CHARACTERIZATION OF MONENSIN

POISONING IN THE

HORSE

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PREFACE

This study characterizes monensin toxicosis in the horse. Clinical signs and physiological parameters were closely monitored in four horses given various dosages of monensin. The most obvious degenerative changes occurred in the myocardium. The changes noted on necropsy examination, histopathologic and electron microscopic examinations are described. These descriptions will be helpful in diagnosing and in developing treatment regiments for monensin poisoning in horses. The toxicity of monensin to horses based on this study is compared to previously reported values.

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CHAPTER I

THE RESEARCH PROBLEM

Monensin is a metabolite of the fungus <u>Streptomyces cinnamonesis</u>. The fungus was first cultured in 1964 from an Arizona soil sample (1). Shumard and Callender (2) reported on the anticoccidial activity of monensin in 1967. Monensin was made commercially available as a poultry coccidiostat in 1971 and is sold under the trade names of Coban[R][1] and Elancoban.[R][2] In recent years monensin has had about 80% of the poultry coccidiostat market in the United States.

The effect of monensin on ruminal fermentation was reported in 1974 (3) (4). Monensin as a feed additive was shown to increase feed efficiency in cattle 10 to 15%. Subsequently monensin was introduced as a feed additive for cattle under the trade name of Rumensin.[R][3] An estimated 60 million cattle are fed monensin annually.

A warning was issued in 1975 to veterinarians and users of Elancoban[R] that the product may be hazardous to horses. The product label was changed to include the statement: "DO NOT FEED TO HORSES OR OTHER EQUINES - MAY BE FATAL."

The oral LD_{50} of monensin for horses is approximately 2 mg/kg (1).

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[1]Coban[R] - Monensin, Elanco Products Company, Indianapolis, IN.

[2]Elancoban[R] - Lilly International, Indianapolis, IN.

[3]Rumensin[R] - Monensin, Elanco Products Company, Indianapolis, IN.

This compares with an oral LD_{50} for cattle of approximately 22 mg/kg, 17mg/kg for swine, and 200 mg/kg for chickens (37). The reasons for such variation in species susceptibility is not completely understood. Concentrations of monensin used in medicated poultry feed, premixes for cattle feed, and in cattle feed supplements are great enough to cause death in horses when consumption of small to moderate amounts occurs. The mechanisms of action of monensin poisoning in horses is not known. The extensive use of monensin as a feed additive and coccidiostat makes the likelihood of accidental exposure to horses great. This makes monensin a hazardous compound for horses.

An effective treatment for monensin poisoning has not been found and few reports describing symptomatology, pathology and diagnosis have appeared in the literature.

This study was undertaken to investigate monensin poisoning in horses. The project includes clinical observations, hematologic and clinicopathologic analyses of blood and serum, electrocardiographs, necropsy, histopathologic and electron microscopic studies of tissues of experimental horses given varied dosages of monensin, and control horses. It was believed that such a study would result in a better understanding of monensin toxicosis in horses.

CHAPTER II

REVIEW OF THE LITERATURE

Monensin is an antibiotic that inhibits gram positive bacteria and is effective in controling coccidia in chickens. It belongs to the class of antibiotics called polyether carboxylic ionophores.

Ionophores In General

Ionophores have been defined as compounds possessing the ability to form lipid-soluble complexes that provide a vehicle for cations to traverse lipid barriers such as biological membranes (5). Harned et. al. (6) characterized the first ionophore (Nigericin) in 1951. The unique ion-transporting properties of the ionophores were discovered seredipitously by Moore and Pressman (7) (8) (9) (10) in 1964. They investigated the inhibition of mitochondria by guanidine derivatives and found that the oxidative phosphorylation uncoupling action of valino- mycin (an ionophore derived from a Streptomyces fungus) was different from that of classical uncouplers such as dinitrophenol. Valinomycin produced a much greater acidification of the mitochondria containing medium than could be explained by the hydrolysis of adenosine triphos- phate (ATP). When a potassium selective electrode was inserted into the medium, loss of K^+ was approximately equivalent to the quantity of hydrogen ions that appeared. Valinomycin apparently catalyzed an energy linked K^+ for H^+ exchange. With increased K^+ concentration within the

mitochondria there was a corresponding increase in mitochondrial volume and rate of respiration (11).

