Brit. J. Pharmacol. (1949), **4**, 120.

STUDIES ON THE METABOLISM AND MODE OF ACTION OF DDT

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(Received November 9, 1948)

Despite the world-wide employment of 2 : 2-bis (*p*-chlorophenyl) - 1 : 1 : 1-trichloroethane (DDT) as an insecticide, comparatively little interest has been shown in its mode of action and few investigations have been carried out on its metabolism, absorption, and excretion in contaminated animals. The present paper deals with the latter topics in an attempt to find out how DDT acts on living cells as well as on the organism as a whole.

METHODS

DDT and 2 : 2-bis(*p*-chlorophenyl)acetic acid (DDA) were estimated spectrophotometrically by the method of Schechter, Soloway, Hayes, and Haller (1945). Organically bound chlorine was determined simultaneously by the method of the writer (1948); urinary sulphur partitions by the volumetric benzidine method as modified by Elson, Goulden, and Warren (1945); urinary glycuronic acid by the method of Kapp (1940); urinary nitrogen, plasma protein, and non-protein nitrogen by the micro-Kjeldahl method; blood lactic acid by the method of Barker and Summerson (1941); glucose by the Hagedorn-Jensen method or by the technique of Nelson (1944); liver glycogen by the method of Good, Kramer, and Somogyi (1933); serum potassium by the method of Hoffman (1937); serum sodium by that of Kramer and Gittleman (1924) as modified by Rourke (1928); serum calcium, magnesium, and inorganic phosphate were estimated by the method of Briggs (1924), ascorbic acid being used as the reducing agent. Respiratory exchange *in vitro* was measured in the Warburg apparatus. Details of experiments with particular enzyme systems, etc., are given in the text.

Diphosphopyridine nucleotide (coenzyme 1) was prepared by the method of Sumner, Krishnan, and Sisler (1947); cytochrome C by that of Keilin and Hartree (1937).

The barium salt of adenosinetriphosphate (ATP) was obtained from Messrs. Boots, Nottingham, and purified as necessary.

Adenylic acid was obtained from Messrs. Light, Wraysbury.

Pure DDT (m.p. 108°) was administered in arachis oil in 10 per cent (w/v) solution, either orally by stomach tube or intraperitoneally by injection. The emulsion consisting of 1 part of the oily solution and 9 parts of a suspension of lecithin in 0.9 per cent NaCl solution, as recommended by Philips and Gilman (1946), was found to be satisfactory for intravenous injections.

DDA was used as the Na salt, prepared by the method of White and Sweeney (1945).

Most of the experiments were carried out on white rats and rabbits, but a few frogs were employed.

RESULTS

Tissue distribution of DDT

Table I shows the distribution of DDT and DDA in rats and rabbits after intraperitoneal, oral, and intravenous administration of 1,000 mg., 500 mg., and 50 mg. per kg. body weight respectively. Table II gives data on the recovery of DDT and DDA from the whole bodies of rats 3 hr. after intravenous injection of 50 mg. DDT/kg. Although the typical symptoms of DDT poisoning described by Cameron and Burgess (1945) appeared as a rule, there was some variation according to the route of administration: thus symptoms appeared within 6 hours of intraperitoneal injection, the animals becoming hyperexcitable, often displaying mild tremors for 48 hours but seldom going into convulsions; convulsions were the rule with oral and intravenous administration, after a latent period of 2 hours and 20 minutes respectively; death took place some 2–3 hours after the first convulsion.

From the results in Tables I and II it is evident that the only notable degradation product is DDA, and that this accumulates in tissues to a small extent. *p*-Chlorobenzoic acid was sought for, the analytical method used being the production of a rose-red colour by the dinitro compound under the conditions defined by Schechter *et al.* (1945): DDA is converted into tetranitrodichlorobenzophenone by intensive nitration, so that this neutral compound can be separated from dinitrochlorobenzoic acid after nitration of the

TABLE I

THE DISTRIBUTION OF DDT AND DDA IN RAT AND RABBIT TISSUES

(Rat tissues except where otherwise stated)

Tissue	Dosage	No. of observations	DDT mg./100 g. tissue			DDA mg./100 g. tissue		
			Mean	Range	S.D.	Mean	Range	S.D.
Liver	i.p. 1,000 mg./kg.	6	8.67	4.0–14.0	3.96	0.89	0.00–2.00	0.69
Kidney	,,	6	4.60	2.5–14.0	4.40	1.10	0.00–3.00	1.06
C.N.S.	,,	6	5.30	1.4–7.6	2.14	0.0	—	—
Muscle	,,	7	5.76	2.0–10.0	3.18	4.00	0.20–11.00	3.72
Heart	,,	1	4.80	—	—	0.0	—	—
Fat	,,	3	92.00	63.0–110.0	25.0	0.0	—	—
Blood	,,	6	3.40	1.7–5.6	1.70	0.0	—	—
C.N.S.	i.v. 50 mg./kg.	5	5.48	5.0–10.0	2.70	0.0	—	—
C.N.S. (rabbit)	,,	3	4.70	4.5–5.0	0.20	0.0	—	—
Cerebellum	,,	4	5.25	4.5–7.0	1.20	0.0	—	—
Heart	,,	5	7.60	2.0–20.5	7.30	0.0	—	—
Heart (rabbit)	,,	4	9.90	7.5–12.0	1.85	0.0	—	—
Muscle	,,	5	4.10	2.4–5.0	1.11	0.16	0.00–0.25	0.11
Blood	,,	5	11.60	9.0–15.0	2.46	0.0	—	—
Fat	,,	7	25.00	15.0–40.0	9.18	0.0	—	—
Liver	,,	10	12.80	6.0–20.0	5.09	0.0	—	—
Liver (rabbit)	,,	3	17.00	12.0–21.0	4.58	0.0	—	—
Kidney	,,	5	6.30	5.7–7.7	0.26	0.57	0.42–0.90	0.13
Kidney (rabbit)	,,	3	5.90	5.4–6.4	0.51	0.0	—	—
Adrenal (rabbit)	,,	6	20.00	17.0–25.0	2.96	0.0	—	—
C.N.S.	oral 500 mg./kg.	7	3.61	1.3–5.7	1.79	0.0	—	—
Liver	,,	6	7.96	7.0–9.0	0.84	0.0	—	—
Kidney	,,	6	10.60	8.0–15.0	2.05	0.0	—	—
Muscle	,,	7	6.00	3.0–9.0	3.09	1.43	0.00–4.80	2.07
Fat	,,	7	21.40	6.0–36.0	9.80	0.0	—	—
Blood	,,	5	1.50	0.8–2.0	0.44	0.0	—	—

