SUMMARY

1. The oxidation of IPA and IBA by oxygen is catalysed by highly purified horseradish peroxidase preparations in the presence of Mn^{2+}.

2. The mechanism of the reaction has not been elucidated but the formation of hydrogen peroxide as an obligatory intermediate is suggested by the inhibiting effect of catalase.

3. It is suggested that the previously reported oxidation of IPA and IBA by plant extracts may have been due to the presence of peroxidase and Mn^{2+} in the extracts.

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Absorption and Metabolism of [^{14}C]Pyrethroids by the Adult Housefly, *Musca domestica* L., *in vivo*

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The pyrethrins represent the most important group of pyrethroid insecticides of natural origin and are widely used for the control of various insect pests, particularly houseflies. The flowers of the plant *Chrysanthemum cinerariaefolium* provide a rich source of the natural insecticide, e.g. by extraction with light petroleum (LaForge & Haller, 1935). The natural pyrethrins are a mixture of the four principal esters (indicated in Fig. 1) and their stereoisomers (Harper, 1949).

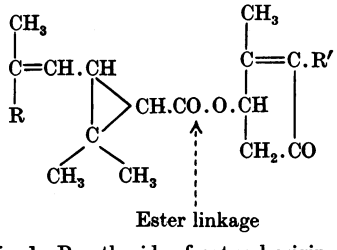

Fig. 1. Pyrethroids of natural origin.

Ester	Structure
Pyrethrin I	R = CH_3; R′ = —CH_2.CH:CH.CH:CH_2
Cinerin I	R = CH_3; R′ = —CH_2.CH:CH.CH_3
Pyrethrin II	R = CO.O.CH_3; R′ = —CH_2.CH:CH.CH:CH_2
Cinerin II	R = CO.O.CH_3; R′ = —CH_2.CH:CH.CH_3

In this paper the esters derived from the *cyclo*propanemonocarboxylic acid (chrysanthemic acid, R = CH_3) and the *cyclo*pentenone alcohol are called chrysanthemic esters, whereas those derived from the *cyclo*propanedicarboxylic acid monomethyl ester (pyrethric acid, R = CO_2Me) are called the pyrethric esters. Several insecticidally active analogues and homologues of the chrysanthemic esters have been synthesized in recent years (Schechter, Green & LaForge, 1949; Crombie, Edgar, Harper, Lowe & Thompson, 1950). Allethrin is the name approved by the U.S. Interdepartmental Committee on Pest Control for the synthetic allyl homologue (R = CH_3; R′ = CH_2.CH:CH_2) of cinerin I and is an economically important ester. It is more or less toxic than the natural esters to various insect species (Gersdorff, 1949; Bottger, 1951) and appears to have greater stability (Blackith, 1952). The toxicity of the natural pyrethroids to insects is enhanced by the prior or simultaneous application of suitable synergists, particularly certain compounds containing the methylenedioxyphenyl group (Schroeder, Jones & Lindquist, 1948; Chamberlain, 1950; Kerr, 1951). The synergists are themselves relatively non-insecticidal. The toxicity of allethrin

is similarly but less effectively enhanced by these compounds (Gersdorff, Nelson & Mitlin, 1951, 1953). Chamberlain (1950) studied the synergism between the natural pyrethroids and the synergist piperonyl butoxide, which contains principally α-[2-(2-butoxyethoxy)ethoxy]-4:5-methylenedioxy-2-propyltoluene. He concluded that the synergism did not involve physical factors such as an increased permeability to the insecticide of the insect cuticle, and suggested that the synergist might inhibit a pyrethroid detoxication mechanism of the insect. He considered that lipase action was a likely detoxication mechanism because of its wide distribution in insect tissue and the ester linkage (Fig. 1) of the pyrethroids. His experiments to demonstrate this were inconclusive. The experiments described in this paper were designed to determine the nature and extent of pyrethroid metabolism by the adult housefly, *Musca domestica* L. *in vivo* and to determine whether or not this metabolism was inhibited in the presence of the synergist piperonyl cyclonene [a mixture of 3-*iso*pentyl-6-ethoxycarbonyl-5-(3:4-methylenedioxyphenyl)-2-*cyclo*hexen-1-one and 3-*iso*pentyl-5-(3:4-methylenedioxyphenyl)-2-*cyclo*hexen-1-one]. Preliminary studies were made by one of us (F.P.W.W.) in the Insect Toxicology Laboratory of the University of California, Berkeley, in the spring of 1952, using [^{14}C]pyrethroids prepared biosynthetically by Pellegrini, Miller & Sharpless (1952), and they have been briefly reported (Wiseringham, 1952*a*). Later studies were made with fully synthetic [^{14}C]allethrin (see below). In all cases the labelled insecticide was injected into or applied topically to adult houseflies, either alone or in the presence of the synergist. After a period the insects were homogenized and extracted, and the breakdown of the pyrethroid in the extract was examined. In all cases the pyrethroid had partly decomposed *in vivo*, this decomposition being inhibited by the synergist, less effectively with allethrin. Concurrently with this preliminary work, the same biosynthetically labelled pyrethroids have been independently used for studying their metabolism in the American cockroach, *Periplaneta americana* L. (Zeid, Dahm, Hein & McFarland, 1953) and in the housefly, *Musca domestica* L. (Earle, 1952). All the results are briefly compared and discussed below.

