amount of products which are not precipitable by trichloroacetic acid.

2. The enzyme responsible for this proteolytic activity has been purified by adsorption of inactive material on diethylaminoethylcellulose and carboxymethylcellulose.

3. Chromatography on diethylaminoethylcellulose has demonstrated the existence of only one enzyme in the purified preparation.

4. The optimum pH and the Michaelis constant of this enzyme are the same as those of the proteolytic activity in the crude preparation. It was therefore concluded that this enzyme is responsible for the proteolytic activity of the crude spleen extracts.

5. A comparative study of the immunoelectrophoresis patterns given by human-serum albumin, degraded with the crude and purified spleen extracts, has shown that it is this enzyme which is responsible in both cases for the splitting of the antigenic structure of human-serum albumin.

We wish to thank Dr P. Grabar for his advice and Mrs J. Cavillon for her technical assistance. One of us (C.L.) acknowledges the support of the Institut National d'Hygiène, Paris. This work was done while T.W. was a

post-doctoral research fellow of the Life Insurance Medical Research Fund.

REFERENCES

- Grabar, P. & Williams, C. A., jun. (1955). *Biochim. biophys. Acta*, **17**, 67.
 Hedin, S. G. (1903). *J. Physiol.* **30**, 155.
 Lapresle, C. (1955*a*). *Bull. Soc. Chim. biol., Paris*, **37**, 969.
 Lapresle, C. (1955*b*). *Ann. Inst. Pasteur*, **89**, 654.
 Lapresle, C., Kaminski, M. & Tanner, C. E. (1959). *J. Immunol.* **82**, 94.
 Lapresle, C. & Webb, T. (1960). *Ciba Foundation Symp., Cellular Aspects of Immunity*. London: J. and A. Churchill Ltd.
 Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
 Peterson, E. A. & Sober, H. A. (1956). *J. Amer. chem. Soc.* **78**, 751.
 Press, E. M., Porter, R. R. & Cebra, J. (1960). *Biochem. J.* **74**, 501.
 Sober, H. A. & Peterson, E. A. (1958). *Fed. Proc.* **17**, 1116.
 Tallan, H. H., Jones, M. E. & Fruton, J. S. (1952). *J. biol. Chem.* **194**, 793.
 Weichselbaum, T. E. (1946). *Amer. J. clin. Path. tech. Suppl.* **10**, 40.
 Willstätter, R. & Bamann, E. (1929). *Hoppe-Seyl. Z.* **180**, 127.

Biochem. J. (1960) **76**, 543

Effects of the Insecticides DDT and Dieldrin on Phosphorus Metabolism of the Adult Housefly *Musca domestica* L.

By F. P. W. WINTERINGHAM, G. C. HELLYER AND M. A. MCKAY
Agricultural Research Council, Pest Infestation Laboratory, London Road, Slough, Bucks

(Received 23 February 1960)

DDT [1:1:1-trichloro-2:2-di-(*p*-chlorophenyl)ethane] and dieldrin (1:2:3:4:10:10-hexachloro-6:7-epoxy-1:4:4*a*:5:6:7:8:8*a*-octahydro-*exo*-1:4-*endo*-5:8-dimethanonaphthalene) respectively represent two important classes of insecticides. Both compounds are toxic to mammals as well as to insects at comparable tissue concentrations, but comparatively little is known of their mode of action in terms of one or more specific biochemical lesions. There is some evidence that DDT may affect oxidative metabolism in a way that is not explained by the exhaustion of endogenous reserves (see review by Winteringham & Lewis, 1959).

The application of a ³²P-labelled-pool technique for studying changes in the relative concentrations of the soluble phosphorus compounds of intact adult houseflies as a result of flight, starvation, etc., has been described (Winteringham, 1960). The use of the same technique for studying the effects of DDT and dieldrin is now reported.