alkali-soluble fraction; no evidence of the formation of chlorobenzoic acid was obtained. The possibility that DDA might be decarboxylated to the corresponding ketone was also excluded. Since it has been suggested that DDT owes its toxic action to the liberation of HCl in tissues, evidence was sought that dehydrochlorination did take place. The product of this reaction, dichlorodiphenyl-dichloroethylene, yields dichlorobenzophenone on intensive nitration, which gives a red colour in the spectrophotometric test (Schechter *et al.*, 1945). If only part of the DDT were to react, a discrepancy between the value of alkali-labile Cl (Gunther, 1945; Judah, 1949) and the spectrophotometric determination would have arisen. No evidence for such a transformation could be obtained.

TABLE II

RECOVERY OF DDT AND DDA FROM WHOLE RATS INJECTED INTRAVENOUSLY WITH 4.6 MG. DDT/RAT

Rat No.	DDT mg.	DDA mg.
1	4.50	0.13
2	4.25	0.15
3	4.35	0.20
4	4.45	0.10
5	4.40	0.12
6	4.30	0.20

The Tables also show that there is great variation from animal to animal, even when the drug is injected intravenously, an observation which agrees with the findings of Smith and Stohlman (1945). Since some evidence exists that the symptoms of DDT poisoning are cerebellar in origin, estimations were made on the cerebrum and cerebellum separately in 5 animals. However, the concentrations of DDT were identical in both tissues, suggesting a uniform distribution of the compound throughout the central nervous system, with no special affinity of the cerebellum for the drug. Since Philips and Gilman (1946) found that ventricular fibrillation sometimes occurred in DDT intoxication, special attention was

directed to the concentration of DDT in the heart. The figures were not high, except in one rat which died within 20 minutes of an intravenous injection, presumably of ventricular fibrillation.

The amounts of DDT found in body fat were always high, an observation which confirms the finding of Woodward, Ofner, and Montgomery (1945).

Toxicity of DDA

Though DDA was seldom found in tissues after intravenous and oral administration of DDT, it was thought possible that this compound might be the toxic factor. However, rats can tolerate intravenous doses of 100 mg./kg. body weight of the acid, administered as the sodium salt. Estimations of amounts in tissues 18 hours after injection showed higher concentrations of DDA than were usual in acute DDT poisoning (Table III).

TABLE III

DDA IN RAT TISSUES 18 HOURS AFTER INTRAVENOUS INJECTION OF 100 MG. DDA/KG.

Tissue	DDA mg./100 g.			No. of observations
	Mean	Range	S.D.	
Liver ..	1.4	0.6–3.0	1.29	4
C.N.S. ..	0.4	0.0–0.75	0.33	4
Muscle ..	0.3	0.0–0.50	0.23	4
Kidney ..	1.6	1.0–2.50	0.76	4
Fat ..	—	—	—	4
Blood ..	2.0	0.3–3.25	1.05	4

Absorption and Excretion of DDT

Table IV gives some data about the absorption from the gut of DDT administered in nut-oil to 6 rats by stomach tube. The absorption rate is exceedingly slow, and it is paralleled by the excretion rate. Fig. 1 shows the excretion of DDT and DDA by rats after an intravenous dose of 2.5 mg. DDT. It can be calculated from these experiments that about 10 per cent of the total dose is excreted in 5 days. No DDT is excreted in the urine, which is in keeping with the observation of White and Sweeney (1945). A similar experiment on the excretion of DDT and DDA, after intraperitoneal injection of DDT, carried out on 12 rats showed that only 2.5 per cent of a dose of 1,000 mg./kg. body weight was excreted in 5 days. The total amounts excreted in this experiment were, of course, considerably higher than after intravenous injection.

TABLE IV

ABSORPTION OF DDT FROM THE GUT OF RATS

(3 hr. after administration by stomach tube)

Rat No.	DDT administered mg.	Found in gut mg.
1	72.0	64.0
2	90.0	80.4
3	125.0	103.0
4	125.0	90.0
5	125.0	95.0
6	125.0	91.0

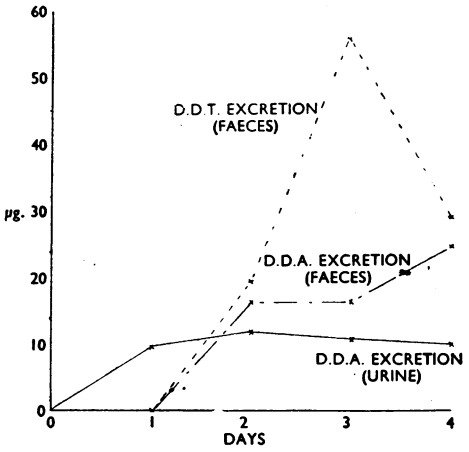

FIG. 1.—Faecal and urinary excretion of DDT and DDA by rats poisoned with 25 mg./kg. body weight of DDT i.v. Mean values for 6 rats.

