INTERACTION OF DIELDRIN WITH THE SUBCELLULAR COMPONENTS OF BOTH RESISTANT AND SUSCEPTIBLE STRAINS OF *AEDES AEGYPTI* L.

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The mechanism of dieldrin action and dieldrin-resistance in insects is unknown. Dieldrin is a very stable and unreactive compound with lasting residual effects. This persistence has caused recent public controversy. Accordingly, the metabolic differences among strains as the cause of dieldrin-resistance have never become an important question: i.e., both resistant and susceptible individuals have been found to produce only negligible amounts of metabolites from dieldrin (Winteringham and Harrison, 1959). Nor did studies on the fate of dieldrin in the body with respect to its distribution, excretion, storage, penetration through the cuticle and the nerve sheath, etc., uncover any significant intrinsic strain differences that might have accounted for dieldrin resistance (Winteringham and Harrison, 1959; Ray, 1963; Perry *et al.*, 1964).

In contrast, the genetic aspects of dieldrin-resistance are relatively well known. Busvine (1954) was the first to notice that dieldrin-resistance constituted an entirely different resistance group from that of DDT-resistance. Davidson (1956) found that the dieldrin resistance of *Anopheles gambiae* Giles was caused by a single gene allele and extends to other cyclodiene compounds and γ-BHC. This and other evidence (Brown, 1960; Davidson and Mason, 1963) clearly support the view that dieldrin resistance arises as a result of Darwinian selection for preadaptations, and that it segregates as a partially dominant allele in mosquitoes. Klassen and Brown (1964) were successful in differentiating two closely linked resistance factors, namely DDT- and dieldrin-resistance, in the Isla Verde strain, which also showed a simple genetical pattern for both dieldrin and DDT.

The purpose of this paper is to report on aspects of physico-chemical studies concerning the behavior of dieldrin in the nervous system of resistant and susceptible mosquito larvae. The above mentioned Isla Verde strain was chosen for this purpose since its genetic identity has been carefully studied. Particular attention was paid to studying the process of dieldrin-nerve component interactions in resistant and susceptible strains.

MATERIAL AND METHODS. A CSMA susceptible strain was obtained from the Wisconsin Alumni Research Foundation. The resistant Isla Verde strain was kindly supplied by Dr. A. W. A. Brown, University of Western Ontario, Canada, and was the original parental stock of which the LC_{50} was 1 p.p.m. (see Klassen and Brown, 1964). Dieldrin C^{14}, labeled at the ring carbons, was kindly supplied by the World Health Organization of the United Nations, and the radio-activity of each sample in 0.5 ml. of water was measured by a Tricarb liquid scintillation spectrophotometer (Packard Instrument Co.) with a 10 ml. aliquot of counting solution: a mixture of toluene (0.5 liter), ethyleneglycol monomethyl ester (0.5 liter), PPO (5.5 g.) and dimethyl POPOP (300 mg.).

To study the subcellular distribution of dieldrin *in vitro*, the heads (or the whole bodies in some experiments) of the fourth instar larvae were collected and homogenized in 0.25 M sucrose at 0° C. using small Teflon Potter-Elvehjem homogenizers. The homogenate concentrations will be specified later. C^{14} dieldrin in acetone was added to the 4 ml. aliquot of homogenate to make the final concentration of 1×10^{-5}M (final acetone concentration 1 percent) in a 10 ml. glass centrifuge tube, and the system was maintained at 24° C.

for 1 hour. The reaction was stopped by transferring the vial into an ice-bath.

Four subcellular fractions were obtained therefrom by centrifuging the system at 600 g. for 10 min. (crude nucleus fraction), at 800 g. for 10 min. (mitochondrial fraction) and at 20,000 g. for 2 hrs. to yield the final sediment (microsomal fraction) and the supernatant (supernatant fraction). The supernatant fraction from each strain was first poured into a Sephadex G-50 (fine) column of 1 x 30 cm. (O'Brien and Matsumura, 1964) and each component was eluted carefully with distilled water. Two ml. each of eluate were collected in a test tube at a time and 0.5 ml. was used for radioassay as before. Other subcellular fractions were washed once with 4 ml. of fresh sucrose solution and resuspended in a 4 ml. aliquot of fresh sucrose solution. The radioactivity remaining in the final suspension was assayed as before. Any quenching effect caused by the presence of protein and other organic matter was corrected with check solutions. For *in vivo* distribution studies the method of Matsumura and Brown (1961) was adopted. The internal components were further separated into sediment and supernatant by centrifugation at 20,000 g. for 2 hours as before.

