Effects of a Thiocarbamate Herbicide Compound (Pebulate) on Magnesium: Calcium Ratio and Blood Urea Nitrogen Levels in Exposed Sheep and Cattle

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Pebulate[1] (S-propyl-butylethylthiocarbamate) is a selective pre-emergence control of broadleaf weeds such as henbit, hairy nightshade and pigweed.

Several herbicide compounds were used for toxicologic studies, and a few produced an enlargement or congestion of the thyroids and the kidneys (6). It is reasonable, therefore, to expect that calcium levels would be lowered as hypocalcemia is one of the most constant and characteristic features of diminished parathyroid function. In man, hypermagnesemia occurs primarily in chronic renal insufficiency (4) and was investigated, as was Blood Urea Nitrogen (BUN), for this condition.

Because of common pathways for the gastrointestinal absorption and renal transport for magnesium and calcium (4), it was thought that oral exposures of the herbicide might affect absorption or elimination of one or both of these elements. Therefore, a study of Mg:Ca ratio change was investigated.

The biological roles of calcium (Ca) and magnesium (Mg) are already well established and were quantitated by a reliable method of analysis (5).

Materials and Methods

Test Animals. Diet. Sheep and cattle on test were fed mixed grain and hay with free choice of minerals (1/2 bone meal and 1/2 salt).

Eight yearling sheep and 7 yearling cattle were used. Two sheep were controls, 3 were dosed daily with the regular commercial formulation of 76% emulsifiable concentrate (EC), 2 were dosed with technical material (95.4%) and 1 sheep was dosed with the inerts (materials used in the emulsifiable concentrate formulation without S-propyl-butylethylthiocarbamate).

Each sheep was given a 250 mg./kg. dose daily.

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[1] Stauffer Chemical Co., New York, N.Y.

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Two yearling cattle were controls, 2 were given regular commercial formulation, 2 were given technical material, and 1 yearling was given formulation inerts. Each was given 100 mg./kg. daily.

Chemical Analyses. Blood samples were taken from the jugular vein for 3 days before initiating oral exposures. Samples were taken daily after the trials began. Fresh blood was heparinized and the plasma used for Ca and Mg determinations by atomic absorption spectrophotometry (3).

Muscle biopsies for Ca and Mg determinations were taken before the experiment, as well as at the time of greatest Mg:Ca ratio changes in the plasma and at necropsy. Samples of muscle tissue (0.5 gm.) were dry-ashed at 600° C. for 18 hours. The ash was digested with 5 ml. of Hcl. A 1:100 dilution of the tissue was made for the Ca determinations and a 1:1000 dilution for Mg. The dilutions were made with a 1:100 dilution of lanthanum chloride. Determinations of Ca and Mg were made by atomic absorption spectrophotometry (1). Calcium determinations were read at 4227 A with the flame in a highly reducing mode:

Fuel: Acetylene at 16 p.s.i.

Oxidant: Air at 12 p.s.i.

Magnesium determinations were read at 2835 A with the flame in a reducing mode:

Fuel: Acetylene at 15 p.s.i.

Oxidant: Air at 10 p.s.i.

All samples, both tissue and plasma, were analyzed in duplicate.

Results

Normal Values.

Sheep:

Ca - 10.26 $\pm$ 0.17 mg./100 ml. range 7.2 - 12.0 mg./100 ml.
Mg - 2.62 $\pm$ 0.002 mg./100 ml. range 1.9 - 3.9 mg./100 ml.

Cattle:

Ca - 10.03 $\pm$ 0.16 mg./100 ml. range 9.0 - 11.4 mg./100 ml.
Mg - 2.31 $\pm$ 0.009 mg./100 ml. range 1.8 - 3.2 mg./100 ml.

Blood urea nitrogen (BUN) determinations were conducted concurrently with the plasma Ca and Mg using Echols ultra-micro method for urea-nitrogens (2).

TABLE I

Effects of Repeated Oral Doses of Pebulate and Its Major Components to Cattle and Sheep

Animals and Treatment	No. of Doses To Produce Ill Effects	No. of Doses Total Administered	Final Biopsy After	Results and Remarks
Cattle - 100 mg./kg. Dosages				
EC[a]	2	5	3 doses	Died after 5 doses
EC[a]	2	6	3 doses	Died after 6 doses
Tech.[b]	None	6	6 doses	Survived
Tech.[b]	None	6	6 doses	Survived
Inerts[c]	None	6	6 doses	Survived
Control	----	----	3 days	
Control	----	----	7 days	
Sheep - 250 mg./kg. Dosages				
EC[a]	2	3	3 doses	Killed 6 days later
EC[a]	2	3	3 doses	Died 6 days later
EC[a]	37	56	56 doses	Survived
Tech.[b]	6	15	15 doses	Killed 4 days later
Tech.[b]	None	15[d]	15 doses	Survived
Inerts[c]	None	15	15 doses	Survived
Control	----	----	3 days	
Control	----	----	16 days	

[a] Emulsifiable concentrate (76%) formulated by Stauffer Chemical Co. as Tillam .

