4. Anomalies in the behaviour of the enzyme in relation to the Michaelis-Menten theory are explained by the assumption that the enzyme has two sites for the substrate, one of which is catalytically active, the other activating or inhibiting the enzyme, depending on the substrate employed.

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Absorption and Metabolism of [¹⁴C]Allethrin by the Adult Housefly, *Musca domestica* L.

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In an earlier paper (Winteringham, Harrison & Bridges, 1955) the absorption and metabolism of ¹⁴C-labelled pyrethroids were studied. It was shown that in 24 hr. topically applied pyrethrins or allethrin (the synthetic allyl analogue of the natural insecticide cinerin I) was almost completely absorbed by the adult housefly, *Musca domestica* L., and that significant fractions of the absorbed insecticide were detoxified enzymically. The detoxication was inhibited when the synergist 'piperonyl cyclonene' was applied simultaneously with the insecticide, but less effectively when allethrin was applied. This suggested that the synergistic mechanism involved an interference with the natural detoxication process of the insect. These studies have now been extended so that the absorption and metabolism of allethrin by a normal laboratory strain of the adult housefly *M. domestica* L. could be compared with those of the natural pyrethrins and of DDT and its bromine analogue

(Winteringham, Loveday & Harrison, 1951; Winteringham, 1952*b*) under identical experimental conditions. An attempt has been made to demonstrate the metabolism of allethrin in insect homogenates.

MATERIALS AND METHODS

The preparation of the [¹⁴C]allethrin used in the present work, together with the techniques for its application to insects, subsequent recovery and radioanalysis by paper chromatography have already been described (Winteringham, 1952*a*; Winteringham *et al.* 1955).

Metabolism of [¹⁴C]allethrin in vitro. An ether-extracted acetone-dried powder containing lipase was prepared from 3- to 4-day-old male adult houseflies as described by Chamberlain (1950). Male housefly lipase powder (0.1 g. equivalent to approx. 33 male flies) was incubated in a vial with 100 µg. of [¹⁴C]allethrin in 1 ml. of phosphate buffer, pH 7.5 (84.1 ml. of m/15-Na₂HPO₄; 15.9 ml. of m/15-KH₂PO₄), with 50 µg. of Triton X-155 (supplied by Rohm and Haas Co., Philadelphia) at 37° under continuous agitation for 5 and 24 hr. periods. Control vials, in which the lipase

powder was heated for 10 min. at 100° to inactivate the enzyme before incubation, were given similar treatment. At the end of the incubation period, 0.5 g. of anhydrous Na_2SO_4 was added to each vial to discourage emulsion formation and the contents were extracted three times with 2.5 ml. portions of A.R. diethyl ether. The ether extracts were combined, reduced to approximately 0.5 ml. *in vacuo* at room temperature and examined by the normal phase (N.P.) paper-chromatographic techniques described by Winteringham (1952*a*) and Winteringham *et al.* (1955). The relative proportions of the recovered ^{14}C -activity behaving as unchanged allethrin and as metabolites or decomposition products could thus be determined. The powder was tested qualitatively for lipase activity by incubating 0.1 g. of powder with 500 μg . of male insect lipid under the same conditions and the resulting extracts examined chromatographically. Enzymic hydrolysis of the lipid was indicated qualitatively by the production of unsaturated material behaving as free acid under the conditions of paper chromatography.

Whole tissue homogenates were used in incubation experiments with [^{14}C]allethrin. The thoraces were removed from approx. two hundred 3- to 5-day-old female adult houseflies and extracted at 0° with approx. 7 ml. of an isolation medium consisting of 0.318 M-sucrose, 0.005 M-phosphate buffer pH 7.4 (prepared by dilution from 0.4 M-phosphate buffer, pH 7.4) and 0.01 M-ethylenediaminetetra-acetate. The homogenate was passed through muslin and 1 ml. samples equivalent to approx. 30 thoraces were incubated at 25° in Warburg flasks with 1 ml. of 0.4 M-phosphate buffer at pH 7.4 (80 ml. of 0.4 M- Na_2HPO_4 ; 20 ml. of 0.4 M- KH_2PO_4), 100 μg . of [^{14}C]allethrin, 50 μg . of Triton X-155, 0.1 ml. of 0.84 M-succinate and the volume made up to 2.8 ml. with distilled water. In addition, 0.2 ml. of 10% KOH was placed in the centre well of the flask. In some experiments 100 μg . of piperonyl cyclonene was also added. In control experiments the homogenate was first inactivated by heating in water at 100° for 10 min. At the end of the incubation period of 4–5 hr. 1.5 g. of anhydrous Na_2SO_4 was added to each flask, which was extracted three times with 4 ml. portions of A.R. diethyl ether. The ether extracts were reduced in a stream of dry air, examined by N.P. chromatography and assayed as described for extracts of lipase homogenates. Similar experiments were carried out with corresponding abdominal homogenates.

