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# Low Dose Lead Effects in Calves Fed a Whole Milk Diet

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Cattle are the farm species most sensitive to lead (Pb) poisoning and the number of cattle exposed to above normal amounts of Pb each year could be as high as 150,000 world wide (Botts 1977). Because of this exposure, it is important to establish the minimum toxic level of Pb which will cause fatal poisoning. Previous studies have indicated that doses of 5 mg Pb/kg b.w./day for 7 days or 2.7 mg Pb/kg b.w./day for 21 days are highly toxic to young calves fed a whole milk diet (Bratton et al. 1981; Zmudzki et al. 1983). These results further show that the amount of Pb necessary to produce chronic Pb intoxication is less than the minimum toxic levels defined in earlier studies (Allcroft and Blaxter 1950; Hammond and Aronson 1964).

The present study was designed to evaluate the effects of 1 mg Pb/kg b.w./day on young calves fed a whole milk diet. Clinical assessment, tissue Pb distribution, and hematological indicators of exposure to Pb were investigated.

## MATERIALS AND METHODS

Eight Holstein bull calves (2-3 weeks old) weighing 44 4 kg were housed on concrete in individual wire pens and fed twice/day with a commercial milk replacer diet (Formula 300, manufactured by Milk Specialties Co. for AMPI Corporation, Houston, TX). This milk replacer was balanced in all known dietary needs (National Research Council 1978) and allowed the calves to grow and develop at a rate compatible with good management standards (0.24±0.02 kg/day). Lead levels in the milk replacer were below 0.1 mg Pb/kg dry diet.

Following a 7-day acclimatization period, all calves were eating well, were judged to be clinically normal, and had normal hematological parameters (Tennant et al. 1974). They were individually

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dosed with 1 mg Pb/kg b.w./day for 56 days. Lead was given by nursing bottle as Pb acetate, Pb $(C_2H_3O_2)_2 \cdot 3H_2O$ (Fisher Scientific Corporation, Houston, TX) dissolved in 100 ml of water. Dosing occurred approximately 2 hours after each morning feeding.

Blood samples were collected from the external jugular vein of all calves 3 days prior to the initial dose of Pb and then at 1, 3, 6, 12, and 24 hours after the first, the 8th, and the 22nd dose of Pb. Single samples were collected on days 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, and 56 of the experiment. Blood samples were collected in 50 ml heparinized plastic tubes and then divided to determine blood Pb (BPb), erythrocyte δ-aminolevulinic acid dehydratase (ALAD) activity, zinc protoporphyrin (ZPP) levels in erythrocytes, hemoglobin (Hb) levels in erythrocytes, hematocrit (PCV), total erythrocytes (RBC), total leukocytes (WBC), and serum bilirubin, iron, zinc, copper, magnesium, calcium, and phosphorus. Physical examinations were recorded at each daily feeding and consisted of an assessment of alertness, body posture, gait, behavior, and eating habits.

All surviving calves were killed by an IV injection of pentobarbital (Nembutal sodium, Abbott Laboratories, North Chicago, IL) on the 57th day of the experiment. The following samples were collected, washed, and frozen for Pb analysis: left kidney, left lobe of liver, spleen, pancreas, left biceps femoris muscle, left ventricle, cerebellum, brainstem, cerebrum, thoracic spinal cord, left caudal lung lobe, left femoral head, left 4th rib, 50 ml of urine, and the maximum amount of bile obtainable.

ALAD was measured by the colorimetric method of Burch and Siegel (1971) which has been shown to work well in cattle (Bratton and Zmudzki 1984b). ZPP was measured on a human hematofluorometer calibrated to display ZPP as µg ZPP/g Hb (AVIV ZPP Meter, AVIV Corporation, Lakewood, NJ). PCV, Hb, total RBC, and total WBC were measured by standard methods (Coulter Equipment and Methods Manual, Coulter Electronic, Inc., Hialeah, FL). Bilirubin was measured by the blue azobilirubin method (Gilford Diagnostic, Subsidiary of Gilford Instrument Lab., Inc., Cleveland, OH). Serum iron, zinc, copper, magnesium and calcium were measured by atomic absorption spectroscopy (Analytical Methods for Atomic Absorption Spectroscopy, Perkin Elmer, Norwalk, CN). Pb was measured in blood and tissues by flame atomic absorption spectroscopy following chelation and organic extraction (Yeager et al. 1971; Zmudzki 1977).