EXPERIMENTAL

Treatment and analysis of insect material. Female adult houseflies (*Musca domestica* L.) not less than 3 days old at the time of extraction were used throughout. The strain used was susceptible to the action of DDT or dieldrin. The principal soluble phosphorus compounds were uniformly labelled with ³²P *in vivo*, extracted and assayed exactly as explained by Winteringham (1960). Any special treatment of the insects before extraction, such as exposure to insecticides, is indicated in the tables. Insecticides were applied topically to the dorsal surface of the thorax in acetone solution, while the insect was under mild cyclopropane anaesthesia. Aqueous glucose was injected intrathoracically by means of an Agla micrometer syringe (Burroughs Wellcome and Co., London) fitted with a 27 s.w.g. needle. Treated insects were always kept at 27–30° in aerated glass chambers without food or water in artificial light. The injection of water alone or the topical application of acetone alone was without detectable effect on phosphorus distribution (Winteringham, 1960).

Tabulation and significance of the data. The results are collected in Tables 1-5. The phosphorus of each compound or fraction (assayed as ^{32}P) is expressed in units % of the total soluble phosphorus recovered from the head (rich in nervous tissue, poor in muscle) or thorax (mainly flight muscle). The significance of differences between the results of single experiments and the means of several experiments has been estimated by the *t* test and corresponding levels of probability (*P*) are indicated. In applying the *t* test it has been assumed that the variance in single experiments with treated insects would be the same as that found in the corresponding groups of control experiments and indicated by the standard deviations (s.d.) of Table 1. A single experiment relates to a group of ten insects.

The description of a fraction in the tables as a particular compound must not be taken to exclude the presence of other unidentified compounds in relatively small but possibly variable proportions. Unresolved composite fractions are indicated by brackets in the tables.

Abbreviations. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), glucose 6-phosphate (G 6-P), arginine phosphoric acid (Arg-P), inorganic phosphate (PO_4), phospholipid recovered under the conditions of extraction (P-lipid). The unidentified compounds UC. 1, UC. 2 and UC. 3 are partly or completely resolved under the routine conditions of paper chromatography used but await identification.

RESULTS

Soluble phosphorus compounds in normal insects. In Table 1 are the mean distributions of soluble phosphorus in the head and thoracic tissues of normal insects which were either active or briefly anaesthetized at the instant of plunging into liquid nitrogen before extraction, etc. The level of thoracic α -glycerophosphate of untreated houseflies was lower when they were active than when they were brought to rest by the use of minimal cyclopropane anaesthesia (Winteringham, 1960). *cycloPropane* was not finally used in some experiments, in which the insects were alive but unable to escape on account of the toxic effects of an in-

secticide. In comparing the results of treated insects with those of untreated controls account must be taken therefore of whether or not terminal anaesthesia was applied (see Table 1).

Effects of DDT. The results of experiments to determine the soluble phosphorus distribution at different stages of DDT poisoning are compared in Table 2. *cycloPropane* anaesthesia was applied finally in these experiments. The results in Table 2 should therefore be compared with those of the anaesthetized groups of Table 1.

Changes in phosphorus distribution were apparently related to the condition of the poisoned insects rather than to the time of treatment. For example, the group of insects used in Expt. 1 of Table 2 was evidently less susceptible to the action of DDT than the group used in Expt. 3. Although exposed for a shorter time the insects of Expt. 3 displayed more advanced signs of poisoning and the phosphorus distribution was significantly different (see below) from that of the corresponding control in Table 1. When the insects were 'knocked down' (Expts. 3 and 4, Table 2) the thoracic α -glycerophosphate remained at a significantly low level ($P < 0.05$) despite terminal *cyclopropane* anaesthesia. At the later prostrate stage (Expt. 4, Table 2) there was a significant fall ($P < 0.01$) in thoracic ATP and corresponding accumulation of PO_4 and possibly of AMP.

