Reduction of Dieldrin Storage in Rat Liver: Factors Affecting *in Situ*

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The accumulation of chlorinated hydrocarbon insecticides in the animal body is one of the most serious problems associated with the use of these chemicals for the control of insect pests. These insecticides may accumulate in vital organs such as the liver and the nervous system and could cause tissue damage. Among them, chlorinated cyclodiene insecticides, namely dieldrin, chlordane, etc., represent the group of compounds which shows the highest rate of storage in animal tissues.

Recently it has been reported (1) that the amount of stored dieldrin can be greatly reduced by administering DDT to the animal in vivo. Subsequent studies by the same workers indicated that a variety of drugs which had previously been shown to have the ability to stimulate the detoxication mechanisms (i.e. "induction ability") also reduced dieldrin storage (2). Since it had already been shown by Hart and Fouts (3) and by Gillett et al. (4) that in vivo administered DDT and chlordane could stimulate microsomal drug metabolism in vitro, natural conclusion for the reduction of dieldrin

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storage appeared to be the increased dieldrin metabolism stimulated by these inducer drugs (2). Dieldrin is, however, a very stable compound and it is degraded only slowly, if at all, in various organisms (5, 6). In fact, Gillett et al. (4) based their assay method for microsomal oxidation activity upon the quantitative epoxidation of aldrin to dieldrin. Incubation of dieldrin with microsomes with NADPH and/or reduced glutathione at various protein levels for up to two hours gave recoveries of 95 ± 5% of unchanged dieldrin. By contrast the latest information provided by Street and Chadwick (7) indicated that DDT-treated rats produced much more metabolites from dieldrin in vivo. So far all the experimental evidence indicating clear reduction of dieldrin storage by various inducer drugs has been conducted in vivo. In the absence of solid evidence of microsomal stimulation of dieldrin degradation it was felt that the above phenomena of apparent reduction of dieldrin should be re-examined under more controlled experimental conditions.

Experimental

For this purpose we used isolated liver tissues from the male rat. The liver tissue was quickly frozen at -60° C and slices were made with razor blades to obtain thin squares of approximately 1 x 1 x 0.3 cm. Each slice was weighed and the ones which were in the range of 300 to 400 mg were used throughout the study. For diffusion study, the liver slices were immersed in C^{14} dieldrin solution (final concentration 10^{-6} M in saline) for 1 hour at 24°C.

