Insecticide Mode of Action. Effect of Dieldrin on Ion Movement in the Nervous System of *Periplaneta americana* and *Blattella germanica* Cockroaches

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Dieldrin initially increased the rate of al ion exchange processes in the nervous tissues of both American and German cockroaches. After this initial excitation period, sodium ions accumulated in the dieldrin-treated nerve cells while the potassium ion influx decreased. The situation superficially resembles that of DDT poisoning. Dieldrin also caused a mild condition of hypocalcemia at the late stage of poisoning. This phenomenon, however, does not seem to be causally related to the mode of dieldrin resistance in the German cockroach strains.

The precise mode of action of chlorinated hydrocarbon insecticides is not known, though it is generally acknowledged that they attack the nervous system, especially the central nervous system, as their primary target of toxication. From the electrophysiological data, Narahashi and Yamasaki (1960) have postulated that the immediate cause of the disturbed functions of the DDT-poisoned nervous system is interference with potassium transport. That is, DDT causes a state of hypopotassemia by reducing the axonic membrane permeability towards the entry of external potassium ions. This in turn results in the high accumulation of sodium ions in the nervous system. Despite the early prediction (Welsh and Gordon, 1947) that hypocalcemia inside the nerve membrane should be the major cause of DDT poisoning, Narahashi and Yamasaki could not substantiate the theory with experimental evidence. By using a radioisotope technique, Matsumura and O'Brien (1966) confirmed that the efflux of potassium ion was severely blocked in the DDT-treated abdominal nerve cord of the American cockroach, sodium ions accumulated in the nerve cord, and the effect of DDT upon calcium ion transport was relatively small. With respect to dieldrin, a more potent chlorinated hydrocarbon insecticide against roaches than DDT, Yamasaki and Narahashi (1958a) studied the electrophysiological symptomology of the dieldrin-poisoned nerve cord of the American cockroach and noticed that the nerve cord showed spontaneous bursts of action potential. The same authors also observed that the intact nervous system of resistant houseflies showed a much longer latent period between the application of dieldrin and the appearance of discharge than that of the susceptible individuals (Yamasaki and Narahashi, 1958b). This approach of using the resistant insects in the study of the action mechanism of dieldrin may prove to be a successful one, for the dieldrin-resistant insects show no other biochemical peculiarities which could account for the enormous decrease in their susceptibility to this insecticide. The present study was designated to determine the effect of dieldrin upon the ion-transport mechanism of the insect nervous system and to search for the major symptomological differences in the dieldrin-poisoned nervous system of the resistant insects from that of the susceptible ones.

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Experimental

The insects were adult male American cockroaches, *Periplaneta americana* of the Wisconsin laboratory strain (originally obtained from the University of Minnesota), and adult male German cockroaches, *Blattella germanica* of the CSMA susceptible strain sometimes known as the Hazard strain, the Fort Rucker strain (originally from Fort Rucker, Alabama: a medium chlordane-resistant strain), and the London strain (originally from Canadian Army and Air Force camps, reared at the University of Western Ontario: a highly chlordane-resistant strain).

The nerve cord of the American cockroach was obtained by dissecting the male roach in cockroach saline solution (Yamasaki and Narahashi, 1959) and each abdominal nerve cord, inclusive of the first and the last abdominal ganglia, was transferred into saline solution with radioactive ions with and without nonradioactive dieldrin. For the experiments on German roaches, a head was cut into halves and used as nerve material. In each case, the corresponding nerve tissue was taken from the radioactive saline solution following the initial 30-minute incubation period, briefly washed with nonradioactive saline, blotted by a filter paper to remove the excess liquid, and then transferred into 10 ml. of nonradioactive fresh saline solution. At the end of various incubation periods, a 0.5-ml. portion of the ambient solution was radioassayed to measure the amount of radioactive ions effluxed from the nerve tissue (Treherne, 1962). For the absorption experiments, the nerve tissues were maintained in the radioactive saline solution for various incubation periods prior to the radioassay of the tissues themselves.

