Blood Porphyrin Determination : A Rapid Field Test for Lead Poisoning in Cattle*

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Introduction

Lead poisoning in cattle presents challenging diagnostic problems requiring laboratory confirmation (1,3,6,12,16,18). It is reported to occur most commonly following a single ingestion of material containing toxic quantities of lead; however, a chronic form is also described when pastures are contaminated with lead from industrial operations (1). The diagnosis is generally based on clinical signs, necropsy findings and analysis of blood or tissue for lead content. Tissue lead analysis is expensive and not readily available during acute outbreaks in the field. Recent developments in methods for the detection of plumbism in children have led to studies on other screening tests which could be used as alternative tests to blood lead determination (5,20). Most of these tests are based on well known metabolic effects of lead on heme biosynthesis. Recent studies would suggest that changes in heme biosynthesis accompanying acute lead poisoning in cattle are similar to those seen in man but may have certain unique features associated with the intensity and duration of lead exposure which should be considered in diagnosis (10,18).

It is the purpose of this report to describe the changes in blood porphyrin seen during experimental lead poisoning in the bovine animal. In these studies we have intoxicated cattle by a single intravenous injection of lead acetate. This produced an acute form of intoxication similar to that seen when animals ingest a large quantity of lead over a short period.

Qualitative blood porphyrin determination has become a valuable screening test for the detection of lead poisoning in our laboratory (11). This test requires minimal quantity of specimen and has simple methodology and instrumentation. The marked elevation of blood porphyrin frequently seen in lead poisoned cattle can be easily demonstrated by this test and therefore is of value in screening suspected cattle for lead intoxication in the field.

Further work on blood porphyrin levels in field cases of lead poisoning and other anemias will be required to establish limits of expected fluorescence in the bovine animal. Preliminary results obtained on clinical cases of lead intoxication in cattle have indicated that in addition to blood porphyrin, decreased blood δ-aminolevulinic acid dehydrase (ALAD) activity is a highly specific test of value in the diagnosis of lead poisoning (10). Decreased ALAD activity is of diagnostic value during the early course of lead intoxication since toxic effects in the central nervous system may be apparent before porphyrin-laden erythrocytes are formed and released from the bone marrow. In herds where positive cases of lead poisoning have been found, blood porphyrin screening could also be useful in the detection of asymptomatic animals with increased exposure to lead that may benefit from chelation therapy. The reader is referred to the case report for an example of the use of these laboratory tests in the evaluation of an outbreak of acute lead poisoning in cattle.

Materials and Methods

Experimental

Eight Hereford calves, 6-18 months of age, were obtained from the Veterinary Research Station, Pawhuska, Oklahoma. One group of four calves (Group PLX) was intravenously injected with 1 mg/lb lead acetate four months previous to the

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onset of this study. The remaining four calves (Group NLX) were not exposed to lead and had no history of lead exposure prior to day 0. Subsequently at day 0, all eight calves were intravenously injected with 2 mg/lb lead acetate and blood samples were analyzed periodically for blood lead and blood porphyrin over the next two months. Clinical signs of lead intoxication (convulsions, depression, anorexia) were seen during the first week. Some inappetence and weakness were noted during the second week. Thereafter, the animals appeared clinically normal during the remainder of the study.

Technique

Methodology for Qualitative Determination of Blood Porphyrin

The method of Haining, et al. (11), was found to be satisfactory for use in cattle. It is repeated here for the convenience of the reader.

One ml of whole blood is added to 3 ml of a mixture of one part glacial acetic acid and four parts ethyl acetate. The mixture is stirred well, shaken for one minute and centrifuged for two minutes. The supernatant solution is decanted into a second tube containing 0.5 ml of three normal hydrochloric acid. The two phases are shaken thoroughly for one minute, allowed to separate and the lower HC1 layer is examined for *orange-red porphyrin fluorescence utilizing ultraviolet light.* A Woods light has been found suitable as the source of ultraviolet light in a darkened room.

The fluorescence is graded as follows: Negative - no fluorescence; Trace - faint pink fluorescence; 1+ - definite orange-red fluorescence; 2+ - bright orange-red fluorescence. Reagents required can be prepared in advance and stored until needed. Although quantitative methods are available for the determination of blood porphyrins, the exact measurements are not necessary for screening in most cases of lead poisoning.