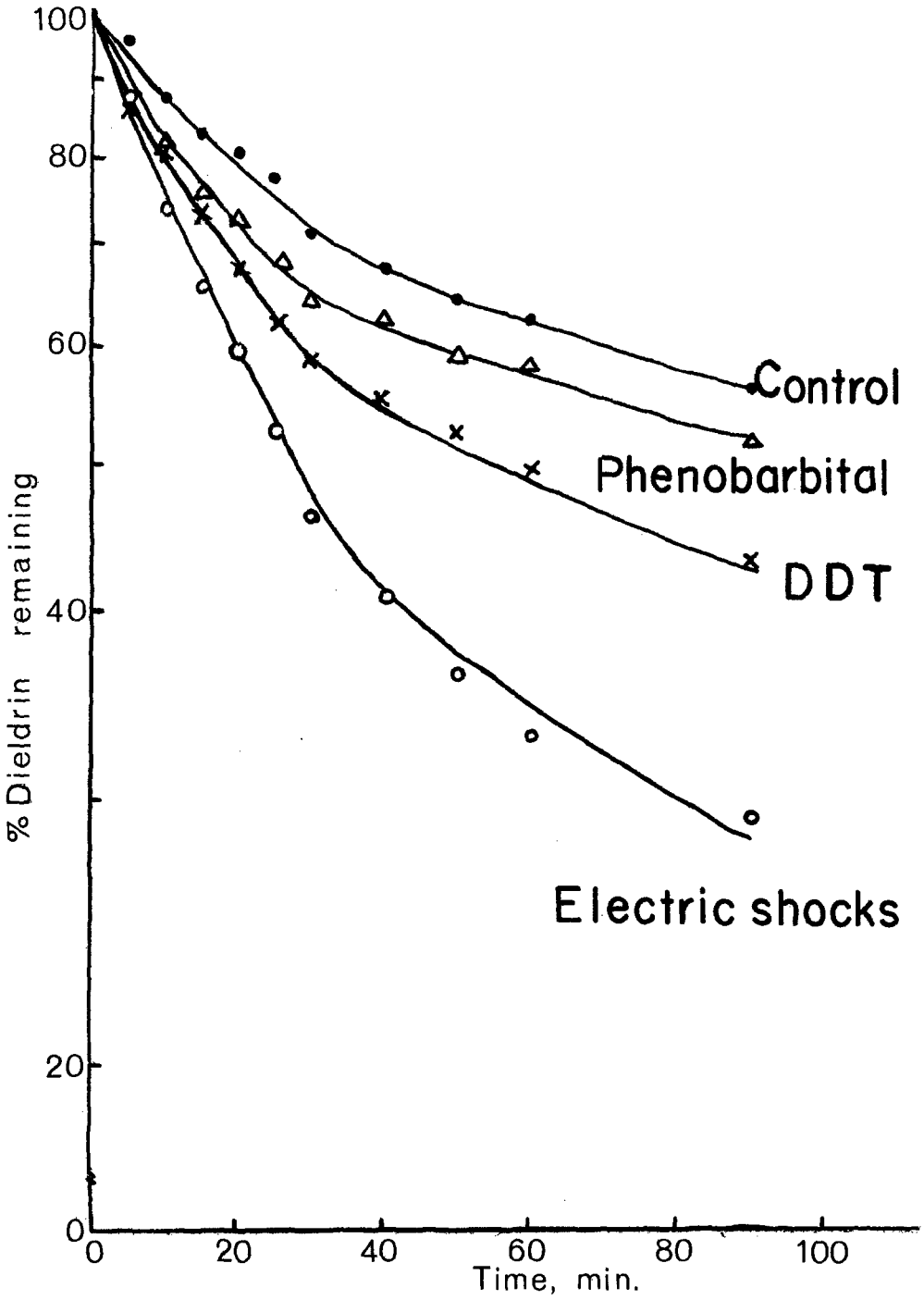

Fig. 1. Effects of in situ treatment of the liver slices with phenobarbital, DDT, and electric shocks upon the rate of dieldrin discharge from the tissue to the external saline medium.

They were picked up, washed briefly with fresh saline for a moment, carefully blotted with a filter paper and transferred to 10 ml of fresh saline solution, with or without interferring drugs. After a given time interval a 0.5 ml portion of the ambient saline solution was sampled for radio-assay to detect the amount of dieldrin diffused into the external medium. In some cases the electric stimuli in terms of series of square pulses were given to the slices prior to the treatment with C^{14} dieldrin by using a stimulator (Grass Instrument) with the aid of an oscilloscope. For absorption study, the liver slices were treated with 10^{-5} M of interferring drugs in saline for 30 minutes, and then the slices were incubated with 1 ml of C^{14} dieldrin containing saline solution at 10^{-5} M for 30 minutes. In all cases the amount of dieldrin was determined by assaying both the ambient medium as well as the liver tissues themselves. The latter assay was done by homogenizing the slices directly in the counting solution for liquid scintillation spectroscopy.

The results in Fig. 1 indicated that the liver slices thus treated normally released 38% of dieldrin within 1 hour, and that the presence of DDT and phenobarbital stimulated the rate of dieldrin release. By far the most conspicuous effect was obtained, however, with the slices that were stimulated by mild electric shocks. That the above process of releasing of dieldrin did not involve degradation of dieldrin could be shown by both thin-layer and gas chromatographic analyses of the sample recovered from both the liver tissue and the

TABLE I

Effect of Liver Treatment on the Rate of Dieldrin Absorption in situ.

Treatments	Relative Absorption of dieldrin (control as 100)[a]
In vivo	
DDT 50 mg/kg - 24 hrs.	76.7 ± 2.7[b]
Phenobarbital 75 mg/kg - 24 hrs.	83.7 ± 19.2
Aminopyrine 150 " "	111.1 ± 8.6
Tolbutamide 5 " "	124.3 ± 18.7
In situ	
DDT 10^{-5} M: 30 min.	91.8 ± 0.9[b]
Electric shock[c] 200 mv:2 min.	91.4 ± 1.2[b]
Phenobarbital 10^{-5} M: 30 min.	117.0 ± 14.6
NADPH 2 mg/5 ml: 30 min.	105.4 ± 15.1
Triton X-100 1%: 30 min.	140.0 ± 12.9 [b]
Tetrodotoxin 0.3 uM: 30 min.	146.1 ± 1.7 [b]

a/ The data expressed in relative value ± standard error.

b/ Statistically significant differences at 5% level. Average of 3 to 5 replicates.

c/ Continuous, each pulse duration and delay being 10 m second and 1 second respectively.

external medium.

The effect of the same treatments of the liver slices upon the rate of dieldrin absorption was then studied. It was found (Table 1) that the liver slices from the DDT-treated rat absorbed significantly less dieldrin than did the control. Phenobarbital, as in the case with diffusion tests (Fig. 1), produced a similar but less profound effect. The same phenomenon could be reproduced by pretreating the liver slices with DDT or electric shocks. Addition of NADPH, a known co-factor for dieldrin metabolism in the rat liver homogenate (8) did not affect the rate of dieldrin absorption. On the other hand tetrodotoxin, an inhibitor for the transport mechanism of sodium ions across the cell membrane (9) and triton X-100, a surface activating agent enhanced the rate of dieldrin uptake by the liver slices.

It may not be correct to conclude from the above data that DDT and other inducing compounds do not activate the dieldrin degradation system in the liver. The data presented here cover only the short term responses in terms of minutes and hours, whereas the in vivo phenomenon observed by Street and his co-workers (1,2) was the result of excretion for 10 consecutive days. To relate the above data to others, we have repeated the same absorption experiments as in TABLE I with the liver slices from the rats that were treated with DDT, phenobarbital, aminopyrine, and tolbutamide for 8 consecutive days. The results, however, did not indicate any significant changes in the rate of dieldrin absorption that could be

ascribed to the treatments with these drugs. Furthermore preliminary data obtained in this laboratory (8) indicated that the liver homogenate thus induced by phenobarbital did not show any higher degradation activities towards dieldrin in vitro with or without the presence of NADPH. At present, therefore, one cannot logically explain the meaning of the long term effects of these inducing agents upon the liver systems and their roles in reducing the amount of dieldrin storage.

The major purpose of this report is to show that the liver tissue could be influenced by a number of stimulating, surface activating, as well as inhibitory treatments, to change the absorption and desorption behavior toward dieldrin, without involving any biochemical degradation processes against the insecticide.

Although the phenomenon could be explained in terms of competitive absorption towards the same binding site between the dieldrin and DDT molecules, for instance, the results of tests with mild electric stimuli strongly support the view that the liver tissue at an excited state can retain much less dieldrin than does the liver tissue at a resting or inhibited state.

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