Dieldrin, Effect on the Ion Transport Activities in Liver Tissues

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The precise modes of action of persistent chlorinated hydrocarbon insecticides are not known. Because of their repeated use in controlling insect pests and resulting build-up in our environments, however, it has become increasingly important to know the effects of chronic exposure to those chlorinated hydrocarbon insecticides in animals, including man.

There are a number of reasons to believe that the nervous system and the liver is one of the most sensitive organs against chronic exposure to many chlorinated hydrocarbon insecticides: the most notable histopathological and electrophysiological changes being confined in the liver and the nervous system respectively (1,2). That chlorinated hydrocarbon insecticides, even at low doses, affect the biochemical conditions of liver tissues has been reported recently. In brief, chlorinated hydro-

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carbon insecticides can stimulate microsomal drug metabolism (3,4) and also can reduce the amount of dieldrin storage in various tissues (5).

The purpose of this paper is to report another effect upon the liver tissue by one of the potent chlorinated hydrocarbons, dieldrin, with respect to the ion transport activities.

Materials and Methods

The rat liver was obtained from a male albino rat supplied locally. The liver tissues were sampled immediately after stunning the animal with a mild cerebral concussion, and were frozen immediately. Liver slices of approximately 1 x 1 x 0.3 cm^3 were made with a razor blade and were weighed.

To a 5 ml flat-bottom vial 0.2 ml of saline solution (6) containing either Ca^{45}, Na^{22}, Cl^{36}, or K^{42} ions, dieldrin in 2 ul of acetone (or 2 ul of acetone alone for control) was added to make the final concentration of dieldrin at 10^{-5} M. A liver slice was transferred into the vial, immersed, and maintained at 24° C for 1 hour. The slice was picked up, briefly washed in a 10 ml aliquot of fresh saline solution, blotted by a filter paper to remove the excess liquid, and then transferred into another 10 ml aliquot of fresh saline solution (7). 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 liver tissue. The experiment procedures employed for the ion influx measurements were the same as above except that the liver slices themselves were immediately homogenized with a 10 ml

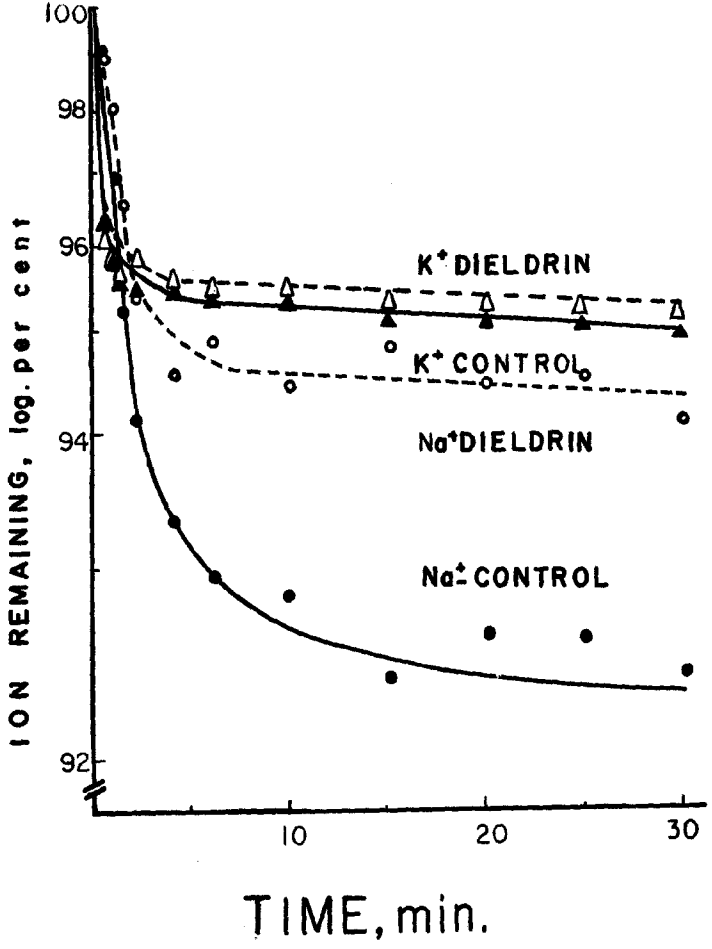

Fig.1. Effect of 1 x 10^{-5}M dieldrin on the rate of sodium (Na^{22}) and potassium (K^{42}) ion efflux from the tissue slices of rat liver.

