Dieldrin Resistance

Biochemical Mechanisms in the German Cockroach

Fumio Matsumura and Mamoru Hayashi

Nerve components of the dieldrin-resistant German cockroach have less binding capacity for dieldrin than those of the susceptible cockroach. The nerve component which is responsible for the difference was partially purified. An attempt was made to confirm the relationship between dieldrin resistance and the binding phenomenon by a genetical experiment. The strain obtained by crossing the resistant strain with susceptible insects for four generations and by selecting the resistant individuals at each generation showed a decreased binding pattern with dieldrin in vitro.

The mechanisms of dieldrin poisoning and dieldrin resistance in insects are totally unknown. Dieldrin is a very stable and unreactive compound from a chemical point of view, and its acute toxic action in the biological system could not be explained by a conventional biochemical reaction theory. Accordingly, the metabolic differences among strains as the major cause of dieldrin resistance have never become an important question (Winteringham and Harrison, 1959) despite the fact that dieldrin is extensively degraded in some animal species (Korte *et al.*, 1962; Oonnithan and Miskus, 1964). 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 differences that might have accounted for dieldrin resistance in those insect colonies (Khan and Brown, 1966; Perry *et al.*, 1964; Ray, 1963).

Because of the general unreactivity of chlorinated hydrocarbon insecticides with the biochemical systems, researchers have attempted to explain their mode of action from their physicochemical properties. For instance, Mullins (1955) suggested that several chlorinated hydrocarbon insecticides owe their insecticidal properties to precise fit in a hypothetical intermolecular lattice, and Gunther *et al.* (1954) proposed that van der Waals binding to a protein in the nervous system is involved. That binding of these insecticides to the nerve components should play an important role in the process of poisoning was pointed out by Soloway (1966), who suggested the importance of general molecular shapes and two symmetrical electron-negative centers for the insecticidal activity, and by Matsumura and O'Brien (1966), who suggested the formation of charge-transfer complexes by the insecticides with the nerve components as the first step of poisoning.

The above reasoning, that the interaction of chlorinated hydrocarbon insecticides with the nervous system is of primary importance, comes mainly from the electrophysiological observations. For instance, a direct relation of the action of dieldrin to the central nervous system was observed by Yamasaki and Narahashi (1958a), who discovered that dieldrin-poisoned nerves of the American cockroach (*Periplaneta americana*) showed spontaneous bursts of action potential; they also noticed that the nervous systems of resistant houseflies showed much longer latent periods between the application of dieldrin and the appearance of discharge than the systems of susceptible flies (Yamasaki and Narahashi, 1958b). If such a mechanism is directly implicated in dieldrin poisoning, nerve components of dieldrin-resistant cockroaches should have a different binding pattern with this insecticide from that of the susceptible counterparts. The present investigation was designed to study such nerve-binding phenomena by using various dieldrin-resistant German cockroach strains.

Department of Entomology, University of Wisconsin, Madison, Wis. 53706

EXPERIMENTAL

Throughout this investigation only male cockroaches were used, since the females possess nonspecific natural resistance factors against dieldrin. The German cockroach strains were: the CSMA-susceptible strain (S) and the Fort Rucker strain (FR) from the Wisconsin Alumni Research Foundation, and the London stock strain (L) from the University of Western Ontario. Also from the London stock strain, a dieldrin-super-resistant (LG_3) strain was obtained by selecting the resistant stock colony for three generations with dieldrin through a contact method for 1 to 3 days.

For the binding studies, the head parts from each strain of the German cockroach (*Blattella germanica* L.) were homogenized with a small Teflon Potter-Elvehjem homogenizer, in 0.25*M* sucrose solution at 0° C., at a concentration of three heads per milliliter. Prior to homogenization the head samples from each strain were carefully weighed to ensure equality of the homogenate concentrations among the strains: London, Fort Rucker, and CSMA strains averaged 2.517, 2.508, and 2.458 mg. per head, respectively.

