1:1:1-Trichloro-2:2-di-(*p*-chlorophenyl)ethane (DDT) and Rat Adipose Tissue

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The universal use of 1:1:1-trichloro-2:2-di-(*p*-chlorophenyl)ethane (DDT) and the knowledge that it readily accumulates within mammalian fat tissue clearly indicates that the health hazards of this insecticide cannot be ignored. Thus it has been demonstrated that DDT and its derivative, 1:1-di-(*p*-chlorophenyl)-2:2-dichloroethylene, can be found in the fat depots of humans who have had no occupational contact with the compounds (Hayes, Quinby, Walker, Elliott & Upholt, 1958). Further, they accumulate steadily during prolonged feeding experiments on man (Hayes, Durham & Cueto, 1956).

In recent years, experimental studies have shown that fat tissue, far from having a purely passive role of fat storage, is metabolically as active as most other tissues. In view of this active state it was of interest to examine the possibility that DDT *in situ* may affect the metabolism of fat tissue. In the present work, the behaviour of adipose tissue from rats which had been fed with DDT in their diet was examined. Other experiments are reported in which DDT was added to adipose tissue *in vitro*. The potential capacity of DDT and related compounds to influence cellular metabolism is shown by experiments in which these substances were added to rat-liver mitochondria.

EXPERIMENTAL

Male albino rats were used. Fat tissue was taken from the groin and epididymal regions and 'brown' fat was taken from the interscapular depot.

Composition of diet. Control diet was 41B powder (Bruce & Parkes, 1956) containing arachis oil added to a concentration of 1.3%.

DDT and Dieldrin diets were 41B powder containing 1.3% (v/w) of arachis oil in which had been dissolved DDT or Dieldrin to give final concentrations of 200 or 50 p.p.m. respectively.

Carbohydrate-rich diet was as described by Itzhaki & Wertheimer (1957), but with arachis oil as the fat component.

Special chemicals. 1:1:1-Trichloro-2:2-(*p*-chlorophenyl)-ethane, *pp'*-isomer, (DDT) was a gift of Geigy Co. Ltd.; for experiments with mitochondria this was recrystallized from 70% ethanol. 1:1-Di-(*p*-chlorophenyl)-2:2-dichloroethyl-

ene was prepared from DDT (Grumitt, Buck & Stearns, 1945). Di-(*p*-chlorophenyl) acetic acid was prepared from di-(*p*-chlorophenyl)dichloroethylene (Grumitt, Buck & Jenkins, 1945). 1:2:3:4:10:10-Hexachloro-6:7-epoxy-1:4:4a:5:6:7:8:8a-octahydro-1:4:5:8-dimethanonaphthalene (Dieldrin), a gift of Shell Petroleum Co. Ltd., London, was recrystallized from ethanol. 1:2:3:4:5:6-Hexachloro-cyclohexane α -isomer, a gift of Imperial Chemical Industries Ltd., was also recrystallized from ethanol. Adrenaline acid tartrate, B.P., was obtained from Burroughs Wellcome and Co. Ltd., London, and insulin, 40 units/ml., from Allen and Hanbury Ltd., London. Bovine-plasma albumin, fraction V was supplied by The Armour Laboratories, Hampden Park, Eastbourne, Sussex.

Glycogen synthesis (Tuerkischer & Wertheimer, 1942.) One-month-old rats were fed for 3 months on a diet containing 200 p.p.m. of DDT. A second group of rats were fed on the control diet only. At the age of 4 months the rats were separated in individual cages and the quantity of food was restricted so that each rat received 6–7 g. of the appropriate diet daily. This weight of diet represented about 60% of the normal daily consumption. The rats had free access to water. At the end of 10 days the rats had lost approximately 20% of their previous weight. Two rats from each group were killed and samples of the groin-fat depots and of interscapular fat were taken for glycogen analysis (Somogyi, 1937). The rest of the rats were then given unrestricted access to 'carbohydrate-rich' diet until the end of the experiment. A number of rats (figures in parentheses, Fig. 1) from both the experimental and control groups were killed on each consecutive day of the refeeding period. Groin fat and interscapular fat were dissected from each animal and the glycogen content was determined. The results are expressed as g. of glucose/100 g. wet wt.

