Technical Papers

The Detoxification of DDT by Resistant Houseflies and Inhibition of This Process by Piperonyl Cyclonene

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It has been found by numerous experimenters that the common housefly (*Musca domestica*) becomes less susceptible to the toxic action of DDT after one or more seasons' exposure. The marked increase in effectiveness of pyrethrin solutions when various so-called synergists are added has led to the hope that synergists might be found for DDT, but no marked effect has been reported to date.

TABLE 1

EFFECT ON MORTALITY OF FLIES OF ADDING VARYING AMOUNTS OF PIPERONYL CYCLONENE TO DDT

	DDT μg/fly	Piperonyl cyclonene μg/fly	% Mortality
A. Berkeley laboratory strain	0.05	0.0	43.3
	"	0.1	45.6
	"	1.0	42.9
	"	5.0	24.4
	"	10.0	18.2
	0.0	10.0	0.0
B. Laton strain	0.5	0.0	50.6
	"	0.1	58.5
	"	1.0	72.5
	"	5.0	93.4
	"	10.0	100.00
	0.0	10.0	0.0
C. Bellflower strain	5.0	0.0	42.1
	"	1.0	51.2
	"	5.0	70.4
	"	10.0	76.6
	"	25.0	87.9
	"	50.0	76.8
	0.0	50.0	0.0

A study of several synergists for pyrethrin showed that piperonyl cyclohexenone (piperonyl cyclonene) markedly increases the toxicity of DDT for the DDT-resistant strains of houseflies, but has little or no effect with the ordinary susceptible strain when acetone solutions are applied topically. Typical results with groups of 50 females flies are shown in Table 1. The three strains are: a nonresistant one taken in Berkeley and called "Berkeley laboratory strain," a moderately resistant one, "Laton strain," and a highly resistant one, "Bellflower strain." Preliminary results with DDD and with methoxychlor show similar effects.

The above mortalities were obtained by applying the chemicals together in acetone solution and hence might be interpreted as resulting from more rapid or complete penetration of the integument by DDT when the synergist was present. This is not the case, for separate application to different parts of the body resulted in the same increase in mortality. In fact, larger amounts used jointly appear to retard penetration and decrease mortality of the Berkeley strain (cf. Table 1). Hence an explanation was sought in changes undergone by DDT after absorption.

TABLE 2

AMOUNTS OF INTERNAL DDT AND DDE RECOVERED FROM DDT-SUSCEPTIBLE AND RESISTANT STRAINS

	DDT applied μg/fly	Internal DDT recovered		Internal DDE recovered	
		μg/fly	Percent of amount applied	μg/fly	Percent of amount applied
Berkeley laboratory strain					
(a) dead	0.05	0.026	52.0	0.024	48.0
(b) living	0.05	0.015	30.0	0.033	66.0
Laton strain					
(a) dead	0.5	0.093	18.6	0.088	17.6
(b) living	0.5	0.0	0.0	0.250	50.0
Bellflower strain					
(a) dead	5.0	0.490	9.8	0.874	17.5
(b) living	5.0	0.376	7.5	1.654	33.1

Twenty-four hours after application the flies were thoroughly rinsed in chloroform to remove adhering DDT and then were ground and again extracted with chloroform. DDT was determined colorimetrically by the Schechter-Haller method. In some instances a reddish color was produced in addition to the customary blue color. This is an indication of some degradation product, e.g., the acetic acid derivative (DDA), the ethylene derivative (DDE) or perhaps dichlorobenzophenone. The substance giving this color appears not to be removed by dilute alkali and hence the simplest assumption is that it is the ethylene derivative DDE. Some typical results are given in Table 2.

It is obvious that increased ability to convert absorbed DDT into DDE is characteristic of the resistant strain. In a given experiment, the survivors on the average always had converted more DDT than those that died and hence ability to make this conversion is a major factor in variation in resistance within individuals of a given strain.[1]

Similar analyses on flies that had been treated with the DDT-piperonyl cyclonene combination showed that the conversion to DDE is largely prevented. Hence the synergism is, at least in part, an interference with the

[1] Dr. C. W. Kearns of the University of Illinois has stated in correspondence that similar results obtained in his laboratory are now in press.