The brain homogenate of the German cockroach was prepared by homogenizing the isolated head parts in the cockroach saline solution using a Teflon-glass homogenizer connected to the rod of a motor driven stirrer (at 1000 r.p.m.) for 3 minutes. From this homogenate, the large cuticular and other tissue debris such as muscle fragments were first removed by passing the crude homogenate through borosilicate glass wool.

The filtrates were incubated with Ca^{45} (added as 10 μl. of original stock solution per 2 ml. of homogenate) for 1 hour, then both cellular and subcellular particles were collected by centrifuging the filtrate at 0° C. The sediment was washed once with fresh saline and resuspended in the same amount of fresh saline as the initial homogenate preparation to assess the amount of Ca^{45} absorbed into these tissue components. All radioactivities were measured by a liquid scintillation spectrometer (Tri-Carb 314E, Packard Instrument Inc.) with a toluene–methyl Cellosolve base counting solution (Matsumura and Hayashi, 1966). The levels of the specific activity of the radioactive ions at the time of the actual experimental processes were in the same order as indicated by Treherne (1962).

Results

The results of susceptibility tests on the males indicated that the LT_{50} values for the German cockroach strains (at 1 mg. of dieldrin per jar, inner surface 200 sq. cm.) were 4 hours for CSMA-susceptible, 15 hours for the Fort Rucker–resistant, and 48 hours for the London-resistant strains, respectively. The LT_{50} value for the American cockroach under the identical test condition was 13 hours.

Effect of Dieldrin in Vivo. In this series of experiments, 0.003 μmole of nonradioactive dieldrin in 3 μl. of saline solution which contained radioactive sodium, potassium, calcium, or chlorine ions was injected into the abdomen of live male German roaches. After various incubation periods, the head parts were collected and washed briefly with fresh saline solution. Excess liquids were removed with filter paper. The heads were then homogenized in a 10 ml.-aliquot of the liquid scintillation counting medium to assess the extent of ion intake. The controls were provided to estimate the rate of normal ion exchange processes in the nerve tissue of the cockroach, by injecting only 3 μl. of saline solution containing radioactive sodium, potassium, calcium, or chlorine ion into the cockroach and repeating the above experiment under the identical conditions. The results (Table I) indicate that the rate of sodium and potassium uptake was greatly increased by the presence of dieldrin in the injection medium at the first 30-minute period, and after 1 hour of reaction, the whole system seems to have attained an equilibrium as to the internal levels of sodium and potassium ions as compared with the control figures. The dieldrin appeared to cause a relatively low calcium level at the end of the 2-hour poisoning period in all strains and accelerated the rate of absorption of chlorine ions at the initial 30 minutes in the susceptible tissue only. The inhibitory effect of dieldrin on the process of chlorine influx became apparent only after the first hour of incubation.

Effect of Dieldrin on Isolated Nerve Tissues. Na^{22} TRANSPORT. Experiments with the isolated nerve cord of the American cockroach indicated that the total uptake of Na^{22} for the first hour of dieldrin poisoning was accelerated by the presence of simultaneously administered $1 \times 10^{-5}M$ dieldrin in vitro, the actual values being 171.9 for the treated and 141.2 μmoles per gram of tissue for the untreated nerve cords. When the cords were pretreated with dieldrin 1 hour before the addition of radioactive sodium ions, however, the rate of uptake of sodium ion was reduced to 97.6 μmoles per gram. To study the rate of sodium efflux, six cords were first maintained in Na^{22}-containing saline for 1 hour, and then were transferred into plain saline solution to assess the efflux rate of Na^{22} with the external medium. The rate of diffusion (efflux) also was largely stimulated at the initial hour (Figure 1). The experiments with the nerve tissues of the German cockroaches indicated that only the susceptible tissue picked up more sodium ions in the presence of $1 \times 10^{-5}M$ dieldrin than did the untreated controls during the first 1-hour period of dieldrin poisoning in vitro (Table II). To study the effect of dieldrin on the rate of sodium efflux, the brain slices were made from 10 German roach heads, and were immersed in Na^{22}-containing saline

