenyl)ethane (II)	0.0005	0.10	0.017
1:1:1-Trichloro-2:2-(<i>p</i> -methoxyphenyl)ethane (III)	0.0005	0.15	0.010
1:1-Dichloro-2:2-di-(<i>p</i> -chlorophenyl)ethylene (IV)	> 0.25	0.17	0.012
Di-(<i>p</i> -chlorophenyl)acetic acid (V)	> 0.5	0.60	0.014
1:1:1:2-Tetrachloro-2:2-di-(<i>p</i> -chlorophenyl)ethane (VI)	0.03	0.07	0.012
1:1-Di-(<i>p</i> -chlorophenyl)ethanol (VII)	0.03	0.18	0.013
1:1:1-Trichloro-2:2-diphenylethane (VIII)	> 0.25	0.07	0.012
1:1-Di-(<i>p</i> -chlorophenyl)-2:2:2-trifluoroethanol (IX)	0.03	0.05	0.015
1:1-Di-(<i>p</i> -chlorophenyl)-2:2:2-trichloroethyl acetate (X)	> 0.2	0.07	0.013

In the present study the sensitivity of the ATP- ^{32}P exchange reaction to DDT and to some related compounds has been compared in systems *in vitro*. A close correspondence has been revealed between the extent of inhibition of ATP- ^{32}P exchange produced by various analogues of DDT and their concentration in the respiratory particles proper. This finding recalls the action of narcotic substances. According to Meyer (1937), the latter exert their physiological effect as a result of their preferred solubilities in the lipids of the cell. It is likely that the pronounced sensitivity of the ATP- ^{32}P exchange reaction and that of the oxidative phosphorylation to DDT and its analogues is merely an expression of the fact that the phosphorylating electron-transport particles are rich in lipids. Various analogues of DDT which have similar partition characteristics, like compounds (I), (II), (III), (VIII) and (X), differ widely in their toxicity toward insect larvae. Compound (VIII) and compound (X) are approximately 1000 times less toxic than DDT. Thus the effect *in vivo* reflects not only the variation in lipid solubilities. One has to conclude either that the effect of DDT on the phosphorylative processes has no physiological significance at all or alternatively that, *in vivo*, unknown factors impede selectively the access of some of the analogues to the respiratory particles.

SUMMARY

1. The effect of 1:1:1-trichloro-2:2-di-(*p*-chlorophenyl)ethane (DDT) and its analogues on the adenosine triphosphate- ^{32}P exchange reaction in mosquito sarcosomes has been compared.

2. All analogues of DDT tested inhibited the exchange reaction by nearly the same extent when their concentration in the particles reached the same level.

3. No correlation was found between the toxicity for mosquito larvae of the compounds tested and their effect *in vitro* on the exchange reaction.

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Synthesis of Glucosylfructoses by the Action of a Yeast α -Glucosidase

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It has been reported (Herzog, 1913; Isaiev, 1926) that hydrolysis of maltose by yeast maltase is slightly inhibited by D-fructose. Similarly Avigad (1958) in a study of a hybrid yeast α -glucosidase has shown that D-fructose partially inhibited liberation of glucose from suitable α -glucoside substrates (sucrose, methyl α -glucoside or maltose).

It was of interest to inquire whether this inhibitory effect might be due to an ability of D-fructose to combine with the glucon-binding site of the enzyme, as suggested by Gottschalk (1950), or to an ability of fructose to compete with water as an acceptor for transferred α -glucosyl residues. Experiments described below show that the latter