Ionophores have been used extensively to study ionic transport of biological membranes (11) (7) (5) (12) (13) (14) (15). The rate of complexation, membrane-transport, decomplexation exceeds that of most macromolecular enzymes and is in the range of hundreds-to-thousands per second (12). With a specific ionophore, ion specificity ratios observed in membrane transport studies depends upon the choice of membrane. In studies of the K+ transport rate across mitochondrial versus erythrocytic membranes, valinomycin was a highly specific transporter of K+ across both membranes. However, the erythrocytic turnover rate was two orders of magnitude lower than the value obtained when rat hepatocytic mitochondria were used (12). Differences observed were believed to be due to decreased mobility of the complex in erythrocytic membranes due to their high cholesterol content or some absorption (and thus inactivation) of the ionophore to hemoglobin or membrane proteins (12).

Naturally occurring ionophores have recently been isolated from mitocondria of bovine myocardium (5). Ionophores may be the universal charge-separating species used by all energy-coupling systems (5).

Ionophores are of moderate molecular weight (200-2000). They form complexes which are highly specific for certain cations (K+, Na+, Ca++, Mg++) and amines (norepinephrine, serotonin, dopamine, epinephrine).

Ionophoric molecules in general consist of a backbone of diverse structures that contain strategically spaced oxygen atoms (7). The structure is capable of assuming a shape in solution that orients oxygen atoms centrally to form a hydrophilic cavity capable of complexing with cations whose diameter and charge are compatable with that of the

cavity. Various hydrocarbon groupings are located on the exterior of the molecule. The solubility characteristics of ionophores are due to the lipid solubility of peripheral hydrocarbon groups and, because such groups shield the polar interior of the molecule, delocalization of the cation charge occurs (7). In the case of carboxylic ionophores, neutrality of the complex is partially due to the cancellation of the central cationic charges by the negative charge of the carboxyl group (16).

Ionophores are presently being used to study the mechanisms of oxidative phosphorylation (7) (17). Investigation of the erythrocytic transport of ions across membranes utilizing ionophores has established that the transport of Cl^- and presumably other anions as well, is mediated through exchange-diffusion carriers and not through channels as was previously believed (11).

Effects of Ionophores on Cardiac
and Skeletal Muscle

Ionophores specific for various cations and amines have been shown to have profound effects on cardiac and skeletal muscle (18) (19) (20) (21) (22). A calcium ionophore known as X-537A caused rapid release of accumulated calcium from vesicles of the sarcoplasmic reticulum and had marked contractile effects when applied to isolated preparations of skeletal, cardiac and vascular smooth muscle at uM concentrations (18).

An ionophore known as A204, which was obtained from the fermentation of the fungus Streptomyces albus, has a primary myotoxic effect on skeletal and cardiac muscles (19). Clinical signs of neuromuscular toxicosis in dogs prompted Todd et al. to administer A204

to rats. The substance was given by gavage at doses of 2, 4, and 8 mg/kg. Significant histopathologic lesions were found in skeletal and cardiac muscle. The most extensively damaged muscles were those with the highest level of activity, such as, the diaphragmatic, abdominal and cardiac muscles. Twenty-four hours after a single toxic dose, affected muscle fibers were swollen and had an increased eosinophilia and homogenous-staining cytoplasm. Later, affected fibers were more numerous. Fragmentation, vacuolation and loss of cross striations was evident. By 48 hours post-treatment, a cellular infiltrate that consisted mostly of macrophages was evident. Cardiac damage produced by A204 were similar to those described in potassium-depleted rats (19). Further study of A204 led to the conclusion that A204 blocks re-entry of cellular K+ (22).