Table I. Effect of in Vivo Injection of Dieldrin on the Rate of Ion Uptake by the Live German Cockroach Brains

All data expressed in μmole equivalent of ions picked up by a gram of tissue

Ion	Hours after Injection	Dieldrin Treated[a]			Untreated Control		
		L	F	S	L	F	S
Na^{22}	0.5	17.08	10.70	11.46	3.53	3.29	3.32
	1	17.52	8.85	11.71	12.14	10.65	10.50
	2	11.91	10.72	9.40	11.84	10.52	10.65
K^{40}	0.5	0.0321	0.0397	0.0329	0.0169	0.0103	0.0098
	1	0.0123	0.0116	0.0145	0.0108	0.0138	0.0137
	2	0.0111	0.0132	0.0112	0.0129	0.0125	0.0125
Ca^{45}	0.5	0.0278	0.0297	0.0222	0.0275	0.0279	0.0268
	1	0.0178	0.0162	0.0160	0.0178	0.0196	0.0139
	2	0.0155	0.0154	0.0144	0.0217	0.0203	0.0169
Cl^{36}	0.5	14.71	11.99	15.49	14.42	10.48	9.31
	1	11.60	8.97	11.43	12.77	12.17	11.72
	2	10.11	12.13	12.44	14.71	12.39	13.92

[a] L, F, and S represent the London-resistant, Fort Rucker–resistant, and CSMA-susceptible strains of German cockroaches, respectively.

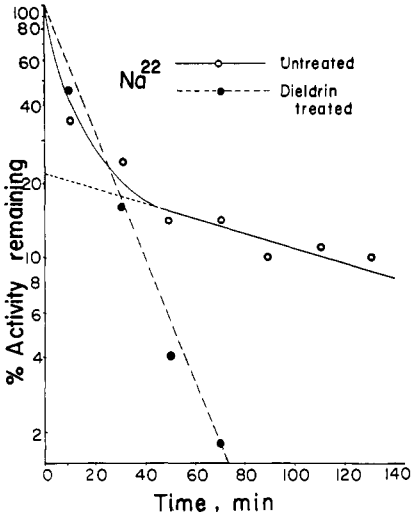

Figure 1. Efflux of Na^{22} ions from the isolated nerve cords of the American cockroach

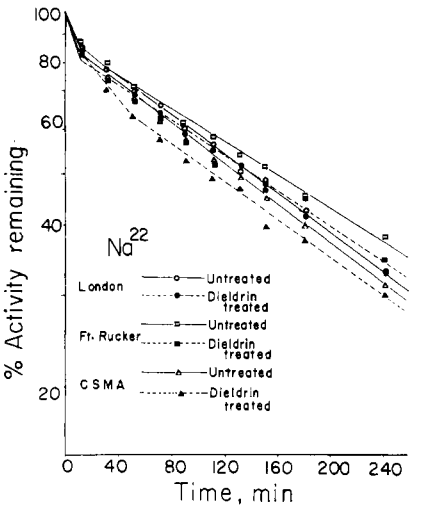

Figure 2. Efflux of Na^{22} ions from the isolated brain tissues of the German cockroach

solution for 30 minutes. They were briefly washed and were then transferred into plain saline solution with or without dieldrin. The rate of diffusion was accelerated by dieldrin for the first 1-hour period and the tendency was more marked in the susceptible strain (Figure 2). The balance of evidence indicated that these nerve tissues picked up more sodium ions than they lost at the end of the initial 1-hour incubation period with dieldrin. For example, with the nerve cord of the American roaches, the average rate of sodium uptake increased by 21% while the rate of diffusion rose by 7 to 8%. The same figures for the nerve tissues of the susceptible German roaches were 27% and 13%, respectively.