Insects at the prostrate stage as a result of DDT treatment could sometimes be revived by the injection of aqueous glucose. However, insects revived in this way never recovered the muscular co-ordination of the normal insect. Recovery was associated with a return of ATP and PO_4 towards normal levels in both head and thoracic tissues. The effects of glucose and water injections are shown in Table 3. *cycloPropane* was not finally applied in these experiments. The results may therefore be compared with those of the un-

Table 1. Mean soluble phosphorus distribution of the active or anaesthetized housefly

Fraction	Phosphorus of fraction as percentage of the total soluble phosphorus recovered from the head or thorax $\pm$ s.d.			
	Normal active insect		Insect lightly anaesthetized	
	Head (7 expts.)	Thorax (9 expts.)	Head (11 expts.)	Thorax (49 expts.)
ATP + UC. 1	32.0 $\pm$ 5.4	32.2 $\pm$ 4.4	35.9 $\pm$ 6.8	34.7 $\pm$ 4.8
ADP	5.0 $\pm$ 1.8	4.7 $\pm$ 1.9	4.9 $\pm$ 2.2	3.7 $\pm$ 2.4
AMP + UC. 2	4.0 $\pm$ 2.3	2.5 $\pm$ 1.3	4.3 $\pm$ 1.4	3.6 $\pm$ 1.9
G 6-P	8.7 $\pm$ 2.7	8.3 $\pm$ 2.8	10.6 $\pm$ 2.8	9.1 $\pm$ 3.2
UC. 3	2.0	0.7	0.8	Trace
Arg-P	9.3 $\pm$ 6.7	13.4 $\pm$ 3.9	6.2 $\pm$ 1.7	10.5 $\pm$ 3.4
α -Glycerophosphate	13.0 $\pm$ 3.6	12.7 $\pm$ 3.1	13.0 $\pm$ 3.8	20.9 $\pm$ 4.6
PO_4	21.7 $\pm$ 4.1	20.4 $\pm$ 3.8	20.9 $\pm$ 4.2	16.8 $\pm$ 4.7
P-lipid	4.3	5.1	3.4	0.7
	100	100	100	100

Table 2. *Soluble-phosphorus distributions in adult houseflies at different stages of DDT poisoning*

Expt. 1: 1.0 μ g. of DDT was applied topically. Insects were left about 10 hr. without food or water. Insects were hyperactive but still upright. Terminal anaesthesia was applied.

Expt. 2: 1.0 μ g. of DDT was applied topically. Insects were left about 5 hr. without food or water. Insects were 'knocked down' and showed unco-ordinated hyperactivity. Terminal anaesthesia was applied.

Expt. 3: 1.0 μ g. of DDT was applied topically. Insects were left for 5 hr. without food or water. Insects were definitely 'knocked down' but showed considerable leg activity, etc. Terminal anaesthesia was applied.

Expt. 4: 1.0 μ g. of DDT was applied topically. Insects were left 10 hr. without food or water. All were prostrate and showed only feeble movements, tarsal tremors, etc. Terminal anaesthesia was applied.

Fraction	Phosphorus of fraction as percentage of the total soluble phosphorus recovered from the head or thorax							
	Expt. 1		Expt. 2		Expt. 3	Expt. 4		
	Head	Thorax	Head	Thorax	Thorax	Head	Thorax	
ATP + UC. 1	28.7	36.2	26.3	30.3	35.9	30.7	14.5	
ADP	3.3	4.8	5.0	10.1	0	2.3	1.7	
AMP + UC. 2					1.4		6.2	
G 6-P	22.3	19.0	25.2	16.6	16.1	24.0	6.7	
UC. 3					Trace		0	
Arg-P					12.2		9.2	
α -Glycerophosphate	9.7	14.2	10.9	21.1	9.1	8.6	10.5	
PO ₄	28.2	18.4	32.6	21.9	25.3	34.4	51.2	
P-lipid	7.8	7.4	Trace	Trace	Trace	0	Trace	
	100	100	100	100	100	100	100	

Table 3. *Effects of injecting aqueous glucose into adult houseflies at the prostrate stage of DDT poisoning*

Expt. 1: 1.0 μ g. of DDT was applied topically. Insects were left approx. 6 hr. without food or water. All were prostrate but showed slight leg movements. No terminal anaesthesia.