A calcium ionophore known as Ro 2-2985 (X537A) had the following effects on the cardiovascular system (21): When administered intravenously at a rate of 1 mg/kg to Beagles, Ro 2-2985 produced a significant increase in cardiac output. Total body venous compliance was decreased and there was a significant increase in heart rate and mean arterial pressure. A significant decrease in total peripheral resistance was also observed. Ro 2-2985 altered aortic distensibility allowing the accommodation and delivery of larger volumes of blood without altering arterial pressures. Thus it increases myocardial efficiency with only a slight increase in oxygen consumption.

In 1972 it was observed that divalent cationic polyether ionophores were potent inotropic agents (5). This inotropic effect was ascribed to the release of myocardial Ca++ ions. A single dose (2 mg/Kg i.v. in anesthetized dogs) of lasalocid (a calcium ionophore) caused a two-fold

increase in contractility, doubled the cardiac output, slightly increased blood pressure and heart rate, decreased peripheral resistance and decreased coronary resistance. Lasalocid was found to transport such amines as serotonin, dopamine, norepinephrine and epinephrine as well as Ca++. Thus, it possibly affected the cardiovascular system by altering membrane permeability to amines and/or Ca++.

The relative inotropic activity of six monovalent cation ionophores in decreasing order of potency is: A-204A, X-206, monensin, dianemycin, nigericin, lasalocid (5). The mechanism of this inotropic effect is not fully understood, but it cannot be explained entirely on the basis of Ca++ and catacholamine transport because monensin and X-206 do not transport either species across membranes in vitro.

Other Uses of Ionophores

Ionophores have been used in chemistry to dissolve highly reactive inorganic compounds in solvents of low polarity (7). Such use can tremendously accelerate saponification, oxidation and reduction reactions of certain organic compounds.

Ionophore-doped membranes form the basis of many ion-selective electrodes (7). The best glass-based K+ selective membranes available show a 20:1 preference for K+ over Na+. Valinomycin-based electrodes exhibit 4000:1 K:Na selectivity preference.

Calcium ionophores have been used to study the calcium dependent release of histamine from isolated mast cells (7). Calcium induced exocytosis appeared to be a general mechanism for release of cellular secretory products by promoting fusion of secretory granules with plasma membranes.

Properties of Monensin

Possible Effects of Monensin on Mitochondria
and ATP Production

Adenosine triphosphate (ATP) serves as an "energy currency" which can be used to power the various functions of cells. In eukaryotic cells, oxidative phosphorylation of adenosine diphosphate (ADP) to form ATP takes place only in the mitochondrial membrane. The reaction of carbohydrates with oxygen to yield energy, carbon dioxide, and water in biological systems is called respiration. In mitochondria, hydrogen is extracted from carbohydrates and is delivered to nicotinamide adenine dinucleotide (NAD+) in the citric acid cycle.

Nicotinamide adenine dinucleotide hydride (NADH) the reduced form of NAD+ is the intermediary between the citric acid cycle and the enzymes within the inner mitochondrial membrane. These enzymes, known as the respiratory chain eventually deliver electrons to oxygen, forming water and ATP. How the transfer of electrons through the respiratory chain is coupled to oxidative phosphorylation is not fully understood. The chemiosmotic theory was proposed by Peter Mitchell (17) in 1961. He suggested that the flow of electrons through the respiratory chain resulted in an electrochemical proton gradient across mitochondrial membranes. The proposed gradient consisted of a difference of hydrogen ion concentration (pH) and a difference in electric potential. The phosphorylation of ADP was driven by a reverse flow of protons down the gradient. The outer mitochondrial membrane is permeable to hydrogen ions allowing passive diffusion of H+ to the cytoplasm.