Urinary chemistry in DDT intoxication

Since DDT is a halogenated hydrocarbon, the urinary sulphur partition and glycuronic acid excretion were studied in 12 rats after intraperitoneal injection of 1,000 mg. DDT/kg. body weight. The animals were kept on the standard diet described by Elson *et al.* (1945). No change attributable to DDT intoxication was observed in the excretion of sulphur or of glycuronic acid. Partition chromatography failed to disclose any alteration in the excretion of urinary amino-acids. Daily examination of the urine of these rats and of 6 others injected intravenously with 25 mg. DDT/kg. body weight gave no evidence of ketones, reducing substances, or of protein. Total urinary nitrogen increased abruptly in the group

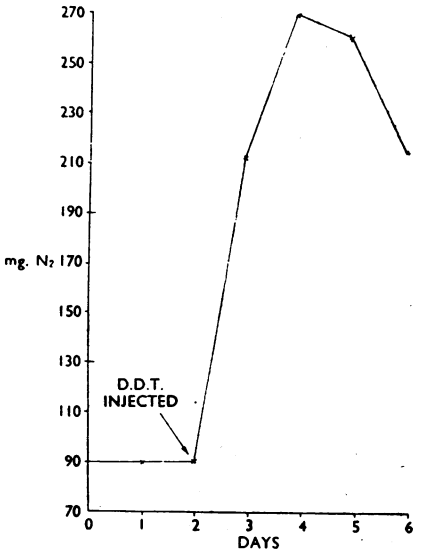

FIG. 2.—Urinary total nitrogen excretion of 6 rats given a dose of 25 mg./kg. body weight of DDT i.v.

injected intravenously (Fig. 2) and at the same time the rats lost weight, despite the fact that they were allowed food *ad libitum*; they were somewhat hyperexcitable and tremulous for the first 48 hours after injection.

Blood chemistry in DDT intoxication

The serum concentrations of potassium, calcium, sodium, magnesium, and inorganic phosphorus were determined in rabbits 2.5 hours after intravenous injection of a lethal dose of DDT. Relatively few animals were studied, since it was found difficult to obtain samples of blood when symptoms were well established. Of the 14 animals used, 8 provided samples which were so badly haemolysed as to be useless.

Table V illustrates the results. It is clear from these that the claim of Vaz *et al.* (1945) that DDT causes hypocalcaemia is groundless. The mean serum Ca was normal, but one animal showed the high value of 19.0 mg./100 ml. Cameron and Burgess (1945) also reported high serum Ca in some of their animals. The high serum K content may explain the cardiac irregularities described by Philips and Gilman (1946). During the acute phase in rabbits, auscultation of the chest revealed cardiac arrhythmias in nearly all animals. No objective method of recording the heart rhythm was available.

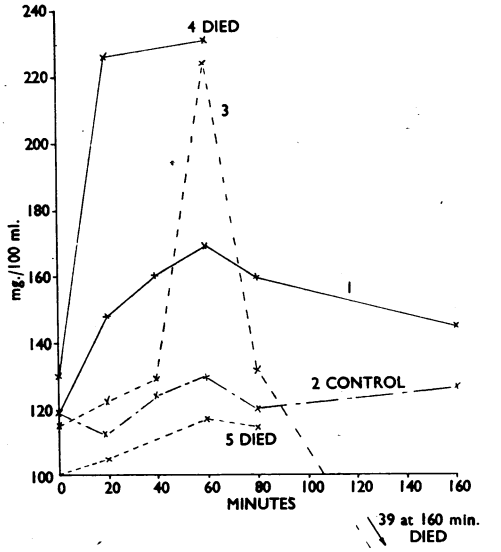

FIG. 3.—Blood sugar changes of rabbits poisoned with DDT. No. 2, control. No. 1, 25 mg./kg. DDT i.v. Nos. 3, 4, and 5, 50 mg./kg. DDT i.v.

Serum proteins were estimated in 6 rats receiving a lethal dose of DDT orally 2.5 hours after symptoms had started, but no change in protein concentration was observed.

The blood sugar and lactate were estimated in rats and rabbits during acute DDT intoxication. The results are presented in Figs. 3 and 4 and

TABLE V

SERUM ELECTROLYTES OF RABBITS POISONED WITH DDT (50 MG./KG. I.V.) AND KILLED 2½ HR. AFTER INJECTION

Substance	Control mg./100 ml. serum		Experimental mg./100 ml. serum		Number of observations	
	Mean	Range	Mean	Range	Control	Experimental
Sodium	320.0	316–320	328.0	321–336	4	6
Potassium	16.6	14.5– 20	45.0	20– 68	4	6
Calcium	14.0	14– 14.1	16.5	14.7– 19.8	4	6
Magnesium	2.5	2.0– 2.9	4.0	2.1– 6.0	4	6
Inorganic phosphorus	2.5	1.6– 3.3	4.0	2.5– 4.9	4	6

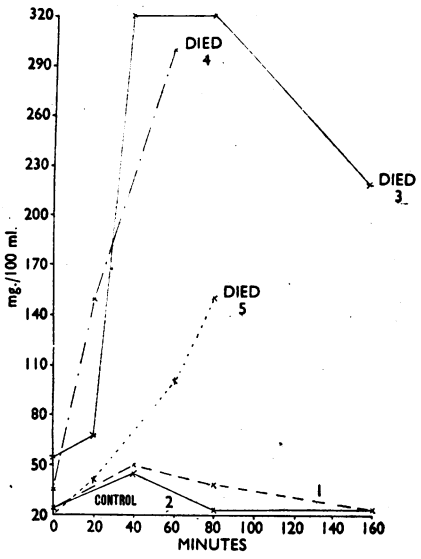

FIG. 4.—Blood lactate changes of rabbits poisoned with DDT. Numbering of curves identical with Fig. 3.