The large tissue fragments were first removed by filtering the crude homogenate through borosilicate glass wool. The filtrates were incubated with $1 \times 10^{-5}M$ C^{14} dieldrin (specific activity 9 mc. per mmole), added

to the homogenate with 10 μl. of acetone (or ethanol in some cases) per ml. of homogenate for 1 hour at 24° C. The reaction was stopped by transferring the reaction tube into an ice bath.

Four subcellular fractions were obtained by centrifuging the system at 650 × G for 10 minutes (crude nuclear fraction), 8000 × G for 10 minutes (mitochondrial fraction), and 20,000 × G for 2 hours to yield the final sediment (microsomal fraction) and the supernatant (supernatant fraction). Each sediment fraction was washed twice with fresh 0.25*M* sucrose solution before radioassay.

In some cases nerve cords were used instead of heads as experimental material by preparing the homogenate at a concentration of five nerve cords per milliliter of cockroach saline solution (Yamasaki and Narahashi, 1959) and incubating it with C^{14} dieldrin for 10 minutes at 24° C.

For the density gradient centrifugation studies, the subcellular particles were first collected from C^{14} dieldrin-incubated homogenate at 20,000 × G for 45 minutes at 0° C. The sediment was suspended in 6 ml. of 0.8*M* sucrose and transferred to the top layer of a 34-ml. centrifuge tube filled with 6 ml. each of 1.8, 1.5, 1.2, and 1.0*M* sucrose solution to form distinct layers. The tube contents were then subjected to centrifugation at 90,000 × G (maximum force) for 2 hours by using an ultracentrifuge (Spinco L2, Beckman) with a swing-bucket rotor (SW 25.1) at 0° C. Sucrose layers were separated by pipetting each layer with a syringe specially designed for this purpose.

The radioactivity of each sample in 0.5 ml. of aqueous solution was measured by a liquid scintillation spectrophotometer (Tricarb, Packard Instrument Co.) with a 10-ml. aliquot of counting solution: a mixture of toluene (0.5 liter), ethylene glycol monomethyl ester (0.5 liter), PPO (5.5 grams), and dimethyl POPOP (300 mg.).

The total lipid contents of the roaches were assayed by the method of Folch *et al.* (1957).

RESULTS

The results of susceptibility tests by a topical application technique with 1 μl. of acetone and by a contact method with 1 mg. of dieldrin in a 500-ml. jar are summarized in Table I. To study the metabolic fate of dieldrin, 11.43 μg. of C^{14} dieldrin in corn oil was injected into the abdomen of each male roach. The insects were kept in a 200-ml. jar for 24 hours at room temperature. Ten individuals each from the susceptible and the London (LG_3) strain were homogenized in 10 ml. of 0.2% trichloroacetic acid, and immediately extracted with chloroform three times. The solvent was evaporated, and the residue was picked up in a 1-to-1 mixture of *n*-hexane–acetonitrile. After vigorous shaking, the acetonitrile phase was collected and further condensed to 1 ml. through evaporation of the solvent. This condensed sample was spotted on a thin-layer chromatographic plate of silica gel H, and developed with a mixture of *n*-hexane–ether (1 to 9). The resulting chromatogram (Figure 1) indicated that a small portion of dieldrin was metabolized. Some interstrain difference in the rate of dieldrin metabolism was observed. The results of in vivo studies, however, could be misleading, since the physiological conditions of resistant and susceptible individuals were different. Furthermore, it is doubtful whether this small interstrain difference in metabolism can account for such discrete differences in their susceptibilities toward dieldrin.