Expt. 2: exactly as in Expt. 1 but 2 μ l. of water was injected intrathoracically after 5 hr. Insects showed slight recovery of leg activity after injection of water. Insects were finally extracted 6 hr. after DDT treatment without terminal anaesthesia.

Expt. 3: exactly as in Expt. 1 but 2 μ l. of aqueous 10% glucose was injected intrathoracically after 5 hr. Insects showed considerable recovery after injection of glucose but seemed unable to walk properly. Insects were finally extracted 6 hr. after DDT treatment without terminal anaesthesia.

Expt. 4: 1.0 μ g. of DDT was applied topically. Insects were left approx. 9 hr. without food or water. All were prostrate but showed slight leg movements. No terminal anaesthesia.

Expt. 5: exactly as in Expt. 4 but 2 μ l. of aqueous 10% glucose was injected intrathoracically after 8 hr. All insects showed considerable recovery after injection of glucose but appeared to lack muscular co-ordination. Insects were finally extracted 9 hr. after DDT treatment without terminal anaesthesia.

Fraction	Phosphorus of fraction as percentage of the total soluble phosphorus recovered from the head or thorax									
	Expt. 1		Expt. 2		Expt. 3		Expt. 4		Expt. 5	
	Head	Thorax	Head	Thorax	Head	Thorax	Head	Thorax	Head	Thorax
ATP + UC. 1	32.5	26.6	24.5	25.8	33.1	35.7	24.8	20.4	27.2	28.8
ADP	5.0	5.4	6.4	6.9	7.6	3.4	9.3	6.0	12.7	3.9
AMP + UC. 2								0		0
G 6-P	19.3	25.4	25.1	25.3	20.7	28.4	20.6	13.9	25.6	19.0
UC. 3								0		Trace
Arg-P								9.8		10.5
α -Glycerophosphate	5.6	7.8	6.8	8.5	9.0	6.4	7.2	9.4	6.0	12.0
PO ₄	25.9	23.6	29.3	22.1	23.6	21.2	33.4	31.2	23.0	19.3
P-lipid	11.7	11.2	7.9	11.4	6.0	4.9	4.7	9.3	5.5	6.5
	100	100	100	100	100	100	100	100	100	100

anaesthetized groups of Table 1. It will be seen that in both the head and thoracic tissues of the DDT-treated insects the α -glycerophosphate levels were lower than those found in the normal unanaesthetized insects. The results of Expts. 2, 3 and 5 (Table 3) suggest that neither water nor aqueous glucose was effective in restoring α -

glycerophosphate to the levels of the normally active insects (Table 1). The results in Table 4 show that DDT-treated insects spared the typical hypermuscular activity by sustained cyclopropane anaesthesia are not protected from a significant fall in ATP ($P < 0.01$). These results are in agreement with some reported earlier (Winteringham, 1956).

[illegible]

changes in phosphorus distribution were found until the stage of flight convulsions (see Table 5). At this stage the level of thoracic α -glycerophosphate was as low as or even lower than that of the normal unanaesthetized insect, and, as with DDT poisoning, cyclopropane anaesthesia failed to restore the α -glycerophosphate to the resting level (compare Expts. 5 and 6 of Table 5). Instead, it remained significantly low ($P < 0.01$). In one experiment there was a fall in thoracic ATP which was just significant ($P < 0.05$) and a corresponding rise in AMP and PO_4 (Expt. 2, Table 5). If the insects were chilled in liquid nitrogen at the instant of wing fanning during the stage of flight convulsions (Expt. 5, Table 5) there was no evidence of the increase in thoracic PO_4 observed during normal flight (Winteringham, 1960). The results of Table 5 may be compared with those of Table 1.

