Endrin Induced Trace Metal Alterations Following Acute Exposure

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Introduction

The presence of chlorinated pesticides in human tissues throughout the world attests to the almost universal application of and exposure to these materials. One of the most important insecticide groups is the cyclodienes including aldrin, its stero-isomer - - isodrin, its epoxide - - dieldrin, and dieldrin's stero-isomer - - endrin. In general, the cyclodienes are highly toxic to man, animals and insects through their action on the central nervous system. Like other chlorinated hydrocarbons, they are deposited in fatty tissue where they remain until they are metabolized and/or excreted in the milk, urine and/or feces (1). The most consistent observations following repeated exposure of experimental animals have been changes in the liver and kidneys (1). Endrin, one of the most toxic members of this group, has induced symptoms such as abdominal discomfort, vertigo, insomnia and weakness of the legs during mild intoxication, and nausea and diarrhea during acute exposure. Moderate doses have been observed to produce, without warning, a marked loss of appetite, loss of weight and convulsions (1). Due to

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this comparatively high toxicity, the Federal Government permits no endrin residual on crops that are to be consumed by humans or marketable animals.

Even though much is known about the pharmacological action of the cyclodienes (1,2,3) relatively little is known concerning their molecular base of action. Recent studies (4,5) concerning similar disease states (intoxication and/or shock) have indicated that shifts in certain trace metals at the molecular level result in observed clinical phenomena. Such trace metal shifts may not only result from but contribute to the toxic state and as such could provide a basis for the detection and evaluation of intoxication before gross physiological responses are manifested. Thus, an evaluation of trace metal mobilization appears to be essential to a basic knowledge of the toxic reaction to this environmental contaminant.

The purpose of the present investigation was to determine if a mobilization of some of the most biologically significant metals, iron, magnesium, zinc and copper, occurs in those organs, tissues, and body fluids considered most responsive to a single oral dose of endrin.

Procedure

The experimental animals consisted of adult male Holtzman albino rats (300 to 425 g each with an average weight of 403 g) purchased from the Holtzman Company, Houston, Texas. The control group which received no endrin consisted of 10 rats while the exposure group was composed of 12 rats. An endrin solution was pre-

pared by dissolving 5 g in 100 ml of commercial grade peanut oil and was administered with a stomach catheter in a single dose of 25 mg endrin per kg body weight. Sacrifice was effected at the initiation of the third convulsion. Since the lapsed time between administration of the endrin and the occurrence of the third convulsion was rather constant (2 ± 0.5 hours), all the exposed animals were closely grouped at the same degree of intoxication. The animals were sacrificed by first placing each under anesthesia with diethyl ether, then opening the abdominal cavity and exsanguinating the animal by inserting a needle attached to a syringe into the abdominal aorta just above the iliac arch. The syringes, needles, test tubes and capillary tubes were pretreated with heparin.

After the animals had been bled, the liver, right kidney, spleen, brain and heart were removed from the body, blotted dry, placed in tared containers and the wet weight determined. Then the tissues were dried in an oven at 105^{o} C for 16 hours, placed in a dessicator overnight, and the dry weight determined. The moisture weight was derived from the differences between the organ total wet weight and the subsequent dry weight. Next the tissues were ashed at 500^{o} C for 8 hours. The ash weight was determined after dessication overnight. The weight lost during the ashing process was termed volatile weight and was derived by the differences between the dry and ash weight. The ash samples were dissolved by adding 1 ml of 0.0324 N HCl for each mg of ash.

The blood sample was separated into plasma and red blood cells

(RBC) by centrifugation at 15,000 x g for 5 minutes. One ml of packed RBC was withdrawn and diluted with 35 parts of the dilute acid.

All trace metal analyses were accomplished with a Jarrell-Ash Atomic Absorption Spectrophotometer Model 82-362 utilizing a 0.5 meter Ebert mount monochrometer having a grating of 30,000 lines per inch in the ultraviolet range. The hollow cathode tubes were operated at 6.0 milliamperes for zinc, 11.6 for iron, 10.0 for magnesium, and 7.0 for copper. A direct aspiration Hetco burner was utilized using hydrogen and air at the following pressures: (a) ainc, hydrogen 15 psi and air 21 psi; (b) iron, hydrogen 12.5 psi and air 15 psi; (c) copper, hydrogen 12.5 psi and air 15 psi; and (d) magnesium, hydrogen 5.0 psi and air 15 psi. An RCA photomultiplier tube, Model IR 106 was operated at the following voltages and wavelengths: (a) zinc, 450 and 2138 Å; (b) copper, 450 and 3247 Å; (c) iron, 760 and 2483 Å; and (d) magnesium 450 and 2851 Å.