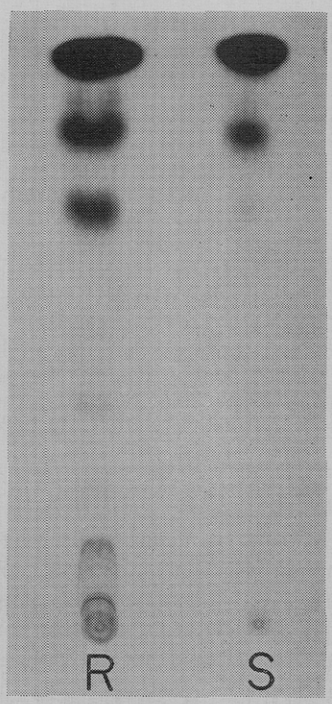

Figure 1. Radioautographic representation of thin-layer chromatograms of C^{14}-dieldrin and its metabolites produced in vivo by London superresistant (LG_3 shown as R) and CSMA-susceptible (shown by S) cockroaches

Table I. Susceptibility of the German Cockroach Strains to Dieldrin

Strains	Contact LT_{50}, Hr.	Resistance Level (-fold)	Topical LD_{50}, μg./Male Adult	Resistance Level (-fold)
London	56	14	54	270
Fort Rucker	12.5	3	1.5	7.5
CSMA	4	—	0.2	—

To study the lipid contents of the cockroach strains, 10 males from each strain were first homogenized in a solvent mixture of chloroform-methanol (2 to 1). The exact volume of solvent used was derived by multiplying the total weight of the roach sample in grams by 20 (Folch *et al.*, 1957). The result summarized in Table II indicated no significant differences in lipid contents among the strains.

To study the distribution of dieldrin among various

Table II. Total Lipid Contents of Resistant and Susceptible Strains of the German Cockroach

Fractions	Lipid Contents, Mg./10 Male Adults		
	London	Fort Rucker	CSMA
Free lipid	15.4	19.3	17.3
Bound lipid	2.8	3.7	3.7
Total lipid	18.2	23.0	21.0
Body weight, μg./10 male adults	587.9	480.7	505.9

components of the cockroach body, the homogenate was prepared from the whole cockroach at a concentration of 1.5 male roach equivalents per milliliter of saline solution. After removal of the large tissue fragments through borosilicate glass wool, a 2-ml. portion of the homogenate was incubated with $1 \times 10^{-5}M$ C^{14}-dieldrin at 24° C. for 1 hour. Four subcellular fractions were prepared by using the centrifugal separation method described above. The results in Table III indicate that a large quantity of dieldrin was taken up by these subcellular particles. That these absorption processes did not involve changes in the molecular structure of dieldrin was shown by the fact that only unchanged dieldrin was recovered from each fraction upon extraction with a mixture of chloroform and methanol. The above experiments confirmed the previously held view that dieldrin itself is a biochemically unreactive compound, being absorbed by the biological materials without altering its original molecular structure, and that no significant interstrain differences may be observed in the gross pattern of dieldrin behavior between the whole body constituents of the resistant and susceptible cockroaches.

To investigate the fate of dieldrin in the nervous system, the target tissue itself, the roaches were topically treated with 0.381 or 3.81 μg. per insect of C^{14}-dieldrin with 1 μl. of acetone. After various time intervals the roaches were successively surface-washed with saline solution and acetone, and dissected to isolate the abdominal nerve cords. Each nerve cord was directly homogenized in the counting solution with 0.5 ml. of cockroach saline to assay the amount of dieldrin taken up by the nerve cords. The comparison of the amounts of dieldrin taken up by the resistant and the susceptible roaches at identical poisoning time periods (Table IV) indicates that susceptible nerve cords generally accumulated more dieldrin than the resistant counterparts. That

Table III. Absorption of Dieldrin by Subcellular Components of the Whole Body of German Cockroach[a]

Fractions	Resistant Strains		Susceptible Strain, CSMA
	London	Fort Rucker	
Crude nuclear	34.9	39.5	37.9
Mitochondrial	17.4	17.1	17.3
Microsomal	17.2	17.3	15.3
Supernatant	30.6	26.2	29.6

[a] Data expressed in percentages of given dieldrin (10 nanomole/ml.) recovered in each fraction.