## RESULTS AND DISCUSSION

During the first three weeks of Pb dosing all calves ate well and presented no clinical abnormalities. During the fourth week, 4 calves became less active, spent more time lying down, and seemed to be less alert at feeding time. Two calves had difficulty sucking and swallowing (hypoglossal and pharyngeal paresis), and one calf appeared blind.  These signs appeared and disappeared throughout the remainder of the experiment. At the end of the 8th week (day 56), one calf became severely depressed, developed a trunkal ataxia and died during a grand mal seizure (status epilepticus). The experiment was terminated the following day. All signs appeared to be related to malfunction of the nervous system, and no gastrointestinal abnormalities were noted. In general, the calves continued to eat and gain weight throughout the study.

All calves accumulated Pb in the tissues at levels compatible with the diagnosis of Pb poisoning (Buck et al. 1976; Clarke and Clarke 1967). Lead concentrations in 18 different tissues are shown in Table 1.

Table 1. Lead Concentrations in Calf Tissues (mg/kg wet tissue).

| | 1 mg Pb/kg b.w.-8 wks | | | 5 mg Pb/kg b.w -1 wk* | | |
|---|---|---|---|---|---|---|
| Tissue | $\bar{X}$ | SE | RANGE | $\bar{X}$ | SE | RANGE |
| Rib | 97.79 | 12.64 | 57.14-150.05 | 47.24 | 3.30 | 19.20-83.87 |
| Femoral H. | 110.56 | 11.20 | 76.43-156.19 | 68.21 | 6.02 | 31.43-127.74 |
| Kidney | 105.39 | 24.64 | 23.64-247.25 | 95.84 | 7.37 | 41.38-157.94 |
| Liver | 17.04 | 3.14 | 7.66-32.43 | 29.62 | 3.26 | 11.30-73.47 |
| Spleen | 1.21 | 0.25 | 0.65-2.82 | 1.70 | 0.23 | 0.51-5.58 |
| Pancreas | 2.82 | 0.38 | 1.47-4.68 | 5.38 | 0.60 | 1.75-12.91 |
| Lung | 0.90 | 0.14 | 0.43-1.42 | 1.63 | 0.17 | 0.77-4.44 |
| Testes | 0.62 | 0.17 | 0.18-1.12 | 1.32 | 0.09 | 0.72-2.28 |
| Muscle | 0.22 | 0.05 | 0.10-0.46 | 0.32 | 0.03 | 0.17-0.62 |
| Heart | 0.23 | 0.03 | 0.15-0.39 | 0.55 | 0.05 | 0.29-1.04 |
| Nerve | 0.43 | 0.05 | 0.20-0.62 | 0.88 | 0.04 | 0.47-1.35 |
| Cerebellum | 0.71 | 0.08 | 0.43-1.09 | 0.68 | 0.04 | 0.27-1.09 |
| Brain Stem | 0.93 | 0.09 | 0.57-1.28 | 0.72 | 0.05 | 0.36-1.07 |
| Cerebrum | 1.30 | 0.15 | 0.70-2.03 | 0.81 | 0.05 | 0.38-1.18 |
| Spinal Ccrd | 0.40 | 0.06 | 0.24-0.71 | 0.34 | 0.03 | 0.15-0.66 |
| Urine | 0.12 | 0.02 | 0.08-0.26 | 0.34 | 0.06 | 0.07-1.03 |
| Bile | 0.93 | 0.16 | 0.49-1.70 | 2.37 | 0.32 | 0.87-6.67 |
| Blood | 1.50 | 0.08 | 1.24-1.91 | 1.44 | 0.12 | 0.90-3.21 |

* The tissue Pb levels in calves (n=22) given 5 mg Pb/kg b.w./day for 7 days are included in this table for comparison. These results were reported by Zmudzki et al. 1983 and Bratton and Zmudzki 1984a; all experimental parameters were exactly the same as those defined in this study.

The highest Pb levels were found in bone and kidney. After eight weeks of Pb dosing, the amount of Pb was approximately the same in each of these tissues and exceeded 100 mg/kg wet tissue. Liver accumulated about six times less Pb than did bone or kidney. The pancreas contained the fourth highest Pb level as has previously been reported (Zmudzki et al. 1983). Calves dosed with 1 mg Pb/kg b.w./day for 56 days accumulated more Pb in bone and cerebrum than did calves dosed with 5 mg Pb/kg b.w./day for 7 days ($P < 0.05$). In other tissues, the Pb levels were either the same or less than in tissues from calves dosed with 5 mg Pb/kg b.w./day for 7 days. The higher levels were anticipated based on the total dose of Pb given, but the lower levels were unexpected. The lower dose and longer excretion time in calves dosed with 1 mg Pb could easily explain a difference in body burden of Pb between these calves and those dosed with 5 mg Pb. However, the fact that only some tissues were affected warrants further investigation and cannot be answered by the present experiment.