Potassium Ion Transport. The same experiment was repeated with K^{42} (a short-life isotope) by using the head parts of the German cockroach. The results (Table III) indicated that the rate of potassium influx was hardly affected by dieldrin at the very beginning and then was inhibited in both strains at the end of the 3-hour incubation period. The study on the effect of dieldrin upon the process of potassium efflux (the method of experiments as Na^{22}-efflux tests) revealed a moderate activation effect in both strains (Figure 3). Further examination of the data indicated that the rate of potassium ion efflux, at the first 1-hour poisoning period, was activated by 14% in the susceptible nervous system. Since under the same conditions dieldrin stimulated the rate of potassium uptake by only 2%, the net effect of dieldrin poisoning at the end of the initial 1-hour period must be a state of hypopotassemia.

Chlorine Ion Transport. In agreement with the above test with sodium ions, the results of a diffusion test with three strains of German cockroaches (Figure 4) indicated that the rate of chlorine ion efflux was markedly stimulated by the presence of dieldrin in all cases.

Calcium Ion Transport. The effect of dieldrin on the rate of calcium diffusion from the abdominal nerve cord of the American cockroach was rather simple: dieldrin activated the rate of calcium efflux (Figure 5). Yet the initial rate of calcium uptake apparently was not markedly influenced by the same treatment: total uptake by the treated cord was 1.23, and the untreated one was 1.18 μmoles per gram.

A similar test with the nerve tissue of the German cockroach strains indicated that dieldrin drastically

Table II. Effect of Dieldrin on the Rate of Sodium Ion Uptake by the Isolated German Cockroach Brains

Data expressed in μmole equivalent of ions picked up by a gram of tissue

Hours of Incubation	Dieldrin Treated[a]		Untreated Control	
	L	S	L	S
0.5	8.351	12.140	8.222	9.820
1	23.128	20.315	27.866	16.184
2	33.904	23.318	33.331	24.817
3	32.758	27.391	33.165	25.882

[a] L represents the London-resistant strain of the German cockroach. S represents the corresponding CSMA-susceptible strain.

Table III. Effect of Dieldrin on the Rate of Potassium Ion Uptake by the Isolated German Cockroach Brains

Data expressed in μmole equivalent of ions picked up by a gram of tissue

Hours of Incubation	Dieldrin Treated[a]		Untreated Control	
	L	S	L	S
0.5	0.045	0.048	0.056	0.047
1	0.052	0.057	0.057	0.057
2	0.056	0.077	0.066	0.078
3	0.091	0.077	0.109	0.102

[a] L represents the London-resistant strain of the German cockroach. S represents the corresponding CSMA-susceptible strain.

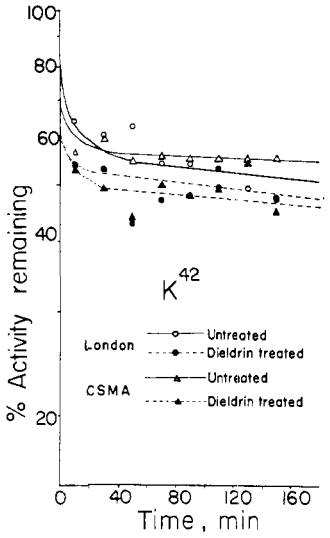

Figure 3. Efflux of K^{42} ions from the isolated brain tissues of the German cockroach

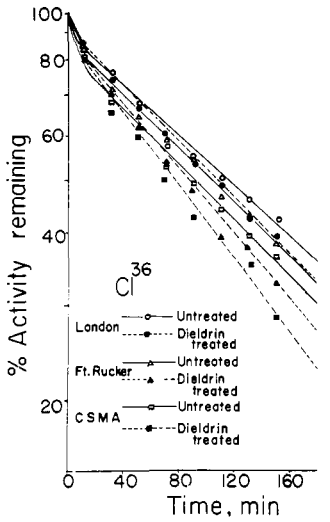

Figure 4. Efflux of Cl^{36} ions from the isolated brain tissues of the German cockroach