Metabolism of [^{14}C]allethrin in vivo. Groups of ten 2- to 5-day-old male or female houseflies under cyclopropane anaesthesia were injected intrathoracically or intra-abdominally with 1 μl . of A.R. acetone containing the weights of [^{14}C]allethrin indicated in Table 1. Further treatment, preparation of controls and analysis of extracts were essentially similar to those described above and earlier (Winteringham *et al.* 1955). In order to throw some light on the enzyme systems involved in allethrin detoxication some groups of flies were treated with various inhibitors before injecting them with allethrin. Groups of flies were exposed to a saturation concentration of methyl bromide vapour for 30 sec. (Table 1, Expt. 2c) and 3 min. (Table 1, Expt. 1c) and others to a saturation concentration of hydrogen cyanide for 15 sec. (Table 1, Expt. 2d) and 1 min. (Table 1, Expt. 1d) before injection with allethrin. Chamberlain (1950), using rat pancreatic and cockroach lipase extracts, considered that the inhibitory actions of benzaldehyde and piperonyl butoxide on the detoxication of pyrethrum were

similar. In order to compare the action of benzaldehyde and piperonyl cyclonene on the detoxication of allethrin, groups of female flies were injected with benzaldehyde plus allethrin simultaneously (Table 1, Expts. 3c, 3d, 3e) and in another experiment flies were injected with piperonyl cyclonene 2½ hr. (Table 1, Expt. 4c) and 3½ hr. (Table 1, Expt. 2e) before injection with allethrin. In an attempt to localize the site of allethrin detoxication, the abdomens from female flies were severed from thoraces and heads 30 min. after injection and, in another group, immediately before extraction. The abdomens were extracted and analysed separately (Table 1, Expts. 5c to 6f).

Absorption of [^{14}C]allethrin applied topically to houseflies. Groups of ten female adult houseflies, under cyclopropane anaesthesia, were treated topically on the dorsal thorax with doses varying from 0.6 to 12.0 μg . of [^{14}C]allethrin/fly in 1 μl . of A.R. acetone. During the penetration period flies were kept at 25° or 30° . Groups of ten flies and the exposure jars were rinsed with A.R. acetone 5 hr. after the application of allethrin. The rinses were concentrated *in vacuo*, and samples were applied to Whatman no. 1 filter paper and the ^{14}C -activity was assayed by direct counting below a thin end-window Geiger-Müller counter or a specially constructed sensitive $4\pi\beta$ -radiation counter; geometrical conditions were standardized so that replicate analyses usually agreed to within 3–4% (Harrison & Winteringham, 1955). Similarly, groups of ten female flies were rinsed 10 hr. after application of allethrin.

RESULTS

All the results are summarized in Tables 1 and 2 and Fig. 1. Approximately 60% of the ^{14}C -activity applied was recovered in the extracts. Variation in the amount of ^{14}C -activity recovered occurred in both live and heat-killed flies and homogenates but was independent of the amount of decomposition. In previous experiments Winteringham *et al.* (1955) were unable to detect any loss of ^{14}C -activity from allethrin-treated flies by excretion or respiration. Blum (1955) was also unable to detect any volatile metabolites from houseflies treated topically with [^{14}C]pyrethrum. No attempt was made to recover the ^{14}C -activity remaining in the residues after extraction. It was assumed that the allethrin:non-allethrin ratios found in the extracts were representative of the ratios present in the tissues of the fly or homogenates at the time of extraction and that the retention was due to some non-selective physical process. The net weight of [^{14}C]allethrin metabolized has been obtained by subtracting the weight decomposed in heat-inactivated flies or homogenates from that of live flies or homogenates.