Glucose accumulation by epididymal fat. The rats were killed by a blow on the head and exsanguinated. The distal portion of each epididymal-fat pad was removed by a single cut, weighed on a torsion balance and dropped into a 10 ml. conical flask containing 1.6 ml. of Krebs-Ringer bicarbonate solution (Umbreit, Burris & Stauffer, 1959) containing 0.55 M-glucose. To each flask was added 0.2 ml. of insulin or water. The final concentration of insulin was 2 units/ml. The flasks were sealed with Parafilm (A. G. Gallenkamp and Co. Ltd., London, E.C. 2) and then gassed for 10 min. with O₂ + CO₂ (95:5) through syringe needles piercing the Parafilm. After gassing, the flasks were resealed with a second piece of Parafilm and shaken for 4 hr. at 37° in a Dubnoff metabolic shaker. Duplicate 0.25 ml. samples of medium were then taken for glucose

determination. The adipose tissue was removed from the medium, defatted and dried (Itzhaki & Wertheimer, 1957). Results are expressed as mg. of glucose taken up/10 mg. of dry defatted tissue/hr. In some experiments 20 mm-methylene blue replaced insulin.

Release of free fatty acids and the adrenaline effect. The initial stage of these experiments was as described in the preceding paragraph except for the composition of the incubation medium. Each 10 ml. conical flask contained 5 ml. of Krebs-Ringer phosphate solution (Umbreit *et al.* 1959) containing 1% of bovine albumin and adjusted to pH 7.4 (Gordon & Cherkas, 1958). Adrenaline (0.1 ml.) was added from a solution containing 50 μ g./ml. After incubation at 37° for 4 hr. the adipose tissue was removed, defatted and dried as before. Samples of the medium were taken for analysis of free fatty acids. The results are expressed as μ equiv. of fatty acid released/mg. of dry defatted tissue/hr.

Oxygen uptake. Oxygen uptake of fat tissue was measured in a conventional Warburg apparatus at 37° with air as the gas phase. Groin fat was removed as rapidly as possible after killing the animal and chilled on ice. Slices were cut on a Stadie-Riggs-type microtome, the lower part of which had been constructed of stainless steel. The microtome stood on a block of ice while the fat was sectioned. The fat slices were weighed on a torsion balance and placed in a Warburg flask containing 3.0 ml. of Krebs-Ringer phosphate solution, pH 7.4, with 0.01 M-glucose and 0.01 M-succinic acid; 0.15 ml. of 20% KOH was in the centre well. After an equilibration period of 10 min., O_2 consumption was measured at intervals for 3 hr. At the end of the experiment fat tissue was removed from the flasks and defatted and dried. Consumption of O_2 was expressed as μ l. of O_2 /mg. of dry defatted tissue/hr.

Each rat was decapitated and the brain quickly removed into ice-cold Krebs-Ringer phosphate solution. The cerebellum and hind brain were cut off, the hemispheres were divided and most of the white matter was removed from the lower surface. Two slices were cut with a Stadie-Riggs microtome, from each hemisphere, and placed directly into the main compartment of Warburg flasks. In each flask there was 3.0 ml. of Krebs-Ringer phosphate solution, pH 7.4, containing 0.01 M-glucose. Additions of DDT and of other compounds were made as 0.03 ml. of their solutions in dimethylformamide. Dimethylformamide (0.03 ml.) was added to the control tissues. The centre well contained 0.15 ml. of 20% KOH. The temperature was 37°. The flasks were gassed for 5 min. with O_2 , equilibrated for 10 min. and manometric readings were taken for 60 min. The brain slices were then removed and dried on weighed watch glasses for 24 hr. at 105°. The results are expressed as μ l. of O_2 uptake/mg. dry wt./hr.

Experiments with liver mitochondria. Liver mitochondria were prepared and experiments on oxidative phosphorylation, respiration in the presence of pyruvic acid and adenosine triphosphatase were performed as described by Aldridge (1958). Additions of DDT and other compounds were made as 0.03 ml. of their solutions in dimethylformamide and the same quantity of dimethylformamide was added to the control experiments.

Analytical methods. Glucose was determined by the method of Nelson (1944) as revised by Somogyi (1952). 1:1:1-Trichloro-2:2-di-(p-chlorophenyl)ethane was determined by the method of Schechter, Soloway, Hayes & Haller (1945) and unesterified fatty acids were determined

by the method of Grossman, Palm, Becker & Moeller (1954).