[b] Technical material as S-propyl-butylethylthiocarbamate (95.4%).

[c] Inerts (24%) in Tillam formulation.

[d] Last dose only partial due to shortage of technical material.

Toxicologic. Preliminary toxicity screening trials with this herbicidal formulation were carried out in cattle and sheep as oral encapsulated doses. Dosages were calculated on the basis of milligrams of technical content to kilograms of body weight (mg./kg.) These trials indicated that two consecutive daily doses to cattle at 100 mg./kg. resulted in initial signs of poisoning with death following an additional 4 doses or less.

Consecutive daily doses to sheep at 250 mg./kg. resulted in an increased tolerance to the compound or death.

Necropsy observations (gross) on the fatalities included congestion of the thyroids and hemorrhagic kidneys. To investigate further the observed deleterious effects of this chemical compound and its components, a study was initiated to relate its occurrence to physiologic functions. Toxicologic observations on randomly selected yearling cattle and sheep are summarized in Table 1.

Microscopy. Tissues for histologic examination were fixed in 10% formalin, routinely processed and stained with hematoxylin and eosin.

The prominent renal lesion in the cattle and sheep was an acute toxic tubular nephritis. Many of the glomeruli were increased in size and cellularity and glomerular capillary endothelium was hypertrophic. Bowman's space contained an eosinophilic precipitate. Coagulative necrosis of tubular epithelium occurred in scattered cortical and medullary convoluted tubules but was more noticeable in the cortical tubules. The cytoplasm of the renal convoluted and collecting tubules was moderately to extensively vacuolated. Cytoplasm of the renal convoluted tubules were more extensively involved. Desquamation of tubular epithelium was minimal and tubular lumens contained an eosinophilic precipitate. Congestion was mild in the glomerular tufts and interstitial vasculature. Regenerative changes were present as evidenced by hyperchromatic and hypertrophic epithelial cells predominantly in cortical renal convoluted tubules. These regenerative cells in cattle tend to be in clusters whereas in sheep they were scattered.

Hepatic lesions in cattle and sheep were dilated central veins and coagulative necrosis and mild fatty metamorphosis of scattered hepatocytes in the centrilobular areas.

Mild congestion occurred in the adrenal glands, spleen, thyroid glands and lung parenchyma. In the sheep there was focal interstitial pneumonitis.

Biochemical.

1. *Cattle.* The results are reported as change in ratios of Mg to Ca, *i.e.*, pre-experiment Mg - 2 mg./100 ml.:Ca - 12 mg./100 ml., hence a ratio of 1:6; post-experiment Mg - 3 mg./100 ml.:Ca - 9 mg./100 ml., hence a ratio of 1:3 or a 50% ratio decrease.

TABLE II

Effects of Pebulate and Components on Plasma Magnesium:Calcium Ratios and the Blood Urea Nitrogen Levels in Cattle and Sheep

Animal and Treatment	Days on Test	Pre-treatment[a] Mg:Ca[c] Ratio	Greatest Decrease[a] Mg:Ca[c] Ratio	Decrease Percent	Day Greatest Decrease	BUN[b] Pre-treatment	BUN[b] Maximum
Cattle - 100 mg./kg. Dosages							
EC[d]	(died) 5	2.4:9.4 / 3.91	4.1:10.3 / 2.51	36	4	2	73
EC[d]	(died) 6	2.4:9.7 / 4.04	4.9:11.2 / 2.29	44	5	2	130
Tech.[e]	7	2.0:11.1 / 5.55	2.3:10.0 / 4.35	22	7	8	12
Tech.[e]	7	2.4:10.9 / 4.54	2.6:10.8 / 4.15	9	5	6	12
Inerts[f]	7	1.9:10.3 / 5.42	2.2:10.3 / 4.68	14	7	7	11
Control	7	2.3:10.9 / 4.73	2.5:10.4 / 4.16	12	7	8	10
Control	13	2.15:9.8 / 4.55	2.4:10.0 / 4.17	6	4	1	3
Sheep - 250 mg./kg. Dosages							
EC[d]	(killed) 9	2.4:11.3 / 4.71	2.8:9.9 / 3.53	24	4	7	19
EC[d]	(died) 9	2.3:10.3 / 4.48	2.9:9.8 / 3.37	23	4	10	48
EC[d]	56	2.7:10.5 / 3.89	3.6:11.1 / 3.08	21	8	17	26

