Trace Metal Alterations Following Sub-Acute Exposure to Endrin

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Introduction

Recent studies (1,2,3) concerning various states of intoxication have indicated that shifts in certain trace metals occur prior to and/or during manifestation of clinical phenomena. Such trace metal shifts may not only result from but also contribute to the toxic state and as such could provide a basis for the detection and evaluation of intoxication, hopefully; before gross physiological responses are obvious or irreversible. It was with this goal in mind that a series of investigations into the role of trace metals in certain disease states was undertaken. The purpose of the present study, the second in a series on endrin intoxication, 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 indicated (4) to be most responsive to sub-acute oral exposure to endrin.

Procedure

The experimental animals consisted of adult male Holtzman albino rats having an average weight of 402 g. The animals were randomized into two groups of 10 rats each, placed in individual metabolism cages and allowed to receive water and Rockland Mouse Breeder diet *ad libitum*. The control group received no endrin while the ex-

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posed animals received, over an 8-day period, a total oral dose of 8 mg of endrin per kg of body weight. This pesticide, which was prepared by dissolving 5 g of endrin in 100 ml of commercial grade peanut oil, was administered, via a stomach catheter, in four doses starting with 3 mg/kg on the first day, 2 mg/kg on the third and fifth days and 1 mg/kg on the eighth day. This group, along with the control group, was sacrificed on the ninth day.

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 heparinized syringe into the abdominal aorta just above the iliac arch. After the animals had been bled, the liver, right kidney, spleen, brain and heart were removed, blotted dry and the wet weight determined. The dry weight was determined after 16 hrs. at 105° C and the ash weight after 8 hrs. at 500° C. The weight loss during drying was termed moisture while the volatile weight was determined as the loss during ashing. The ash residue was dissolved in 1 ml of 0.0324 N HCl per mg of ash. Urine and feces from the control and exposure animals were collected on alternate days and pooled. The air dried feces were ashed and solubilized in the same manner as the tissue samples while the urine was prepared for analysis by dilution with 5 parts of the dilute acid.

The blood samples were separated into plasma and RBC by centrifugation at 15,000 x g for 5 min. One ml of plasma was then withdrawn and diluted with 5 parts of 0.0324 N HCl and 1 ml of packed RBC was withdrawn and diluted with 35 parts of the dilute acid.

Preparation of the tissues, urine, feces and blood fractions in this manner permitted direct aspiration of the samples into a Jarrell-Ash Atomic Absorption Spectrophotometer. Operational parameters for the various metal analyses are described in detail in an earlier publication (3).

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

Observations and Discussion

Tissue weights from the control as well as the exposed animals are shown in Table 1, and the organ metal contents (total amounts as well as concentrations) for both groups are shown in Tables 2 and 3. For all tables, the means ($\overline{x}$) and variances (s^2) were calculated using data from 10 control and 10 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 exhibited minor, if any, changes in size following sub-acute exposure. The fact that the liver and heart demonstrated significant increases in all organ size parameters during acute exposure (3) but were the least responsive during sub-acute exposure indicates that these organs react quickly to the initial intoxication but are able to counteract during sub-lethal exposures. Conversely, the spleen, which was the only organ to remain unchanged in size following acute exposure, demonstrated significant decreases in all organ size parameters examined following sub-acute

TABLE 1

TISSUE WEIGHTS FOR CONTROL AND EXPOSED RATS

Tissues		Control Weights, Grams				Exposed Weights, Grams			
		Wet	Ash	Moisture	Volatile	Wet	Ash	Moisture	Volatile
Liver	$\bar{x}$	10.26456	0.19005	6.929	3.15	9.82125	0.21491	6.647	2.96
	s^2	1.26126	0.00190	0.593	0.13	2.01366	0.001963	0.996	0.16
Kidney	$\bar{x}$	1.11244	0.01600	0.786	0.340	1.11445	0.01416*	0.815	0.286
	s^2	0.00588	0.0000021	0.0027	0.0112	0.01249	0.0000011	0.0079	0.00056
Spleen	$\bar{x}$	0.64758	0.01290	0.477	0.157	0.53951*	0.00935*	0.397*	0.133*
	s^2	0.00896	0.0000019	0.0061	0.00033	0.01607	0.0000051	0.0102	0.00056
Brain	$\bar{x}$	1.55128	0.02549	1.174	0.341	1.63062	0.02471	1.251*	0.352
	s^2	0.01468	0.000016	0.0093	0.00111	0.00843	0.0000034	0.0058	0.00033
Heart	$\bar{x}$	1.12381	0.01412	0.842	0.267	1.09027	0.01202	0.811	0.262*
	s^2	0.00777	0.0000012	0.0044	0.00056	0.01290	0.0000019	0.0082	0.00067

*indicates statistical significance at the 0.05 level.

