CHANGES IN BLOOD PARAMETERS IN BREEDER TOMS FOLLOWING SUBCUTANEOUS INJECTION WITH CADMIUM

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ABSTRACT

Alterations in blood biochemical values of yearling breeder toms following parenteral administration of cadmium (Cd) were evaluated. Each treated member of a pair (total 24 pairs) of toms received a single dose of 4.5mg of Cd per kg. body weight (0.04-mole). The birds were sacrificed at 0,6,24 and 192 hours post-treatment. Highly significant ($P < 0.0001$) increase in serum Cd concentration was observed in the Cd-injected toms. Reduction in serum Cd ($P < 0.05$) at the successive post-necropsy periods indicated systmematic clearance of the metal from the fluid system. Nearly 50 percent of the original loading was eliminated by 24 h post-treatment. Serum glutamic pyruvic transaminase (SGPT) was similarly increased ($P < 0.05$) in the Cd-treated birds and was presumed to have originated mainly from damaged skeletal muscle tissues. The cadmium treatment did not affect ($P > 0.05$) serum testosterone level, total protein, total haemoglobin, lactate dehydrogenase, packed cell volume or serum glutamic oxaloacetic transaminase. It was concluded from these observations that the Cd treatment did not seem to have interferred seriously with renal or hepatic functions, iron, copper and zinc metabolism or caused significant damage to myocardial tissue.

Key words: Biochemical values, blood parameters, toms, cadmium

INTRODUCTION

Cadmiun (Cd) is a relatively rare metal which does not occur naturally in the pure state. However, increased demand for commercial purpose has led to its heightened in recent years and this has contributed immensely to its relative abundance in the biosphere. The high toxicological potential of the metal to plant and animal life has been well established from several experiemtal studies. (Jacob *et. al*, 1969; Kobayashi *et al.*, 1970; Friberg *et al.*, 1971; Stowe *et al.*, 1972; Richardson *et al.*, 1974; Singhal *et al.*, 1976). Among the major target organs in animals are the liver, kindney and testis. Evaluation of the hematological and biochemical constituents of the blood have been used to produce useful evidence of tissue damage in avain species (Ibrahim *et al.*, 1980; Driver, 1981; Rivertz and Bogin, 1982 and mandal and Banerjee, 1982). The variety of physiological and histological changes observed in these target organs are due to the toxic effects of the metal.

Little information exist on the effect of exposure of turkey to this metal. The purpose of the experiment reported in this paper was to evaluate the changes in some hematological and blood biochemical parameters of breeder toms following a single subcutaneous (SC) Cd injection.

MATERIALS AND METHODS

Animals, Location and Management

An initial population of 72 Diamond hybrid large white breeder toms were used in the study. The birds were raised in floor pens at the United States Department of Agriculture, (USDA) Avian Physiology Laboratory facility in Beltsville, Maryland U.S.A. They were brooded for 6 weeks and then transferred to a grow-out building at 10 weeks of age following blood test. Corn-soyabean diets (composition described by Cecil, 1984) containing 30, 26, 19 and 17% CP were fed *ad libitum* at 0-4, 4-8, 8-12 and 12-17 weeks of age respectively. A corn diet containing 8% protein was fed from 17 weeks till the end of the experiment.

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Lighting was provided for 24 hours during the first 2 days and then stepped down systemmatically by 2 h daily till a level of 14 h/day was attained at 7 days. This regimen was maintained till 10 weeks of age when 12h (of 54 1x) incandescent light was introduced up till 26th week from which 14h (of 6.5 1 x) of lighting was provided till the birds were sacrificed.

Experimental Design

A total of 48 toms were finally selected and paired (24 pairs). A 2x4 factorial experimental design in a randomized complete block was used with Cd (at 0 and 4.5 mg/kg body weight) applied respectively to the members of each pair, as one factor and post- treatment necropsy periods (of 0,6,24 and 192 h) as the second factor. The treatment combinations were randomized within each block and replicated six times.

Cadmium Injection.