RESULTS. Table 1 indicates the results of *in vitro* experiments on dieldrin distribution among the subcellular fractions in the larval body homogenates (10 mg. wet weight/ml. of 0.25 M sucrose solution). It was noticed that the susceptible "crude nucleus fraction" absorbed more dieldrin than did the corresponding resistant fraction, and that the recovery of dieldrin in the resistant-supernatant was less than that in the susceptible counterpart. A similar experiment was repeated with the heads from each strain (100 heads homogenized in 5 ml., and using 4 ml. of homogenate for the test).

The results of six independent tests (Table 2) indicated that the susceptible particle fractions, "crude nucleus fraction" and "mitochondrial fraction" absorbed more dieldrin than the corresponding resistant fractions. The interstrain differences between the supernatant fractions were not as clear as with those obtained for the whole body homogenates. On the other hand, the supernatant fractions still contained a certain amount of free dieldrin besides bound dieldrin. The supernatant fractions from the above experiments were therefore treated by Sephadex columns to obtain the bound dieldrin free from unbound dieldrin. Figure 1 represents a typical elution pattern of the supernatant fraction from each strain on a Sephadex column. The peaks 1, 2, and 3 represent: dieldrin bound to large protein molecules, organic matter exclusive of large proteins, and free dieldrin respectively. Though it was shown that dieldrin binds with proteins and organic matter, the relative peak heights between the two strains did not seem to be significantly different. Results of five to seven independent column tests for each strain indicated the relative ratio of the column height (R/S) for the peaks 1, 2 and 3 to be 1.05, 1.19 and 1.11 respectively.

To study the *in vivo* behavior of dieldren, 100 larvae from each strain were kept in 1×10^{-5}M of C^{14} dieldrin suspension

TABLE 1.—Absorption and distribution of C^{14} dieldrin among the subcellular fractions from the whole larval body homogenate.

Strains	Percent of administered dieldrin*			
	Crude nucleus fraction	Mitochondrial fraction	Microsomal fraction	Supernatant fraction
Isla Verde resistant	41.4	20.2	9.2	29.3
CSMA susceptible	61.7	11.6	8.0	18.7

* Average of two independent tests. Incubated with 1×10^{-5}M of C^{14} dieldrin at 24° C. for 1 hr., before the separation of each fraction.

TABLE 2.—Absorption and distribution of C^{14} dieldrin among the subcellular fractions from the larval head homogenate.

Strains	Percent of administered dieldrin*			
	Crude nucleus fraction	Mitochondrial fraction	Microsomal fraction	Supernatant fraction
Isda Verde resistant	18.1±1.3	10.9±2.2	1.5±0.4	69.5±3.1
CSMA susceptible	19.6±0.8	12.4±1.9	1.9±0.7	66.2±2.1

* Average of six independent tests. Conditions as Table 1.

(0.384 p.p.m.) for 1 hour. At the end of the incubation period 3 percent of the resistant and 10 percent of the susceptible larvae were dead. The larvae were thoroughly washed with distilled water and homogenized in 5 ml. of sucrose solution. Afterward, 4 ml. of the resulting homogenate was used for testing. The result (Table 3) indicated that the resistant individuals picked up nearly twice as much dieldrin from the surrounding media, but the ratio of dieldrin distribution between the sediment and the supernatant hardly differed from each other.

DISCUSSION. That dieldrin binds with some cellular components and proteins has already been demonstrated by Moss and Hathway (1964). By means of electrophoretic and radioscanning techniques the above authors showed that dieldrin could be bound tightly with protein molecules in various tissue components of the rat. Binding of DDT to the nerve components was also studied by O'Brien and Matsumura (1964) who indicated that the process might involve "charge-transfer complex" formation between DDT and the nerve components and that this process of complex formation could account for the main toxic action of DDT.

In this paper it is shown that (1) dieldrin indeed bound the nerve and some body components, that (2) the rate of *in vitro* binding with the resistant particulate components of the whole body as well as the nervous system was relatively lower than that of corresponding susceptible components, and that (3) the resistant individuals picked up twice as much dieldrin as the susceptible ones *in vivo*. Dieldrin being a very poor compound for ultraviolet assay, no attempts have been made to show that these processes of dieldrin binding are related to the formation of "charge-transfer complex" as in the case of DDT. Nor is there any proof that the modest *in vitro* interstrain differences in the rate of dieldrin absorption by the nerve components of each strain are causally related to the final expression of dieldrin-resistance in the Isla Verde strain. Obviously much more genetic and biochemical data are needed to prove this point. However, the important fact here is that dieldrin is shown to strongly bind with the nerve components; which should

TABLE 3.—Absorption and distribution of C^{14} dieldrin by the larvae *in vivo:* expressed in percent of administered dieldrin.*

Strains	Recovery from ambient water	Recovery from the larval body	
		Particulate** fractions	Supernatant fractions
Isla Verde resistant	40.1± 2.4	45.9±7.2	14.0±0.8
CSMA susceptible	71.1±11.1	21.9±3.1	7.0±0.2

* Average of three independent tests. The larvae were maintained at 0.384 p.p.m. level at 24° C. for 1 hr., and used for assay as Table 1.