RESULTS

The amount of DDT present in groin fat and interscapular fat was determined in three rats fed on a diet containing 200 p.p.m. of DDT for 3 months. The average concentrations of DDT in groin fat and in interscapular fat were 700 and 239 μ g./g. respectively.

Wertheimer (1945) demonstrated that in rats which were semi-starved for several days and then given a carbohydrate-rich diet, there occurred a deposition of glycogen in the adipose tissue. This effect, although pronounced during the first 1 or 2 days of the refeeding régime, was transitory and was followed by fat deposition. As the change in fat and glycogen involved both synthetic and anabolic processes it might be sensitive to a toxic substance stored in the fat. This experiment was repeated on groups of DDT-fed and control rats. Comparison of the mean and standard error of the mean between DDT and normally fed rats on corresponding days of the refeeding period showed that DDT had no effect on the sequence or extent of the glycogen deposition (Fig. 1).

Most studies of the effect of DDT on oxidative metabolism have been performed on insect tissue and of the few concerned with mammalian tissue, in particular liver and brain slices, the results are variable and even contradictory (cf. Johnston, 1951). The effects *in vitro* on rat-brain-slice respiration of DDT, and of two other chlorinated insecticides, Dieldrin and 1:2:3:4:5:6-hexachlorocyclohexane, are shown in Table 1. In the same table,

Fig. 1. Glycogen deposition in fat of rats fed on a carbohydrate-rich diet after a period of partial starvation. Glycogen is expressed as glucose. Figures in parentheses refer to number of rats and other figures are S.E.M. ■, Rats fed on 41 B diet containing 200 p.p.m. of DDT; □, rats fed on 41 B diet only.

the respiration of fat slices from DDT-fed rats is also recorded. There is no statistically significant difference between any of the experimental groups and the corresponding controls.

The metabolism of adipose tissue is influenced by insulin, both *in vivo* (Wertheimer, 1945) and *in vitro* (Itzhaki & Wertheimer, 1957; Winegrad & Renold, 1958). The last-named authors also describe experiments which suggest that, in adipose tissue, glucose may be metabolized by the phosphogluconate oxidative pathway. Brin & Yonemoto (1958), working with erythrocytes, demonstrated that the rate of glucose metabolism by this route could be stimulated by oxidation-reduction dyes such as methylene blue. In Table 2 are the results of experiments in which the glucose uptake of epididymal fat from DDT- and Dieldrin-fed rats was determined with and without the addition of insulin. The table also gives the result of adding methylene blue to the epididymal fat of DDT-fed rats. There was the expected increase of glucose uptake after the addition of insulin but the small increase after the addition of methylene blue was not significant. The presence of DDT or Dieldrin in

the diet did not, under any condition, influence the uptake of glucose.

The factors influencing the mobilization of lipids from fat depots have been studied by Gordon (1957) and Dole (1956). The liberation of unesterified fatty acids from fat tissue *in vitro* was accomplished by Gordon & Cherkes (1958), who also demonstrated that the addition of adrenaline stimulated an increase of these acids. It was of interest to investigate the possibility that adipose tissue from DDT-fed rats might be different from normal fat in its response to adrenaline. Table 3 shows that the presence of DDT had no effect upon the release of fatty acids either in the presence or absence of adrenaline.

The preceding experiments indicate that metabolic activity of adipose tissue is insensitive to the presence of DDT. The ability of DDT and related substances to influence metabolic processes of cellular constituents is shown in the next experiment (Table 4). Also investigated were the main metabolites of DDT: 1:1-di-(*p*-chlorophenyl)-2:2-dichloroethylene and di-(*p*-chlorophenyl)acetic acid. All the compounds affect oxidative phosphoryl-

Table 1. *Effects of DDT, Dieldrin and hexachlorocyclohexane on respiration of tissue slices*

Q_{O_2} (μ l. of O_2 uptake/mg. dry wt./hr.) is given with s.e.m. The last two columns give Student's *t* values and probabilities (*P*). Expt. 1, DDT fed to rats at 200 p.p.m. of diet for 3 months. Expt. 2, DDT and other insecticides added *in vitro*.