Table VI. The blood lactate rises abruptly in both species during the first hour of symptoms, while the blood sugar at first rises and then falls, sometimes to hypoglycaemic levels. However, this is neither the cause of DDT convulsions nor the explanation of death owing to DDT poisoning, for several animals maintained a normal blood sugar level, even 3 hours after a lethal dose, and despite pronounced convulsive seizures. Of course, it is possible that hypoglycaemia may contribute to a fatal termination in any given animal.

It is noteworthy that rats are far more resistant than rabbits to the derangement of blood sugar level.

TABLE VI

BLOOD SUGAR, LACTATE, AND LIVER GLYCOGEN OF RATS AFTER 50 MG./DDT/KG. I.V.

	Mean	Range	S.D.	No. of observations
	BLOOD SUGAR (mg./100 ml.)			
1st hr. experimental	124.3	114–141	12.0	6
1st hr. control	107.0	105–110	2.9	3
3rd hr. experimental	76.2	39–145	31.6	12
3rd hr. control	100.25	99–110	4.7	4
	LACTATE (mg./100 ml.)			
1st hr. experimental	72.0	53–100	22.2	6
1st hr. control	36.3	36– 37	0.4	3
3rd hr. experimental	74.3	36–100	27.0	12
3rd hr. control	29.8	27– 34	3.1	4
	LIVER GLYCOGEN (g./100 g.)			
3rd hr. experimental	0.2	0.00–0.40	0.2	5
3rd hr. control	3.9	3.0 –4.5	0.8	3

Liver damage and DDT intoxication

Liver glycogen was determined in 5 rats during acute DDT intoxication. After an intravenous injection of 50 mg./kg. body weight, the liver glycogen fell to vanishing point within 3 hours, at which time the animals were moribund (Table VI). It is interesting that, despite this, 3 of these animals had a nearly normal blood sugar level.

A further 6 animals (4 rabbits, 2 rats) were given the same dose of DDT and developed very severe symptoms and died within 0.5–1 hour. In each of these animals extensive, pre-existing liver damage of long standing was found on post mortem examination. In order to find out whether impairment of liver structure modifies the response of animals to DDT 12 rats were given subcutaneous injections of 0.2 ml. CCl_4/100 g. body weight, and this was followed 24 hours later with 50 mg. DDT/kg. intravenously. The effect was the opposite to that expected. All the CCl_4 group survived for at least 3 hours with only moderate symptoms, whereas the control group of an equal number of animals died within this time with severe symptoms. Liver damage was confirmed by autopsy and microscopical examination, and was assessed by Dr. K. K. Cheng as varying from 30–90 per cent of the total organ. To exclude the possibility of the trapping of DDT by necrotic liver cells, 4 of the livers were assayed for DDT. The content was uniform throughout healthy and damaged tissues and within the limits for normal animals receiving the same dose of DDT. No explanation can be offered for this paradoxical result.

The effect of calcium gluconate on DDT intoxication

Because Vaz *et al.* (1945) claimed that calcium gluconate prevents the symptoms of DDT poisoning, it was used in the treatment of rats given lethal doses of DDT. Three groups, each of 6 animals, were selected, together with an equal number of controls. In the first group, calcium gluconate (0.4 ml./100 g. body weight of a 10 per cent (w/v) solution) was injected intravenously 1.5 hours after 50 mg. DDT/kg. had been administered intravenously. No amelioration of symptoms was observed up to 1.5 hours after calcium

gluconate had been injected, but all the animals survived whereas the controls died. In the second group of rats, the same doses of calcium gluconate and DDT were used, but the former was injected a few minutes before the DDT. In this group, the animals developed only mild symptoms, and seemed almost normal 3 hours after injection, whereas the control rats were moribund at this time. The rats treated with calcium gluconate remained normal for the 48 hours during which they were observed. In the third group, calcium gluconate was administered, in the usual dose, before DDT, but the DDT dose was doubled. On this occasion no protective action was apparent. Both treated and untreated rats were dead within 90 minutes. No explanation of this calcium gluconate effect is obvious, but further reference will be made to it later, in connection with the effect of DDT on enzymes.

The effect of DDT on tissue respiration

Since the distribution experiments had given no information about the site and mode of action of DDT, preliminary experiments were made with slices of brain, liver, and kidney ; in addition, the effect of DDT on the respiration of rat diaphragm was investigated. Experiments were always run in duplicate. DDT was added from the side-arm of the Warburg flask after a preliminary control period of 20–40 minutes, to give final concentrations ranging from 0.001–0.0001 *M*. For these experiments, the emulsion used for intravenous injection was the best vehicle for adding the compound. When necessary, the emulsion was diluted with Krebs-Ringer solution. Controls were always carried out with the emulsion alone, but no effect due to it was ever recorded. The conditions of the experiments were those usual to tissue-slice work (Dixon, 1943).

Table VII shows the effect on the aerobic respiration and anaerobic glycolysis of liver, diaphragm, and brain cortex. Fig. 5 illustrates a typical experiment on the anaerobic glycolysis of muscle and liver ; the aerobic respiration of kidney cortex was also studied, but not its anaerobic glycolysis. The results show a very marked effect of DDT on the glycolysis of liver and muscle, but, paradoxically, not on that of brain. The aerobic respiration of all the tissues except kidney was little affected. In the latter, a 20 per cent inhibition was observed on the 3 occasions that kidney cortex slices were studied.