DISCUSSION

Fall in adenosine triphosphate. It has been reported (Winteringham, 1960) that in houseflies deprived of food and water to the point of prostration there was a significant fall in the ATP of the head tissues and, like the fall in thoracic ATP of DDT-treated insects (see above), this could be reversed by injecting aqueous glucose. Later, when the insect could not be revived by glucose injection, most of the ATP had disappeared from both head and thoracic tissues. The results in Table 4 show that protection of the DDT-treated insect from physical exhaustion by cyclopropane anaesthesia failed to prevent a fall in thoracic ATP. It has also been found that cyclopropane anaesthesia failed to protect adult houseflies from the enhanced desiccation associated with DDT poisoning. This is shown by the results of Fig. 1. This may also explain why cockroaches exposed to DDT but spared the characteristic hyperactivity and consequent carbohydrate depletion by sustained cyclopropane anaesthesia went on to die as though they had not been so protected (Merrill, Savit & Tobias, 1946). Ingram (1955) concluded that the enhanced loss of water by DDT- or by pyrethrum-treated insects was not correlated with hyperactivity or increased respiration. Beard (1958) found ATP depletion in *Galleria* larvae only when prostration was apparently associated with a loss of water by regurgitation.

The accelerated desiccation caused by DDT may also account for the fact reported earlier (Winteringham, 1956) that DDT-induced prostration and the fall in ATP occur before the exhaustion of endogenous reserves as indicated by total oxygen consumption. Heslop & Ray (1959) have similarly observed that prostration of cockroaches exposed to DDT or physical stress occurred when total

oxygen uptake indicated only partial depletion of endogenous reserves. Thus the fall in ATP and in rate of oxygen uptake in DDT-poisoned houseflies may be due to loss of the normal ability to draw upon endogenous reserves as a result of desiccation and not due to the direct action of the insecticide.

Levels of α -glycerophosphate. The results of Expts. 3 and 4 of Table 2 and of Expts. 4 and 6 of Table 5 show that in both DDT- and dieldrin-poisoned insects the thoracic α -glycerophosphate remained at a lower level than is found in the normal anaesthetized insect. Moreover, this effect occurred before any significant fall in ATP and, in the dieldrin-poisoned insects, before there was likely to be serious desiccation (Fig. 1).

The simultaneous formation and removal of α -glycerophosphate by a soluble diphosphopyridine nucleotide-dependent dehydrogenase and structurally bound oxidase respectively may provide an important method of transporting hydrogen from the cytoplasm to the respiratory chain of the mitochondria of insect flight muscle (Bücher, Klingenberg & Zebe, 1958; Zebe, Delbrück & Bücher, 1959). The operation of this ' α -glycerophosphate cycle' is indeed consistent with the observed changes in α -glycerophosphate levels at the instant of rest or activity (Winteringham, 1960). Bücher *et al.* (1958) have suggested that *in vivo* the α -glycerophosphate cycle is under respiratory control,

Fig. 1. Loss in weight of groups of 20 adult female houseflies in aerated glass chambers at 27°. No food or water was given. Initial treatments: ●, 1 μ l. of acetone; ▲, 2 μ l. of acetone; ○, 0.37 μ g. of dieldrin/2 μ l. of acetone; △, 1 μ g. of DDT/1 μ l. of acetone; ■, 1 μ g. of DDT/1 μ l. of acetone and continuous cyclopropane anaesthesia. Insects which were exposed to acetone alone behaved normally until they had lost about 30% of their initial weight (A), when signs of complete exhaustion rapidly appeared. All insects that were exposed to DDT or to dieldrin were 'knocked down' within 2 hr. (B).

whereas Sacktor (1958) has postulated a direct control of enzyme activity by means of an endogenous chelating agent. In this context the failure of *cyclopropane* to restore thoracic α -glycerophosphate of the DDT-poisoned insects, and, more especially, of the dieldrin-poisoned insects to their resting levels is of interest. *cycloPropane* quickly suppresses visible motor activity and, presumably, the increased respiration associated with motor activity (Merrill *et al.* 1946). Yet the α -glycerophosphate remains at a level normally found in the active or flying insect. If the enzymes of the α -glycerophosphate cycle are under nervous control, e.g. through the agency of a neurosecretion or hormone, it is possible that the well-established unstabilization of nervous tissue by DDT or dieldrin is also associated with an interference in the regulation of carbohydrate metabolism at the α -glycerophosphate level. There is some evidence that particulate α -glycerophosphate-oxidase activity may be under thyroid-hormone control (Lee, Takemori & Lardy, 1959).

