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# Toxicity of *Alpha*-Ethoxyethylglyoxal Dithiosemicarbazone in Cattle

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## SUMMARY

*Alpha*-ethoxyethylglyoxal dithiosemicarbazone,<sup>a</sup> administered 10 consecutive days at the dose rate of 5 mg./kg./day, caused axonal and myelin degeneration of the vagus nerve in 2 of 7 calves. Of the 7 experimental calves, 6 died of tympanites.

*Alpha*-ethoxyethylglyoxal dithiosemicarbazone is a relatively new chemotherapeutic compound for treating anaplasmosis. The compound is a member of the thiosemicarbazone group which was shown to have antiviral activity.<sup>11</sup> A member of the same group of compounds, diphenyl thiosemicarbazone, produced retinal degeneration and detachment in dogs.<sup>3</sup>

*Alpha*-ethoxyethylglyoxal dithiosemicarbazone was reported to cause intoxication in cattle, especially when used in an attempt to eliminate anaplasmosis

infection from carrier cattle by giving them large doses for extended periods.<sup>b</sup> The compound was used in a single dose of 5 mg./kg. by other investigators<sup>1,2,9</sup> to treat anaplasmosis; deleterious reactions of intoxication did not occur. Elimination of the anaplasmosis infection from the carrier animal by giving inoculations of tetracyclines for 10 consecutive days or longer has been reported.<sup>4,5,8,10</sup> Kuttler and Adams<sup>6</sup> used a similar pattern of repeatedly inoculating *alpha*-ethoxyethylglyoxal dithiosemicarbazone to eliminate *Anaplasma marginale* carrier infections, but encountered some evidence of toxicity when the drug was used for 10 consecutive days at the rate of 5 mg./kg. given intravenously.

The purpose in the present experiment is to determine the clinical and pathologic manifestations induced by *alpha*-ethoxyethylglyoxal dithiosemicarbazone in cattle.

## Materials and Methods

Seven Holstein-Friesian and Romosinuano anaplasmosis-carrier calves, 10 to 13 months old, were given *alpha*-ethoxyethylglyoxal dithiosemicarbazone intravenously for 10 consecutive days at the dosage of 5 mg./kg./day. The group included 1 female and 6 castrated males; 5 of the latter were splenectomized. Four splenectomized anaplasmosis carrier calves were used as controls. All experimental calves were kept under identical conditions. Clinical observations were made each day, packed cell volumes were determined, and blood smears were collected every 3 days.

Brain, lung, adrenal gland, liver, heart, urinary bladder, lymph node, hemal lymph node, kidney, intestine, pancreas, aboma-

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<sup>a</sup> Drug No. 356-C61, Burroughs-Wellcome and Company, Tuckahoe, N.Y.

<sup>b</sup> Roberts, G. I., Burroughs-Wellcome & Company, Tuckahoe, N.Y.: Personal communication, 1968.

sum, spinal cord, bone marrow, rumen, thyroid gland, skeletal muscle, esophagus, skin, bone, gallbladder, tongue, spleen, and peripheral nerve tissues were fixed in buffered 10% formalin, sectioned, and stained with hematoxylin and eosin. Certain tissues were stained with Prussian blue and Holmes' silver nitrate-Luxol fast blue methods.<sup>7</sup> Blood agar and tryptose broth bacteriologic cultures were made from the tissues of 4 of the treated calves.

## Results

*Clinical Observations.*—The drug caused a temporary collapse in some of the calves, usually after the 5th injection, even if it was administered slowly; sometimes the drug caused temporary collapse after the first injection. Tympanites occurred in all treated calves as early as 1 day and as late as 10 days after the 10th injection, with tympanites occurring usually 3 days after the 10th injection.

The tympanites was initially slight, but gradually became more severe until the time of death. Mortality due to tympanites occurred in 6 of the 7 calves as early as 1 day and as late as 41 days after the 10th inoculation was given (av., 15½ days after the 10th inoculation). The tympanites persisted until the time of death and was not cured by ordinary methods (trocarization or antifoam agents) of treatment. One treated calf survived after 2 weeks of chronic tympanites.