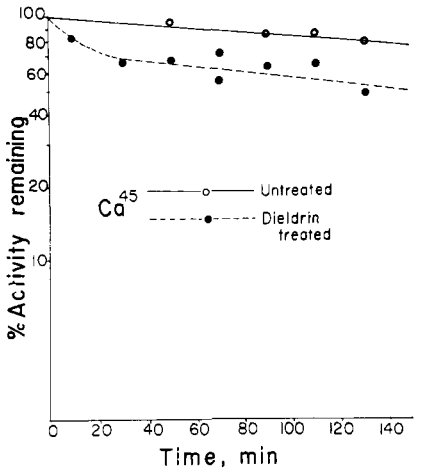

Figure 5. Efflux of Ca^{45} ions from the isolated nerve cords of the American cockroach

affected the efflux process of Ca ions at the first 2- to 3-hour period (Figure 6). To study the effect of dieldrin on the rate of calcium influx, the brain slices from 10 male roaches were immersed in 1 ml. of Ca^{45}-containing saline solution for various time intervals, and the amount of Ca^{45} taken up by the brain tissues was measured (Figure 7). The data involving the later part of dieldrin poisoning—e.g., after 2 or 3 hours of incubation in the dieldrin—in the above influx experiment (Figure 7), however, might not represent the actual effect of dieldrin, for the period of incubation of the tissue with Ca^{45}-containing saline solution differed in each test for dieldrin poisoning. Therefore, an experiment was designed to study the initial rate of Ca^{45} absorption at different stages of dieldrin poisoning (Table IV). The results clearly indicate that the initial effect of dieldrin poisoning is stimulation of Ca uptake by the nervous system. In contrast, at the end of the initial 1-hour incubation period, dieldrin appeared to inhibit the rate of Ca uptake by the nervous system. Though the total effect was more drastic with the susceptible nerve tissues, the corresponding resistant tissues also showed a similar tendency.

The results of an in vivo poisoning experiment (Figure 8), in which the insects were exposed to residually applied dieldrin film (1 mg./200 sq. cm.) in a jar for 5 hours prior to the identical test as in Figure 6, indicated that the rate of calcium ion exchange from the isolated nerve tissue was markedly inhibited in the susceptible individuals that were at the early prostrate stage of poisoning. The last experiment also indicated that all of the nerve tissues that were pretreated with dieldrin in vivo accumulated fewer calcium ions at the end of the 4-hour incubation period than did the untreated control tissues. Thus, after the initial excitation stage of poisoning, the treated nerves must gradually be prevented from taking up external calcium ions.

Rate of Ion Exchange in the Nerve Homogenates. To ascertain that the above phenomenon is caused by intrinsic nerve cell absorption and not by an external cause such as circulation of the body fluid, live German cockroaches were treated topically with 10 μg. per

Table IV. Effect of Dieldrin on the Rate of Ca^{45} Ion Uptake by the Isolated German Cockroach Brains

Incubation period with Ca^{45}-containing saline solution was kept constant for 30 minutes for all experiments

Total Dieldrin Exposure Time, Min.	Preincubation Time with Nonradioactive Dieldrin ($1 \times 10^{-5}M$), Min.	Incubation Time with Ca^{45}-Containing Saline with Dieldrin ($1 \times 10^{-5}M$), Min.	Incubation Time with Ca^{45}-Saline (without Dieldrin), Min.	L,[a] μmole/G.	S,[b] μmole/G.
0	0	0	30	0.00785	0.00752
30	0	30	0	0.00790	0.00825
60	30	30	0	0.00343	0.00242
90	60	30	0	0.00437	0.00297
120	90	30	0	0.00325	0.00212
180	150	30	0	0.00260	0.00287

[a] L represents the London-resistant strain of the German cockroach.
[b] S represents the corresponding CSMA-susceptible strain.