A curious effect was noticed when DDT in very low concentration (2×0.00001 *M*) was tested against brain and muscle in the absence of sub-

TABLE VII

THE EFFECT OF DDT ON THE RESPIRATION OF TISSUES

Tissue	Conc. of DDT $\times 10^{-4}M$	No. of exp.	Percentage inhibition (−) or acceleration (+) of O_2 uptake	Percentage inhibition of anaerobic glycolysis
Liver	10.0	2	Nil	80
,, ...	5.0	4	Nil	75
,, ...	2.5	4	Nil	Nil
Brain cortex ..	10.0	2	Nil	Nil
,, ,, ..	5.0	2	Nil	Nil
,, ,, ..	2.5	2	Nil	Nil
,, ,, .. (without substrate)	1.0	2	+100	—
Brain cortex .. (without substrate)	0.5	2	+100	—
Kidney cortex ..	2.0	2	−20	—
,, ,, ..	1.0	2	−20	—
Diaphragm (rat)	10.0	4	+25	50
,, ,,	5.0	4	+19	30
,, ,,	2.5	2	Nil	Nil

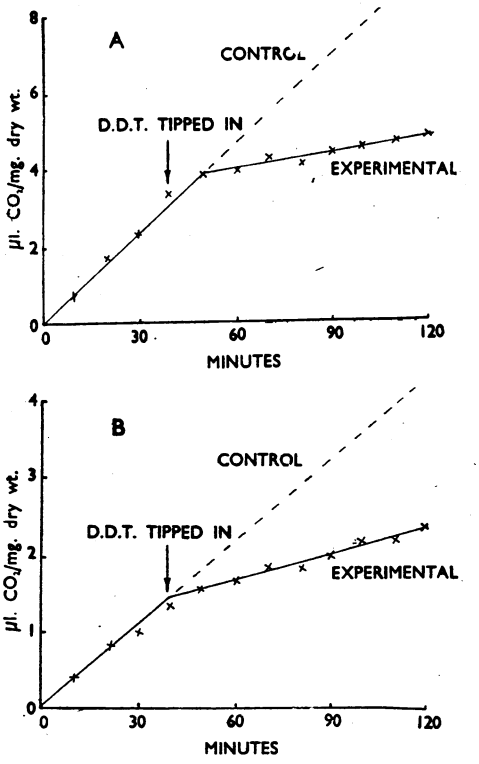

FIG. 5.—Effect of DDT ($10^{-3}M$) on anaerobic glycolysis of (A) liver slice, (B) rat diaphragm.

strate. Activations of oxygen uptake of up to 200 per cent were recorded. These experiments were not carried out on anaerobic glycolysis, and no explanation can be offered for them. The extra oxygen uptake was not due to oxidation of DDT. Assays on the content of the flasks revealed little breakdown. The latter problem also received attention during these experiments in the hope of finding the site and mechanism of DDT oxidation. Although experiments were carried on for 2.5 hours, little evidence of DDT breakdown was obtained. Indeed, with liver, diaphragm, kidney, and brain incubated with 100 μg. of DDT, 95 per cent of the compound could be recovered unchanged, and the remainder accounted for as DDA. Chemical estimations of the aerobic accumulation of lactate and pyruvate in liver slices and diaphragm showed that DDT had no effect on aerobic glycolysis.

Since DDT was found to exert a powerful effect on muscle and liver glycolysis, but none on that of brain, it was necessary to decide whether the absence of effect on the latter could be due to poor penetration of DDT into the brain slices; indeed, *in vivo* experiments suggest that brain always shows a poor concentration of DDT. In order to test this possibility, the effect of DDT on brain homogenates was investigated, since in these the fine state of division of the tissue should rule out permeability factors. Two types of homogenate were used, one prepared by the method of Elliott and Libet (1942), in which the tissue is homogenized in isotonic, calcium-free media at 37° C., and the other by the technique of Potter (1947) and Utter, Reiner, and Wood (1945), in which homogenization is carried out in ice-cold distilled water and the preparation fortified with ATP and coenzyme 1. The activity achieved by the latter type of homogenate is far greater than with slices. The former is not fortified, and has an activity comparable with that of cortex slices. DDT was found to have no effect on the oxygen uptake or anaerobic glycolysis of brain homogenates prepared by either method, and the results were confirmed for anaerobic glycolysis by chemical estimation of lactate produced in the Warburg flasks.

Despite these negative results, DDT was also tested against a variety of enzymes. DDT was without effect when tested against the following enzymes: aldolase (Dounce and Beyer, 1948); adenosine triphosphatase of brain and liver (Dubois and Potter, 1943); choline oxidase (Bernheim, 1940); glutamic acid dehydrogenase; glycolytic cycle in brain homogenates (Utter *et al.*, 1945); hexokinase; lactic dehydrogenase (Green and Brosteaux, 1936); malic dehydrogenase (Potter, 1946); pyruvic oxidase (Passmore *et al.*, 1933), succinoxidase (Schneider and Potter, 1943); triose phosphate dehydrogenase (Jandorff *et al.*, 1941); creatine phosphokinase (Lohmann enzyme) (Lehmann and Needham, 1937); aerobic phosphorylation (Loomis and Lipmann, 1948; Green *et al.*, 1948). In every test, the final concentration of added DDT was 5×10^{-4} *M*, except in that of the glycolytic cycle, where concentrations of 10^{-2} *M* and 10^{-3} *M* were also used; and in that of pyruvic oxidase, triosephosphate dehydrogenase, and the aerobic phosphorylation system, where concentrations of 10^{-3} *M* were also employed. The references cited give for each enzyme the methods used.

