Effects of Aflatoxin B_1 on Distribution of Fe, Cu, Zn, and Mn in Rat Tissues

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Stress-related acute and chronic infectious diseases (PEKAREK et al. 1969,), endotoxemia (KAMPSCHMIDT and UPCHURCH 1962), and cadmium toxicosis (DOYLE and PFANDER 1975) lead to a redistribution of essential trace metals throughout the body. Little is known concerning the pathophysiological mechanisms involved in the redistribution. However, we do know that the changes are rapid host responses to the stresses and that the end result may vary with types of stress.

Because aflatoxin B_1 is another known stressor to animals, is a potent hepatotoxin and carcinogen (GOLDBLATT 1969), and interacts with infectious diseases (SILLER and OSTLER 1961), its role in trace element metabolism in animals is of interest. Currently, little is known concerning the interaction of aflatoxins with essential trace elements. Therefore, we decided to investigate the effects of injected aflatoxin B_1 on the Fe, Cu, Zn and Mn distribution in rat tissues.

MATERIALS AND METHODS

Two experiments were conducted. In the first experiment, 24 Sprague-Dawley male rats, approximately 30 days old and weighing 130 g, were allotted at random into four groups of six animals. Group 1 was the control and groups 2, 3, and 4 were injected intraperitoneally with 5 ml of dimethyl sulfoxide (DMSO)/kg of body weight, 5 mg of AFB_1 in DMSO/kg of body weight, or 7 mg of AFB_1 in DMSO/kg of body weight, respectively. All animals were killed after 76 hours. Samples of liver, kidney, and spleen were collected from all animals, freeze dried and digested by the nitric acid-perchloric acid method (A.O.A.C. 1970). Fe, Cu, Zn, and Mn concentrations of liver, kidney, and spleen were determined by atomic absorption.

The second experiment compared the effects of injecting 10 mg of AFB_1 in DMSO/kg of body weight versus injecting DMSO alone (5 ml/kg of body weight) in 100 g

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Sprague-Dawley male rats. All animals were killed after 54 hours, and liver, kidney, spleen, and pancreas collected. Tissue samples were bulked within treatments, digested by the nitric acid-perchloric acid method and Fe, Cu, Zn, and Mn determined for all tissues by atomic absorption.

Analysis of variance and the LSD (STEEL and TORRIE 1960) were used to ascertain differences among treatments in experiment 1. Standard errors of the means were calculated for all data. The weights of all animals in both experiments were determined both pre- and post-experiment. Purina Lab Chow** was fed ad libitum throughout the experiments.

RESULTS AND DISCUSSION

In experiment 1 there were marked differences in body weight gain between groups. The control and DMSO groups gained 13 and 15 g/rat, respectively, over the experimental period and the 5 mg of AFB_1/kg and the 7 mg of AFB_1/kg groups lost 1 and 8 g/rat, respectively. In experiment 2, the control rats gained 8 g/rat and the 10 mg of AFB_1/kg group lost 20 g/rat. No animals died in either experiment.

The above data indicate that body weight loss during aflatoxicosis is dose related and reinforce the report by WYATT et al. (1975) that aflatoxin reduced body fat and the earlier reports by TUNG et al. (1972) and DONALDSON et al. (1972) that alterations in lipid metabolism may be a primary effect of aflatoxicosis. However, the body weight loss may be in part or in toto a function of decreased feed intake. The 20 g/rat loss in body weight by the 10 mg of AFB_1/kg of body weight group over a period of 54 hours is not likely to be due solely to decreased or even zero feed intake. Hence, we can plausibly suggest that body weight loss in animals during aflatoxicosis may be due in part to reduced body fat and in part to reduced feed intake.

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Iron. In experiment 1 the Fe concentration in the liver was significantly greater ($p<0.05$) in group 4 (7 mg AFB_1/kg of body weight) than in group 1 (control) Table 1). In addition, the Fe concentration in the kidneys was markedly greater in group 4 than in group 1. Splenic Fe concentration was significantly greater ($p<0.05$) in group 2 and group 4 than in group 3 (Table 1). The Fe data obtained in experiment 2 (Table 5) support the data of experiment 1 and in addition show that the pancreatic Fe is also increased greatly as a result of alfatoxicosis.

The significance of this marked rise in hepatic Fe is unknown, but some observations are appropriate. We know that Fe accumulates in the liver as a result of infection and endotoxemia and that Fe accumulates in conjunction with the decline in serum Fe. Serum Fe may similarly decline during aflatoxicosis and although this possible decline might be a defense mechanism, the accumulation of Fe in the liver and other organs may possibly be a contributing factor toward the known lethality of aflatoxicosis. In this regard, Newberne and Conner (1974) reported that 1 µg/g of dietary selenium protected against the acute toxicity of AFB_1. Possibly, the selenium in some unknown way, presents the influx of Fe into the liver in large quantities or facilitates the rapid elimination of excess Fe from the liver, thereby preventing any possible lethality due to the Fe.