MATERIALS AND METHODS

Biosynthetic [^{14}C]*pyrethroids.* These were extracted in light petroleum from the heads of pyrethrum flowers allowed to mature in an atmosphere containing $^{14}CO_2$ by Pellegrini *et al.* (1952). The sample used in the present work consisted of a solution of 20 mg. of the 'purified' natural mixture of chrysanthemic and pyrethric esters dissolved in 20 ml. *n*-hexane, and was made available through the kindness of Dr A. C. Miller of the Gulf Research and Development Company, Pittsburgh, Pa. Reversed-phase paper chromatographic analysis (Fig. 3*a*) indicated on the basis of uniform ^{14}C-labelling that the mixture contained 41% of the chrysanthemic esters, 26% of the pyrethric esters and 33% unidentified impurities. After storing for 6 months at 0° the composition indicated was 50% of the chrysanthemic esters, 26% of the pyrethric esters and 24% unidentified impurities. The apparent increase in the chrysanthemic esters may have been due to decomposition in the earlier chromatograms. The specific activity of the crude mixture has been estimated to be 108 μc/g. (Pellegrini *et al.* 1952). For insect application a solution of either the chrysanthemic esters separated by reversed-phase paper chromatography or the unresolved mixture in A.R. acetone was used throughout.

Preparation of [^{14}C]*allethrin.* The labelled ester was prepared by the route indicated in Fig. 2. Asterisks denote the positions of isotopic carbon atoms. [1:3-$^{14}C_2$]Acetone (VII) (1·5 mc), purchased from the Radiochemical Centre, Amersham, Bucks, was diluted to 2·5 ml. with A.R. acetone. 3·8 g. purified selenium dioxide was dissolved in 20·0 ml. redistilled peroxide-free dioxan and 1·4 ml. water at 50° under a reflux condenser. The labelled acetone was added and the mixture gently refluxed for 4 hr. The mixture was distilled in nitrogen at atmospheric pressure. Water and dioxan were removed from the distillate under vacuum at 0°, when the labelled pyruvic aldehyde (VIII) remained as a clear syrup. The yield of aldehyde, determined volumetrically in rehearsal runs with unlabelled materials by the method of Friedemann (1927), was 40% based on the acetone. Ethyl 2-oxohex-5-ene-1-carboxylate (I) (2·27 ml.) was saponified with 9·3 ml. 10% KOH as described by Crombie *et al.* (1950) and the resulting acid (II) condensed with the total yield of (VIII) as described by the same authors. Provision was made here, however, for the quantitative recovery of CO_2 (IV), which proved to be unlabelled. The remaining stages—(V), (IX) and (X) to the final labelled ester (VI)—were conducted on about one-twenty-fifth of the original scale (Crombie *et al.* 1950) by using conventional high-vacuum microdistillation techniques. Oxidative losses during ether extractions were reduced by using peroxide-free ether containing 0·01% quinol. On the reduced scale yields tended to be slightly lower than those originally reported and fraction cutting of distillates was made less rigorous.

(±)-*trans*-Chrysanthemic acid (IX) was used for the acid chloride (X). The total yield of crude allethrin (VI) was 441 mg. This was purified as required by reversed-phase paper chromatography (see below), followed by elution of the allethrin zone in 95% ethanol at 5°. Radio-assay of paper-chromatograms (Fig. 3*b*) indicated that the crude ester contained 47% allethrin of specific activity approx. 57 μc/g. of pure ester. This specific activity was somewhat lower than that calculated on the basis of the labelled starting material. However, the labelled acetone had been stored for some months in a warm climate before dilution with inactive carrier and it seems likely that some decomposition had occurred.

The ester fraction was identical with (±)-*cis–trans*-allethrin when chromatographed by normal and reversed-phase techniques (see below) and was of comparable toxicity to the adult housefly when chromatographic eluates were bio-assayed by the technique of Hoskins & Messenger (1950). From the known stereochemistry of

allethrin the labelled product (VI) used in the present studies would be expected to be the racemic mixture (±)-allethronyl (±)-*trans*-chrysanthemate (Elliott, 1954). The preparation of the labelled ester was very greatly facilitated by the generous gifts of intermediates (I) and (IX) by Dr S. H. Harper. After a preliminary communication of this work had been published (Winteringham & Harrison, 1954), Acree, Roan & Babers (1954) also independently reported the synthesis of specifically labelled allethrin.

