

Effect of Allethrin on Feeding Behavior of Insects.* (30557)

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Although as early as 1921 Cory(3) observed that leaf-feeding insects avoided plants treated with an alcohol extract of pyrethrum flowers, the effects of synthetic pyrethrin-like compounds have not been studied on insect behavior in relation to laboratory animals. The current investigation was undertaken to investigate the feeding behavior of yellow fever mosquitoes when exposed to biological baits treated with allethrin and like compounds.

Methods. All chemicals were obtained commercially. Adult mosquitoes, *Aedes aegypti*, were reared according to standard procedure. The feeding deterrent properties of the test compounds were determined using live mice as well as citrated human blood.

The *in vivo* mouse procedure has been described(5). Essentially the test compounds were applied to the skin of anesthetized mice. A fluorescent dye and radioiodinated serum albumin were injected intravenously into the mice, and 5 minutes later the mice were exposed to starved adult female mosquitoes for 30 minutes. The mosquitoes that fed were separated under ultraviolet light, and the amount of blood ingested was determined by radioassaying the mosquitoes and the mouse blood.

For the *in vitro* testing, Tarshis's procedure (6) of mosquito feeding was modified. The test compound and the indicator substances were homogenized in outdated citrated pre-treated human blood. Ten ml of the blood mixture was added to a glass cylinder covered on one end with a preboiled Badruch capping membrane, through which mosquitoes sucked the blood for 1 hour. The blood was maintained at $34 \pm 1^\circ\text{C}$ while the mosquitoes were kept at room temperature.

The test compounds were diluted with acetone for the *in vivo* tests but were homogenized with Tween 80 for the *in vitro* tests. Neither Tween 80 nor the indicator sub-

stances prevented mosquitoes from feeding.

Results. Table I shows the effect of allethrin and allethrin-like compounds on mosquitoes feeding on live mice. Allethrin at a concentration of 0.001 mg/ml effectively prevented mosquitoes from feeding, but dimethrin, barthrin, and allethrolone were effective only at a concentration of 1 mg/ml. The ethyl ester of chrysanthemumic acid was effective at a concentration of 10 mg/ml.

Table II shows the effect of allethrin and allethrin-like compounds on mosquitoes feeding on human blood through an animal membrane. The results are qualitatively similar to those obtained with mice as bait.

Diethyl-m-toluamide, until now considered to be the most potent insect repellent(4), loses its feeding deterrent properties at a concentration of 0.1 mg/ml (unpublished data).

Discussion. Experiments using quantitative procedures showed that allethrin is more than a hundred times more effective than diethyltoluamide in preventing mosquitoes from ingesting blood. Exposure of mosquitoes to the lower concentrations of allethrin was not lethal to the mosquitoes. In view of the low mammalian toxicity(1) and the high feeding deterrent potency of allethrin we are now designing appropriate experiments to evaluate its insect repellent properties.

Walkden and Nelson(7) reported that grain treated with pyrethrin was not attacked by insects for as long as 2 summer seasons. However, the insecticidal action of pyrethrin persisted for only one month. Similar findings for allethrin on grains were recently reported by Berndt(2). These observations indicate separation of feeding deterrents and insecticidal properties of the chemicals. Our studies with allethrin and its structural analogues further indicate the disassociation of two distinct properties of these compounds. Structural analogues of allethrin, such as barthrin and dimethrin, are not as potent feeding deterrents as allethrin, but their insecti-

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cidal properties are similar. All of these considerations suggest that the feeding deterrent property of allethrin is independent of its insecticidal action and is therefore likely to possess a different biochemical mechanism of action.

Summary. Allethrin in tracer quantities prevented mosquitoes from ingesting blood from a living mouse or from a cylinder covered with animal membrane. Potent insecticidal structural analogues of allethrin were not potent feeding deterrents. Disassociation

TABLE I. Effect of Allethrin and Allethrin-Like Compounds Applied on Mouse Skin on Mosquito Biting.