The tissue and blood samples were aspirated directly into the burner of the atomic absorption unit. Standard solutions were prepared over the indicated ranges: (a) zinc, 0.25 to 2.0 ug/ml; (b) copper, 0.25 to 5.0 ug/ml; (c) iron, 1 to 25 ug/ml; and (d) magnesium, 0.1 to 15 ug/ml.

The data were subjected to analysis of variance for unequal replications. The term "significant" as used herein implies statistical significance at the 0.05 level.

Observations and Discussion

TABLE 1

TISSUE WEIGHTS FOR CONTROL AND EXPOSED RATS

Tissues		Control Weights, Grams				Exposure Weights, Grams			
		Wet	Moisture	Volatile	Ash	Wet	Moisture	Volatile	Ash
Liver	$\bar{x}$	10.26456	6.929	3.15	0.19005	13.07622*	8.871*	3.86*	0.34942*
	s^2	1.26126	0.593	0.13	0.00190	2.44518	1.144	1.01	0.01131
Kidney	$\bar{x}$	1.14244	0.786	0.340	0.01600	1.24330*	0.917*	0.309	0.01698
	s^2	0.00588	0.0027	0.01122	0.0000021	0.01156	0.0082	0.00045	0.0000038
Spleen	$\bar{x}$	0.64758	0.477	0.157	0.01290	0.69270	0.525	0.155	0.01304
	s^2	0.00896	0.0061	0.00033	0.0000019	0.01114	0.0061	0.00073	0.0000029
Brain	$\bar{x}$	1.55128	1.174	0.341	0.02549	1.65609*	1.280*	0.349	0.02697
	s^2	0.01468	0.0093	0.00111	0.000016	0.01046	0.0063	0.00036	0.0000063
Heart	$\bar{x}$	1.12381	0.842	0.267	0.01412	1.29144*	0.976*	0.299*	0.01574*
	s^2	0.00777	0.0044	0.00056	0.0000012	0.00879	0.0050	0.00045	0.0000028

*statistically significant at the 0.05 level.

TABLE 2

TOTAL, MEAN CONCENTRATION AND VARIATION OF ZINC AND COPPER IN ASH OF INDICATED TISSUE

Tissues		ZINC				COPPER			
		Control		Exposed		Control		Exposed	
		Total ug	ug/mg	Total ug	ug/mg	Total ug	ug/mg	Total ug	ug/mg
Liver	$\bar{x}$	182.77	0.96	259.26*	0.74	26.77	0.14	10.58*	0.030*
	s^2	494.52	0.022	2488.07	0.12	19.56	0.0010	11.04	0.00002
Kidney	$\bar{x}$	16.31	1.02	28.22*	1.66*	6.23	0.39	7.4	0.44
	s^2	7.4	0.0156	17.30	0.127	5.21	0.0194	5.37	0.0222
Spleen	$\bar{x}$	9.94	0.77	17.52*	1.34*	0.92	0.07	0.62*	0.048*
	s^2	1.91	0.0022	4.88	0.44	0.04	0.00034	0.0036	0.00014
Brain	$\bar{x}$	14.14	0.56	18.84*	0.70	3.51	0.14	1.74*	0.065*
	s^2	6.35	0.008	2.35	0.008	0.49	0.00027	0.09	0.00014
Heart	$\bar{x}$	13.48	0.96	22.00*	1.40*	4.27	0.30	2.33*	0.148*
	s^2	1.57	0.0033	4.53	0.044	0.92	0.0038	0.38	0.0019
RBC	$\bar{x}$		17.4[a]		11.1[a]*		0.40[a]		0.30[a]
	s^2		61.75		6.81		0.0042		0.0332
Plasma	$\bar{x}$		1.27[a]		1.21[a]		0.20[a]		0.49[a]*
	s^2		0.0478		0.2518		0.00049		0.0168

[a]values expressed in ug/ml. *statistically significant at the 0.05 level.

TABLE 3

TOTAL, MEAN CONCENTRATION AND VARIATION OF MAGNESIUM AND IRON IN ASH OF INDICATED TISSUE

Tissues		MAGNESIUM				IRON			
		Control		Exposed		Control		Exposed	
		Total ug	ug/mg	Total ug	ug/mg	Total ug	ug/mg	Total ug	ug/mg
Liver	$\bar{x}$	1335.6	7.0	1662.1	4.8*	392.65	2.07	527.75*	1.51
	s^2	89740.	0.4178	193720.	1.6645	6743.2	0.23	11512.	0.25
Kidney	$\bar{x}$	121.12	7.6	156.80*	9.2*	19.33	1.21	40.40*	2.38*
	s^2	209.63	0.2378	227.61	0.9527	80.80	0.25	115.2	0.45
Spleen	$\bar{x}$	83.54	6.5	121.4*	9.3*	50.16	3.89	86.6*	6.64*
	s^2	416.03	1.4489	120.9	0.8154	266.6	2.18	1187.	8.28
Brain	$\bar{x}$	151.2	5.9	119.4*	4.4*	11.66	0.46	10.38	0.38
	s^2	824.7	0.9222	226.1	1.0327	6.36	0.002	16.10	0.023
Heart	$\bar{x}$	129.1	9.1	169.6*	10.8*	28.08	1.99	48.25*	3.07*
	s^2	384.3	0.3244	51.67	2.1636	10.35	0.047	133.6	0.61
RBC	$\bar{x}$		23.5[a]		17.5[a]*		1049.4[a]		1052.[a]
	s^2		22.5		12.59		259.6		540.2
Plasma	$\bar{x}$		8.3[a]		7.4[a]*		7.43[a]		6.99[a]
	s^2		0.32		0.61		2.70		2.40