			No. of rats	Q_{O_2}		t	P
Compound	Tissue	Concentration		Control	Experimental		
			Expt. 1				
DDT	Groin fat	200 p.p.m. in diet	5	9.2±2.6	6.2±2.6	1.1	> 0.3
			Expt. 2				
DDT	Brain	200 μ M	9	9.8±0.5	10.2±0.3	0.7	> 0.5
Dieldrin	Brain	200 μ M	4	7.9±2.3	11.4±4.2	0.7	> 0.5
Hexachloro- cyclohexane	Brain	200 μ M	2	9.2±1.4	10.8±1.1	0.9	> 0.5

Table 2. *Effect of insulin and methylene blue on glucose uptake of epididymal fat*

Results are given as mean values with s.e.m. Figures in parentheses are Student's *t* values and probabilities (*P*). Final concentrations were insulin, 2 units/ml., and methylene blue, 100 μ M.

Expt.	No. of rats	No. of samples	Glucose uptake (mg. of glucose/10 mg. of dry defatted tissue/hr.)	
			No insulin	Insulin
Control	3	11	1.42 ± 0.36	2.94 ± 0.40
DDT (200 p.p.m.)	3	12	1.15 ± 0.13 (<i>t</i> 0.7, <i>P</i> > 0.4)	2.48 ± 0.29 (<i>t</i> 0.9, <i>P</i> > 0.3)
Control	3	12	0.84 ± 0.08	2.11 ± 0.19
Dieldrin (50 p.p.m.)	3	12	1.07 ± 0.11 (<i>t</i> 0.9, <i>P</i> > 0.3)	2.56 ± 0.25 (<i>t</i> 0.8, <i>P</i> > 0.4)
			No methylene blue	Methylene blue
Control	3	12	0.63 ± 0.18	1.03 ± 0.17
DDT (200 p.p.m.)	3	12	0.79 ± 0.07 (<i>t</i> 0.7, <i>P</i> > 0.4)	1.34 ± 0.23 (<i>t</i> 1.1, <i>P</i> > 0.3)

Table 3. *Release of unesterified fatty acids from epididymal fat*

Epididymal fat (300–500 mg.) was incubated in 5 ml. of Krebs–Ringer phosphate (pH 7.4) containing 2 % of bovine albumin. Adrenaline concn. was 1 μ g./ml. of medium. Results are given as mean values with s.e.m. Figures in parentheses are Student's *t* values and corresponding probabilities (*P*) calculated by comparison of appropriate experiments.

Expt.	No. of rats	Adrenaline	Fatty acid released (μ equiv./mg. of dry defatted tissue/hr.)
Normal diet	8	Absent	0.063 $\pm$ 0.0097
		Present	0.179 $\pm$ 0.027
DDT diet (200 p.p.m.)	6	Absent	0.081 $\pm$ 0.0097 (<i>t</i> 1.4, <i>P</i> 0.18)
		Present	0.203 $\pm$ 0.022 (<i>t</i> 0.7, <i>P</i> 0.5)

Table 4. *Concentrations of chlorinated insecticides and metabolites of DDT affecting liver mitochondria*

Oxidative phosphorylation: concentrations affecting inhibition of P uptake and O_2 uptake during oxidation of pyruvic acid. Oxidation: concentrations affecting oxidation of pyruvic acid in the absence of hexokinase and glucose. Adenosine triphosphatase (ATPase): concentrations producing maximal stimulation of adenosine triphosphatase in the absence of substrate. Figures in parentheses refer to μ moles of phosphate liberated/mg. of protein/hr. Control values were 1.03 $\pm$ 0.40 μ mole/mg. of protein/hr. For details of reaction mixtures see Aldridge (1958).

Compound	Concn. (μ M)	Oxidative phosphorylation		Oxidation (μ M)	ATPase (μ M)
		Inhibition of phosphate uptake (%)	Inhibition of oxygen uptake (%)		
DDT	35	62	64	40 (67 % stimulation)	200 (7.3)
Dieldrin	35	62	49	—	—
Hexachlorocyclohexane	90	50	37	— (Inhibition)	—
Di-(<i>p</i> -chlorophenyl) acetic acid	80	55	37	40 (77 % stimulation)	40 (5.2)
Di-(<i>p</i> -chlorophenyl)dichloroethylene	40	31	24	40 (50 % stimulation)	200 (7.3)