Drug	Conc, mg/ml	Total No. of mosquitoes tested*	Biting, % of un- treated $\pm$ S.E.†	
			No. engorged	Blood ingested
Allethrolone	1.0	100	16	14
	.1	100	83	85
	.01	100	51	53
	.001	100	66	95
Chrysanthemumic acid, ethyl ester	10.0	100	22	21
	1.0	100	100	96
	.01	100	98	96
Chrysanthemumic acid, 2,4-dimethylbenzyl ester (Dimethrin)	1.0	100	23	12
	.1	50	87	73
	.01	50	85	77
Chrysanthemumic acid, 6-chloropiperonyl ester (Barthrin)	1.0	100	20	15
	.1	50	82	64
	.01	50	96	100
Chrysanthemumic acid, dl-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one ester (Allethrin)	1.0	50	0	0
	.1	200	15 $\pm$ 8	7 $\pm$ 2
	.01	400	21 $\pm$ 11	23 $\pm$ 8
	.001	300	34 $\pm$ 4	26
	.0001	150	100	97
	.00001	50	97	95

* Not more than 50 adult female mosquitoes were exposed at a time.

† Results of the treated mice were compared with the untreated mice and mosquitoes tested on the same day. More than 90% of the mosquitoes bit the untreated mice. Two hundred to one thousand mosquitoes were used for untreated mice.

TABLE II. Effect of Allethrin and Allethrin-Like Compounds Emulsified in Blood on Mosquito Feeding.

Ester of chrysanthemum, monocarboxylic acid	Conc, mg/ml	Total No. of mosquitoes tested*	Feeding, % of untreated†	
			No. engorged	Blood ingested
Ethyl	10.00	100	0	0
	1.00	100	33	28
	.01	100	81	78
2,4-Dimethylbenzyl (Dimethrin)	1.0	100	52	22
	.1	100	76	49
6-Chloropiperonyl (Barthrin)	1.0	100	30	13
	.1	100	74	48
dl-2-Allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one (Allethrin)	1.0	50	0	0
	.1	50	0	0
	.01	400	16 $\pm$ 5	8
	.001	400	60 $\pm$ 12	42 $\pm$ 12

* Not more than 50 adult mosquitoes were exposed at a time.

† Results of the treated mice were compared with the untreated mice and mosquitoes tested on the same day. More than 90% of the mosquitoes ingested the untreated blood. Two hundred to one thousand mosquitoes were used for untreated blood.

of feeding deterrent properties and insecticidal action of these compounds is suggested.

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Combined Effect of Cobalt and Testosterone on Erythropoiesis. (30558)

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It has long been known that cobalt increases erythropoiesis in man and experimental animals(1,2,3,4), and that it achieves its erythropoietic effect by initiating erythropoietin formation(5,6). The clinical effectiveness of testosterone therapy in various refractory anemias has led to investigations which suggest that one method by which this hormone promotes erythropoiesis is also by stimulating the elaboration of erythropoietin(7). In this report we shall describe the combined effects of cobalt and testosterone on erythropoiesis in transfusion-induced polycythemic mice, and on plasma erythropoietin levels in normal mice.

Methods. All experiments were carried out on CF No. 1 female mice, 6 to 10 weeks of age and weighing 20-30 g.

Polycythemia was induced according to the method of DeGowin *et al*(8) by intravenous injection of an 80% suspension of washed mouse RBC's on 2 consecutive days. All polycythemic mice had hematocrits between 55-75% at the end of the experiment.

Testosterone was administered subcutaneously in the form of testosterone propionate in sesame oil in a concentration of 25 mg/ml.

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Cobalt was administered subcutaneously in the form of cobaltous chloride in normal saline from stock solution in a concentration of 25 μ moles/ml.

The rate of Fe^{59} uptake into newly formed RBC's was determined as follows: One-half (0.5) μ c of Fe^{59} chloride was given intravenously 60 hours after cobalt injection. Three days after injection of Fe^{59} , the animals were killed and 0.25 ml of blood was removed by cardiac puncture. Radioactivity was measured in a well-type scintillation counter, and the percentage of the injected dose of Fe^{59} appearing in the peripheral red cells was calculated from these counts(8).

Plasma erythropoietin titers were determined using the polycythemic mouse assay described by DeGowin *et al*(8).

Results. *Erythropoietic effect of testosterone and varied doses of cobalt:* The following experiment was performed to determine the combined effects of testosterone and various doses of cobalt on erythropoiesis in polycythemic mice. Fig. 1 illustrates the results.

On day 1 three groups of mice received single injections of 2.5 mg of testosterone in oil, while the appropriate controls received single injections of 0.1 ml of sesame oil. Sixty hours later, on day 3, single injections of either 1.0 or 2.5 μ moles of cobalt or nor-