Trace levels of porphyrins have been found in normal cattle utilizing quantitative techniques (15), however we have found that normal cattle do not show porphyrin fluorescence utilizing the described technique. Preliminary studies of various responding anemias in cattle have revealed rare cases with trace level fluorescence.

Results

Blood lead concentration (mean ± standard deviation) and blood porphyrin rating (number of animals at respective levels of fluorescence) are given in Table 1. Previous to lead intoxication on day 0, higher blood lead concentration and porphyrin fluorescence were noted in group PLX, which had been exposed to lead. Student's "t" test revealed a significant difference ($p < .005$) in blood lead concentration between groups at this time. These findings reflect a greater total-body lead burden in group PLX and support present concepts that lead excretion occurs slowly over a prolonged period of time. Following intoxication, clinical signs gradually diminished over a two week period, despite a continuing high blood lead concentration in both groups. The pre-treatment difference in blood lead concentration between groups (approximately 20 μg%) appeared to be also reflected during the latter portion of the period of study and may account for greater levels of fluorescence in group PLX on days 44 and 64.

Following lead administration, average blood

Table 1

Changes in Blood Lead and Blood Porphyrin Following Experimental Lead Poisoning in Cattle

	Group PLX[a]					Group NLX[b]				
	Blood Lead[d] (mean + S.D.)	Blood Porphyrin[e]				Blood Lead[d] (mean + S.D.)	Blood Porphyrin[e]			
		Neg	Trace	1+	2+		Neg	Trace	1+	2+
PRI[c]	34 + 8	0	2	2	0	14 + 4	4	0	0	0
DAY 1	133 + 6	0	2	2	0	132 + 27	4	0	0	0
DAY 3	112 + 6	0	2	2	0	116 + 40	2	2	0	0
DAY 5	N.D.*	0	1	2	1	N.D.*	0	1	2	1
DAY 8	140 + 24	0	0	4	0	145 + 45	0	1	0	3
DAY 15	134 + 16	0	0	3	1	130 + 54	0	1	0	3
DAY 22	124 + 8	0	0	2	2	84 + 30	0	0	1	3
DAY 29	126 + 12	0	0	0	4	102 + 21	0	0	0	4
DAY 44	80 + 19	0	0	0	4	59 + 10	0	0	4	0
DAY 64	72 + 8	0	0	1	3	54 + 6	0	2	2	0

*N.D. = Not determined.

(a) Group PLX consisted of 4 animals injected with 1 mg/lb lead acetate 4 months previous to Day 0.

(b) Group NLX consisted of 4 animals with no previous history of lead exposure.

(c) Represents the average of 3 determinations/animal taken over a 2 week period prior to Day 0.

(d) Expressed in μ g%.

(e) Expressed as number of animals with a given level of fluorescence.

porphyrin levels were noted to increase in both groups reaching 1+ fluorescence by day five and 2+ fluorescence by day 29. After day 29, blood porphyrin levels decreased more rapidly in group NLX which reflected the lower blood lead concentration in this group.

Discussion

Ingested lead is poorly absorbed by the ruminant, although the age of the animal and the solubility of the lead product may influence the degree of absorption from the intestinal tract (2). Absorbed lead is concentrated in various soft tissues, primarily liver, kidney and erythrocytes; however, its level in the brain seems critical to the development of nervous symptoms typically seen in affected cattle (23). The precise nature of lead's effect on cellular metabolism in the brain and other soft tissues remains unclear. Miller, et al. (19), have shown that feeding lead to lactating rats produced commensurate reductions of both blood and brain (ALAD) activity in suckling rats. Goyer, et al. (9), have demonstrated decreased mitochondrial function in lead affected renal tubular cells. These studies suggest that impaired energy metabolism in the central nervous system may play a critical role in the development of clinical signs.

Significant lead accumulation also occurs in the bone marrow where its well known effects on erythropoiesis are manifested. Bone marrow lead levels up to 50 times blood levels have been demonstrated in man (25). The multiple effects of lead on heme biosynthesis include inhibitions of ALAD, coproporphyrinogenase and ferrochelatase (8). These effects lead to increased excretion of δ-aminolevulinic acid (ALA) and coproporphyrin in urine and accumulation of protoporphyrin in erythrocytes. Less well understood is impaired erythroid maturation leading to a variable anemia in lead toxicity (21). The release of basophilic stippled erythrocytes has been shown to be related to abnormal ribosomal agglutination in developing erythroid precursors (13). This parameter has received much emphasis in the diagnosis of lead poisoning in dogs (27). Basophilic stippling, however, is not sufficient evidence for the diagnosis of lead poisoning since this phenomenon is common to several anemias in the bovine animal (24), and has not been found to be uniformly present in lead poisoning (10).