A total of 9.128gr. of cadmium chloride ($CdC1_2.2.5.\ H_20$) (Fisher, Pittsburg, PA) was dissolved completely by continuous stirring for one hour using an automatic stirrer, in 50 ml distilled deionized water and administered in a single dose of 0.04 m-mole corresponding to 4.5mg/kg body weight, to each Cd-treated tom. To achieve this dosage level of Cd, calculations were made on the basis of the molecular content of Cd in $CdC1_2$. Aliquots of 0.05ml of the solution per kg body weight were injected subcutaneously into the interscapular region of the dorsal presentation of the toms. Control (Omg/kg body weight) birds were sham injected with equivalent volumes of deionized water in proportion to their respective body weights. At the post-treatement periods of 0,6,24 and 192 h, the birds were randomly selected for necropsy. Each tom was weighed prior to sacrifice. The birds were euthanized using an overdose of sodium pentobarbital (Somlethal; A.J. Buck and Son Inc. Cockeyville, MD). Necropsy was generally scheduled at approximately the same time for each period of tom sacrifice so as to minimize variability due to diurnal rhythm.

Hematologic and Blood Biochemical Evaluations

Prior to euthanization of the toms, blood samples were obtained from each bird by brachial venipuncture. About 5 ml of blood was drawn into sodium heparinized (Becton-Dickenson, Rutherford N.J) vacutainer tubes. Additional 10 ml was collected into Corvac (Corning Glass Works) tubes without addtivies and allowed to clot over a period of 45 minutes at room remperature. Serum was subsequently harvested by decanting into clean tube and centrifuged at 4800 RCF for 1 $^1/_2$ hr at 5-10°C. Hemolyzed specimens were discarded. The serum and whole blood samples were stored in a refrigerator for subsequent biochemical assays. Blood parrameters measured in the samples included total protein (TP), total hemoglobin (HB), Serum glutamic pyruvic trasnaminase (SGPT), serum glutamic oxaloacetic trasnsaminase (SGOT), Lactate Dehydrogenase (LDH), serum cadmium concentration (SCC), serum testosterone (ST) and packed cell volume (PCV). The whole blood or serum samples were analysed within 24 h of collection. Duplicate samples were determined and the resulting data averaged.

Commercial reagent kits for evaluating LDH, SGOT, SGPT, Hb and TP were used (Sigma Chemical Company, St. Louis, Missouri). The spectrophotometric procedures outlined in Sigma Diagnostic (technical bulletin) Nos. 500, 505, 525, and 540 based on the methods of Cabaud and Wroblewski (1958) Reitman and Frankel (1957) Drabkin and Austin 1935) and Gornall *et. al.*, (1949) respectively were followed. Serum Cd concentrations were determined by the method described by DuVal and Grubb (1986) using a Perkin_Elmer 4000 atomic absorption spectrophotometer, equipped with an HGA-400 furnace and programmer, an electrodeless discharge lamp and argon purge gas. Packed cell volume was measured by the microhaematocrit procedure with haeparinized capillary tubes, model MB centrifuge and on International microcapillary tube reader as described by Mukkur and Bradley (1969).

Statistical Analysis

Serum and Cd concentrations were log transformed prior to statistical anylysis to ensure homogeneous varinaces among the groups. Effects of the variables due to the Cd treatments, time of necropsy or interaction between these variables were analysed by the General Linear Model Procedure type II SS (SAS Institute Inc., 1985). Significant mean values between treatments were compared by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Values obtained for the evaluated aspects of the blood biochemical and haematological profiles in the test birds at the various post-exposure necropsy periods are summarized in Table 1. Highly significant increase in SCC ($P<0.001$) and significant elevation in SGPT ($P<0.05$) attributable to the Dc treatment were observed. Mean SCC value of 288.05 ppm in the Cd-treated toms amounts to about 6 times the value in the control. A significant reduction ($P<0.05$) in SCC was observed at the successive post-necropsy time periods, indicatng a continuous and systemmatic clearance of Cd from the blood serum. It is evident from these data that at 24 h, nearly 50% of the original Cd loading had been eliminated. Nath *et al.* (1984) reported that following Cd injection in experimental animals, the metal occurrence in blood resides predominantly in plasma, but clears up during the subsequent 24 h only to accummulate in blood cells. Earlier, Sell, (1975) observed that 75% of 109 Cd detected in the blood of hens *(Gallus domesticus)* was found in blood plasma and that no radioactivity was noticed in blood at a later sampling time beyond 48 h confirming the observation of Duval and Grubb (1986). These later workers reported a Cd concentration of less than 10% of the value in whole blood in rabbits that received high (1.5 mg/d) and chronic Cd dose over a 28 days period by means of a subcutaneous implanted pump. The present finding agreed with their report. Considering the high Cd clearance rate observed, it is obvious that normal serum Cd level in the bird was attained between 24 and 192 h. post-injection. Similar events have been reported in rats (Fribergy, 1952) and in sheep (Doyle *et al.* 1974). The observed serum clearance phenomenon may be related to the general belief that Cd in plasma is more readily exchangeable between different compartments than erythrocyte Cd (Nordberg *et al.* 1971). In plasma, Cd is normally bound to a variety of protein fractions, including plasma albumin, emoglobin and other high or low molecular weight proteins (Kloke et al. 1983).