The time response of blood Pb (BPb) concentration, erythrocyte ALAD activity, and erythrocyte ZPP concentration is given in Table 2. Mean BPb concentration increased slowly during the entire experiment and reached 150 µg/100 ml at the end of 56 days. Diagnostically significant levels were observed in the blood after 7 days of dosing with 1 mg Pb/kg b.w./day and the levels became even more confirmatory with time.

During the 24 hours following the first dose of Pb, the highest BPb level was observed at 6 hours post dosing. Similar six-hour peaks appeared after the 3rd, 8th, and 22nd doses of Pb, Figure 1. An even more remarkable 6-hour peak was observed in calves dosed with 5 mg Pb/kg b.w. (Zmudzki and Bratton 1984). This 6-hour peak is very important in the diagnosis of Pb poisoning. The time of sampling after the ingestion of Pb can be critical to the utilization of BPb for diagnosis.

Erythrocyte ALAD activity dropped from the baseline value $523.7 \pm 111.9$ nM porphobilinogen (PBG)/ml of erythrocytes (RBC)/hr to about 50% of pretreatment activity in 12 hours. The activity decreased to about 30% by the end of 7 days and remained at that level despite the continued ingestion of Pb. Calves dosed with 1.5 mg Pb/kg b.w. 3 times/week for 7 weeks failed to show a decrease in ALAD activity (Lynch et al. 1976), but a significant decrease was seen in calves dosed with 3 mg Pb/kb b.w. 3 times/week in the same study. When calves were dosed with 5 mg Pb/kb b.w./day for 7 days, the decrease in ALAD was slightly faster and 10% lower than in this study (Bratton and Zmudzki 1984b). However, these differences appear to have little practical application for assessment of rate of exposure, since both dose levels produced a highly significant decrease in ALAD activity in less than 24 hours.

Table 2. Blood Pb levels, erythrocyte ALAD activity and erythrocyte ZPP levels in calves dosed with 1 mg Pb/kg b.w./day for 8 weeks. ($\bar{x}$±SE, n=8)

| Time | Pb µg/100 ml | ALAD % of pretrial value | ZPP µg/g Hb |
|---|---|---|---|
| 0 | 3±0.4 | 100%* | 3.2±0.2 |
| 1 hr | 4±0.4 | 130.5±9.2 | 3.0±0.2 |
| 3 | 12±4.2 | 118.0±16.1 | N.D. |
| 6 | 23±6.0 | 70.1±14.2 | N.D. |
| 12 | 19±3.2 | 53.9±8.3 | N.D. |
| 24 | 19±2.8 | 50.9±5.3 | 3.1±0.2 |
| 2 days | 22±2.5 | 40.6±5.5 | 3.1±0.2 |
| 3 | 33±2.5 | 40.0±3.1 | 2.8±0.1 |
| 7 | 53±4.9 | 31.9±3.3 | 3.9±0.3 |
| 10 | 64±5.3 | 31.6±2.2 | 5.2±0.6 |
| 14 | 71±3.9 | 38.5±4.4 | 8.3±1.0 |
| 17 | 77±5.3 | 33.1±4.1 | 12.5±1.8 |
| 21 | 86±4.6 | 28.7±3.3 | 15.2±2.1 |
| 24 | 96±6.0 | 29.6±3.3 | 19.8±2.9 |
| 28 | 103±7.8 | 26.1±4.1 | 26.1±4.0 |
| 31 | 100±8.1 | 25.1±4.7 | 29.2±4.5 |
| 35 | 108±7.8 | 36.3±5.1 | 42.6±7.8 |
| 38 | 117±6.0 | 25.3±4.2 | 48.5±9.0 |
| 42 | 124±7.4 | 28.7±4.8 | 56.9±10.6 |
| 45 | 119±8.1 | 27.3±4.3 | 60.0±10.8 |
| 49 | 132±7.8 | 26.6±4.3 | 72.7±11.6 |
| 52 | 139±9.9 | 28.8±4.3 | 73.0±11.6 |
| 56 | 150±7.8 | 30.4±5.2 | 78.1±10.6 |

* pretreatment value 523.7±111.9 nM PBG/ml RBC/hr.

A significant negative correlation was found between BPb and ALAD activity during the entire experiment (correlation coefficient r= -0.73 P, < 0.01). This correlation was higher for the first week of the study, r = -0.79 (P < 0.01) and significantly lower for a period from week 2 to 8, r = -0.45 (P > 0.05). These correlations demonstrate the high sensitivity of ALAD to Pb and the maximum depression in activity which occurred at about 50 µg/100 ml BPb.