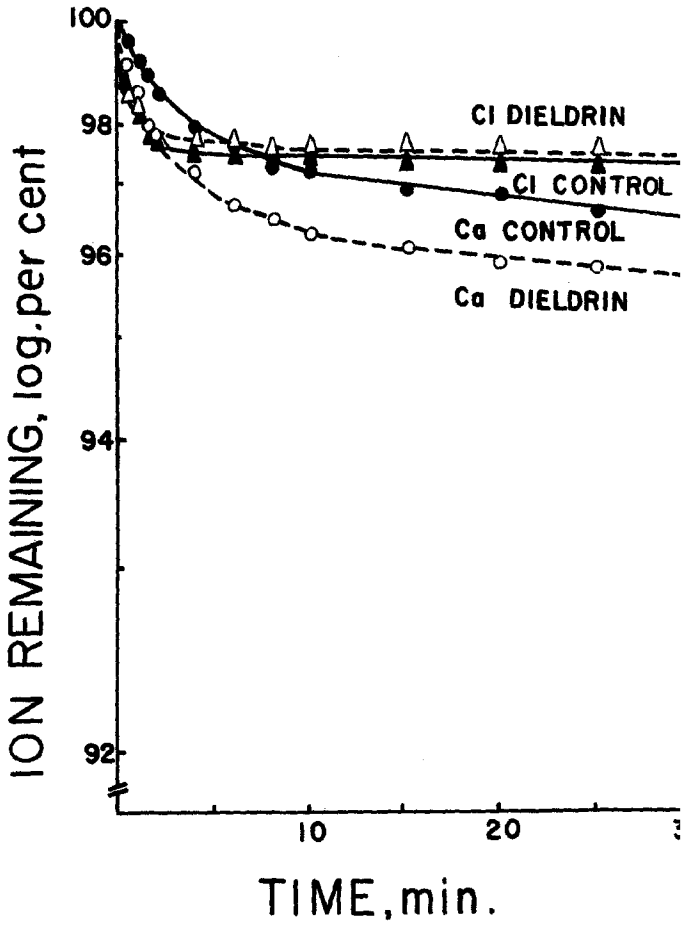

Fig.2. Effect of 1 x 10^{-5}M dieldrin on the rate of calcium (Ca^{45}) and chlorine (Cl^{36}) ion efflux from the tissue slices of rat liver.

aliquot each of counting solution for liquid scintillation assay after the same rinsing and blotting treatment (7).

For preparing Ca^{45} and K^{42} containing saline solutions, the amount corresponding to the radioactive ions were compensated by decreasing the quantity of the corresponding salt to keep the ion composition constant.

Results and Discussion

The results of Na^{22} ion efflux tests (Fig. 1) indicated that the rate of soidum ion efflux from the liver tissue was markedly inhibited. This tendency of dieldrin to inhibit the rate of monovalent cation efflux appeared to be less pronounced for potassium ions: dieldrin treated liver slices released slightly less potassium ions than did the normal tissues (Fig. 1). On the other hand, the rate of efflux of chlorine ions was found to be hardly affected by the same treatment (Fig. 2).

Contrary to the general tendency of dieldrin to inhibit the ion transport systems in the liver, the rate of calcium ion efflux was greatly activated (Fig. 2).

The rates of various ion uptake (influx) by the liver slices were then studied. The results shown in Table 1 indicate that, on the whole, the effect of dieldrin on the ion uptake processes are relatively small: both calcium and sodium ion uptake were slightly activated while the rates of uptake of chlorine and potassium ions slightly decreased.