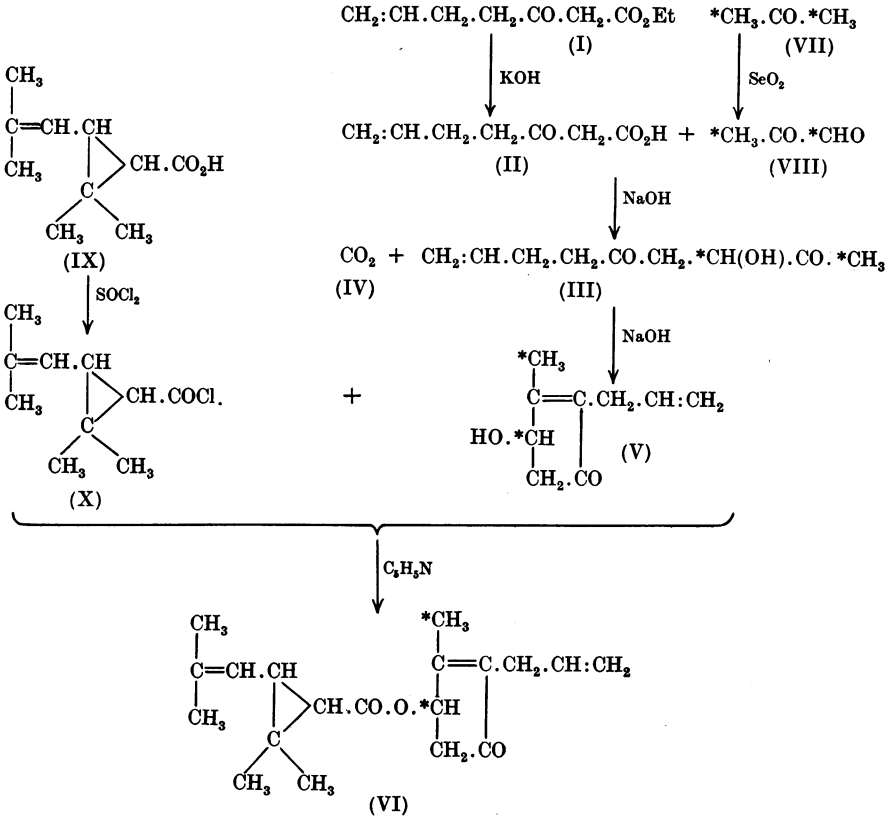

Fig. 2. Preparation of [^{14}C]allethrin.

Paper chromatography; radio-assay and bio-assay techniques. The chromatographic techniques described by Winteringham (1952*b*) were used with slight modifications. Confirmation of R_F values originally reported was made possible by the kind gifts of authentic samples of allethrin and cinerin I by Dr J. B. Moore and Dr M. S. Schechter respectively. Ascending chromatography was carried out in all-glass columns under reduced pressure (Winteringham, Bridges & Hellyer, 1955) at 25°. The original spraying technique for the location of separated fractions has been replaced by a simpler and equally sensitive dipping technique. After drying, the paper strip is dipped once in neutral 0·1% A.R. aqueous $KMnO_4$ and immediately washed free of excess of permanganate in distilled water. When almost dry the strip is dipped in a 0·5% solution (w/v) of A.R. benzidine in dry A.R. acetone containing 5% (v/v) glacial A.R. acetic acid. Pyrethroids and their unsaturated derivatives appear as intense blue zones. Less than 1 μg. of a separated pyrethroid is readily detected. Contamination of the strips (e.g. by fingering) is scrupulously avoided at all stages. Pyrethroids or their derivatives, whether or not recovered from insect tissue, were always applied to paper chromatograms in A.R. ether solution. Acetone, or some impurity in acetone, interfered with the separation of chrysanthemic acid from the alcohols derived from the pyrethroids in the simple two-stage technique described (Winteringham, 1952*b*). Labelled compounds were located and determined on the paper chromatograms by the methods described elsewhere (Winteringham, Harrison & Bridges, 1952). A sensitive 4π scanning technique was used for very low ^{14}C-activities (Harrison & Winteringham, 1955). In tissue samples ^{14}C was assayed as $Ca^{14}CO_3$ after wet oxidation of the sample (Winteringham, Harrison, Bridges & Bridges, 1955). For separating either the chrysanthemic esters of the biosynthetic sample or pure allethrin from the crude product (see above) it was found that up to 1 mg. of the mixture could be applied to a single 1·125 in. paper chromatogram without seriously interfering with the separation of the required esters. For the bio-assay of separated fractions by their toxicity to adult houseflies the fractions were eluted with 95% aqueous ethanol into the small vials used for

exposing the flies, the solvent was removed *in vacuo* and the residue bio-assayed by the method of Hoskins & Messenger (1950). Blank paper was similarly eluted for controls.