** Centrifuged at 20,000 g. for 2 hrs. to obtain all particulate fractions.

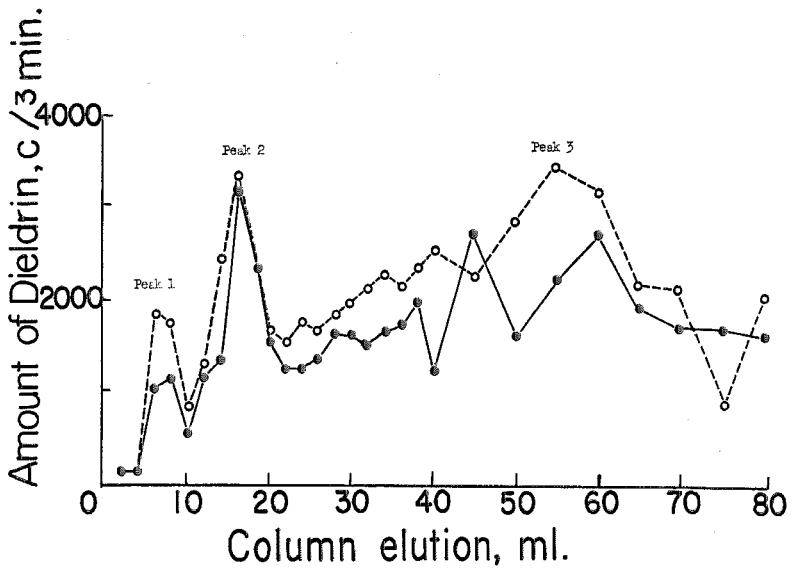

FIG. 1.—Separation of dieldrin complexes on Sephadex G-50 (fine) column. Open circles (broken line) for Isla Verde resistant and closed circles (solid line) for CSMA susceptible strain brain homogenates. Peak 1, 2 and 3 are: dieldrin bound to large proteins, organic matter exclusive of large proteins, and free dieldrin respectively.

be the obvious first step for dieldrin-poisoning in the nervous system.

As a powerful nerve poison, dieldrin must first enter the nervous system and attack the "target" molecule. The primary target sites could very well be a limited area of the whole nervous system, and, therefore, unspecific absorption by substances other than the "target" molecules might mask the important interstrain differences which otherwise could be more clearly shown. At the same time it is very interesting to note that a distinct interstrain difference was found between the rates of dieldrin absorption to the crude nucleus fraction from the whole larval body than to the difference found in the nerve components from heads. It is also possible that the tissue parts which actually play an important role in the dieldrin-resistance mechanism in the Isla Verde strain is in the abdominal and/or the thoracic regions.

The distinct interstrain difference observed in the rate of dieldrin pickup *in vivo* could be attributable to the difference in the physiological conditions between the resistant and susceptible individuals: i.e., the resistant individuals, being less affected by dieldrin, can continue the process of dieldrin absorption, translocation and other related functions, whereas the susceptible individuals may be considerably weakened by dieldrin poisoning and do not carry on the above functions. Contrary to our finding, Gerolt (1965) has recently reported that the resistant larvae (from Puerto Rico) picked up just as much dieldrin as the susceptible individuals from the ambient water at a low dieldrin concentration (0.008 p.p.m.) and with a long exposure time. That is, though the mortality of the susceptible individuals at the end of the exposure period was 45 percent (vs. 0 percent for the resistant strain), the suceptible larvae picked up even slightly more dieldrin than the resistant ones. It is entirely possible that the Isla Verde strain in this laboratory has somewhat different defense mechanisms. It seems likely, however, that the above dieldrin

effect *in vivo* is not causally related to the genuine mechanism of dieldrin resistance as attested by the fact that the distribution ratio of dieldrin between the supernatant and the crude nucleus fractions remained constant in both strains despite the difference in the amount of total dieldrin pick-up.

ACKNOWLEDGMENTS

The authors wish to thank Dr. A. W. A. Brown of the University of Western Ontario for providing the resistant mosquito strain, and the World Health Organization for supplying C^{14} dieldrin. This work was supported in part by a grant ES00067–01 from the Bureau of State Services, U.S. Public Health Service, and from the Research Committee, University of Wisconsin. This paper has been approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

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