Effects of single subcutaneous Cadmium injection on serum biochemistry of breeder toms sacrified at four post injection periods (means +SD) confirming the observations of DuVal and Grubb (1986).

The marketed elevation of SGPT level (16.69% increase over the contrrol) as observed in the Cd-injected toms in the present study, accords with the report of Cain *et al.* (1983) in a feeding experiment with young mallard ducks. Significantly higher SGPT concentrations were observed at 8 weeks of age following acute Cd exposure. The use of plasma or serum enzyme activities as indicators of tissue damage is a common technique in differential clinical diagnoses (Cornelius, 1970) and has been extensively employed in avian species (Duncan and Prasse, 1977; Ibrahim *et al.*, 1980; Holland *et al.*, 1980 and Mandal and Benerjee, 1982). However, in the present study, the specific site of tissue damage or the origin of the surging serum enzyme (SGPT) cannot be precisely ascertained. Serum GPT level was suggested as a specific and sensitive index of hepatic necrosis in waterfowls by Fowler (1970) and Szaro *et al.* (1981). Franson *et al.* (1985), however, cautioned against the use of enzyme activities in one species to establish the norms of another species beccause of the wide interspecific variation. Thus, the observed increase in SGPT level in the Cd-treated toms in this study cannot be interpreted as an indicator of liver damage. More so as Bogin *et al.* (1976) contended that histologic impairment of the liver in the turkey may not

TABLE 1: EFFECTS OF SINGLE SUBCUTANEOUS CADMIUM INJECTION ON SERUM BIOCHEMISTRY AND HEMATOLOGY OF BREEDER TOMS SACRIFICED AT FOUR POST INJECTION [illegible] (MEAN±SD)

	Necropsy			time	intervals	after	cadmium	injection	
Treatment	N	0hr Mean	N	6hr mean	N	24hr Mean	N	192hr Mean	PR F
				Serum Cadmium Concentration (ppm)					
1	5	45.60(15.30)	6	50.50(20.77)	6	56.17(17.50)	4	33.40(9.40)	
2	6	488.00(492.90)	6	387.17(318.94)	5	221.83(154.11)	4	53.20(16.60)	0.0001**
	11	266.80a	12	218.84b	11	138.99c	[illegible]	44.30d	0.0383
				Serum glutamic-pyruvic transaminase ([illegible] Unit/ml)					
1	5	18.98(2.32)	6	17.65(3.18)	6	17.08(12.75)	[illegible]	17.97(2.16)	
	6	18.41(3.15)	6	22.95(7.73)	5	23.46(10.66)	[illegible]	18.82(1.26)	.0368*
	11	18.70	12	20.30	11	20.27	[illegible]	18.40	.5332NS
				Serum Glutamic-exaloacetic trasaminase (SF [illegible])					
1	5	565.10(106.59)	6	698.0(119.59)	6	774.08(209.53)	[illegible]	647.00(131.04)	
2	6	709.16(120.06)	6	824.17(348.26)	5	849.80(353.84)	[illegible]	606.25(156.27)	.2748NS
	11	637.13	12	761.09	11	811.94	[illegible]	626.62]	.1710NS
				Lactate dehydrogenase (B-B Units/[illegible])					
1	5	584.06(247.17)	6	798.99(81.88)	6	644.91(220.84)	[illegible]	580.29(148.26)	
2	6	543.24(151.18)	6	1402.72(1324.78)	5	1324.39(1267.3[illegible]	[illegible]	397.11(244.89)	0.2270NS
	11	563.65	12	1199.86	11	984.65	[illegible]	488.70	0.1329NS
1	5	3.49(0.42)	6	3.64(0.31)	6	3.84(0.37)	[illegible]	3.77(0.34)	
2	6	3.59(0.59)	6	3.94(0.56)	5	3.37(0.60)	[illegible]	4.52(0.33)	0.269NS
	11	3.54b	12	3.79ab	11	3.62ab	8	4.15a	0.0446*
				Serum testosterone (ppm) ng/ml)					
1	5	0.29(0.17)	6	0.79(1.12)	6	1.25(1.12)	4	0.92(0.59)	
2	6	0.46(0.24)	6	0.31(0.42)	5	1.00(0.97)	4	0.70(0.31)	0.4050NS
	11	0.38	12	0.55	11	1.13	8	0.81	0.1222NS