Metabolism in vitro. The net decomposition of [^{14}C]allethrin by male lipase extract was only 8% after 24 hr. incubation and decomposition by female-abdomen homogenates was only 7% after 5 hr. incubation. This percentage decomposition was considered to be of doubtful significance. Piperonyl cyclonene, when added to abdominal

Table 1. *Metabolism of [¹⁴C]allethrin by the adult female housefly*

It has been assumed that the allethrin:non-allethrin ratios found in the extracts were representative of the ratios present in the tissues at the time of extraction. The temperature was 30° in all experiments except nos. 5a-d, when it was 25°. The metabolism time was 24 hr. except where otherwise stated. The net weights of [¹⁴C]allethrin metabolized was obtained by subtracting the weight apparently decomposed in heat-killed flies from the weights decomposed by flies in the remaining groups of the same experiment.

Expt.	Treatment	Estimated distribution of allethrin at time of extraction (%)	¹⁴ C recovered % of total		Net wt. metabolized (μg.)	
			Allethrin	Non-allethrin		
1 (a)	Live flies injected intrathoracically with 3.5 μg. of allethrin	—	46	54	1.6	
(b)	As in (a) but flies first killed in boiling water	—	91	9	—	
(c)	As in (a) but flies exposed to CH ₃ Br for 3 min. before injection with allethrin	—	81	19	0.4	
(d)	As in (a) but flies exposed to HCN for 1 min. before injection with allethrin	—	89	11	<0.1	
2 (a)	Live flies injected intrathoracically with 3.5 μg. of allethrin	—	49	51	1.6	
(b)	As in (a) but flies first killed in boiling water	—	95	5	—	
(c)	As in (a) but flies exposed to CH ₃ Br for 30 sec. before injection with allethrin	—	78	22	0.6	
(d)	As in (a) but flies exposed to HCN for 15 sec. before injection with allethrin	—	89	11	0.2	
(e)	As in (a) but flies first injected with 10 μg. of piperonyl cyclonene	—	80	20	0.5	
3 (a)	Live flies injected intrathoracically with 4.3 μg. of allethrin	—	58	42	1.5	
(b)	As in (a) but flies first killed in boiling water	—	92	8	—	
(c)	Live flies injected with 4.3 μg. of allethrin + 0.5 μg. of benzaldehyde	—	43	57	2.1	
(d)	Live flies injected with 4.3 μg. of allethrin + 1.0 μg. of benzaldehyde	—	68	32	1.0	
(e)	Live flies injected with 4.3 μg. of allethrin + 2.0 μg. of benzaldehyde	—	66	34	1.1	
(f)	As in (a) but 5 hr. allowed only for metabolism	—	65	35	1.2	
(g)	As in (a) but male flies used	—	81	19	0.6	
(h)	As in (b) but male flies used	—	94	6	—	
4 (a)	Live flies injected intrathoracically with 6.0 μg. of allethrin	—	71	29	1.3	
(b)	As in (a) but flies first killed in boiling water	—	92	8	—	
(c)	As in (a) but flies first injected with 10 μg. of piperonyl cyclonene	—	81	19	0.7	
5 (a)	Live flies injected intrathoracically with 4.0 μg. of allethrin	—	46	54	1.6	
(b)	As in (a) but flies first killed in boiling water	—	87	13	—	
(c)	{ As in (a) but abdomens severed from thoraces and heads 30 min. after injection	Abdomens	22	51	49	0.3
(d)		Thoraces + heads	78	85	15	<0.1
6 (a)	Live flies injected intrathoracically with 5.2 μg. of allethrin but abdomens severed from thoraces and heads 30 min. after injection	Abdomens	25	52	48	0.5
		Thoraces + heads	75	84	16	0
(b)	As in (a) but flies first killed in boiling water	Abdomens	—	91	9	—
		Thoraces + heads	—	81	19	—
(c)	Live flies injected intra-abdominally with 5.2 μg. of allethrin but abdomens severed from thoraces and heads 30 min. after injection	Abdomens	92	69	31	1.0
		Thoraces + heads	8	64	36	0
(d)	As in (c) but flies first killed in boiling water	Abdomens	—	90	10	—
		Thoraces + heads	—	56	44	—
(e)	Live flies injected intra-abdominally with 5.2 μg. of allethrin but abdomens severed from thoraces and heads at end of 'metabolism' period before extraction	Abdomens	80	48	52	1.8
		Thoraces + heads	20	17	83	0.6
(f)	As in (e) but flies first killed in boiling water	Abdomens	—	90	10	—
		Thoraces + heads	—	73	27	—