Ruminal atony, gas distension of the rumen, anorexia, severe constipation, recumbency, dyspnea, and central nervous system depression were other accompanying signs. Control calves were not affected. All bacteriologic cultures were test-negative for common pathogens. A significant increase in packed cell volumes occurred in the treated calves. The pretreatment average packed cell volume was 25.4% and the average packed cell volume at post-treatment days 12 to 18 was 33.1% ( $P < 0.05$ ).

*Gross Lesions.*—The ocular, nasal, and oral mucous membranes were extremely congested. The skin was adhesive, thickened, and dehydrated but subcutaneous

edema was evident in the tarsal region of each calf. The tarsal articulations were swollen. The parotid, supratharyngeal, bronchial, and mediastinal lymph nodes were congested and enlarged. The peripharyngeal connective tissue contained a large amount of gelatinous edema.

The rumen was extremely distended and contained a large amount of green aqueous ingesta and gas. The diaphragm was displaced cephalad by the overdistended rumen, thereby greatly compressing the lungs. The trachea and bronchi were often plugged with large amounts of ruminal ingesta. Petechial hemorrhages were evident in the mucosa of the trachea and bronchi. The endocardial and pericardial surfaces of the heart frequently had numerous petechiae and ecchymoses. The liver was small and distinctly gray.

*Histopathologic Changes.*—The respiratory system and lymph nodes cephalad to the diaphragm had changes characteristic of tympanites.

The hepatic capsule was thickened by fibrous connective tissue. Mild periarterial hepatic fatty infiltration and sinusoidal dilation were seen. Several portal veins, as well as hepatic sinusoids, were engorged with mononuclear cells. Marked hyperplasia of Kupffer's cells was observed in 2 calves, as well as a few focal microabscesses which did not have any particular lobular distribution. Hemosiderin deposition was frequently observed.

The arcuate vessels of the kidney were usually surrounded by an intense lymphocytic infiltration. Multiple focal perivascular lymphocytic, histiocytic, and plasmocytic infiltrations associated with small arteries were commonly observed in juxtaposition to glomeruli. A dark brown, nonbirefringent substance was usually in or around the perivascular reaction.

The supratharyngeal, bronchial, and mediastinal lymph nodes were edematous and congested. The medullary sinuses of these lymph nodes often contained neu-

trophilic infiltrations. Deposition of hemosiderin in medullary macrophages was observed in almost all lymph nodes; 1 suprailiac lymph node had severe hemosiderosis. The spleen of the intact calf which died had lymphoid hyperplasia. All lymph nodes examined had lymphoid hyperplasia, as evidenced by a large increase in immature lymphocytes and diminution of the center of lymphatic nodules.

The vagus nerve seemed abnormal in 2 calves. The axons were disrupted and tortuous and often formed bulblike enlargements. The myelin sheaths were fragmented and swollen, indicating a primary degeneration, since neuronal damage was not demonstrable. In cross sections of the vagus nerve, the endoneurium was thickened.

Lesions were not observed in the other tissues examined.

### Discussion

The ruminal atony caused by  $\alpha$ -ethoxyethylglyoxal dithiosemicarbazone was at least partially attributable to myelin and axonal degeneration which occurred in the vagus nerve. The tympanites gradually became worse as the duration of rumen atony lengthened, and the calves finally succumbed to severe, chronic tympanites. Because  $\alpha$ -ethoxyethylglyoxal dithiosemicarbazone has antimicrobial activity, the possibility cannot be overlooked that the compound might also have inhibited the ruminal flora and caused a disturbance in normal bacterial and protozoal activities of the rumen and thereby contributed to the cause of the tympanites. The experimental compound caused signs and lesions only when it was administered in sustained doses. Adverse effects were not observed in calves given the drug 2 or 3 times at 2-day intervals.

The drug is prepared as a suspension of small particles which are birefringent. The fine suspension, when administered

intravenously, may have occluded vessels and caused temporary cerebral anoxia or the compound may have bound electrolytes, such as calcium or magnesium, and caused the immediate temporary collapse. The suspension apparently acted as a foreign substance similar to India ink in eliciting the generalized hyperplasia of lymphocytes and reticulo-endothelial cells seen in most organs.

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