TABLE 1

The Effects of Aflatoxin B_1 on the Distribution of Fe* in Rat Tissues

		Liver	Kidney	Spleen
		µg/g dry tissue		
1	Control	389.6±123.1	316.2±87.8	617.7± 52.3
2	DMSO	341.6±111.7	376.3±83.9	697.8± 74.5
3	5 mg AFB_1/kg BW	389.2± 87.0	335.5±80.0	505.4± 76.1
4	7 mg AFB_1/kg BW	587.6±106.6	401.1±98.8	662.9±175.1
	LSD (0.05)	131.16	105.9	127.3
	LSD (0.10)	108.9	87.6	105.3

*Mean ± SE

Copper. In experiment 1, significant differences were found in liver Cu ($p<0.05$), kidney Cu ($p<0.10$) and splenic Cu ($p<0.05$) between groups 1 and 4 (Table 2). However, in experiment 2 (Table 5) no marked differences were observed in kidney Cu between treated and control groups, but levels of Cu were increased in the liver, spleen, and pancreas of the treated rats when compared with levels in the controls. This finding supports the data obtained in experiment 1.

The increased liver Cu concentrations observed in this study are consistent with our present knowledge of Cu metabolism during periods of acute stress (BEISEL and PEKAREK 1972). Possibly, aflatoxicosis acts like infection and increases serum Cu and synthesis of ceruloplasmin by the liver. As the kidney Cu results of experiment 1 were not repeated in experiment 2, we doubt whether aflatoxicosis causes any marked change in kidney Cu metabolism. The splenic Cu results appear to be due to the action of the DMSO rather than aflatoxicosis.

TABLE 2

The Effects of Aflatoxin B_1 on the Distribution of Cu* in Rat Tissues

Group		Liver	Kidney	Spleen
			µg/g dry tissue	
1	Control	16.02±1.19	21.98±3.33	6.32±0.35
2	DMSO	16.27±1.55	21.80±4.04	7.63±1.02
3	5 mg AFB_1/kg BW	19.02±1.23	20.10±1.16	7.17±0.95
4	7 mg AFB_1/kg BW	22.15±8.00	26.82±6.40	7.77±0.60
	LSD (0.05)	5.02	5.02	0.94
	LSD (0.10)	4.15	4.15	0.78

*Mean ± SE

Zinc. In general, there were no significant differences in Zn concentrations of the liver, kidney or spleen due to aflatoxicosis (Table 3). These results are in

marked contrast to the increased concentration of Zn found in the livers of rats as a result of other stresses (PEKAREK et al. 1972; DOYLE and PFANDER 1975).

TABLE 3

The Effects of Aflatoxin B_1 on the Distribution of Zn* in Rat Tissues

Group		Liver	Kidney	Spleen
			µg/g dry tissue	
1	Control	102.5± 2.2	96.2±12.7	90.5± 3.8
2	DMSO	113.3±20.5	84.8± 9.3	107.0±12.3
3	5 mg AFB_1/kg BW	105.5± 9.8	83.5± 5.8	81.3± 9.4
4	7 mg AFB_1/kg BW	97.8± 8.0	94.8±11.9	81.3±17.6
	LSD (0.05)	14.5	12.4	15.7
	LSD (0.10)	12.0	10.2	13.0

*Mean ± SE

Manganese. In experiment 1, there were significant increases in the Mn concentration of liver, kidney, and spleen of the treated rats compared to the control rats (Table 4). These results were supported by the data obtained in experiment 2 (Table 5). We do not know why Mn increases in body tissues as a result of acute aflatoxicosis. However, we do know that stimulation of the adrenal cortices of mice with adrenocorticotropic hormone (ACTH) results in a Mn shift in the body from the liver to the carcass (HUGHES and COTZIAS 1961). Acute aflatoxicosis may reduce glucocorticoid hormone activity and thereby cause a shift of Mn from the carcass to such tissues as liver, kidney and spleen.

Because no reports on the effects of aflatoxicosis on the distribution of Fe, Cu, Zn, and Mn in rat tissues have been reported previously, no comparisons can be made. However, the preliminary results reported in this paper indicate that the effect is a complex problem involving not only a redistribution of the metals but possibly some hormonal action as well. It remains to be determined whether the redistribution of the metals, especially the massive influx of Fe into the liver, is the actual toxic factor in aflatoxicosis that leads to sickness and death.

TABLE 5

Effects of 10 mg AFB_1/kg BW on Fe, Cu, Zn, and Mn Distribution in Rat Tissues

	Fe		Cu		Zn		Mn	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
	μg/g dry tissue							
Liver	302.5	470.0	17.25	23.25	116.3	110.0	9.75	11.25
Kidney	193.8	290.5	20.28	21.75	108.0	96.0	3.48	4.40
Spleen	513.7	650.0	5.98	6.67	116.3	127.7	1.37	2.57
Pancreas	76.9	150.0	5.54	6.70	94.6	113.0	6.69	9.30

TABLE 4

The Effects of Aflatoxin B_1 on the Distribution of Mn* in Rat Tissues

Group		Liver	Kidney	Spleen
			µg/g dry tissue	
1	Control	8.50±0.74	3.06±0.67	1.12±0.21
2	DMSO	9.04±0.83	3.61±1.06	1.42±0.58
3	5 mg AFB_1/kg BW	8.71±0.89	3.12±0.54	1.13±0.18
4	7 mg AFB_1/kg BW	9.88±2.18	4.19±1.04	1.52±0.18
	LSD (0.05)	1.56	1.00	0.38
	LSD (0.10)	1.29	0.83	0.32

*Mean ± SE

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