TABLE 1. STATUS OF LIVER COMPONENTS AT TIME OF PROTECTION

Agent	Time after injection	Total -SH	Acid soluble -SH	Protein bound -SH	Free -SS-	Mixed -SS-
Cysteamine	10 min	No change	Down	Up	Down	Down
Cysteine	10 min	Down	Up	Down	Down	Up
A.E.T.	10 min	Up	Down	Up	Down	Up
Serotonin	10 min	Up	Down	Up	Down	Down
Na. monofluoracetate	1 hr	Down	Up	Down		Down
Na. monofluoracetate	2–5 hr	Varies	Varies	Varies		Varies

of the protector to cellular protein occurs it is not via -SH groups or that an immediate compensatory, or over compensatory, loss of preexisting mixed disulphides occurs.

The four agents for which data were obtained all caused a fall in free -SS- levels at a time when protection would be expected. This is consistent with the previous finding[1] of higher -SS- levels in radioresponsive than in radioresistant tissues. Whilst under present circumstances it is not possible to envisage any critical role for a molecule such as -GSSG- (oxidized glutathione) in irradiation response it is evident that the problem of tissue disulphides demands further attention.

Acknowledgement—The expenses of this work were defrayed from a block grant from the Cance Research Campaign.

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Biochemical Pharmacology, Vol. 20, pp. 3243–3245. Pergamon Press, 1971. Printed in Great Britain

Inhibition of oligomycin sensitive and insensitive fish adenosine triphosphatase activity by chlorinated hydrocarbon insecticides*†

(*Received* 29 *January* 1971; *accepted* 30 *April* 1971)

IN PREVIOUS reports[1–3] we have shown that chlorinated hydrocarbon pesticides cause inhibition of both Na^+-K^+ ATPase and Mg^{2+} ATPase activities. The inhibition occurred in a wide variety of

* Paper No. 7480, Scientific Journal Series, Institute of Agriculture, University of Minnesota, St. Paul, Minn., U.S.A.

† This investigation was supported by a grant from the U.S. Department of the Interior, FWPCA 16030 ELZ.

tissues and from different species (rabbit, fish, insects). Further, it was demonstrated *in vitro* that DDT produced a greater inhibition of Mg^{2+} ATPase than of Na^+-K^+ ATPase. In order to relate this enzyme inhibition with toxicity, it was necessary to investigate the insecticide specificity with respect to the type of Mg^{2+} ATPase.

Lardy *et al.*[4] and Kagawa and Racker[5] have shown that mitochondrial Mg^{2+} ATPase activity is specifically inhibited by very low concentrations of oligomycin. In this report we show that different types of chlorinated hydrocarbon insecticides have different selectivity as shown by inhibition of oligomycin-sensitive and oligomycin-insensitive Mg^{2+} ATPase activities. Table 1 shows the effects

TABLE 1. OLIGOMYCIN INHIBITION OF ATPASES IN FISH TISSUE PREPARATIONS*

	Per cent inhibition							
	Liver		Kidney		Muscle		Brain	
Oligomycin (μg/Rx Mix)	Mg^{2+}	Na^+-K^+	Mg^{2+}	Na^+-K^+	Mg^{2+}	Na^+-K^+	Mg^{2+}	Na^+-K^+
0·03	60·9	0	51·4	0	15·9	0	35·6	11·1
0·10	67·9	20·4	57·2	4·1	12·9	9	40·0	11·2
0·50	69·7	31·1	61·3	23·4	18·3	0	43·2	28·1
1·00							45·4	32·9
2·50			59·1	60·9			46·1	56·2
Untreated sp. act.†	23·5	2·1	35·1	36·3	60·6	2·9	25·6	29·2
± S.E.	± 2·2	± 0·7	± 1·3	± 2·3	± 6·0	± 1·6	± 1·1	± 1·7

* A 3-ml reaction mixture (Rx) contained: 5 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+ (all as chlorides), 120 mM imidazole buffer pH 7·5, 0·19 mM NADH, 0·5 mM PEP, 0·02% BSA, approximately 9 units pyruvate kinase, and 12 units lactic dehydrogenase, and 100 μl homogenate fraction. Absorbance changes were measured at 340 nm using a Beckman DU spectrophotometer with temperature controlled for 37° in reaction mixture, except for muscle preparations which were at 27°. When used, ouabain was present at 1 mM concentration. Total ATPase activity was determined in the presence of Na^+, K^+, and Mg^{2+}. Mg^{2+} ATPase activity used same mixture plus ouabain. Na^+-K^+ ATPase activity is total activity minus Mg^{2+} ATPase activity.[6] Homogenate fraction was 13,000 *g* sediment (from tissue homogenized in 0·32 M sucrose, 1 mM EDTA, pH 7·5), centrifugation time 20 min, and prepared according to the procedure of Koch[7] and called B fraction. B fraction is crude fraction containing nerve ending particles and free mitochondria.