TABLE 3

EFFECT OF PIPERONYL CYCLONENE ON CONVERSION OF DDT TO DDE

	DDT applied µg/fly	Piperonyl cyclonene µg/fly	Internal DDT recovered µg/fly	Internal DDT recovered Percent of amount applied	Internal DDE recovered µg/fly	Internal DDE recovered Percent of amount applied
Laton strain						
(a) dead	0.5	10.0	0.115	23.0	0.030	6.0
(b) living ...	0.5	0.0	0.0	0.0	0.250	50.0
Bellflower strain						
(a) dead	5.0	25.0	0.793	15.8	0.173	3.4
(b) living ...	5.0	0.0	0.376	7.5	1.654	33.1

detoxification process. Typical data for the Laton and Bellflower strains are shown in Table 3. Similar results were found with other resistant strains.

An indication that DDE is not the only decomposition product formed in some cases is given by attempts to account for all applied DDT. With survivors from the resistant strains, the sum of external DDT, internal DDT, internal DDE calculated back to DDT, plus these compounds excreted or brushed off in the container usually was at least a third less than the amount of DDT originally applied.

The conversion of absorbed DDT to DDE may be an enzymatic process, for flies first killed by heating in air at 80° C for a few minutes absorbed large amounts of DDT but converted none to DDE.

Work on this problem is continuing and will be reported on more fully later.

Biosynthesis of Radioactive Vitamin B_{12} Containing Cobalt^{60}

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Vitamin B_{12} obtained from liver extracts (*6, 9, 11*) and by fermentation from *Streptomyces griseus* (*8*) has been characterized as a cobalt complex (*1, 7*). In a paper by Hendlin and Ruger (*3*), it has been shown that the production of LLD activity and vitamin B_{12} can be increased during fermentation processes by the addition of small amounts of cobalt ions. The addition of radioactive cobalt to a broth inoculated with *Streptomyces griseus* should yield radioactive vitamin B_{12}. Fermentations[2] conducted with this organism using a broth to which cobalt^{60} sulfate[3] had been added actually have yielded vitamin B_{12} containing radioactive cobalt. The vitamin content of the vitamin B_{12} concentrate was evaluated by means of an 8-stage countercurrent distribution, using the system benzyl alcohol-water.[4] In addition to measuring color intensities in the several tubes, the β-radioactivity of aliquots was determined by means of a thin window counter, employing evaporation residues in steel planchets. The counting efficiency of our equipment was $\simeq 6\%$. Activities are reported as measured, i.e., without efficiency corrections.

Preliminary experiments were undertaken to ascertain the feasibility of preparing the radioactive vitamin by fermentation. For this purpose radioactive cobalt, having a specific activity of 1.94×10^5 cpm/mg was added to a fermentation broth at the level of 2 ppm. After fermentation, isolation, and partial purification, a vitamin B_{12} solution with a total activity of 2000 cpm was obtained which was analyzed spectrophotometrically and by countercurrent radioactivity distribution. The vitamin B_{12} content of this solution equivalent to the observed intensity of the 5500-A band (*1, 2*) was computed to be 0.147 mg vitamin B_{12} or 6.6 µg Co (*7, 10*) and therefore would be expected to have a maximum activity of 1280 cpm if added cobalt were utilized preferentially by the organism. This would indicate that the radioactive vitamin B_{12} constituted not more than $\frac{1280}{2000} \times 100$, or 64% of the product (if all radioactive components were of approximately equal molecular weights). The material available was insufficient for a countercurrent color distribution. From countercurrent radioactivity distribution analysis, however, it was ascertained that 19% of the total activity concentrated in the fourth tube as compared to a theoretical value of 29% for pure vitamin B_{12}. This analysis indicates that a maximum of $\frac{19}{29} \times 100$, or 66% of the radioactive cobalt was present as vitamin B_{12}, which maximum agrees with the value of 64% computed from the spectrophotometric determination. The agreement between the maximum vitamin B_{12} contents calculated by these independent methods in experiments of this type clearly pointed to the formation of radioactive vitamin B_{12}.

A subsequent experiment, performed with cobalt of higher specific activity, permitted the isolation of $\simeq 0.5$ mg of once-crystallized vitamin. The visual appearance of these radioactive crystals was similar to that of normal vitamin B_{12}, and the spectrum in aqueous solution exhibited all the absorption bands (*1, 2*) reported for the nonradioactive product, despite the presence of another absorbing component. The presence of activity in the

[1] The authors are greatly indebted to Robert G. Denkewalter and George B. Hughey for their interest in this work.

[2] Fermentations were performed under the supervision of E. O. Karow and B. L. Wilker.

[3] The radioactive cobalt sulfate was obtained from Tracerlab, Inc., on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

[4] A manuscript including details of this analytical method is in preparation and will be published separately by F. A. Bacher *et al.*