1 = Control 2 = Cadmium treatment *Significant (P<0.05) ** Highly significant (P<0.0001)

NS = Not significant

a,b,c,d. Means in the same row bearing similar superscripts are not significantly differnt (P>0.05)

				Packed Cell Volume (Microhematocrit) (%)					
1	5	37.71(2.92)	6	38.78(1.55)	6	38.71(3.40)	4	40.01(2.80)	
2	6	41.60(5.84)	6	41.46(3.93)	5	41.55(7.80)	4	39.56(3.24)	0.108NS
	11	39.,66	12	40.12	11	40.13	8	39.79	0.9897NS
				Total Hemoglobin (g/dl)					
1	5	12.25(0.65)	6	12.68(1.06)	6	12.56(0.9[illegible]	[illegible]	13.35(1.10)	
2	6	13.86(2.23)	6	13.60(1.98)	5	12.80(2.4[illegible]	[illegible]	12.94(1.64)	0.2578NS
	11	13.06	12	13.14	11	12.68	[illegible]	13.15	0.9020NS

1 = Control 2 = Cad,i, treatment NS = Not significant.

elicit any significant change in SGPT because of the relatively low level in this organ. The workers further reported that SGPT activities was highest in the dark skeletal muscle. Based on this position, the elevated SGPT in the present experiment could be presumed to have originated mostly from the skeletal muscle which probably suffered increased cell permeability and muscular necrosis due to the parenteral Cd treatment.

No significant differences ($P > 0.05$) were observed in the values of LDH and SGOT in this study. This is in agreement with Cain *et al.*, (1983) and DuVal and Grubb (1986) who used mallard ducks and rabbits repectively, but contradicts Stowe *et al.* (1972) and Cousins *et al.* (1973) who reported elevated SGOT in rabbit and swine (respectively) exhibiting Cd toxicity. In the turkey, GOT and LDH have been detected in several tissues including, the liver, lungs, spleen, testis, skeletal, breast and cardiac muscles (Bogin *et al.* 1976). The highest concentration of both enzymes were observed in the myocardium. It is therefore possible that the cardiac muscle of the Cd-treated birds in the present study was not affected. Perhaps the Cd body burden generated by the treatment was below the critical myocardial tissue concentration at which damage could occur. The World health Organisation (WHO, 1972) for example established 200 mg/kg wet weight of kidney as the critical renal cortex concentration of Cd beyond which renal damage could be anticipated. Total protein (TP) and ST were similarly unaffected ($P > 0.05$) by the Cd treatment. Unaltered TP levels suggest unimpaired hepatic protein synthesis, normal renal function and possibly, the intergrity of the reticulo-endothelial system. Testosterone, the major hormone of the testis is synthesized in the Leydig cells under the control of luteinizing hormone (LH). The probable mechanism of LH stimulation of Leydig cells was postulated by Ganong, (1971) as involving increased formation of cyclic AMP and increased protein synthesis. Singhal *et al.* (1976) demonstrated that cyclic AMP protein kinase system in rat testis and prostrate gland was markedly altered by Cd treatment. They further suggested that this disruption might be involved in Cd-induced testicular damage. Such damage would be expected to affect the androgen production capacity of the organ. The level of serum testostoerone was not altered by Cd treatment in the study described in this paper.

No significant ($P > 0.05$) alterations in the blood levels of PCV and Hb attributable to the Cd exposure was found in the present study, agreeing with the report of Weber and Reid (1974), White and Finley, (1978) Di Giulio and Scanlon (1984) and DuVal and Grubb (1986). In contrast, to our observation, Cousins *et al.* (1973) however, reported significant changes in hematocrit values which they considered as the most sensitive measure of the toxicity symptoms in growing swines fed graded levels of Cd over a 6 week period. Anemia has also been identified by some workers as a common manifestation of toxicity in some species of animals exposed to chronic Cd. It was suggested that Cd induces such biological changes in animals through alterations in metallic transport processes involving iron, copper and zinc (Underwood, 1977; Mas and Arola, 1985). Our findings suggest no apparent interference with copper or iron metabolism to support these suggestions.

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