TABLE 1

Effect of 1 x 10^{-5} M dieldrin upon the rate of influx (uptake) and efflux of various ions: the data expressed in the per cents of normal exchange rates for each ion by the tissue slices of the liver in 30 minutes.

	Na^+	K^+	Cl^-	Ca^{++}
Influx	100.6	90.3	97.4	109.6
Efflux	85.8	90.0	95.6	120.0
Balance*	+14.8	+0.3	+1.8	-10.4

* Plus signs indicate accumulation of the corresponding ions. The minus sign indicates depletion of the ion in the liver tissue.

Previously (7) the effects of dieldrin in the nervous system was described as activation of all the ion transport mechanisms at the early stage of poisoning ("excitation stage" up to 30 minutes for all ions *in vitro*; 30 minutes for Na^+, K^+ and Cl^-; and 3 hours for Ca^{++} ions *in vivo*). It inhibited both influx and efflux of all ions thereafter ("depression stage").

Since in the present study all the liver slices were incubated for 1 hour prior to the influx and efflux tests, the data obtained in this work should be interpreted to correspond to the "depression stage" for all ion-transport mechanisms except for calcium ions. It appears, therefore, the response of the

tissue slices of the rat liver closely resemble that of the insect nervous system in vivo (rather than the isolated nervous system).

That a number of chlorinated hydrocarbon insecticides attack the nervous system to cause abnormal ion movements across the nerve membrane has been well documented (8). In brief DDT blocks the process of potassium ion influx and sodium ion efflux after depolarization causing a state of hyperexcitation of the nerve cells involved (9). The action of dieldrin in the nervous system superficially resembles that of DDT, but the phenomenon appeared to be accompanied by the depletion of calcium ions from the cell membrane (7). In view of such actions of chlorinated hydrocarbon insecticides upon the nerve membranes, it is not surprising that the membranes of the hepatic cells are also affected by these insecticides in the same manner as the nerve membranes, for all membrane systems are believed to be operated under similar fundamental mechanisms.

The relationship between this finding and other established effects of chlorinated hydrocarbon insecticides upon the liver systems such as induction of microsomal enzymes, or reduction of storage of other insecticides is worthwhile to consider here, since there is a possibility of a close causal relationship among them. Recently it was reported from this laboratory that mild electric stimulation in terms of repeated square impulses (pulse height 200 mV for 10 m sec and 1 second delay for 2 min) could cause a marked decrease in the amount of dieldrin storage in the liver tissue. The effect could also be produced by DDT and other

inducing drugs such as phenobarbital under the same experimental conditions, indicating that the liver cells at an excited state, either by electric stimuli or drugs, can retain much less dieldrin than it does at the normal resting state. Since the state of liver cells under the environment of hypocalcemia should be the excited one, at least the causal relationship could exist between the reduction of stored insecticides and the reduced Ca^{++} uptake by the liver cells. It may be much premature to relate this phenomena with that of induction, for data presented here cover only the short term responses in terms of minutes and hours, whereas induction phenomena usually takes days and weeks to develop. A more recent report (10), however, indicates that the liver cells start responding to the insecticidal stimuli within 6 to 12 hours after the initial treatment by modifying the rate of RNA incorporation and within 18 hours they start producing smooth endoplasmic reticulum (SER) which is believed to be the cause of the "induction" phenomenon. It is possible, therefore, that the marked effect of dieldrin upon Ca^{++} transport systems, though it is a short term response, is eventually related to the induction phenomena.

Summary

The results of a survey on the effects of dieldrin upon the rat liver systems showed that this chlorinated hydrocarbon insecticide markedly affects Na^{+} and Ca^{++} ion transport mechanisms in the liver. Transport mechanisms for other ions appeared to be only moderately affected. The situation resembles

that which occurs in the nerve cells under dieldrin influence *in vivo* (7). The eventual results of such a physiological modification is excitation of liver cells due to the effect of accumulated sodium and depletion of calcium ions.

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