Treatment and extraction of insects. For studying the absorption and metabolism of the labelled pyrethroids *in vivo* the labelled pyrethroid, either alone or with piperonyl cyclonene, was dissolved in A.R. acetone and 1 μl. of this solution, which contained the amounts stated in Tables 1–4, was applied topically to the dorsal surface of the thorax of each of groups of ten to fifty 2- to 5-day-old adult female houseflies (*Musca domestica* L.) while under mild *cyclo*propane anaesthesia. Alternatively, the insecticide was injected intrathoracically in 1 μl. acetone. An Agla micrometer syringe (Burroughs Wellcome and Co., London) fitted with a 27 S.W.G. needle was used. Flies injected with 1 μl. acetone alone almost invariably survived and behaved normally. The flies were transferred to a metabolism chamber of the type used for studying tissue phosphorus (Winteringham, Bridges & Hellyer, 1955), supplied freely with 5% aqueous glucose and observed for a 'metabolism' period of 24 hr. at 25° or 30°. Usually, up to one-half the number of treated insects remained alive during this period. Mortality was always greater in the presence of the synergist. In some cases provision was made for the collection of expired CO_2 during metabolism. This was precipitated as $CaCO_3$ and assayed for ^{14}C as described by Winteringham (1951). Some groups of flies were killed before treatment with the insecticide by immersion in water at 100° to inactivate tissue enzymes. At the end of the metabolism period all the treated flies, whether apparently dead or not, were briefly anaesthetized with *cyclo*propane. One group of ten flies was removed at random and rinsed with acetone. ^{14}C in this 'surface rinse' was determined and represented the unabsorbed pyrethroid. The rinsed flies were homogenized twice at room temperature with 2 ml. portions of ethanol, the homogenate was centrifuged, the clear combined supernatants were evaporated to dryness *in vacuo* and the residue obtained was dissolved in 1 ml. A.R. diethyl ether. Suitable portions of the ether extracts were then examined chromatographically. This enabled the relative proportions of the ^{14}C behaving as unchanged pyrethroids and as metabolites or decomposition products to be determined separately. These results are expressed as per cent total ^{14}C recovered in the extract. Various methods of extracting the absorbed pyrethroid and its decomposition products were tried [e.g. by diethyl ether extraction of acid homogenates by which 95% of a radioactive-bromine analogue of DDT could be recovered (Winteringham, Loveday & Harrison, 1951)], but the best recoveries (40–60% of the total pyrethroid applied) were obtained by simple ethanol extraction. The chemical fate of the unrecovered pyrethroid was not examined, but it could be recovered as $^{14}CO_2$ by wet oxidation of the tissues, and had not therefore been lost by expiration or excretion. In some experiments the surface rinse was also examined chromatographically. In other experiments the toxicities to houseflies of portions of the tissue extracts containing similar ^{14}C concentrations were compared by the bio-assay techniques mentioned.

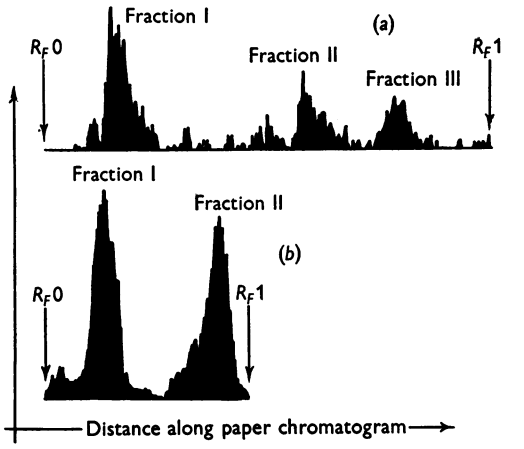

Fig. 3. Radiochromatograms of [^{14}C]pyrethroids. Tracings of original radiochromatograms. Peaks of radioactivity have been inked in for clarity. (*a*) Reversed-phase paper chromatography of natural esters labelled biosynthetically. Fraction I, chrysanthemic esters; fraction II, pyrethric esters; fraction III, non-insecticidal impurities. (*b*) Reversed-phase chromatographic separation of labelled allethrin. Fraction I, pure ester; fraction II, non-insecticidal impurities.