Mitochondria require K+ for phosphorylation of ADP to ATP to occur

(15). The uptake of potassium is an energy dependent reaction. The calculated total free energy of K+ transport approximates the maximum free energy available to mitochondria through hydrolysis of ATP (7). The immediate source of energy for K+ transport is not ATP because K+ transport is not blocked by inhibition of ATPase. This suggests that the two processes are closely coupled. Monensin transports Na+ and K+ ions with an in vitro affinity for Na+ over K+ of 10:1. Monensin's transport of ions across erythrocytic membranes results in a complexation affinity of 5:1 Na+:K+ (23). Thus, the degree to which monensin favors complexation with Na+ over K+ varies with the type of membrane under investigation.

Monensin in vitro inhibits the uptake of K+ by hepatocytic mitochondria of rats (15). The inhibition may be overcome by higher potassium concentrations. Inhibition of K+ uptake was attributed to inhibition of the energy-linked mitochondrial ion pump by monensin-alkali-cation complexes. Monensin's inhibition of K+ transport results in inhibition of ATP hydrolysis and substrate utilization.

Monensin also has been shown to catalyze the release of K+ for H+ in de-energized mitochondria, erythrocytes, and in microsomes of the brain.

In summary, monensin interferes with the transport of alkaline cations across mitochondrial membranes. The exact mechanism of monensin's interference of mitochondrial metabolism is not fully understood.

Effects of Monensin on the Cardiovasculature

Pressman (7) considered monensin to be the most promising of the

carboxylic ionophores for use in cardiovascular applications. The intravenous administration of monensin to anesthetized dogs at the rate of 5-25 ug/kg increased coronary blood flow by 3-5 times (maximum coronary dilation) and decreased total peripheral resistance (24). The response of the coronary blood flow was dose dependent in the 5-100 ug/kg range. Decreased total peripheral resistance was primarily due to the vasodilatory action of monensin. Low doses of monensin (5 and 10 ug/kg i.v.) reduced aortic blood pressure while 25, 50 & 100 ug/kg i.v. caused a marked elevation of aortic blood pressure. Monensin increased cardiac output at all doses tested. Heart rate remained essentially normal throughout the experiment. Duration of increased cardiac output was dose dependent with doses of 5 and 10 ug/kg being transient while doses of 25 ug/kg and larger produced increased cardiac output lasting 5 to 120 minutes.

Coronary blood flow is regulated by a number of factors including oxygen demand resulting from increased heart rate and contractility. Coronary dilatation is thought to be mediated by local release of vasoactive substances such as adenosine, and/or prostaglandins. The vasodilator action of monensin was not blocked by antagonists of histamine, acethylcholine, isoproterenol or inhibitors of prostaglandin synthesis (24). Thus, it was believed that the effect of monensin on coronary blood flow was due to direct relaxation of blood vessels (24).

Monensin's positive inotropic effect may be explained by the antibiotic's ability to transport Na+ across myocytic membranes. Normally low intracellular Na+ concentrations are maintained by a Na+-K+ pump located in the myocytic membrane. Increases in intracellular Na+ leads to an increase in intracellular Ca^{+2}. Although the mechanism for

contractility of cardiac muscle is not completely understood, it is known that increases in intracellular Ca^{+2} result in increased con-tractility. The positive inotropic effect of monensin was only partially blocked by large doses (5 mg/kg IV) of propranolol, a blocker of $Beta_1$ and $Beta_2$ adrenergic receptors (24). This suggested the inotropic action of monensin was in part due to a nonadrenergic mechanism.

Tetraodontoxin, a compound that blocks sodium channels in membranes, had no effect on the positive inotropic action of monensin. Thus, the results obtained from administration of propranolol and tetraodontoxin to cardiac muscle under the influence of monensin lends further support to the theory that monensin's inotropic effect is the result of increased intracellular Na^+ concentrations. These results were believed to be due to monensin's ability to transport Na^+ across myocytic membranes (24).

The positive inotropic effect of monensin has also been demonstrated in guinea-pig and cat myocardia and in rabbit atria (25).