Fig. 1. Absorption of topically applied DDT, its bromine analogue and allethrin by houseflies at 25°. ●, 0.5 µg. of DDT/fly (Sternburg *et al.* 1950); ▲, 2.0 µg. of DDT (bromine analogue)/fly (Winteringham, 1952b); ■, 50.0 µg. of DDT (bromine analogue)/fly (Winteringham, 1952b); ○, 2.0 µg. of allethrin/fly; □, 11.0 µg. of allethrin/fly.

Table 2. Absorption of topically applied $[^{14}\text{C}]$ -allethrin by the adult female housefly at 30°

Dose/fly	Time (hr.)	Percentage of applied dose absorbed
1.0 µg. of allethrin*	24	98
1.0 µg. of allethrin + 5 µg. of piperonyl cyclonene*	24	88
1.0 µg. of allethrin + 10 µg. of piperonyl cyclonene*	24	74
1.2 µg. of allethrin	10	84
0.6 µg. of allethrin	5	53
1.2 µg. of allethrin	5	53
2.4 µg. of allethrin	5	46
12.0 µg. of allethrin	5	22

* Results taken from Winteringham *et al.* (1955).

homogenates, did not inhibit this decomposition. Female-thorax homogenates did not decompose a significant amount of allethrin. These results agree with the results obtained by Matsubara (1954).

Metabolism in vivo. Results indicated that extensive decomposition of allethrin occurred in female flies at 30° (Table 1, Expts. 1a, 2a, 3a, 4a and 6e). In one experiment (Table 1, Expt. 3f) it was shown that 80% of the decomposition taking place in a 24 hr. 'metabolism' period occurred

within the first 5 hr. of that period. Decomposition of allethrin in male flies (Table 1, Expt. 3g) was very much less than that found in female flies. At 25° (Table 1, Expt. 5a) there was no decrease in the amount of decomposition of allethrin. The metabolism of allethrin by flies initially exposed to methyl bromide was significantly reduced, although the flies appeared quite normal at the time of injection (Table 1, Expt. 2c). Hydrogen cyanide strongly inhibited the decomposition of allethrin (Table 1, Expts. 1d and 2d), but the flies appeared not to recover at all and may have been dead at the time of injection with allethrin. Piperonyl cyclonene also brought about a reduction in the decomposition of allethrin. Results of experiments in which abdomens were extracted separately from thoraces and heads indicated that the amount of decomposed allethrin found in the abdomen was much greater than that in the thoraces and heads. When the abdomens were severed 30 min. after injection of allethrin no significant decomposition took place in the thoraces and heads. Also, when allethrin was injected intra-abdominally, the total amount of decomposition was increased by 60% above the amount of allethrin decomposed when the injection was made intrathoracically. These results strongly suggest an abdominal site of detoxication.

Absorption. At all doses there was an initial rapid absorption of allethrin over the first 5–10 hr., which was followed by a more gradual absorption period. At the end of 24 hr. absorption of allethrin at low doses, e.g. 1.0–2.0 µg./fly, was almost complete, but at higher doses, e.g. 11.0 µg./fly, only half of the allethrin applied had penetrated into the tissues (Table 2 and Fig. 1). There did not appear to be any significant difference in the weights of topically applied allethrin absorbed at 25° and 30°.