TABLE II (Continued)

Effects of Pebulate and Components on Plasma Magnesium:Calcium Ratios and the Blood Urea Nitrogen Levels in Cattle and Sheep

Animal and Treatment	Days on Test		Pre-treatment[a] Mg:Ca[c] Ratio	Greatest Decrease[a] Mg:Ca[c] Ratio	Decrease Percent	Day Greatest Decrease	BUN[b] Pre-treatment	BUN[b] Maximum
Tech.[e]	(killed)	16	2.1:10.2 / 4.86	2.8:10.5 / 3.75	23	9	16	25
Tech.[e]		16	2.1:11.0 / 5.24	3.2:11.4 / 3.56	32	11	20	29
Inerts[f]		16	2.1:10.7 / 5.10	2.8:10.2 / 3.64	29	15	19	26
Control		14	2.4:11.0 / 4.58	2.3:9.8 / 4.26	7	4	7	9
Control		16	2.2:10.0 / 4.55	3.0:9.9 / 3.30	27	9	21	24

[a] Figure in column is the ratio of magnesium to calcium which is always 1 (*i.e.*, 1:4.66). Upper figure represents Mg:Ca; lower figure represents the ratio.

[b] Blood urea nitrogen in mg./100 ml. of plasma.

[c] Magnesium and calcium in mg./100 ml.

[d] Commercially available emulsifiable concentrate (76%) formulated by Stauffer Chemical Co. as Tillam®.

[e] Technical material as S-propyl-butylethylthiocarbamate (95.4%).

[f] Inerts (24%) in Tillam formulation.

TABLE III

Effects of Pebulate and Components on Muscle Magnesium:Calcium Ratios in Cattle and Sheep

Animal and Treatment	Day of Biopsy	Day of Necropsy	Pre-treatment[a] Mg:Ca[b] Ratio	Biopsy[a] Mg:Ca[b] Ratio	Necropsy[a] Mg:Ca[b] Ratio	Percent Greatest Decrease
Cattle - 100 mg./kg. Dosages						
EC[c]	(died) 3	5	110:20.5 / 0.186	115:24.0 / 0.208	125:27.5 / 0.220	0
EC[c]	(died) 3	6	115:30.0 / 0.260	110:30.5 / 0.277	130:27.0 / 0.207	20
Tech.[d]	7	----	96:16.4 / 0.170	88:15.0 / 0.170	----	0
Tech.[d]	7	----	96:16.0 / 0.166	100:15.0 / 0.150	----	10
Inerts[e]	7	----	96:13.6 / 0.141	95:15.7 / 0.165	----	0
Control	7	----	90.4:16.4 / 0.181	93:16.4 / 0.176	----	3
Control	3	----	110:24.5 / 0.222	105:23.5 / 0.223	----	0
Sheep - 250mg./kg. Dosages						
EC[c]	(killed) 3	9	125:24.5 / 0.196	110:27.0 / 0.245	122:24.0 / 0.196	0
EC[c]	(died) 3	9	115:27.0 / 0.234	115:23.5 / 0.204	112.5:23.5 / 0.208	13
EC[c]	56	----	102:18.8 / 0.184	104:18.2 / 0.175	----	5

TABLE III (Continued)

Effects of Pebulate and Components on Muscle Magnesium:Calcium Ratios in Cattle and Sheep

Animal and Treatment	Day of Biopsy	Day of Necropsy	Pre-treatment[a] Mg:Ca[b] Ratio	Biopsy[a] Mg:Ca[b] Ratio	Necropsy[a] Mg:Ca[b] Ratio	Percent Greatest Decrease
Tech.[d]	(killed) 16	20	82.4:18.8 0.228	86:20.0 0.232	103:26.3 0.255	0
Tech.[d]	16	----	100:16.4 0.164	95:15.4 0.162	----	1
Inerts[e]	16	----	96:17.6 0.183	93:16.3 0.175	----	4
Control	3	----	110:22.0 0.200	108:23.0 0.212	----	0
Control	16	----	92:15.6 0.169	91:15.4 0.210	----	0

[a] Figure in column is the ratio of magnesium to calcium which is always 1 (*i.e.*, 1:4.66). Upper figure represents Mg:Ca; lower figure represents the ratio.