Other Biological Effects of Monensin

Monensin was first reported to be moderately active against gram-positive organisms and certain mycocobacteria by Haney et al. (26) in 1967. Monensin reduces the production of lactic acid and the concomitant drop in rumen pH in cattle fed high grain rations (27). Monensin is effective in controlling coccidiosis in chickens at a dosage of 110 grams per ton of feed (2).

Hammond, Carlson et al. (28) studied the effect of monensin on the conversion of tryptophan to 3-methylindole. In the rumen, L-tryptophan

(a naturally occurring constituent of some forages) is converted to indole-acetic acid and then to 3-methylindole. When absorbed into the systemic circulation in sufficient quantities 3-methylindole produced lesions characteristic of bovine atypical intersitital pneumonia (AIP). Monensin, as a feed additive, reduced the formation of 3-methylindole in vivo and in vitro. Thus, monensin might be of some value in preventing some forms of bovine AIP.

Monensin, as a feed additive, increased feed efficiency of feedlot cattle (4). Monensin alters ruminal microflora by selecting for succinate-forming bacteria that produce proportionally larger amounts of proprionate from carbohydrates and correspondingly less acetate, butyrate and methane. When carbohydrates are converted to acetate and subsequently to butyrate, a pyruvate carbon is converted to methane. The energy associated with breaking the C-C bond in pyruvate is wasted. Thus, any compound that shifts the composition of ruminal volatile fatty acids to greater amounts of proprionate and lesser amounts of acetate and butyrate conserves energy and increases feed efficiency. The energy savings associated with a 10 moles/100 moles increase in ruminal proprionic acid is approximately 5.5%. Monensin increased the molar proportion of propionic acid by approximately this amount. However, monensin increases feed efficiency by 10-15% (4). Thus, monensin apparently acts in other ways to produce an additional 7-9% increase in feed efficiency. It is believed that monensin might increase feed efficiency by lowering the heat increment, sparing protein that would normally be used in gluconeogenesis, stimulating protein synthesis, changing the composition of ingesta reaching the small intestine and increasing the extent of digestion (4).

Witt et al. (27) found an increased efficiency of 4.8% in Oklahoma feedlot cattle receiving monensin supplementation. This increase in feed efficiency is more in line with the theoretical increase mentioned above. This study involved six feeding trials, 716 cattle, and 16 different rations. Although increased feed efficiency was not as great as that reported by Raun et al. (10-15%), it was concluded that monensin supplementation was economically advantageous.

Monensin Tissue Distribution and Metabolism

The distribution of monensin in the tissues of several species was studied by the use of monensin that had been labeled with tritium or carbon-fourteen (29) (3) (30). The route of administration in each study was oral. Radioactivity levels in tissues of chickens were less than 2% of the total dose of radioactivity administered as tritiated (^{3}H) monensin (3). Ninety-eight percent of remaining radioactivity was recovered from feces and urine. Most of the radioactivity of the tissues was associated with the water of the tissue indicating metabolic breakdown of tritiated monensin. Tissue levels of radioactivity dropped rapidly after withdrawal of medication.

Radioactive monensin (^{14}C) was administered orally to steers and fecal and urinary radioactivity was monitored daily (30). At least 75% of the dose was excreted in the feces within three days. All radioactivity excreted was in the feces; none was detected in urine. The following tissues were collected from steers fed radioactive monensin and were analyzed for carbon-14: liver, muscle, fat, kidney, heart, lung, spleen and blood. Liver was the only tissue that contained radioactivity levels greater than those of untreated controls. The

quantity of radioactivity in liver was the equivalent of 0.59 ppm monensin. The liver was then assayed and found to contain 5-15 ppb monensin. This indicated that 97+% of the radioactivity in the liver was incorporated within metabolites of monensin.

In a comparative study of the metabolism of monensin in monogastric animals (rats) versus ruminants (steers), rats metabolized more than 95% of orally administered (^{14}C) monensin whereas steers metabolized 50-60% of the monensin (29). The difference was assumed to be due to differences in absorption of monensin. A large number of metabolites were produced by both species. Absorbed monensin was excreted entirely in bile. Bile from a steer fed [^{14}C] monensin contained radioactivity equivalent to 14 ppm monensin. By fractionation of the bile, 97% of the radioactivity was found to be associated with monensin metabolites while only 3% consisted of unaltered monensin.

