PROBLEMS IN AERIAL APPLICATION:

II. EFFECTS OF CHLORINATED HYDROCARBONS ON SUBSTRATE-LINKED PHOSPHORYLATION

63-4

FEDERAL AVIATION AGENCY
AVIATION MEDICAL SERVICE
AEROMEDICAL RESEARCH DIVISION
CIVIL AEROMEDICAL RESEARCH INSTITUTE
OKLAHOMA CITY, OKLAHOMA

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2. Crop Dusting
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FOREWORD

The mechanism of the harmful effects of toxic substances on the performance of aerial applicator personnel lies at the cellular level. Effective field detection of early toxicity and the application of sound preventive methods can only be achieved following an understanding of the biochemical mechanisms involved.

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ABSTRACT

In a search for an explanation of an earlier finding that dieldrin decreased the rate of methionine uptake by cardiac muscle, chickens and rats were exposed to this compound and the substrate-linked phosphorylation phenomenon was studied during the anaerobic degradation of glucose. Exposure to dieldrin reduced the esterification of inorganic phosphate 50% but did not affect the production of lactic acid. Four other chlorinated hydrocarbons, lindane, heptachlor. isodrin, and endrin did not produce this effect.

In a recent report from this laboratory, data were given which support the concept that some of the hepatic changes resulting from chlorinated-hydrocarbon intoxication could be attributed to a reduction in active membrane transport (1). Since this is an energy dependent phenomenon, a study of the effects of chlorinated hydrocarbons on mechanisms involved in the synthesis of adenosine-triphosphate (ATP) seemed a means of describing in greater detail the influence of such compounds on biological systems.

EXPERIMENTAL

White Rock chickens, obtained locally when 1 week old, were kept in the laboratory on Ralston Growena until they had attained a weight of at least 400 g. At the time of assaying the chlorinated hydrocarbon effect, randomly selected animals were weighed and were given dieldrin (hexachloro-epoxy-octahydro-dimethanonaphthalene) *per os*. The dieldrin had been previously dissolved in acetone, introduced into gelatin capsules and the acetone allowed to evaporate before administration to the chickens. Dieldrin was also given by mouth, dissolved in corn oil (Mazola), with identical results.

The male rats used in part of the study were of the Sprague-Dawley strain and weighed in excess of 300 g. at the time of their exposure to the insecticide. In these tests the exposure was accomplished by intraperitoneal injections of dieldrin dissolved in corn oil (Mazola). Controls received the oil carrier alone. The animals received two injections, each containing 15 mg. of the insecticide.

The present assays on the effect of the exposures were carried out on 10% brain homogenates (in water) incubated in Warburg flasks at 38° C. The incubation medium consisted of those components known to support anaerobic glycolysis in an homogenate system (2, 3). Brain tissue was chosen for these studies as it represents the only normal adult tissue that can adequately phosphorylate glucose in an homogenate system. This was of importance in the present investigation as this type of system was also to be used in other studies. Of equal consideration was the fact that earlier work had shown that the DNA content of brain (indicative of the content of enzymatically active material or nucleated cells) is not affected by exposures to chlorinated hydrocarbons (1, 4). Determinations of total nitrogen, some of which are included in Table 1 below, are further evidence of the refractiveness of brain protein content to the insecticide. Inorganic phosphate was determined by the method of Fiske and SubbaRow (5); total nitrogen by standard micro-Kjeldahl techniques (6); protein by the method described by Lowry et al (7); and DNA by analysis for 2-desoxyribose (8) following tissue fractionation by methods of Schneider (9) and Schneider and Klug (10).

Adenosine-triphosphatase activity was measured in an incubation mixture consisting of Na ATP, Mg ions, KF, tris buffer (pH 7.4) and brain homogenates. The reaction was stopped at 30 min. (37°C) with TCA.

RESULTS AND DISCUSSION

In Table 2 are shown certain of the results of exposing chickens to a single dose of 100 mg/kg of dieldrin, given 24 to 48 hours prior to the assay. This dosage must closely approximate the LD_{100}, since all of the unused test animals died within 72 hours. It may be seen that acid production was not significantly affected by the insecticide. Separate tests have demonstrated that the acid responsible for the evolution of CO_2 was lactic acid. It may be concluded that the anaerobic degradation of glucose, *per se*, was not influenced by the acute exposures to dieldrin. In contrast to this, the disappearance rate of inorganic phosphate was quite obviously affected by the exposure, being reduced to approximately the 50% level. As may be observed from the data given in Table 2, neither sex nor age appeared to be significant factors in this aspect of the response to the insecticide. It was observed, however, that significant variations did occur between individual chickens in terms of (1) the measurable rate of glycolysis (in both controls as well as test animals) and (2) the toxicological response to a particular dose level. These variations will undoubtedly constitute a source of continuing difficulty in obtaining reproducible results on different individuals and in adequately controlling the dose level to obtain "predicted" results. Similar variations in other areas of (poultry) biochemistry have been observed in other work (2).

Additional evidence of this effect of dieldrin in uncoupling inorganic phosphate esterification is given in Table 1. These data represent the results of glycolysis and phosphorylation measurements on the pooled brain homogenates of 3 control and 3 dieldrin-exposed rats in two separate experiments. The dieldrin was given, as described above, on the day preceding the time of assay. It will be seen that the effect produced by dieldrin exposure was identical with that observed in chickens and that the individual measurements closely approximate those shown in Table 2.