Table IV. Dieldrin Take-Up by Nerve Cords of German Cockroach Males in Vivo[a]

Applied Amount of Dieldrin, μg./Male Adult	Time after Application, Hr.	Strains	
		London	CSMA
0.381	5	22 (alive)	26 (alive)
	24	84.5 ± 16.7 (alive)	175.5 ± 24.4 (LT_{90})
	120	98.0 ± 14.7 (LT_{50})	—
3.81	5	163.4 ± 45.5 (alive)	186.8 ± 69.1 (LT_{50})
	120	679.0 ± 80.0 (LT_{90})	—

[a] Data expressed in C^{14} counts/3 minutes (1 μg. dieldrin = 63,000 counts/3 minutes. Average of two to four experiments ± standard error.

the London individuals could tolerate much higher amounts of dieldrin in the nerve cords than the susceptible insects became evident, however, when the amounts of dieldrin accumulated in the nerve cords from these strains were compared at LT_{90} for each strain.

It has been shown that the crude nuclear fractions from the susceptible strains absorbed more dieldrin than those from the resistant strain, and that the rates of dieldrin recovery from the resistant supernatants were much higher than from the susceptible counterpart (Matsumura and Hayashi, 1966).

Further fractionation attempts were made to separate the above crude nuclear fraction through a density gradient centrifugation technique (McIlwain and Rodnight, 1962). The crude nuclear fraction previously treated with C^{14} dieldrin could be separated into five subfractions, and the interstrain difference was distributed among the subfractions which sedimented between 0.8 and 1.5M sucrose solution (Figure 2). To study the distribution of such subfraction materials in the nerve cord, instead of the brain, 15 cords inclusive of the second thoracic and the last abdominal ganglia were obtained from male cockroaches. The ganglia were separated from axonic portions. Homogenates were

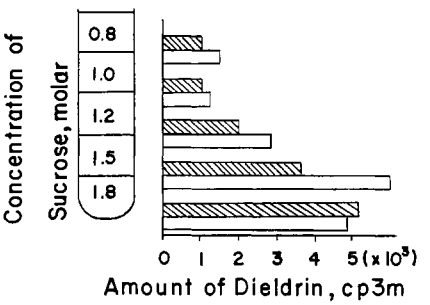

Figure 2. Interstrain comparison of binding patterns of C^{14}-dieldrin among head particulate fractions of crude nucleus fraction separated by sucrose density gradient centrifugation technique. White histograms for CSMA, shadowed for London strain

then made in saline solution from each material, and the particles were collected by brief centrifugation at 20.000 × G for 45 minutes. The density gradient centrifugation technique was then applied to the particulate material. The result (Figure 3) indicates that the amount of bound dieldrin was generally less in the subfractions from the London strain than in the corresponding fractions from the susceptible strain. The interstrain differences appear to be high in the axonic fractions, especially in the fractions with 0.8*M* (approximately threefold) and 1.8*M* sucrose (approximately twofold) solutions.

The above biochemical evidence suggests the presence of a factor which could be causally related with the phenomenon of dieldrin resistance in this species. To study the genetical aspect of the same problem, the London strain was crossed with the susceptible for four successive generations with a mild dieldrin selection at the end of each generation [at the adult stages prior to crossing (Matsumura *et al.*, 1967)]. The resulting strain was designated as "the London pure strain." To study the absorption behavior of dieldrin in the nervous system of the London pure strain, the binding and centrifugation experiments were repeated. The results (Table V) indicated that all the particulate components of the neural material of the London pure strain had less binding capacities than the corresponding susceptible counterparts.

DISCUSSION

That dieldrin is absorbed by various nerve components of the German cockroach was demonstrated in this study. The large portion of dieldrin absorbed (Table III) is not likely to play any significant role in the actual intoxication processes of dieldrin poisoning. Indeed only a fraction of administered dieldrin is expected to come in contact with the actual target sites in the nervous system, to intoxicate the insect (Table IV). The small interstrain differences found between the amount of dieldrin bound with certain nerve components of the susceptible and resistant strains, therefore, could account for the enormous differences in their susceptibility levels. The fact that the interstrain difference in binding becomes large at lower dieldrin concentrations supports the above view (Matsumura and Hayashi, 1966).