RESULTS

All the results have been summarized in Tables 1–4 and are expressed to the nearest unit per cent in Tables 1, 2 and 4; greater precision was not justified by the statistical errors of the ^{14}C assays, these being a principal source of error throughout. The work was handicapped by the low specific activities of the labelled pyrethroids. For example, 1 μg. labelled allethrin was equivalent to 40 counts/min. on a background rate of 25 counts/min. in the 4π counter used (Harrison & Winteringham, 1955). Typically, 1 μg. allethrin or its metabolites was assayed as a chromatographed fraction spread over three 1 cm. sections of Whatman no. 1 paper. Each section was counted for 5 min. so that the coefficient of variation associated with the assay was $\pm 15\%$. Another cause of variation was the chemical instability of the pyrethroids under the conditions of extraction, concentration etc. of microgram quantities. Decomposition was likely to be greater during reversed-phase (R.P.) chromatography, which required 24 hr. or so, than the two-stage normal-phase (N.P.) technique, which could be completed within 1 hr. Moreover, the separation of 'non-pyrethroids' from pyrethroids was invariably more complete in the normal-phase technique. Only results obtained by the same chromatographic technique should therefore be compared. This has been done in Table 3, in which the net weight of absorbed or injected pyrethroid metabolized per fly has been obtained by subtracting the weight decomposed in the heat-killed flies from that in the live flies as estimated by the same chromatographic technique. It seems, however, that the results *in toto* are consistent.

Absorption. In all cases the absorption of topically applied pyrethroid was nearly complete in 24 hr. and was nearly double that of a bromine analogue of DDT under similar conditions (Wiseringham, 1952*c*). The absorption was invariably reduced by the addition of piperonyl cyclonene (Table 1, Expts. 3*a* and 3*b*; Table 2, Expts. 3*a–d*, and 4*a–f*. See also Table 3). There was no evidence of selective absorption of the esters from an applied mixture (Table 1, Expts. 2 and 3).

Metabolism. All the results show an extensive decomposition of the applied or injected pyrethroid within 24 hr. This was invariably considerably less in flies which had been killed in hot water. The decomposition *in vivo* is believed therefore to be enzymic. Despite its greater chemical stability *in vitro*, allethrin was apparently metabolized *in vivo* to an extent comparable with that of the natural esters at a dose of about 1 μg.

The decomposition in the heat-killed flies was probably non-enzymic and occurred during application, extraction or exposure. A single result in Table 1, 2 (*b*) showed some anomalous decomposition of the esters in the heat-killed flies; not only were the results obtained by the two different chromatographic techniques in poor agreement but the decomposition was unusually high in all the extracts of this experiment. It seems likely that a

Table 1. *Metabolism of biosynthetic* [^{14}C]*pyrethroids by the adult housefly at* 25°

Expt.	Treatment	Metabolism time (hr.)	Pyrethroid absorbed (%)	^{14}C recovered (% of total)		Paper chromatographic technique*
				Unchanged pyrethroid	Non-pyrethroid	
1 (*a*)	1·5 μg. chrysanthemic esters applied topically	18	81	65	35	N.P.†
(*b*)	As in (*a*) but flies first killed in hot water	18	—	100	0	N.P.†
2 (*a*)	2·0 μg. natural mixture of esters applied topically	48	92	14 20	86 80	N.P.‡ R.P.‡
(*b*)	As in (*a*) but flies first killed in hot water	48	90	16 65	84 35	N.P.‡ R.P.‡
3 (*a*)	6·2 μg. natural mixture of esters applied topically	48	87	—	—	‡
(*b*)	6·2 μg. natural mixture + 10 μg. piperonyl cyclonene applied topically	48	79	—	—	‡
(*c*)	As in (*a*) but flies first killed in hot water	48	88	—	—	‡
4 (*a*)	0·7 μg. chrysanthemic esters injected intrathoracically	48	—	55	45	N.P.
(*b*)	As in (*a*) but flies first killed in hot water	48	—	100	0	N.P.
5 (*a*)	1·2 μg. chrysanthemic esters injected intrathoracically	48	—	69 40	31 60	N.P. R.P.
(*b*)	As in (*a*); duplicated expt.	48	—	74 57	26 43	N.P. R.P.
(*c*)	1·2 μg. chrysanthemic esters + 10 μg. piperonyl cyclonene injected intrathoracically	48	—	93 84	7 16	N.P. R.P.
(*d*)	As in (*a*) but flies first killed in water	48	—	92 87	8 13	N.P. R.P.
6 (*a*)	2·0 μg. chrysanthemic esters injected intrathoracically	24	—	50 46	50 54	N.P. R.P.
(*b*)	2·0 μg. chrysanthemic esters + 10 μg. piperonyl cyclonene injected intrathoracically	24	—	100	0	R.P.
(*c*)	As in (*a*) but flies first killed in hot water	24	—	95 87	5 13	N.P. R.P.

* R.P., reversed phase; N.P., normal phase in two stages (see text). These abbreviations are also used in Tables 2 and 3.
† Surface rinse examined by R.P. chromatography; no significant decomposition of chrysanthemic esters.
‡ All surface rinses examined by R.P. chromatography; no significant change in composition from that applied.