ation but DDT differs from the others in that O_2 uptake is inhibited to the same extent as P uptake, so that the oxidative phosphorylation quotient (P/O) remains unaltered. The other compounds inhibit P uptake more than O_2 uptake and thus to some extent lower the P/O ratio. DDT and its metabolites stimulate respiration of mitochondria with pyruvate as substrate in the absence of hexokinase and glucose. Dieldrin had no effect and hexachlorocyclohexane inhibited respiration. DDT and di-(*p*-chlorophenyl)dichloroethylene stimulated adenosine triphosphatase activity at concentrations more than 10 times as high as the concentrations affecting oxidative phosphorylation.

DISCUSSION

The accumulation of DDT in fat tissue has been shown in animals (Laug, Nelson, Fitzhugh & Kunze, 1950) and in humans (Hayes *et al.* 1958). The greatest amount of DDT found in the fat of

humans who were not deliberately exposed to DDT was 50 p.p.m. (Pearce, Mattson & Hayes, 1952). By including DDT in the diet of volunteers for 1 year, Hayes *et al.* (1956) were able to increase this figure to an average of 340 p.p.m. without ill effect. This level can be compared with that of 700 p.p.m., the concentration in fat of male rats fed with DDT at 200 p.p.m. During the experimental period these rats showed no symptoms of DDT poisoning. Evidence that the DDT is stored in a pharmacologically active form is derived from an earlier experiment in female rats. After a period of DDT feeding followed by a few days of semi-starvation these rats exhibited symptoms of DDT poisoning and most of them died. It is known that female rats store nearly twice as much DDT as male rats (Ortega, Hayes, Durham & Mattson, 1956).

The experiments described in this paper do not reveal any deleterious effects of DDT on the metabolism of adipose tissue. The concentration of DDT (700 p.p.m.) in groin fat of rats fed with DDT for

3 months is equivalent to a concentration of approximately 2 mM. The experiments of Johnston (1951) and those reported here on liver mitochondria show that this concentration is much higher than that necessary to affect intracellular enzymic processes. The insensitivity of the enzymes of fat cells may be due to the protective effect of the fat within the cells. Johnston (1951) has demonstrated that the effect of DDT on the succinoxidase system is greatly reduced if the DDT is added in an oil emulsion instead of in an ethanol solution. The lack of influence of DDT on a variety of enzymes studied by Judah (1949) can also be attributed to the use of an oil emulsion as the vehicle by which DDT was added to the enzyme systems. This objection was met in the present experiments by using dimethylformamide, a solvent which is miscible with water. The protection *in vivo* afforded by fat in relationship to drug action has been emphasized by Brodie & Hogben (1957). These authors describe experiments in which a short-acting barbiturate, *N*-methylthiopentone, which has a high distribution coefficient in favour of lipid, was given in repeated doses to dogs. After each recovery from anaesthesia the dose could be repeated a number of times without affecting the depth or duration of anaesthesia. This the authors attribute to fat acting as a seemingly bottomless reservoir for the drug.

It is concluded that fat not only protects the whole animal from possible effects of DDT but that it also protects fat cells themselves even when the metabolism of these cells is increased. There is no evidence that the amounts of DDT likely to accumulate in human fat present any toxicological hazard.

SUMMARY

1. The deposition of glycogen in rat adipose tissue, which occurs after refeeding partially starved rats, was not affected by previously feeding the rats on 1:1:1-trichloro-2:2-di(*p*-chlorophenyl)-ethane (DDT) at 200 p.p.m.

2. Previous feeding of DDT did not affect the respiration *in vitro* of groin-fat slices or the glucose uptake and unesterified fatty acid release by epididymal fat. The glucose uptake of epididymal fat was unaffected by the presence of 1:2:3:4:10:10-hexachloro-6:7-epoxy-1:4:4 a:5:6:7:8:8 a-octahydro-1:4:5:8-dimethanonaphthalene (Dieldrin) in the diet.

3. Oxidation of glucose by brain slices occurred at normal rates in the presence of DDT, Dieldrin or

1:2:3:4:5:6-hexachlorocyclohexane. In contrast, enzymic activities of rat-liver mitochondria were modified by the addition *in vitro* of DDT, Dieldrin, hexachlorocyclohexane or two metabolites of DDT.

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