Chemical Properties of Monensin

Monensin was the first polyether antibiotic whose molecular structure was determined (31). The molecular formula of monensin is $C_{36}H_{62}O_{11}$ (molecular weight 670) (32). The sodium salt has a molecular formula of $C_{36}H_{61}O_{11}$ Na and a molecular weight of 692.4 (32). It is further characterized by the absence of ultraviolet absorption. Monensin is somewhat unique because the acid and salt forms are readily extracted from water by organic solvents (26). Monensin and its sodium salts are slightly soluble in water (0.1 mg/ml), and are more soluble in hydrocarbons. Monensin is stable in alkalis and unstable in acids. Donoho et al. (29) investigated the extent of degradation of monensin in the alimentary canal of ruminants by incubating monensin for 2 days in

cattle feces (37°C); for 5 hours in ovine abomasal fluid (38°); and overnight in ruminal fluids from steers (38°C); at least 90% of the monensin was recovered in all cases. Thus, monensin was not extensively degraded in the alimentary canal of ruminants.

The pKa of monensin is 6.65 in 66% N,N-dimethylformamide. Transformations involving the hydroxyl groups bring about considerable change in the pKa as exemplified by the acetylation of primary and secondary hydroxyl groups resulting in a pKa of 7.6.

Monensin is extracted with methanol from the fermentation broth of cultures of Streptomyces cinnamonensis. It is obtained as a mixture of at least four related molecular species differing only by a few CH_2 groups (16). Monensin (Monensin A) is predominant. Stark, Nox and Westland (33) were able to increase monensin yields from 150 mg/ml of broth to 5000 mg/ml by selecting certain optimum strains of the fungus, increasing aeration of the broth, optimizing temperature of the culture, adding selected minerals to the medium and by supplementing the medium with certain oils.

The monensin molecule is a carbon chain with a terminal carboxylic group. Five oxygen atoms form five and six membered rings in the carbon chain. Branching off the carbon chain are seven methyl-, one ethyl-, three alcohol-, and one methoxy- groups.

Monensin complexes with the following cations in order of decreasing affinity: Na, K, Rb, Li, Cs. The rate of complexation to sodium compared to potassium is about 10:1, Na:K. The radius of monensin's hydrophilic cavity allows it to complex with monovalent cations whose diameter is within the range of 1.0 to 1.45A^o and slightly beyond (16).

CHAPTER III

MATERIALS AND METHODS

Six healthy horses of different breed and of different sexes, 3 males and 3 females, 2 to 10 years of age were used in this study. The horses were wormed with 2 gm/100 pounds body weight thiabendazole one week before monensin dosage and allowed to acclimatize in new quarters for 30 days. The horses were fed Omalene^R [1] mixed grain diet (0.75 lb/100 lb of body weight) and prairie hay free choice. Blood was drawn from the jugular vein for complete blood counts (CBC) and serum chemistry tests 24 hours before monensin administration.

Complete physical examinations and electrocardiograms (ECG) were done 24 hours before dosing. Horses 1, 2, and 3 were given single doses of monensin. Monensin was administered _via_ stomach tube in a premix (60 grams monensin/pound of premix) mixed with water to make a slurry. The horses were dosed according to the following schedule.

        Horse #1   -   25 mg monensin/kg body weight

        Horse #2   -   5 mg monensin/kg body weight

        Horse #3   -   2 mg monensin/kg body weight

Horse #4 was given monensin in the feed (318 mg of premix containing 60 gm monensin/pound of premix) twice daily for 30 days. The

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[1] Ralston Purina Company: Checkerboard Square, Saint Louis, Missouri 63188.

monensin containing premix was mixed by hand into 3.5 pounds of grain ration at each feeding. This diet provides a daily dosage of monensin of approximately 0.3 mg/kg. Prairie hay was available free choice. Horses #5 and 6 were used as controls for histologic and ultrastructural studies.