'control' extract was run as a 'live' extract in error in this experiment. In all cases piperonyl cyclonene appeared to inhibit the pyrethroid metabolism, but less effectively with allethrin. Unfortunately the synergist was injected with the natural esters and applied topically with allethrin. However, since most of the allethrin was absorbed, presumably with the synergist, the difference in inhibition was probably significant.

the point of application *A*. The reversed-phase technique will resolve the esters but not the hydrolysis products. Except in one or two doubtful cases the non-pyrethroid ^{14}C invariably behaved as an alcohol and remained at *A* (Fig. 4*b*). Allethrin, however, was labelled specifically in the alcohol portion of the molecule (Fig. 2), so that acid produced by hydrolysis would not be indicated by ^{14}C at *B*. This would not apply to the biosyn-

Table 2. *Metabolism of* [^{14}C]*allethrin by the adult housefly at* 30°

Expt.	Treatment	Metabolism time (hr.)	Pyrethroid absorbed (%)	^{14}C recovered (% of total)		Paper chromatographic technique
				Unchanged pyrethroid	Non-pyrethroid	
1 (*a*)	1 μg. allethrin applied topically	24	94	—	—	*
(*b*)	As in (*a*) but flies first killed in hot water	24	95	—	—	*
2 (*a*)	1 μg. allethrin applied topically	25	98	22 10	78 90	N.P.* R.P.*
(*b*)	As in (*a*) but flies first killed in hot water	25	82	76 92	24 8	N.P.* R.P.*
3 (*a*)	1 μg. allethrin applied topically	24	96	19	81	N.P.
(*b*)	1 μg. allethrin + 10 μg. piperonyl cyclonene applied topically	24	65	34	66	N.P.
(*c*)	As in (*a*) but flies first killed in hot water	24	95	65	35	N.P.
(*d*)	As in (*b*) but flies first killed in hot water	24	66	72	28	N.P.
4 (*a*)	1 μg. allethrin applied topically	24	98	18	82	N.P.
(*b*)	1 μg. allethrin + 5 μg. piperonyl cyclonene applied topically	24	88	40	60	N.P.
(*c*)	1 μg. allethrin + 10 μg. piperonyl cyclonene applied topically	24	82	53	47	N.P.
(*d*)	As in (*a*) but flies first killed in hot water	24	99	81	19	N.P.
(*e*)	As in (*b*) but flies first killed in hot water	24	93	72	28	N.P.
(*f*)	As in (*c*) but flies first killed in hot water	24	86	78	22	N.P.

* Expired CO_2 collected; no ^{14}C-activity detected.

Nature of the metabolites. The simple two-stage chromatographic technique (normal-phase as opposed to reversed-phase) proved to be the more useful technique for measuring the pyrethroid/non-pyrethroid ratio in the tissue extracts. In this technique (Winteringham, 1952*b*) the chromatogram is first developed for a few minutes with acid light petroleum (boiling range 40–60°), dried and then redeveloped for a longer period with ammoniacal light petroleum (boiling range 40–60°). Typical two-stage chromatograms are shown in Fig. 4*a* and *b*. The unchanged esters run with the ammoniacal solvent-front to *C* and free chrysanthemic acids with the acid solvent front to *B*, while allethronyl alcohol, for example, remains at

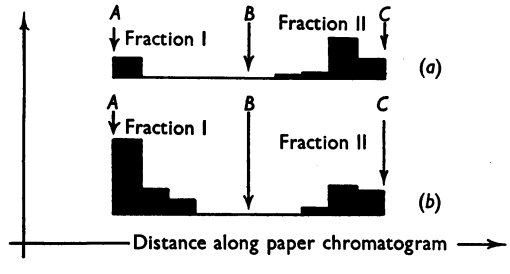

Fig. 4. Radiochromatograms of [^{14}C]pyrethroids. (*a*) Two-stage chromatography of allethrin etc. recovered from heat-inactivated insects. Fraction I, decomposition products; fraction II, unchanged ester. (*b*) As in (*a*) for live insects.

thetically labelled esters since they are labelled in both the alcohol and acid portions of the molecule, but there was no evidence of free chrysanthemic acid in ether extracts of acid homogenates, which are known to recover, partly at least, added unlabelled chrysanthemic acids. Radiochromatographic analysis of an alkaline hydrolysate of the biosynthetically labelled esters indicated, however, that the alcohol portion of the ester contained a larger proportion of ^{14}C than the acid portion of the molecule (cf. Earle, 1952). The evidence for the lack of free acids in the extracts is therefore only qualitative. Although little more than half the tissue ^{14}C was recovered in the ethanol extracts it is likely that the composition found was representative, the retention being due to some non-selective physical process. Recoveries in ethanol were incomplete from live or heat-inactivated tissues, and was apparently independent of the pyrethroid decomposition. This is illustrated in Table 4, which shows the ^{14}C-distribution in the fractions of Expt. 4, Table 2. It will be seen that 85–98 % of the ^{14}C applied as allethrin was accounted for in live and heat-inactivated tissues. This indicates

Table 3. *Net weights of tissue pyrethroids metabolized*

Weights have been estimated from the difference between the per cent of non-pyrethroid ^{14}C found in the extracts of live and of heat-killed flies respectively by the same chromatographic technique. It has been assumed that the non-pyrethroid/pyrethroid ratio found in the extract fully represents that which obtained in the tissues and that the absorption by heat-killed flies of topically applied pyrethroids is the same as that by live flies.