[b] Magnesium and calcium in mg./100 ml.

[c] Commercially available emulsifiable concentrate (76%) formulated by Stauffer Chemical Co. as Tillam®.

[d] Technical material as S-propyl-butylethylthiocarbamate (95.4%).

[e] Inerts (24%) in Tillam formulation.

Plasma Ratios: The ratios of the EC-dosed yearlings did not return to the pre-experiment level after the first exposure, but averaged a ratio decrease of 40% on the day of death. The Mg levels were above the maximum for normal values. The average of the technical-dosed cattle decreased 15.5% and the inert-dosed yearling and controls had a ratio decrease of 14 and 9%, respectively (Table 2).

Tissue Ratios: An average of the ratios of the EC-dosed yearlings decreased 10% and the technical-dosed yearling dropped 5%. The inert-dosed yearling had no decrease and the controls averaged only 1.5% (Table 3).

Blood Urea Nitrogen (BUN): Both yearlings dosed with EC had a significant increase (73 and 130 mg./100 ml.) and both died (Table 2).

2. *Sheep.* The results are presented as previously described for cattle.

Plasma Ratios: The controls had an average ratio decrease of 17%. The average ratio drop for sheep dosed with EC, technical and inert formulations was 23.6, 27.5 and 29%, respectively (Table 2). Both Mg and Ca values remained within normal range.

Tissue Ratios: An average of the ratios of EC-dosed and the inert-dosed sheep decreased 5.3 and 4%, respectively. The technical-dosed and the controls had no decrease (Table 3).

Blood Urea Nitrogen (BUN): One sheep dosed with EC died. It was the only sheep to have a significant BUN increase (48 mg./100 ml.) (Table 2).

Discussion

Pebulate, as the EC formulation or technical material, at a 250 mg./kg. dose level, did not significantly reduce Mg:Ca ratios in sheep plasma when compared to controls. The number of sheep in test were too small to be of any value for statistical evaluation. The increases in Mg were not necessarily reciprocated by a decrease in Ca. The Mg was not elevated significantly even in the sheep showing a marked increase BUN. Even though this sheep died, the other two treated sheep that were killed showed approximately the same histopathological findings. All three sheep had acute toxic tubular nephritis, hepatic damage, mild congestion in the spleen, adrenal and thyroid glands, lung parenchyma and pneumonitis.

The only explanation offered for all the animals is this test group, including the controls, showing increases in their Mg levels is an increased intake of Mg in their diet while on test.

Both yearling cattle dosed with EC, 100 mg./kg. dose, had marked decrease in Mg:Ca plasma ratio, acute toxic tubular nephritis, hepatic damage and mild congestion in the spleen,

adrenal and thyroid glands and lung parenchyma. They expired too soon after dosage for tissue ratios to be significantly altered. The technical- and inerts-dosed cattle showed no ill effects clinically and no notable change in Mg:Ca ratio or BUN levels.

The method of analysis used for BUN determinations was accurate, stable and reproducible. It was used instead of nesslerization or aeration methods because of greater stability, speed and better reproducibility, especially when using sheep blood.

Summary

S-propyl-butylethylthiocarbamate (pebulate) caused no significant change in Mg:Ca ratio in the plasma of experimental sheep, regardless of whether the sheep were given an emulsifiable concentrate or a technical material. However, when emulsifiable concentrate was given to cattle there was a significant decrease in the Mg:Ca ratios and kidney damage, but there was no effect due to the technical material and formulation inerts.

References

1. BRADBURY, M. W. B., KLEEMAN, C. R., BAGDOYAN, HELEN, BERBERIAN, ALICE. J. Lab. and Clin. Med., *71*, 884 (1968).

2. CURTIN, W. H. & CO. Technical Data No. 37815 (1967).

3. FISHER SCIENTIFIC. Technical Data Nos. 186 and 187 (1967).

4. GOODMAN, L. S. The Pharmacological Basis of Therepeutics. Third Edition, 802-804, MacMillan Co., New York (1965).

5. HUNT, L. M. and GILBERT, B. N. Amer. J. Vet. Clin. Path., *2*, 231 (1968).

6. PALMER, J. S. USDA, ARS, Vet. Scis. Res. Div., Tox. Res. Lab. Personal Communication (1967).