TABLE 2

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

		ZINC				COPPER			
		Control		Exposed		Control		Exposed	
Tissues		Total ug	ug/mg	Total ug	ug/mg	Total ug	ug/mg	Total ug	ug/mg
Liver	$\bar{x}$	182.77	0.96	225.30*	1.05	26.77	0.14	24.66	0.11*
	s^2	494.52	0.022	2045.47	0.051	19.56	0.0010	24.24	0.0022
Kidney	$\bar{x}$	16.31	1.02	17.76	1.25*	6.23	0.39	9.09*	0.64*
	s^2	7.4	0.0156	6.9	0.043	5.21	0.0194	4.76	0.0133
Spleen	$\bar{x}$	9.94	0.77	8.60	0.92	0.92	0.07	0.59*	0.06
	s^2	1.91	0.0022	11.42	0.109	0.04	0.00034	0.0188	0.00062
Brain	$\bar{x}$	14.14	0.56	13.63	0.55	3.51	0.14	3.57	0.14
	s^2	6.35	0.008	4.15	0.005	0.49	0.00027	0.23	0.00074
Heart	$\bar{x}$	13.48	0.96	11.72	0.96	4.27	0.30	4.03	0.34
	s^2	1.57	0.0033	3.46	0.0075	0.92	0.0038	0.68	0.0058
RBC	$\bar{x}$		17.4[a]		10.3*[a]		0.40[a]		0.27*[a]
	s^2		61.75		8.64		0.0042		0.0029
Plasma	$\bar{x}$		1.27[a]		0.63*[a]		0.20[a]		0.63*[a]
	s^2		0.0478		0.0133		0.00049		0.0120

[a]expressed in ug/ml. *indicates statistical significance at the 0.05 level.

TABLE 3

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

Tissues		MAGNESIUM				IRON			
		Control		Exposed		Control		Exposed	
		Total ug	ug/mg	Total ug	ug/mg	Total ug	ug/mg	Total ug	ug/mg
Liver	$\bar{x}$	1335.63	7.0	1853.50*	8.62*	392.65	2.07	533.31*	2.48
	s^2	89740	0.4178	106245	0.4122	6743.2	0.23	11283	0.82
Kidney	$\bar{x}$	121.12	7.6	115.68	8.17	19.33	1.21	26.16	1.85*
	s^2	209.63	0.2378	160.79	0.1400	80.08	0.25	30.55	0.15
Spleen	$\bar{x}$	83.54	6.5	64.11*	6.86	50.16	3.89	53.14	5.68
	s^2	416.03	1.4489	283.44	0.2578	266.6	2.18	355.40	1.71
Brain	$\bar{x}$	151.2	5.9	165.7	6.71	11.66	0.46	10.06	0.41
	s^2	824.70	0.9222	221.80	0.1889	6.36	0.0022	1.68	0.0033
Heart	$\bar{x}$	129.1	9.1	114.5*	9.53	28.08	1.99	33.63	2.80*
	s^2	384.27	0.3244	142.81	0.1222	10.35	0.047	37.35	0.16
RBC	$\bar{x}$		23.5[a]		21.9[a]		1049.4[a]		1078.0[a]
	s^2		22.5		19.62		259.6		68.2
Plasma	$\bar{x}$		8.3[a]		7.8[a]		7.43[a]		5.82*[a]
	s^2		0.32		0.16		2.70		2.44

[a]expressed in ug/ml. *indicates statistical significance at the 0.05 level.

exposure, thus indicating the effects of endrin on the circulatory system to be more prolonged. These observations are in general agreement with the physiological mechanisms reported in a prior study (4).

On an organ by organ basis of comparison, the liver indicated significant increases in the total and unit concentration of magnesium. Since the organ size parameters remained unchanged, the mobilization of this metal into the liver cannot be attributed to physical changes within the organ. It should be pointed out that during acute intoxication (3), the liver not only demonstrated increases in all organ size parameters but also mobilization of all metals studied with the exception of magnesium.

The most meaningful changes occurring in the kidney were observed as changes in the total organ content as well as the unit concentration of copper. Since the organ size parameters remained essentially unchanged, a marked mobilization of this metal is indicated. It is noteworthy that this was the only metal studied that did not show significant changes in the kidney during acute treatment. The increase in the unit concentration but not total organ contents of iron and zinc are understandable, if these metals were retained in the kidney in spite of a loss of non-volatile components as indicated by the loss in ash weight.

The spleen, in spite of significant decreases in all organ size parameters, demonstrated changes (decreases) in only the total organ contents of magnesium and copper. The lack of change in the unit concentrations of these two metals indicates that the losses in total organ content were in proportion to the loss in the organ size.

Considering the physiological function of the spleen, this is understandable. The reaction of copper during prolonged exposure was consistent with the observation made during acute treatment, indicating a release of this metal by the spleen. However, the decrease in magnesium during sub-acute exposure was in opposition to the increase noted during the acute study, thus suggesting that this metal is stored in the spleen during the initial intoxication for release later. The lack of change in either the totals or concentrations of iron and zinc indicates a maintenance of these metals regardless of the drastic decrease in organ size. This could be attributed to the sizable increase in these two metals known to occur during the initial stages of intoxication (3).

The brain demonstrated no meaningful changes in organ size or trace metal translocations during sub-acute exposure, indicating those shifts observed in the brain following acute exposure are readily overcome and/or are very markedly reduced in their severity.