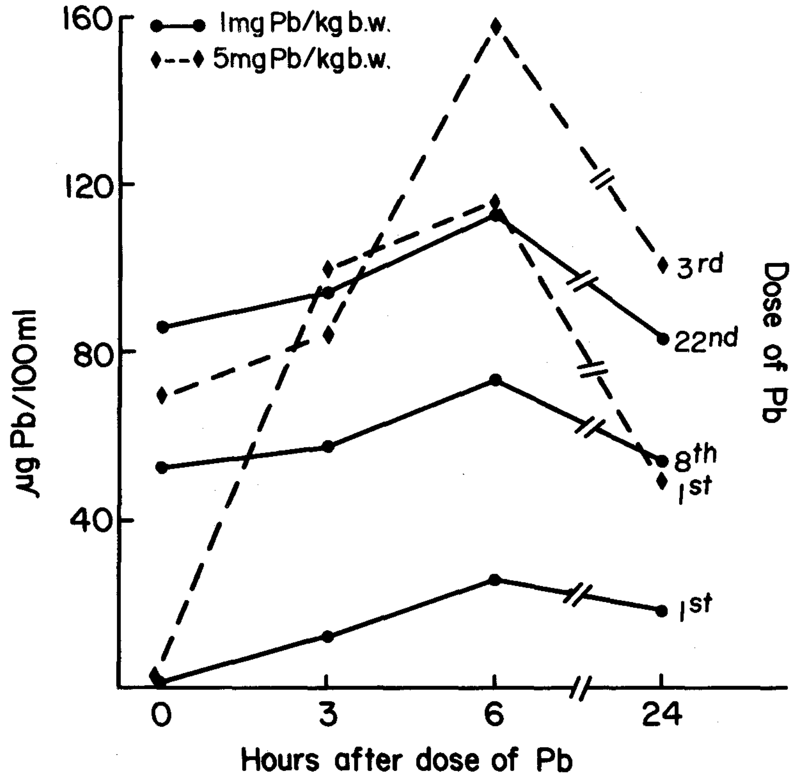


Figure 1. Mean blood Pb levels within 24 hours after the ingestion of Pb. The BPb levels in calves (n=22) given 5 mg Pb/kg b.w./day for 7 days are included for comparison. These results were reported by Zmudzki and Bratton 1984; all experimental parameters were exactly the same as those defined in this study.

Measurement of ALAD in Pb toxicity where cattle are exposed acutely to high Pb levels has little value compared to BPb measurement.

Erythrocyte ZPP concentration did not change significantly until 10 days after the initial dose of Pb, Table 2. From this point, ZPP continued to rise and reached 25 times the base levels at the end of 56 days. ZPP levels were highly correlated with BPb concentration, $r = 0.89$, ($P < 0.01$) for the entire experiment and higher if the 7-day time lag at the beginning was ignored, $r = 0.97$, ($P < 0.01$). A combination of BPb, ALAD, and ZPP would be beneficial in the assessment of acute Pb exposure while BPb and ZPP would be valuable in chronic exposure. The rate at which ALAD and ZPP return to normal after the termination of Pb exposure should be studied in order to establish useful parameters for treatment.

Hematological parameters (PCV, Hb, RBC and WBC) decreased slightly during the experiment as expected from the loss of blood, but the decrease was not significantly different when compared to base levels ($P > 0.05$). All values were within the range considered normal for calves of this age at all points measured (Tennant et al. 1974). Serum Zn, Cu, Fe, Ca, Mg, P, and total bilirubin remained unchanged from prelead exposure levels at all points measured. In addition, all trace elements persisted within the range of normal (Underwood 1978).

The results of the present study show that a dose of 1 mg Pb/kg b.w./day can be lethal in approximately 60 days to calves consuming a milk diet. These data are in agreement with studies in other species where milk has been shown to increase the absorption and accumulation of Pb in tissues (Bell and Spickett 1981; Bushnell and DeLuca 1983; Kello and Kostial 1973). These data further support a recent report showing that calves fed a milk diet accumulated 12 times more Pb in bone and 25 times more Pb in kidney than calves the same age fed a diet of grain and hay (Bratton and Zmudzki 1984a). Diet influences on low doses of Pb in calves should be further assessed prior to final conclusions as to effects.

The amount of Pb which produced intoxication in this study can be found in many Pb polluted areas in the United States. Further investigation with low doses of Pb would be beneficial to define the minimum dose that can cause clinical and subclinical toxicosis.

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