The system employed for oxidative phosphorylation requires further description. The "cyclophorase system" of Green, Loomis, and Auerbach (1948) was used according to the method of Loomis and Lipmann (1948), but in place of yeast hexokinase the transphosphorylation was accomplished through adenylic acid to fructose by means of a crude preparation of brain hexokinase (200 mg of acetone dried brain triturated with 4.0 ml. of ice-cold water and centrifuged; 0.1–0.2 ml. of the supernatant was added to each flask). This system esterifies inorganic phosphate at a brisk rate, but DDT did not affect either the oxygen uptake or the esterification. This experiment excludes the possibility that DDT might have an action similar to that of dinitrophenol in inhibiting synthetic reactions.

These findings did not rule out the possibility that DDT might act on a specific enzyme *in vivo*. Experiments were therefore undertaken to assay the activity of the glycolytic enzymes in tissues, and also to determine the activity of certain other systems.

Table VIII shows the results of such investigations. The same procedure was always followed: the animals were injected with DDT (50 mg./kg.) intravenously; 1 hour later they were killed and the enzyme assays carried out immediately. Controls were run in each experiment, and consisted of an equal number of animals, litter mates of the experimental group.

The assays gave surprisingly uniform results. Estimations of brain glycolysis (Meyerhof and Geliazkowa, 1947) in 6 rats agreed within 2 per cent, and showed no change between experimental and control animals, despite the fact that very severe symptoms were apparent in the poisoned

TABLE VIII

THE ASSAY OF ATP-ASE, AEROBIC RESPIRATION, AND ANAEROBIC GLYCOLYSIS IN RAT BRAIN AFTER ACUTE DDT INTOXICATION

	ATP-ase μg. P liberated per mg. wet wt./15 min. (14 observations)		Aerobic respiration Q_{O_2} (30 min.) (6 observations)		Anaerobic glycolysis $Q_{G^2}^N$ (30 min.) (6 observations)	
	Mean	Range	Mean	Range	Mean	Range
Control	5.3	4.2–6.4	9.75	9.0–10.0	17.35	17.2–17.5
Experimental	4.95	3.4–6.4	9.0	8.0–10.0	17.5	16.6–18.0

animals. The agreement between the assays for ATP-ase was not so good. Dubois and Potter (1943) state that an experimental variation of 15 per cent may be encountered. No significant change was observed in a series of 12 animals. This enzyme was studied because it is known that it requires Ca ions for maximal activation *in vitro*, and it was hoped that this might provide an explanation of the calcium gluconate effect. Accordingly, each assay was conducted with and without Ca in the experimental vessels. In 3 rats poisoned with DDT it was found that, without Ca, only 30 per cent activity was obtained, whereas in the control group, both with and without Ca, the activity was maximal, and this was also true in the DDT group with Ca. This result could not be confirmed in a second experiment, carried out on 8 litter mates of the animals used in the first experiment. It must therefore be assumed to be fortuitous.

The aerobic respiration of the brain was assayed in a group of 6 rats. The experiment was carried out on isotonic homogenates prepared by the method of Elliott and Libet (1942), and, as before, negative results were obtained.

Discussion

Though a certain amount of information about the mode of action of DDT is available, considerable gaps still exist in our knowledge of fundamental points. Smith and Stohlman (1944, 1945) devised a method for the estimation of organically bound chlorine and applied it to the estimation of DDT in the tissues of several mammalian species. They also carried out pharmacological experiments with the compound. But their method lacks specificity, and they were unable to determine what fraction of the organic Cl was present in tissues as DDT and what as degradation products. White and Sweeney (1945) demonstrated that animals given DDT excreted in their urine an alkali-soluble compound which they identified as 2 : 2-bis(*p*-chlorophenyl)acetic acid (DDA) by *x*-ray diffraction studies and synthesis. Similar methods failed to disclose any unchanged DDT in the urine of their experimental animals. Stiff and Castillo (1945a, b) devised a method for the estimation of DDT, and applied it to the study of tissue distribution of the compound. Their conclusion that no DDT can be found in tissues may be criticized on the ground that during extraction of the tissues they carried out a saponification with alcoholic alkali, a procedure known to destroy DDT (Gunther, 1945). The degradation product formed by the interaction of DDT and alcoholic alkali (2 : 2-bis (*p*-chlorophenyl) - 1 : 1-dichloroethylene) does not yield a colour with their reagents (unpublished data). Schechter *et al.* (1945) published a specific method for the estimation of DDT and related compounds, depending on intensive nitration followed by the development of colours in benzene solution with methanolic sodium methylate, and subsequent spectrophotometric estimation. This method was later applied to animal tissues by Ofner and Calvery (1945) and by the present writer (unpublished). It is capable of estimating 10 μg. and upwards of DDT and DDA. The author devised a method for the estimation of microgramme amounts of organic chloride which makes it possible to carry out simultaneous determinations by both techniques on limited samples of tissue (Judah, 1949).

Several investigations have been carried out on the mode of action of DDT. Lauger, Pulver, and Montigel (1945) found that the blood sugar of animals exposed to the insecticide first rose and then slowly fell to hypoglycaemic levels. Simultaneously, the blood lactate rose and the CO_2 combining power of the blood fell to about 50 per cent of the normal value. The liver glycogen of rats was depleted within 3 hours of the administration of DDT. These authors maintained that this combination of events is the cause of death in DDT poisoning. On the other hand, Vaz, Pereira, and Malheiro (1945) claimed that DDT intoxication in dogs was relieved by the administration of calcium gluconate, and consequently they concluded that hypocalcaemia was the cause of the symptoms of DDT poisoning. No blood calcium data are included in their paper.