With the exception of a decrease in the total organ content of magnesium and an increase in the unit concentration of iron, the heart manifested no significant trace metal changes during sub-acute treatment. In view of this and considering the high degree of reactivity demonstrated by the heart during acute treatment, the same conclusion drawn for the brain is indicated. The changes noted for iron and magnesium are understandable in view of the significant decrease in the ash weight of the heart. Apparently, magnesium decreased in proportion to the decrease in the non-volatile components, thus affecting the total organ content but not the unit concentration which is based on the ash content. Likewise, the decrease in non-volatile components would effect an increase in the concentration of iron without affecting the total organ content if the iron was retained rather than released by the heart during sub-acute exposure.

The RBC demonstrated significant decreases in the concentration of both zinc and copper but no changes in the iron and magnesium levels. In comparison with the observations made during acute intoxication, the RBC appears to be a source of zinc under both acute and prolonged exposure; a source of copper during prolonged exposure only; a source of magnesium only during the acute stages of intoxication; and stable with respect to iron during both modes of exposure.

The plasma indicated decreases in iron and zinc but an increase in the concentration of copper. Again by comparison with the observations made during acute exposure, the plasma is seen to be a principal target for the mobilized copper while the zinc loss noted during acute treatment was apparently nullified under the conditions of prolonged exposure. Conversely, a loss of iron and zinc, which was not noted during acute exposure, was significant during sub-acute conditions.

Analysis of the metabolic products collected during the investigation indicated that the average daily fecal excretion for the control animals was 1.5 g on a dry weight basis and 0.0202 g on an ash basis while the exposed animals excreted 0.55 and 0.0055 g respectively. The poor absorption rate common for most metals along with the changes in eating habits and moderate dirrahea developed by the exposed animals, complicates interpretation of the fecal trace metal analyses; consequently, quantitative values determined on pooled samples are not reported. However, two trends were obvious; the first was a marked increase in both the total as well as the unit concentrations of magnesium in the feces of the exposed animals and the second was an equally marked decrease in both the total and the concentration of zinc in the fecal excretion from the exposed group. These observations indicate (irrespective of changes in dietary and excretion patterns) a tendency on the part of the intoxicated animal to decrease the absorption and/or body stores of magnesium and at the same time increase the absorption and/or body stores of zinc.

The urinary excretion volumes were observed to average 15.5 ml/day for the control animals and 9.6 ml/day for the exposed group. The trace metal outputs, which are compared in Table 4, exhibited

TABLE 4

URINARY EXCRETION OF SELECTED METALS BY THE ADULT RAT

	Fe		Mg		Zn		Cu	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
Total, ug/day	39	49	1.6	20	23	0.98	2.2	1.3
Conc., ug/ml	2.5	5.1	0.10	2.1	1.5	0.10	0.14	0.14

a somewhat stable excretion of iron and copper. This observation along with a similar observation on the fecal excretions indicate, that while marked shifts of iron and copper may occur from organ to organ within the intoxicated animal, very little, if any, changes in the excretions of these two metals occurred during sub-lethal exposure. On the other hand, magnesium demonstrated a sharp rise in both the total and concentration excreted in the urine, and zinc underwent an even sharper drop in the total as well as rate of excretion. These observations are in full agreement with the corresponding fecal observations and confirm a tendency on the part of the endrin intoxicated animal to not only experience tissue changes in these two metals but also to undergo marked alterations in their absorption and excretion characteristics.

Summary and Conclusions

Sub-acute oral exposure of rats to multiple doses of endrin totaling 8 mg per kg of body weight, produce, over an 8-day period, the following observations: (a). Of the organs studied only the spleen demonstrated meaningful changes in the organ size parameters observed and these were manifested as decreases. By comparison, the results of the acute study reported earlier (3) indicate the spleen to be the only organ studied that did not experience a significant change in size. (b). From the standpoint of trace metal translocation, the number of shifts observed after 8 days of sub-lethal exposure was small compared to those known to occur during the initial intoxication, thus indicating a tendency on the part of most organs to recover. The noteable exceptions included an increase in both the total as well as the concentration of magnesium in the liver and an increase in not only the total but also the concentration of copper in the kidney. It is noteworthy that during the initial stages of intoxication, the magnesium in the liver and the copper in the kidney were the only situations which remained essentially unaltered. It is also noteworthy that the spleen, under acute exposure demonstrated significant changes in all metals studied but no changes in organ size; however, under prolonged exposure, the spleen decreased drastically in size but the number of metal shifts were comparatively small. In brief, endrin intoxication produced, in the initial stages, sharp changes in the size of most organs observed and significant changes in all metals studied. Such alterations were followed by an apparent recovery attempt in which normal size and metal contents were approached. Following this, the delayed reactions occurred and were exemplified by previously unaffected levels of magnesium in the liver and copper in the kidney, and by a sharp decrease in spleen size. (c). The reactions which seem to offer the most promise for the detection of intoxication involve the metabolic products - - urine and feces - - and were manifested as significant increases in the excretion of magnesium along with a significant increase in the retention (decreased excretion) of zinc.

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