The validity of the genetical cleaning procedure at first sight might appear doubtful, as the number of successive crossing experiments was limited to four generations. Nevertheless, considering the fact that the German cockroaches have as many as 23 or 24 chromosomes (Cochran and Ross, 1966), the actual dilution of the London genes by the CSMA genes can be assumed to be close to the theoretical value—i.e., the amount of London genes remaining in the pure strain is close to one sixteenth of the original. The possibility still remains within the probability of 6%, however, that these strain differences of susceptibility toward dieldrin and binding capacity of the nerve components against this compound are purely incidental, in that case perhaps, the latter being due to geographical variation of the roach strains. Final proof, therefore, should come from biochemical or histological evidence to show the presence of a definite structural difference, and/or the importance of such substances in performing the normal function of the nervous system. Unfortunately, the amount of the binding substances present in the nerve preparation of the German cockroach was too small to permit further chemical analysis at this stage.

This work established that dieldrin could bind with various nerve components of the German cockroach nerve, and that the particulate components from the resistant roaches had less binding capacity with dieldrin than the susceptible counterparts. Some genetical and biochemical evidence supporting the view that this property of the resistant nervous system is related to the expression of resistance to dieldrin has been provided.

Table V. Distribution of C^{14} Dieldrin in Fractions from Homogenate of Cockroach Brain and Nerve Cord[a]

Centrifugal Fractions	Brain Homogenate		Density Gradient Fractions (Sucrose Molar)	Nerve Cord Homogenate	
	London[b] pure	CSMA		London pure	CSMA
Crude nuclear	51.2	59.0	0.8	8.3	20.5
Mitochondrial	11.3	11.3	1.0	8.9	12.2
Microsomal	15.6	13.9	1.2	4.6	8.9
Supernatant	22.0	15.8	1.5	6.2	5.5
			1.8	6.6	6.1
			Supernatant	65.4	46.9

[a] Results expressed in percentages of given dieldrin (10 nanomoles per ml.) recovered in each fraction. [b] Genetically purified from London strain.

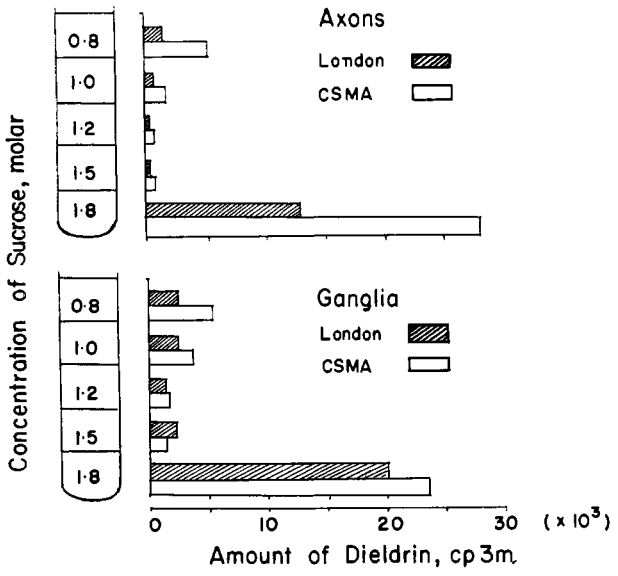

Figure 3. Interstrain comparison of binding patterns of C^{14}-dieldrin among axonic and ganglionic fractions of nerve cord separated by sucrose density gradient centrifugation technique

To find whether this binding phenomenon can actually play an indispensable role in dieldrin poisoning, additional evidence such as structural or biochemical indication of the nature of the binding substance is still needed.

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