The action of DDT on enzymes has received little attention, although Richards and Cutkomp (1945) have shown that the drug is without effect on cholinesterase of brain, and Tobias, Kolros, and

Savit (1946) obtained no evidence that the acetylation of choline *in vitro* is affected by DDT. The latter authors demonstrated that whereas the acetylcholine content of insects in the paralytic stage of DDT poisoning is increased, this is not so in the frog and rat. Lewis and Richards (1945) tested the effect of DDT on tissue cultures, but could find no effect.

These negative results, together with the existence of a long latent period after the oral administration of DDT before toxic symptoms become apparent, suggest the possibility of degradation of DDT to a toxic compound in the body. Philips and Gilman (1946), using their preparation for intravenous injection, found that the latent period after this mode of administration was only 10 minutes. Hence they concluded that DDT is the toxic agent, but they give no estimations of amounts in tissues in support of their claim.

The results of the present investigation may be dealt with in two sections: (1) distribution experiments and (2) the physiological effects and mode of action.

From the former experiments it may be concluded that the main degradation product of DDT is DDA. It cannot be said to be the only one because (1) small amounts of another compound would not affect the recovery of the substance from the whole animal, the inherent error of the chemical estimation being of the order of 5 per cent; (2) agreement between chlorine determination and spectrophotometric data is subject to the same limit, and it is also possible that such a compound would be water-soluble and so escape analysis altogether. The only safe conclusion is that a toxic intermediate if formed must be active in very low concentration, but no evidence for its existence has yet been found.

The distribution figures also throw light on the theory of Martin and Wain (1944) that DDT acts by liberating HCl in tissues. Busvine (1945) studied this and other theories of the mode of action of the drug, and came to the conclusion that the ease with which a compound is dehydrochlorinated has no relation to its insecticidal activity. In the present work no evidence for the dehydrochlorination of DDT in tissues was obtained by direct analysis, and it may therefore be concluded that HCl liberation plays no part in the toxic action of DDT on mammals.

The investigation of the detoxication of DDT was a major part of the present work, and it was found that the breakdown of DDT both *in vivo* and by tissue slices *in vitro* is very slow. No evidence of breakdown by a specific tissue was obtained in either type of experiment; as a result of these findings, the problem of the mechanism of DDT oxidation was not further investigated.

If the rate of degradation of the insecticide is low, the excretion of DDT is exceedingly so. No evidence could be obtained of conjugated derivatives of DDA in the urine of rats, either with amino-acids or with glycuronic acid. This result is not surprising in view of the fact that rats excrete such minute amounts of DDA and DDT. Detoxication mechanisms for DDT must, therefore, be very inefficient. The storage of DDT in body fat may have a protective action, but it has not been possible to test this hypothesis.

The changes in blood chemistry after DDT poisoning are of some interest. Blood sugar and lactate levels of the type found have also been reported by Handler (1945) in a study of the effects *in vivo* of inhibitors of glycolysis. Unfortunately the results with DDT are complicated by convulsions in the experimental animals. These could account for the whole picture without any other hypothesis. If the experiments could be done on previously curarized animals the point could be settled, but this was impossible. Lauger *et al.* (1945) suggest that the action of DDT depends on the changes in blood sugar, lactate, and alkali-reserve already described. However, it has been shown in the present work that while the changes are qualitatively similar to those observed by Lauger *et al.*, they are not always quantitatively sufficient to cause death. The differences in the two investigations may lie in the rate of development of symptoms. The animals in Lauger's work were dosed orally and took about 24 hours to die, whereas in the present work intravenous injection was used for greater precision and death occurred within 3 hours; blood sugar and lactate levels were frequently nearly normal at this time; consequently hypoglycaemia cannot be accepted as a general mechanism for the production of symptoms or the cause of death in DDT poisoning.

The variations in the blood cations can probably also be attributed to the convulsions. Thus Heppel (1940) has shown that muscle during activity loses K, and this could account for the high serum K levels found in convulsing animals. The acidosis caused by lactate accumulation in the blood might result in the occasional high serum Ca values obtained. It should be mentioned that in a few analyses of tissues (muscle and brain) no significant variations in Ca and K levels could be found. The present work throws little new

light on the problem of treating accidental DDT poisoning, but three main points may be made.

(1) Glucose administration has been claimed by Lauger *et al.* to protect their animals from death; no attempt has been made to confirm this in the present work, but it may well be true in any given case. The liver glycogen data also support this view.

(2) Sodium phenobarbitone has been claimed by Philips and Gilman (1946) to protect animals from DDT poisoning. They suggest that the "specific" effect of this barbiturate on the motor cortex is the basis for this action. It would appear likely on *a priori* grounds that suppression of the convulsive episodes would have a beneficial effect. It should be remarked, however, that Philips and Gilman noted ventricular fibrillation in some of their animals despite the fact that they were curarized.

(3) Calcium gluconate has been shown to reduce the severity of DDT symptoms, when given in large doses. It is apparent, however, that protection is partial, and that the best results are obtained when calcium gluconate is administered before DDT. This takes away from its value as a means of treatment in accidental poisoning, but it should be worth a trial should such poisoning take place.

Experiments on the effect of DDT on enzymes and on tissue respiration have not elucidated the mode of action of the drug. It is difficult to explain the discrepancy between the action on liver and muscle, and that on brain cortex, especially as no effect could be demonstrated on the glycolytic enzymes in solution. It is possible that DDT is degraded to an active compound in liver and muscle, but recovery experiments performed on the contents of the Warburg flasks do not support this theory. The possibility of trace substances always exists. Against this possibility are the results of assaying the glycolysis and aerobic respiration of brain homogenates after DDT poisoning of the whole animal. The experiments of Philips and Gilman (1946) with eviscerated preparations support the view that DDT is not degraded in the liver or other visceral organs. These authors showed that the toxicity of DDT to eviscerated animals was as great or greater than to intact animals. A further possibility is suggested by the work of Pilgrim and Elvehjem (1945) on the effect of succinylsulphathiazole on enzyme assays in rat liver; they found that the drug exerted an indirect inhibitory effect on certain enzymes, and attributed this to a great accumulation of Ca in the tissues. It is conceivable that our techniques were such that analogous effects were nullified.