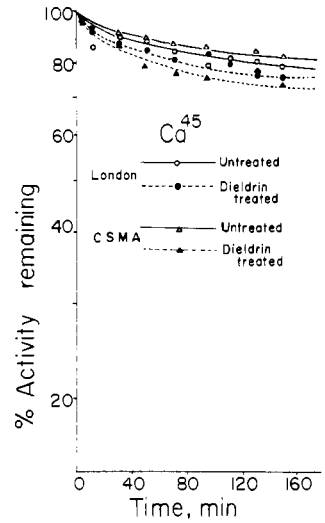

Figure 6. Efflux of Ca^{45} ions from the isolated brain tissues of the German cockroach

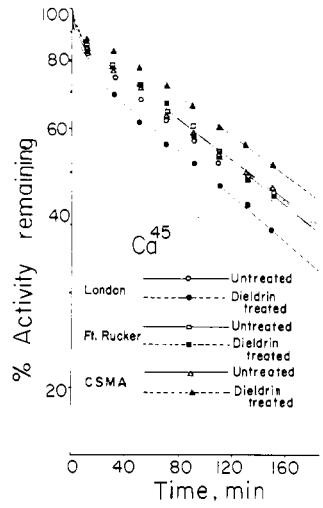

Figure 7. Influx of Ca^{45} ions from the external saline media into the isolated brain tissues of the German cockroach

"Per cent activity remaining" indicates the amount of Ca^{45} remaining in the external media unabsorbed

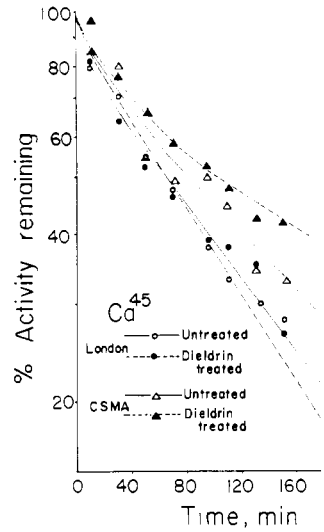

Figure 8. Efflux of Ca^{45} ions from the isolated brain tissues of the German cockroach that was pretreated with dieldrin in vivo

(By a contact method for 4 hours to produce knockdown state for the susceptible roaches)

insect of dieldrin in acetone, and after each experimental time interval, six of them were decapitated to prepare the homogenate with 5 ml. of saline solution per test. The brain homogenate was first incubated with saline solution containing a known amount of Ca^{45} for 1 hour, and then a particle fraction was collected by brief centrifugation at 600 G for 10 minutes at 0° C. This fraction consisted of a large number of small intact nerve cells as well as large nucleus and mitochondrial particles besides a small portion of unidentifiable tissue debris as judged by electron microscopic observations. The amount of Ca^{45} absorbed by these materials was determined by resuspending the sediment in a known amount of saline solution and taking an aliquot of it for radioassay. The results (Figure 9) indicate that the rate of calcium absorption by the particles was stimulated in the dieldrin-poisoned brain tissue at the beginning (about 3 hours for the CSMA and 8 to 9 hours for the London particles) and was inhibited thereafter.

To ascertain that dieldrin itself is an inhibitor of calcium binding to the phospholipids of the nervous system, the experiment of Blaustein and Goldman (1966) was repeated here with commercially available phosphatidyl L-serine (Nutritional Biochemicals, Inc.). The results (Table V) indicated that dieldrin was a mild inhibitor for the binding of calcium to this phospholipid as attested by the decrease of bound Ca^{45} ions that were transferred to the chloroform phase. A similar experiment with the German cockroach phospholipid fractions that were obtained by extracting the brain homogenates with a solvent mixture of chloroform–methanol also showed that dieldrin slightly inhibited the reaction of calcium binding to the brain lipids, though there was no apparent difference in the degrees of inhibition between these two strains.