Studies in man indicate that the concentration of free erythrocyte protoporphyrin (FEP) in lead poisoned children is 25-250 times greater than normal (17). Kammholz, et al., speculated that FEP levels may more closely reflect major metabolic impact of body lead than does actual blood lead (14). Only erythropoietic porphyria in the bovine is associated with comparable elevation in FEP, however, photosensitivity and the demonstration of fluorescence of the teeth distinguish cattle with this rare hereditary disease (15).

Although marked elevation in blood porphyrin is associated with lead poisoning, less pronounced effects are seen with lesser degrees of lead exposure. In this regard, trace level fluorescence may be seen in certain responding anemias, most notably severe iron deficiency anemia (17,26). Prophyrin metabolism in lead poisoning and during chelation therapy has revealed that blood porphyrin remains elevated long after lead has apparently decreased to nontoxic levels (7). This effect has been attributed to the absence of ferrochelatase, a mitochondrial enzyme, in mature erythrocytes. Subsequent inability to convert the elevated protoporphyrin to heme results in the retention of the porphyrins throughout the remainder of the erythrocyte's life span. Alternatively, the studies of Nakao (22) showed that intraperitoneal injection of protoporphyrin in mice led to porphyrin accumulation in erythrocytes. These studies suggest that increased porphyrin synthesis throughout the body may contribute to the net porphyrin content of erythrocytes.

It is difficult to determine the relationship between total body lead and "metabolically-active" lead. Remobilization of lead from inactive storage to active metabolic sites in the body may occur during therapy or during exacerbations of plumbism associated with subsequently encountered disease processes. Evidence indicates that blood lead does not appear to be in equilibrium with the total body burden of lead, however it provides an index of soft tissue or metabolically active lead (4). Other studies have concluded that ALAD determination was the most sensitive test for the average clinical laboratory in the detection of early metabolic effects of lead in man (20). Our preliminary studies also indicate that decreased ALAD activity is seen during the early course of acute lead intoxication in cattle (see case report).

Our studies would suggest that lead intoxication in cattle is associated with porphyrin metabolic defects similar to that reported in man. Blood porphyrin levels were noted to be elevated in Group PLX, four months following a single intravenous dose of lead. Corresponding average blood lead concentration was within normal limits ($< 35\ \mu g\%$). Following experimental lead intoxication, porphyrin fluorescence became apparent at day five and gradually increased to maximal

fluorescence at day twenty-nine. Corresponding blood lead concentrations over this period remained at a high stable level. This may be explained by a continuing accumulation of lead in the marrow with subsequent release of porphyrin-laden erythrocytes. The decrease in blood porphyrin levels seen in Group NLX on days 44 and 64 may reflect the lower net lead exposure in this group or greater availability of inactive sites for lead storage. Progressive elution of porphyrin from erythrocytes or a shortened life span of prophyrin-laden erythrocytes may also be implied in Group NLX since the normal bovine erythrocyte life span has been estimated at 160 days (15).

Although it would appear that blood porphyrin determination may be of limited value during the first days following lead intoxication, our findings have indicated that the blood porphyrins are generally trace level or greater in lead poisoned cattle presented to our clinic. This would suggest that many cases are presented to the veterinarian several days after initial increased lead exposure. Since vague signs of illness can be associated with mild lead exposure, negative findings in animals are also of value in excluding lead poisoning.

Case Report: Acute Lead Poisoning in a Herd of Hereford Cattle

On 2-12-73 the OSU Ambulatory Clinic was called to a local ranch to examine a herd of 26 registered Hereford cattle. On arrival at the ranch, three cows were noted to be showing CNS signs and one cow was dead. Nervous symptoms observed in these cattle included ear twitching, chewing motions, nystagmus, blindness and convulsions. Other animals were noted to be anorectic and depressed. On physical examination no other body systems were noted to be abnormal. From the signs observed a toxicity was suspected, although infectious, embolic meningoencephalitis and polioencephalomalacia were also considered in differential diagnosis. Blood samples were drawn for laboratory analysis and symptomatic treatment was initiated with atropine, calcium disodium edatate and thiamine in cattle showing clinical signs.