[a]values expressed in ug/ml. *statistically significant at the 0.05 level.

Tissue weights from control and exposed animals are shown in Table 1, and organ metal contents (total amounts as well as concentrations) for both exposed and control animals are shown in Tables 2 and 3. For all tables, the means ($\bar{x}$) and varriances (s^2) were calculated using data from 10 control and 12 exposed rats. Asterisks (*) are used to indicate those exposure values that deviate significantly from control observations.

A comparison of the weight data indicates that, with the exception of the spleen, all organs studied experienced increases in size. For the kidney and the brain, these increases were reflected only in the wet and moisture weights. Since these were the only changes observed and since these were at the same direction and magnitude, increases in the intra- and extracellular fluids would account for the increased moisture contents and their accompanying increases in wet weights. For the liver and the heart, all organ size parameters, including the volatile and ash components, increased. This, along with the magnitude of the changes, indicates drastic increases in the sizes of the organs. This is understandable since endrin is known to have marked effects on these two organs (6-10).

On an organ by organ basis of comparison, the heart and the spleen demonstrated the greatest mobilization of metals. In both cases, the total amounts of iron, magnesium and zinc present increased while the total amount of copper decreased. In each case, changes in unit concentrations paralleled those of the total organ contents indicating trace metal shifts over and above that attribut-

able to changes in the organ size parameters.

Of the organs studied, the kidney was intermediate in metal translocation demonstrating significant increases in not only the total organ content but also the unit concentration of iron, magnesium and zinc. It should be pointed out that this was the only organ in which copper did not exhibit a marked decrease.

The principal trace metal shifts experienced by the brain and the liver included decreases in the total and unit concentrations of magnesium and copper in the brain and similar changes in copper in the liver. It is notable that these decreases occurred in opposition to increases in the sizes of both of these organs. The total amounts of zinc present in both organs were observed to increase while the unit concentrations experienced no significant changes, thus indicating that the increases in the zinc levels in the liver and brain occurred in proportion to the increases in organ size. A similar observation was made concerning the iron content of the liver.

On a metal by metal basis of comparison, zinc appeared to be one of the most active elements by exhibiting increases in the total organ content of all five organs studied. In the case of the liver and brain, these increases paralleled increases in organ size; consequently, the unit concentrations in these organs remained unchanged. In the kidney, spleen and heart, the unit concentrations also increased indicating an influx of the metal above that attributable to changes in organ size. The observed decrease in this metal in the red blood cells provides one possible source of

the mobilized zinc.

Copper was observed to change in all organs studied except the kidney. In all cases these changes were in opposition to changes in organ size and were manifested as decreases in the total amount present and were accompanied by corresponding decreases in the unit concentrations. The appearance of increased plasma levels of this metal indicates that copper was mobilized, at least initially, toward the circulatory system.

Magnesium, the most active element studied, increased in the kidney, spleen and heart. In view of the changes in the organ size parameters, the increases in the unit concentrations of this metal indicates extensive migration of magnesium into these organs. The decreases observed in the brain, red blood cells and plasma suggests that magnesium was mobilized at the expense of the circulatory and central nervous systems.

Iron appeared to be the least responsive of the metal studied. It did, however, exhibit changes in the liver, kidney, spleen and heart. In all cases except the liver, these changes were increases in both the total organ content as well as the unit concentration. Considering the organ size parameters, this suggests a comparatively sizable influx of iron into these organs. The source of this mobilized iron could not be attributed to any organ or body fluid studied.

Summary and Conclusions

A single oral dose of endrin (25 mg of endrin per kg of body weight) in rats produced, in two hours, (a) significant increases

in the organ size parameters of the liver, kidney, brain and heart but not the spleen, (b) trace metal shifts typified as increases in the organ levels of iron, magnesium and zinc and decreases in the organ levels of copper, (c) an apparent correlation of the reactions of iron, magnesium and zinc in the kidney, spleen and heart, and (d) indications that the increases in the organ levels of magnesium and zinc occurred at the expense of the circulatory system while the same system received, at least initially, the copper mobilized away from the organs.

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