DISCUSSION

Direct quantitative comparison with published results available is difficult but the present work suggests that allethrin was less effectively metabolized than the natural pyrethrins by housefly tissues *in vitro* (Woke, 1939; Chamberlain, 1950; Earle, 1952; Matsubara, 1953). This indicates that the detoxication mechanisms of the natural and synthetic pyrethroids may not be identical as already suggested by previous *in vivo* studies (Winteringham *et al.* 1955; Winteringham, Harrison & Bridges, 1954).

The present work confirms that allethrin, despite its greater chemical stability (Blackith, 1952), is metabolized to about the same extent as the natural insecticide in intact houseflies. [Compare Table 1 with Table 2, Winteringham *et al.* (1955), and with Table 1 of the present paper; see also Table 3, Winteringham *et al.* (1955).] The present results further confirm that inhibition of the allethrin

detoxication by piperonyl cyclonene *in vivo* (Table 1, Expts. 2e and 4c) is less effective than that of the natural pyrethrins (Table 1, Expts. 5c and 6b and Table 3, Winteringham *et al.* 1955). No difference in metabolism at 25° and 30° was detected. Detoxication of allethrin by female flies was apparently greater than that by male flies, and this may be associated with the greater susceptibility of male flies to insecticides generally (Barber & Schmitt, 1948; Bruce & Decker, 1950; Busvine, 1951).

There was some evidence that benzaldehyde, a strong inhibitor of mammalian pancreatic lipase, was effective in reducing allethrin detoxication at the higher concentrations used (Table 1, Expts. 3c to 3e). Chamberlain (1950) found inhibition by benzaldehyde of his lipase extracts, and he considers that the type of inhibition was similar to the inhibition by piperonyl butoxide and that lipases were responsible, at least in part, for the detoxication of pyrethrins. He considered that the lack of any correlation between other lipase inhibitors and pyrethrum synergists was probably due to the ability of the inhibitors to form fine stable emulsions *in vitro*. A sublethal exposure to methyl bromide, an inhibitor of SH enzymes, partly inhibited allethrin decomposition. This suggests that a non-volatile oil-soluble SH-reagent might be a useful synergist.

A preparation of thoracic tissue rich in respiratory enzymes failed to decompose allethrin. There was evidence from the experiments both *in vitro* and *in vivo* that the site of allethrin metabolism is in the abdominal tissue and that metabolites are not deposited there after being metabolized elsewhere. The results of the present work indicate that the metabolism of allethrin in the housefly is not due to simple hydrolysis and that lipases play only a small, if any, part in the breakdown of allethrin. There is also evidence that simple hydrolysis is not the principal mechanism of pyrethrum detoxication (Winteringham, 1952c; Winteringham *et al.* 1955; Blum, 1955).

From the evidence presented in this and the earlier paper (Winteringham *et al.* 1955) it is clear that the absorption of allethrin and the pyrethrins by the adult housefly is more rapid and more complete than that of DDT (Sternburg, Kearns & Bruce, 1950) or its bromine analogue (Winteringham *et al.* 1951). The absorption data demonstrate clearly that, as with DDT and its bromine analogue, the fraction of applied allethrin absorbed diminishes with increasing dosage. The addition of piperonyl cyclonene to topically applied pyrethroids had a similar effect in diminishing the amount of allethrin absorbed (see Table 2).

SUMMARY

1. Allethrin labelled with ¹⁴C was incubated with enzyme extracts or homogenates or it was injected

into adult houseflies. After a 'metabolism' period any unchanged allethrin and its metabolites were extracted, resolved by paper chromatography and determined radiometrically.

2. A considerable fraction of the allethrin injected into female houseflies was metabolized in a period of 24 hr. The proportion of the metabolism occurring in the first 5 hr. of this period was 80 %. The net weights of allethrin metabolized by a male lipase extract and a female abdomen homogenate were small and of doubtful significance. Metabolism of allethrin by female thoracic homogenates could not be detected.

3. A sublethal exposure to methyl bromide inhibited the metabolism of allethrin. There was some evidence that benzaldehyde, a lipase inhibitor, reduced the metabolism of allethrin *in vivo* at the higher concentrations used.

4. Absorption of topically applied [¹⁴C]allethrin was rapid during the first 5–10 hr. after application. The fraction of the dose absorbed in 24 hr. approached unity at lower doses but fell off significantly at higher doses.

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