A necropsy examination of the dead cow did not reveal significant lesions in the abdominal or thoracic organs. The brain was removed for examination; however, no gross lesions were apparent. Renal tissue was submitted by mail for lead and arsenic analysis. The results of these studies were not expected for 7 to 10 days and therefore were unavailable for diagnosis during the early course of the outbreak. Subsequent microscopic examination of the cerebrum revealed perivascular cuffing with eosinophilic material. This finding has previously been reported in association with lead intoxication in cattle, but should not be considered specific for this condition.

Herd management, health and nutrition were judged to be good. The ranch was primarily a cow-calf operation, most cows having calves at side when the outbreak occurred. No recent change in diet or management practices were reported by the owner. The cattle were pastured on approximately 160 acres of rolling grassland with two small ponds. The vegetation consisted of native pasture with a Bermuda grass base. Some supplementary feeding of alfalfa hay was done in a small corral during the winter months. The pasture was examined for sources of lead and two potential sources were identified. In the week prior to the outbreak an old building had been torn down and a few cattle were noted to be licking paint from some of the boards. There was also a factory immediately adjacent to the pasture that produced lead-containing products. The factory had complied with state environmental safety laws and smoke pollution had never appeared significant in the area. Liquid and/or solid waste products did not appear to drain to the pasture.

Course of Outbreak

During the next week the Ambulatory Clinic treated a total of nine cows in the herd for signs of lead poisoning including two animals that did not respond and subsequently died on days three and seven of the outbreak. Six additional animals were observed to show vague signs of illness; however, they recovered without therapy. Feed consumption of the herd was markedly diminished but gradually returned to normal over a three week period. On day ten of the outbreak blood samples for laboratory studies were collected from three cows that had shown clinical signs of lead poisoning and seven cows that had remained asymptomatic. The results of these studies are given in Table 3. The owner moved the cattle to a different pasture approximately three weeks after the outbreak and to date has had no unusual health problems in the herd. In view of the reported higher incidence of lead poisoning in young calves, it was interesting that only mature cows were involved in this outbreak.

Laboratory Studies

The laboratory results of blood samples collected from two of the cows on day one of the outbreak are given in Table 2. The hemograms of both animals appeared essentially normal except a lymphopenia was present in cow No. 2. SGOT

activity was also markedly elevated in cow No. 2 indicating either hepatic or muscle damage. Elevated blood porphyrin level and decreased ALAD activity are noted in both animals and were considered diagnostic for lead poisoning. Blood lead concentrations were not immediately available and were not obtained until later in the outbreak. Therapy with calcium disodium edatate was therefore continued in cattle showing clinical signs. Rapid abatement of signs and improved appetite following therapy was noted in most cases. The laboratory analysis of kidney tissue from the first dead cow revealed a lead concentration of 2200 μg% which further confirmed the diagnosis of lead poisoning in the herd.

The results of laboratory studies conducted on blood samples obtained from cattle on day ten of the outbreak are given in Table 3. Three of the cows (group 1) had shown clinical signs of lead poisoning and had responded to therapy. Seven of the cows (group 2) had not shown obvious clinical signs during the outbreak, had not received therapy and perhaps most significantly, were thought to be "normal." Hemograms of all cattle were within normal limits and no basophilic stipling of erythrocytes was apparent. Increased lead exposure in all animals was indicated by the elevated blood lead concentration, decreased ALAD activity and elevated blood porphyrin level. No significant difference was noted between groups in either blood lead concentration or ALAD activity utilizing student's "t" test. It is apparent that the correlation is greater between blood lead concentration and blood porphyrin level than between blood lead concentration and blood ALAD activity in these studies. Although all three

Table 2

Laboratory Results (Day 1)

	Cow 1	Cow 2		Cow 1	Cow 2
Hemogram					
WBC (/cmm)	10,800	4,400	BUN[1] (mg%)	18	14
Neutrophils (%)	30	58	SGOT[2] (SF units)	73	4,800
Lymphocytes (%)	63	42	Porphyrin	1+	1+
Monocytes (%)	1	0	ALAD[3] (units/ml)	0	2.1
Eosinophils (%)	6	0			
P.C.V. (%)	39	35.5			
Plasma Proteins (gm%)	8.3	7.9			
Fibrinogen (mg%)	300	500			
Basophilic Stippling	Neg	Neg			

(1) Method of Chaney, et al., Clin. Chem., 8: 131, 1952.
(2) Sigma Technical Bulletin No. 505, Sigma Chemical Co., St. Louis, Mo.
(3) See Table 3 for methodology and normal values.