† Sp. act. calculated as micromoles P_i milligram^{-1} protein hr^{-1}. Each value represents the average from 3 separate preparations.

of different concentrations of oligomycin on the inhibition of ATPase activities for several tissues from blue gill sunfish. Mg^{2+} ATPase from all tissues was highly sensitive to oligomycin at the lowest concentration tested (0·03 μg per 3 ml reaction mixture).

However, the sensitivity of Na^+-K^+ ATPase to oligomycin varies considerably between the tissues (Table 1). The per cent inhibition of Na^+-K^+ ATPase activity in liver and muscle was determined from very small values because in these tissues Na^+-K^+ ATPase represents only a small portion of the total ATPase activity; nevertheless, there appeared to be significant differences in sensitivity to oligomycin. Also, the Mg^{2+} ATPase activity in the muscle preparation contained a much lower sensitivity to oligomycin than the other tissues (Table 1).

The fish brain preparation was used to study the effects of oligomycin-insensitive combinations (Table 2). Three types of chlorinated hydrocarbon insecticides were used: *p,p'*DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and its analog *p,p'*DDE (biphenyls), aldrin (a naphthalene type cyclodiene), and gamma chlordane (a methanoindene type cyclodiene). The results in Table 2 show a difference in total inhibition of Mg^{2+} ATPase activity between the different insecticides, but of greater significance were the differences in inhibition between the oligomycin-sensitive and oligomycin-insensitive Mg^{2+} ATPase activities.

The mitochondrial (oligomycin-sensitive) Mg^{2+} ATPase was almost totally inhibited by DDT and its analog DDE, while the non-mitochondrial (oligomycin-insensitive) Mg^{2+} ATPase was only about one fourth as sensitive to DDT and DDE as the mitochondrial Mg^{2+} ATPase (Table 2).

TABLE 2. EFFECTS OF OLIGOMYCIN-INSECTICIDE COMBINATION ON Mg^{2+} ATPASE ACTIVITIES FROM FISH BRAIN PREPARATIONS*

Insecticide† (10·4 μM)	Type of Mg^{2+} ATPase	Sp. act. (mean ± S.E.)‡	Per cent inhibition§
None	Total	25·3 ± 0·5	
	Non-mitochondrial‖	14·6 ± 0·4	(42·1)§
	Mitochondrial	10·6	
*p,p'*DDT	Total	11·6 ± 0·2	54·1
	Non-mitochondrial‖	10·6 ± 0·2	26·5
	Mitochondrial	0·8	92·0
*p,p'*DDE	Total	11·8 ± 0·3	51·2
	Non-mitochondrial‖	11·0 ± 0·1	24·6
	Mitochondrial	0·7	92·2
Aldrin	Total	17·5 ± 0·6	30·8
	Non-mitochondrial‖	9·3 ± 0·4	36·4
	Mitochondrial	8·2	22·5
Gamma-chlordane	Total	10·6 ± 0·8	56·0
	Non-mitochondrial‖	8·6 ± 0·2	41·1
	Mitochondrial	2·0	79·1

* Reaction conditions same as that given in Table 1.

† Each insecticide was dissolved in ethanol and 1 μl ethanol added to each reaction mixture; no detectable effect on the enzyme has been found at 5 times this amount.

‡ Standard error (S.E.) determined from 3 separate determinations.

§ Per cent inhibition values represent amount of inhibition caused by insecticide listed in left hand column, except for the top inhibition value in parenthesis which shows the amount of oligomycin inhibition of the total Mg^{2+} ATPase activity.

‖ Oligomycin concentration 0·1 μg/3 ml reaction mixture.

Aldrin was the least effective inhibitor of total Mg^{2+} ATPase activity; however, its greatest effect was on the non-mitochondrial Mg^{2+} ATPase, in contrast to all the other insecticides tested (Table 2).

Chlordane was as effective as DDT as an inhibitor of total Mg^{2+} ATPase activity but the amount of inhibition of mitochondrial Mg^{2+} ATPase was lower, while that of non-mitochondrial Mg^{2+} ATPase was higher than that for DDT (Table 2). Previous research[1,2] demonstrated that chlordane was much more effective than DDT as an inhibitor of the Na^+-K^+ATPase activity in nerve tissue preparations.

The differences in sensitivity of the Mg^{2+} ATPase activities demonstrated in our studies by the use of oligomycin may help to explain distinctive characteristics in the mode of action of these chlorinated hydrocarbon insecticides.

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