SUMMARY

1. At the late prostrate stage there is a significant breakdown of thoracic adenosine triphosphate in DDT-poisoned houseflies. This fall could be reversed by injecting aqueous glucose.

2. There was also a significant breakdown of thoracic adenosine triphosphate in DDT-poisoned insects spared the hypermotor activity by *cyclo-*

propane anaesthesia. *cycloPropane* also failed to prevent the enhanced desiccation associated with DDT poisoning.

3. The fall in adenosine triphosphate and respiration rate of DDT-poisoned houseflies is not due to the exhaustion of endogenous reserves or to the hypermotor activity induced by DDT.

4. In both DDT- and dieldrin-poisoned houseflies there was a fall in the level of thoracic α -glycerophosphate, which could not be reversed by *cyclopropane* anaesthesia.

The authors wish to acknowledge the help of Mrs A. Brooks and Dr W. J. Roulston during some of the experiments.

REFERENCES

- Beard, R. L. (1958). *Ent. exp. appl.* **1**, 260.
 Bücher, T., Klingenberg, M. & Zebe, E. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, **12**, 153.
 Heslop, J. P. & Ray, J. W. (1959). *J. insect. Physiol.* **3**, 395.
 Ingram, R. L. (1955). *Ann. ent. Soc. Amer.* **48**, 481.
 Lee, Y.-P., Takemori, A. E. & Lardy, H. (1959). *J. biol. Chem.* **234**, 3051.
 Merrill, R. S., Savit, J. & Tobias, J. M. (1946). *J. cell. comp. Physiol.* **28**, 465.
 Sacktor, B. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, **12**, 138.
 Winteringham, F. P. W. (1956). *Chem. & Ind.* p. 1182.
 Winteringham, F. P. W. (1960). *Biochem. J.* **75**, 38.
 Winteringham, F. P. W. & Lewis, S. E. (1959). *Annu. Rev. Ent.* **4**, 303.
 Zebe, E., Delbrück, A. & Bücher, T. (1959). *Biochem. Z.* **331**, 254.

Biochem. J. (1960) **76**, 548

Metabolism in the New Zealand Strain of Obese Mice

By K. SUBRAHMANYAM*

Department of Biochemistry, Medical School, University of Otago, New Zealand

(Received 15 September 1959)

A mutant strain of mice discovered in the Jackson Memorial Laboratory by Ingalls, Dickie & Snell (1950) exhibits the 'hereditary obese-hyperglycaemic syndrome' which has been studied by Mayer and his collaborators (see Mayer, 1953). Guggenheim & Mayer (1952) found that the obese mice liberated $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ acetate at a slower rate than non-obese mice and Hughes & Tolbert (1958) made a similar observation in respect of $[2-^{14}\text{C}]$ acetate. Other metabolic anomalies that have been reported in obese as compared with

normal mice include increased turnover of hepatic glycogen (Shull & Mayer, 1956) and increased activity of liver phosphorylase (Shull, Ashmore & Mayer, 1956).

The New Zealand strain of obese mice was developed in the Hugh Adam Department of Cancer Research by systematic inbreeding from a pair of mice chosen from a stock colony (Bielschowsky & Bielschowsky, 1953). The New Zealand obese mice exhibit the hyperglycaemic hereditary form of obesity but they differ from the obese strain of the Jackson Memorial Laboratory in several important respects (Bielschowsky & Bielschowsky, 1956).

* Present address: S.C.B. Medical College, Cuttack, Orissa State, India.