Discussion

As pointed out by O'Brien (1966), the method of Treherne (1962) which has been extensively used in this study might not be the perfect test method for nerve-penetration studies. That is, since no isolated nerve tissues can be without some damaged portions through which various ions could freely pass, a part of ion transport activities recorded by this method could be artifactual. Unfortunately, the above criticism cannot be tested by simple experiments. In addition, the

Table V. Effect of Dieldrin on Calcium Binding to Various Fractions Containing Phospholipids

The data expressed in μmole of Ca^{45} ions bound to the phospholipid fraction from 1 μmole of Ca^{45} ions originally added to the reaction media

Fractions	Untreated Control	Dieldrin Treated
No lipid control	0.000	0.000
Phosphatidyl-L-serine	0.886 ± 0.010	0.825 ± 0.018
Roach brain phospholipids from L strain	0.375 ± 0.011	0.351 ± 0.002
Roach brain phospholipids from S strain	0.391 ± 0.011	0.369 ± 0.018

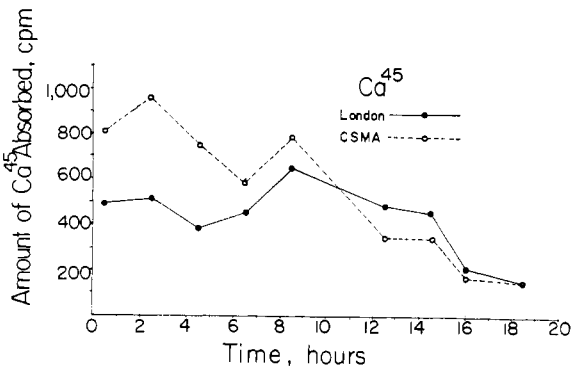

Figure 9. Uptake of Ca^{45} ions from the external saline media by the brain homogenate particles from in vivo dieldrin-treated German roaches

injuries to the nerve tissue also might alter the reaction rates of ion exchange activity between the injured tissue and the external medium. Despite the above problems, there is a direct proof that the relative rates of ion exchange reactions were actually influenced by the presence of dieldrin in the external medium—i.e., the difference in the rate of ion exchange between dieldrin-treated and untreated control tissues always existed. Such alterations, therefore, must come, at least in part, from the action of dieldrin itself, even though the absolute basic values themselves might contain some artifacts.

That dieldrin at the early stage of poisoning stimulates the rate of both uptake and to some extent efflux of sodium ion by the cockroach nerve tissue is clearly established. The rate of potassium ion influx across the nerve membrane is, on the other hand, hardly stimulated by dieldrin from the early stage of poisoning. The balance of evidence suggests that the initial result of dieldrin poisoning appears likely to be similar to that of DDT—i.e., accumulation of sodium ions inside the nerve—though its basic cause must be different as judged by the fact that the inhibition of potassium ion influx started to occur after the above sodium-accumulation phenomenon had taken place, and by symptomological observations by other workers (Busvine, 1954). The effects of dieldrin upon the rate of chlorine ion movement also support the view that at the early stage of poisoning, dieldrin markedly stimulates the activity of sodium transport. In accordance with the case of houseflies (Yamasaki and Narahashi, 1958b), the brain tissues from the susceptible German roaches started to show the increased sodium efflux at much earlier stages than did those of the resistant individuals (Figure 2). That dieldrin increases the rate of calcium exchange at the early stage of poisoning was clearly demonstrated with the nerve cords of American cockroaches (Figure 5). At the later stage of poisoning, the rate of calcium uptake was markedly reduced. With German cockroaches this symptom is not necessarily confined to the susceptible nerve tissues—e.g., dieldrin-treated resistant nerve tissues also exhibited a similar but milder stimulation and subsequent inhibition effect in their rates of calcium ion uptake. The data from the homogenate incubation experiments also indicate that dieldrin stimulates the rate of calcium uptake by the nerve tissue at the beginning, and then inhibits at the later periods of poisoning. The only difference discovered here was that the tendency was much more marked in the susceptible nerve tissues. Therefore, the eventual result of dieldrin poisoning may be inhibition of calcium transport across the nerve membrane. The experimental data obtained with pure phospholipids support the above view that dieldrin could inhibit the rate of calcium uptake by the nervous system. The fact, however, that the same experiments with resistant and susceptible nerve phospholipids showed the same degree of inhibition supports the view that this particular process is not utilized as the major means of defense by the resistant individuals, and/or the inhibitory activity of dieldrin upon the calcium transport system is not the major cause of dieldrin poisoning.

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