Expts.	Net wt. metabolized (μg.)	Chromatographic technique (see Tables 1 and 2)	Treatment of flies (see also Tables 1 and 2)
1 (*a*)–1 (*b*), Table 1	0·4	N.P.	1·5 μg. chrysanthemic esters applied topically
2 (*a*)–2 (*b*), Table 1	0	N.P.	2·0 μg. natural mixture of esters applied topically
	0·8	R.P.	
4 (*a*)–4 (*b*), Table 1	0·3	N.P.	0·7 μg. chrysanthemic esters injected
5 (*a*)–5 (*d*), Table 1	0·3	N.P.	1·2 μg. chrysanthemic esters injected
	0·6	R.P.	
5 (*b*)–5 (*d*), Table 1	0·2	N.P.	1·2 μg. chrysanthemic esters injected
	0·4	R.P.	
5 (*c*)–5 (*d*), Table 1	0	N.P.	1·2 μg. chrysanthemic esters + 10 μg. synergist injected
	0	R.P.	
6 (*a*)–6 (*c*), Table 1	0·9	N.P.	2·0 μg. chrysanthemic esters injected
	0·8	R.P.	
6 (*b*)–6 (*c*), Table 1	−0·3	R.P.	2·0 μg. chrysanthemic esters + 10 μg. synergist injected
2 (*a*)–2 (*b*), Table 2	0·5	N.P.	1·0 μg. allethrin applied topically
	0·8	R.P.	
3 (*a*)–3 (*c*), Table 2	0·4	N.P.	1·0 μg. allethrin applied topically
3 (*b*)–3 (*d*), Table 2	0·2	N.P.	1·0 μg. allethrin + 10 μg. synergist applied topically
4 (*a*)–4 (*d*), Table 2	0·6	N.P.	1·0 μg. allethrin applied topically
4 (*b*)–4 (*e*), Table 2	0·3	N.P.	1·0 μg. allethrin + 5·0 μg. synergist applied topically
4 (*c*)–4 (*f*), Table 2	0·2	N.P.	1·0 μg. allethrin + 10·0 μg. synergist applied topically

Table 4. *Recovery of ^{14}C topically applied to houseflies as labelled allethrin*

Fly group (see Table 2, Expt. 4)	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)
Allethrin applied (μg.)	1·00	1·00	1·00	1·00	1·00	1·00
Piperonyl cyclonene applied (μg.)	0	5·00	10·00	0	5·00	10·00
Initial condition of flies	Live	Live	Live	Dead	Dead	Dead
^{14}C in surface rinse (% ^{14}C applied)	2	10	17	2	6	14
^{14}C retained in tissue (% ^{14}C applied)	53	24	33	36	35	43
^{14}C extracted as allethrin (% ^{14}C applied)	8	21	22	48	38	32
^{14}C extracted as metabolites (% ^{14}C applied)	34	30	19	11	15	9
Total ^{14}C recovered (% ^{14}C applied)	97	85	91	97	94	98
Total ^{14}C extracted in ethanol (% ^{14}C applied)	42	51	41	59	53	41
Severity of symptoms (according to number of crosses) of flies exposed for 1 hr. to residue of evaporated ethanol extract (no symptoms in control group)	+	+ + +	+ + + +	+ +	+ + +	+ + + + +

that loss of ^{14}C by excretion or expiration did not occur or was relatively small. The absence of ^{14}C in expired carbon dioxide was confirmed in Expts. 1 and 2, Table 2, where it was estimated that labelled carbon dioxide equivalent to 3% or more of the applied ^{14}C would have been detected. In the experiments where the insecticide was applied topically it is likely that there were some losses by contact with the walls of the metabolism chamber, but these were not determined. The comparative toxicities to adult houseflies of portions of the ethanol extracts containing equal ^{14}C-activities are also shown in Table 4. The symptoms after 1 hr., i.e. number showing erratic behaviour, 'knock-down', etc., roughly paralleled the proportions of unchanged allethrin indicated chromatographically. Symptoms were always more severe in the presence of the synergist. Similar results were obtained in other experiments using male or female adult houseflies for the bio-assay. This strongly suggests that the metabolites were non-insecticidal and that the pyrethroid decomposition *in vivo* may be described as a detoxication mechanism.