The mechanism of DDT action would not be solved, even by the discovery of a specific enzyme inhibition. The substance is so inert chemically that it is difficult to imagine any action due to it except by some such physico-chemical mechanism as an interference with cell-surfaces. There is some evidence for this possibility; thus Gordon and Welsh (1948) found that DDT causes repetitive firing of the isolated crayfish nerve and showed that the effect was partially reversed by Ca and Mg ions. Furthermore, Fathy (1948) claims that DDT causes expansion of a spread monolayer of myristic acid and cholesterol. These observations are suggestive, but it should be emphasized that loose reference to "surface action" by a drug does not help to elucidate the mode or the mechanism of its effect. Inert substances such as DDT may well have an indirect action on a specific enzyme system by so disorganizing the surface (or surfaces) of the intact cell that the access of co-factors or substrate to the system is disturbed. It may be impossible by present techniques to demonstrate this effect (1) because isolation of the enzyme involves destruction of cell structure or (2) because studies with intact slices permit only overall estimation of gas-exchange, etc., and the enzyme concerned may not contribute significantly to such changes. Advances in cytochemistry may provide means for investigation of such action.

Reference should be made to the evidence that DDT acts on the central nervous system and that symptoms are due to some central disturbance. The most convincing experiments are those of Philips and Gilman (1946), who showed that curarized animals injected with lethal doses of DDT developed typical convulsive episodes as shown by the electro-encephalogram. In addition, it has been found that frogs suffering from the typical symptoms of DDT intoxication lose the signs when the brain is destroyed, nor do they return when normal reflex activity is restored. If the cord transection is carried out before the injection of DDT, symptoms never arise.

In conclusion, it must be stated that the present investigation has failed to explain the mode of action of DDT, its most important objective. Furthermore, it has been impossible to prove or disprove the presence of a toxic intermediate during DDT metabolism by direct chemical estimation. The whole problem of liver damage pro-

duced by chronic DDT poisoning (Cameron and Burgess, 1945) remains unsolved; and the reason for the protective action of calcium gluconate is still to be explained.

Summary

1. The distribution of DDT in tissues and its absorption and excretion have been investigated.

2. Experiments on the metabolic fate of the drug indicate that DDA is the only metabolite. It is pointed out that small amounts of other substances may be produced. No conclusive evidence that DDT itself is the toxic agent can therefore be brought. Direct analysis shows that the HCl liberation theory is untenable in the mammalian species. It has also been found that DDA is relatively non-toxic.

3. Investigation of the blood chemistry in DDT intoxication shows that violent changes occur. The blood sugar rises and then tends to fall. Blood lactate rises to relatively high levels. These results are more pronounced in rabbits than in rats. The serum K of rabbits often presents high values; the serum Ca is never low, and is sometimes raised. Plasma protein, non-protein nitrogen, serum Na, Mg, and apparent inorganic P show no changes. Evidence is presented that the variations are probably secondary to the convulsive seizures suffered by animals poisoned with DDT.

4. Calcium gluconate affords a partial protection against DDT intoxication. It is shown that this is not because DDT causes a hypocalcaemia.

5. The relationship of liver damage to acute DDT intoxication was investigated. Though in some animals with long-standing, pre-existing liver damage DDT toxicity appears to be enhanced, with experimentally produced acute liver damage an opposite and paradoxical effect occurs. The problem of liver damage produced by chronic DDT poisoning remains unsolved. Liver glycogen is rapidly depleted in rats poisoned with a lethal dose of DDT.

6. Experiments with tissue slices and isolated rat diaphragm failed to show any important breakdown of DDT. Kidney, liver, brain, and intact diaphragm oxidized some 5 per cent of the DDT to DDA. The remaining 95 per cent was recovered unchanged. There appears to be no specific site for the breakdown of DDT.

7. Experiments on the respiration of tissues and on enzymes failed to show the mode of action of DDT. Inhibition of anaerobic glycolysis, which was found with liver and diaphragm, could not be demonstrated with brain cortex. DDT was also found to be without effect on the glycolytic enzymes in solution and on a variety of other enzymes. Some acceleration of aerobic respiration was observed with DDT in rat diaphragm and brain cortex slices (when the latter were exposed to the drug in the absence of substrate). The possibility that DDT might have an effect similar to dinitrophenol was shown to be untrue by the lack of effect of the drug on aerobic phosphorylation. Chemical estimation of pyruvic acid and lactate in the Warburg flasks showed that aerobic glycolysis was not increased in liver and muscle.

My thanks are due to Prof. G. R. Cameron, F.R.S., for much advice and encouragement; to Dr. C. H. Kellaway, F.R.S., for the hospitality of the Wellcome Foundation Laboratories where most of the work on enzymes was done; to Dr. H. H. Green, Ministry of Agriculture Veterinary Laboratory, Weybridge, for the determination of blood cations on some of the experimental animals; to Dr. C. E. Dent for carrying out chromatographic analyses on several urine samples; to Mr. H. G. Williams-Ashman and Dr. G. V. R. Born for much assistance and valuable suggestions; and to Messrs. A. Hemming, J. S. Pizey, and J. C. Godding for skilled technical assistance at different times during the investigation.

The writer is indebted to the Medical Research Council for a personal grant and to the Graham Research Fund of the University of London and the Agricultural Research Council for expenses grants.

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