Table 3

Laboratory Results (Day 10)

	Group 1			Group 2**								
Animal No.	28	29	32	$\bar{X}$	10	30	39	60	64	70	90	$\bar{X}$
TEST												
†WBC (/cmm)	7.2	9.0	7.2	7.8	8.2	8.8	10.8	11.0	9.0	7.0	8.2	9.0
†Neutrophils (/cmm)	1.2	3.2	0.9	1.8	2.0	0.9	2.3	1.4	3.1	1.8	1.5	1.9
†Lymphocytes (/cmm)	5.1	5.1	5.6	5.3	5.4	7.2	5.9	8.8	4.0	4.4	5.7	5.9
†Monocytes (/cmm)	0.2	0.1	0.3	0.2	0.5	0.1	0.5	0.2	0	0.3	0.2	0.2
†Eosinophils (/cmm)	0.8	0.6	0.4	0.6	0.3	0.6	2.0	0.6	2.0	0.6	0.9	1.0
P.C.V. (%)[1]	28.0	33.0	36.0	32.3	30.0	32.5	31.5	30.0	36.0	33.5	28.5	31.7
Reticulocytes (%)	0.2	0.1	0	0.1	0	0	0	0	0	0	0.1	0
Basophilic Stippling	–	–	–	–	–	–	–	–	–	–	–	–
ALAD (units/ml)[2]	0.9	1.5	1.7	1.4	0	0	0.8	1.7	0.3	1.1	0	0.6
Porphyrin[3]	2+	2+	trace		1+	2+	2+	1+	2+	1+	1+	
Blood lead (μg%)[4]	101	125	45	90.3	43	68	67	40	98	56	44	59.5

*Cows having received therapy for signs of lead intoxication. **Asymptomatic cows.
$\bar{X}$ = Mean value for group.
(1) Packed cell volume.
(2) ALAD = δ -aminolevulinic acid dehydrase expressed in units/ml; according to the method of Bonsignore, et. al., Med Lavors 56: 199, 1965. Normal values on 85 cattle (mean = 10.2 units/ml; range = 3 to 55 units/ml).
(3) According to the method of Haining, et al., (Ref. 11).
(4) Determined by Atomic absorption spectroscopy.
†10^3/cmm

of these tests were valuable in suggesting lead intoxication, the severity of lead exposure in asymptomatic cases would apparently be best judged by either blood lead or blood porphyrin levels. Further studies on the use of laboratory tests in estimating the severity of lead exposure or the response to therapy are presently in progress.

The diagnosis of this outbreak was therefore confirmed to be acute lead intoxication. The primary source of increased lead exposure has not been definitively established on this ranch. County health officials were notified and several samples of grass, soil, water and paint were analyzed for lead content. The results of these studies did not point to an obvious source that was responsible for the sudden onset of acute intoxication seen in the herd. It should be emphasized that thorough screening of not only clinically affected but also asymptomatic cattle may reveal a greater incidence of generalized lead exposure than suspected in a given outbreak.

Discussion

Qualitative blood porphyrin determination was shown to be a valuable laboratory test in this outbreak as indicated by: 1) its simplicity provided a rapid test for indicating the "probable" diagnosis. The clinician was then able to direct specific therapy for lead intoxication while the diagnosis was being further confirmed by other laboratory tests. This consideration should not be underestimated as early therapy can prevent irreversible damage to organs and ultimately lead to lowered mortality rates or more rapid recovery of affected animals. 2) Its use as a screening test in the herd provided a means of determining increased lead exposure in the entire herd. This information may be of value in judging the merits of therapy in selected individuals and subsequently in improving the health of subclinically affected cattle.

Summary

Qualitative blood porphyrin determination, a rapid field test for the diagnosis of lead poisoning in cattle, is described. Blood porphyrin levels were found to be markedly elevated following experimental lead intoxication in cattle and appeared to reflect the intensity of lead intoxication, as judged by blood lead determination. Certain anemic states, characterized by mild elevation of blood porphyrin, need to be differentiated from increased lead exposure. In these instances blood lead determination, decreased δ-aminolevulinic acid dehydrase (ALAD) activity or examination of erythrocyte morphology may be necessary for accurate laboratory diagnosis. The test also proved to be an invaluable screening test in a herd with several cases of acute lead poisoning.

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