TABLE 1

EFFECT OF DIELDRIN
ON
SUBSTRATE-LINKED PHOSPHORYLATION
IN
RAT BRAIN

Exp. No.	*Animal*	*PO_4*	*CO_2*	*N_2*	*PO_4/CO_2*
I	Control	4.23	3.8	.528	1.11
	Test	1.87	3.8	.480	0.49
II	Control	2.4	2.96	.483	0.81
	Test	1.4	3.22	.483	0.43

PO_4 disappearance in μM/flask/10 min.
CO_2 evolution in μM/flask/10 min.
N_2 in mg./flask

TABLE 2

EFFECT OF DIELDRIN
ON
PHOSPHATE ESTERIFICATION BY CHICKEN BRAIN

Exp. No.		*Animal*	*PO_4*	*CO_2*	*PO_4/CO_2*
I	(m)	Control*	3.50	3.47	1.02
	(m)	Test*	1.69	2.88	0.58
II	(f)	Control*	3.70	3.36	1.05
	(f)	Test*	1.85	3.14	0.59
III	(m)	Control*	5.33	3.60	1.48
	(m)	Test*	1.17	3.65	0.32
IV	(f)	Control*	3.31	3.45	0.96
	(f)	Control*	3.32	3.15	1.05
V	(f)	Control**	2.60	3.30	0.79
	(f)	Test**	1.47	2.50	0.59
Mean		Control	3.62	3.39	1.07
Values		Test	1.54	3.04	0.51

PO_4 disappearance in μM/flask/10 min.
CO_2 evolution in μM/flask/10 min.
* 400-500 g.
** 1300 g.
(m) male
(f) female

Studies were made on the adenosinetriphosphatase activity under conditions similar to those described above. There was no discernible difference between the control and test animals (Table 4). As may be noted, KF actively inhibits ATPase activity. Since KF was included in all of the incubation mixtures used in the study of phosphate esterification, it would not appear that the ATPase system played any role in the differences seen above.

Parker (11) noted a similar effect of DDT and some of its metabolites and dieldrin on phosphate uptake by liver mitochondria using a pyruvate substrate system. It would appear that dieldrin may have a general uncoupling effect on phosphate esterification, whether it is oxidatively or anaerobically supported.

It is apparent from the results of the studies on both chickens and rats that whereas brain glycolysis, as evidenced by lactic acid production, is not affected by the dosage levels used in this work, the esterification of inorganic phosphate, and presumably the synthesis of ATP, is markedly reduced. At present, this effect does not appear to be the result of a simple inhibition by dieldrin, *per se,* since no direct *in vitro* effect has so far been shown (unpublished data). Investigations are now being carried out to identify the active form of the chlorinated hydrocarbon and to test the activity of other chlorinated hydrocarbons. From the effectiveness of the intraperitoneal injections it also would appear that the biological activity of the compound does not depend on chemical alterations involved in or preceding the process of absorption from the intestine.

At this time several other chlorinated compounds of an entirely different molecular configuration have been studied in a preliminary fashion. It is of interest that these compounds have not been shown to inhibit phosphate esterification under conditions comparable to those described herein. Table 3 shows the results of typical experiments, in this case comparing heptachlor with dieldrin. The results with lindane, isodrin, and endrin were practically identical with these shown for heptachlor. While the negative findings with lindane and heptachlor are not too surprising, those obtained following exposure to isodrin and particularly endrin were mildly so, since the latter is the optical isomer of dieldrin. Table 3 also demonstrates, on the basis of total nitrogen and DNA, the comparability of the test and control animals. In no experiments to date has either of these values changed in

TABLE 3

COMPARISON OF EFFECTS
OF
DIELDRIN AND HEPTACHLOR ON
PHOSPHATE ESTERIFICATION BY BRAIN

ANIMAL	*PO_4*	*CO_2*	*PO_4/CO_2*	*Lactic Acid*	*N_2*	*DNA*
Rat (300 g.)			Dieldrin			
Control	2.4	2.96	0.81	2.31	0.48	0.176
Test	1.4	3.22	0.43	2.58	0.48	0.171
Rat (180 g.)			Heptachlor			
Control	2.3	2.68	0.85	......*	0.52	0.128
Test	3.6	3.18	1.13	......*	0.50	0.124
Chick (1100 g.)			Heptachlor			
Control	3.0	3.18	0.94	2.21	0.44	0.118
Test	3.2	3.18	0.94	2.13	0.44	0.125

PO_4 disappearance in μM/flask/10 min.
CO_2 evolution in μM/flask/10 min.
Lactic acid in μM/flask/10 min.
N_2 in mg./flask
DNA in mg./g dry weight as desoxyribose nucleic acid
* samples not estimated

TABLE 4

ADENOSINE-TRIPHOSPHATASE
ACTIVITY OF RAT BRAIN FOLLOWING
EXPOSURE TO DIELDRIN

CONTROL*			TREATED*		
0 Time	*Final No KF*	*Final KF Added*	*0 Time*	*Final No KF*	*Final KF Added*
(1) 2.32**	12.40**	4.13**	2.58**	12.60**	4.40**
(2) 1.90	11.14	3.88	2.02	12.10	3.30

* Two experiments run in duplicate
** μMols of inorganic phosphate per flask

brain. On the basis of the lactic acid determinations, it is further shown that anaerobic glycolysis was actually the system being measured.

Regardless of the mode of action of dieldrin, its effects on those biological phenomena which are energy dependent are apparent. These findings are particularly significant, in view of the reduced rate of S-35 methionine uptake by the heart sarcosomes of dieldrin-exposed animals (1), since methionine is known to be actively transported across biological membranes and interfaces. It now becomes desirable to test the activity of dieldrin (and other such compounds) on energy dependent phenomena in other tissues.

SUMMARY

Acute exposures of chickens and rats to the chlorinated hydrocarbon, dieldrin, have been shown to result in the uncoupling of inorganic phosphate esterification during anaerobic glycolysis. Acid (lactic) production is not affected. Lindane, isodrin, endrin, and heptachlor do not produce this effect.

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