DISCUSSION

In 24 hr. there was almost complete absorption by the adult housefly of topically applied pyrethroids, whether the separated chrysanthemic esters, a natural mixture of the chrysanthemic and pyrethric esters, or the fully synthetic (±)-*trans*-allethrin were applied. It was not surprising therefore that selective absorption of the chrysanthemic esters or pyrethric esters from a topically applied mixture had not occurred. Mammals are far more susceptible to the toxic effects of injected pyrethroids than to those applied percutaneously (Leonard, 1942; Carpenter, Weil, Pozzani & Smyth, 1950), which strongly suggests that the pyrethroids are not rapidly absorbed through the skin of mammals. Their relative contact toxicity to insects is therefore probably due to the relatively greater permeability of the insect integument and not to any particular biochemical susceptibility. There is substantial evidence that many of the halogenated-hydrocarbon contact insecticides similarly owe their selective action to the relative permeability of the insect integument to these substances (Winteringham & Barnes, 1955).

The absorption of topically applied pyrethroids was significantly reduced in the presence of piperonyl cyclonene (Tables 1 and 2). The effect occurred in live and heat-killed flies and was not therefore associated with pyrethroid metabolism which conceivably affected the concentration gradient across the integument. It was probably due to the dilution of the pyrethroid by the relatively large excess of synergist and the consequently smaller proportion of the total non-volatile material applied which the insect was capable of absorbing. The fraction of a labelled-bromine analogue of DDT absorbed by houseflies was similarly reduced at higher dosages (Winteringham, 1952*c*). Earle (1952) has also found that piperonyl butoxide retards the absorption of pyrethroids by houseflies.

Despite its greater chemical stability allethrin was apparently detoxified at 30° to about the same extent in 24 hr. as the natural esters at 25°. This increase in temperature increased the metabolism of a bromine analogue of DDT from 40 to 55% under the same conditions (Winteringham, 1952*c*). Inhibition of the detoxication of allethrin by piperonyl cyclonene was apparently less effective than that of the natural esters. This suggests that synergism of the natural esters is due to an inhibition of the natural detoxication mechanism *in vivo* and that the poorer effectiveness of piperonyl cyclonene as a synergist for allethrin is due to a less effective inhibition of the detoxifying enzyme system. It further suggests that the enzymes responsible for the detoxication of the natural and synthetic esters respectively are not identical. This leaves open the possibility of discovering a more effective allethrin synergist. This conclusion is not in agreement with that of Earle (1952), who compared the hydrolysis of a mixture of the natural esters with that of allethrin by roach lipase *in vitro* and concluded that the natural esters were hydrolysed more rapidly than allethrin. Hydrolysis was measured as unspecified acid formation. Apparent hydrolysis was equivalent to one-tenth or less of the added substrate, and this was less than the proportion of non-pyrethroids almost certainly present in his added substrates. On the basis of bio-assay techniques Earle also concluded that allethrin was more stable *in vivo* than the natural esters, and favoured this as an explanation of the better synergism between the natural esters and piperonyl butoxide.

Zeid *et al.* (1953) have studied the absorption of the biosynthetically labelled esters by the American cockroach, *Periplaneta americana*. These workers found ^{14}C in the carbon dioxide expired by roaches treated with the unfractionated mixture, and apparently assumed that it must have been derived from the 'radioactive insecticides'. The maximum fraction of applied ^{14}C recovered as expired carbon dioxide was 12%, which was less than the proportion of non-pyrethroid ^{14}C clearly present in their applied mixture. The labelled carbon dioxide could therefore have been derived entirely from the non-insecticidal impurities.

SUMMARY

1. A natural mixture of pyrethroids, labelled biosynthetically with ^{14}C, has been resolved by means of reversed-phase paper chromatography

into chrysanthemic esters, pyrethric esters and unidentified non-insecticidal impurities.

2. Allethrin, labelled with ^{14}C in the alcohol portion of the molecule, has been prepared on the millimole scale at a specific activity of about 60 μc/g. of pure esters and purified by means of reversed-phase paper chromatography.

3. Allethrin, the natural mixture of pyrethroids, or the chrysanthemic esters separated chromatographically was injected into or applied topically to adult houseflies. After a metabolism period the unchanged esters and their metabolites were extracted, resolved by paper chromatography and determined by radioactivity assay.

4. Significant and comparable fractions of all the applied pyrethroids were metabolized to relatively non-insecticidal substances within 24 hr. When the synergist piperonyl cyclonene was applied simultaneously with the pyrethroid, the metabolism was substantially inhibited, but least effectively in the case of allethrin. This suggests that the synergism involved an interference with the natural detoxication mechanism of the housefly and leaves open the possible development of a more effective allethrin synergist.

5. Absorption of the pyrethroids applied topically was almost complete within 24 hr. and was apparently non-selective from an applied mixture of esters. The presence of piperonyl cyclonene invariably retarded absorption in 24 hr., presumably by dilution of the pyrethroid on the insect integument.

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