Horses 1, 2, and 3 were observed constantly after dosing. The following observations were recorded hourly: Packed cell volume (PCV), total protein, pulse rate, rectal temperature, respiratory rate, mucous membrane coloration, mucous membrane capillary refill time, and presence or absence of borborygmus. Clinical signs were recorded as they occurred. Electrocardiograms were recorded shortly after clinical signs commenced and at an advanced stage of the poisoning episode. Blood collected from the jugular veins in EDTA and plain tubes was submitted for complete blood count (CBC) and serum emzyme chemistry tests every four to six hours after clinical signs became evident. The following serum enzyme chemistry tests were performed: total bilirubin, direct bilirubin, blood urea nitrogen (BUN), calcium, chloride, creatine phosphokinase (CPK), glucose, lactic dehydrogenase (LDH), magnesium, phosphorus, potassium, protein, serum aspartate aminotransferase (AST) , sodium and triglyceride.

Complete necropsies were performed immediately after each horse expired. Horses 4, 5, and 6 were euthanatized with Sleepaway ^{R} [2] I.V. Brain and spinal cords were fixed in 30% formalin. Other tissues were fixed in 10% formalin.

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[2]Sleepaway^R: Fort Dodge Laboratories, Fort Dodge, Iowa, 10501

Tissues collected for electron microscopy were excised, sectioned and immersed in fixative within 5 to 10 minutes after death. Myocardium, diaphragm, liver and kidney were cut in 1 mm squares and immersed in an adequate volume of buffered glutaraldehyde (10 mg 8% glutaraldehyde, 30 ml .27 M cacodylate buffer, .5 ml acrolein, .02 gm $CaCl_2$, 10ml distilled water, pH adjusted to 7.4 with 1 N HCl, osmolarity = 500) for 2 hours. The tissues were then washed twice in buffer, washed twice in water, postfixed in 2% OsO_4 for 1½ to 2 hours, washed thoroughly in water (four washes for 1 hour), stained in 1% aqueous uranyl acetate overnight at $60^{o}C$, dehydrated and embedded in epoxy with the following schedule:

1.	50% ETOH	15 minutes
2.	70% ETOH	15 minutes
3.	90% ETOH	15 minutes
4.	95% ETOH	15 minutes
5.	100% ETOH	15 minutes
6.	100% ETOH	15 minutes
7.	100% ETOH	15 minutes
8.	Propylene oxide	15 minutes
9.	Propylene oxide	15 minutes
10.	Propylene oxide	15 minutes
11.	1:1 propylene oxide and EPON	4 hours
12.	1:1 propylene oxide and EPON	overnight

13. Embed in 100% EPON -- place in vacuum oven at $60^{o}C$ for approximately 48 hours.

Thick sections were examined by light microscopy. Areas of the

blocks were selected for thin sectioning. Viewing was done with a Philips 200 transmission electron microscope[3].

Tissues fixed in buffered formalin for examination by light microscopy were blocked preparatory to processing and embedment, washed in six baths of absolute Cellusolve[4], three baths of toluene and 3 baths of paraffin. Tissue washing was done in a Lipshaw Trimatic[5], automatic tissue processor. Tissues were then embedded in paraffin, sectioned with a microtome and stained with Hematoxylin and Eosin with a Ames Histotech Automatic Slide Stainer[6]. The following tissues were examined by light microscopy: cerebrum, cerebellum, spinal cord, pituitary, thyroid, esophagus, thymus, prescapular lymph node, lung, heart, mediastinal lymph node, diaphragm, stomach, duodenum, jejunum, ileum, cecum, dorsal colon, adrenal gland, kidney, urinary bladder, spleen, pancreas, liver, and choroid plexus.

The following samples were collected for monensin analysis: urine (when available), kidney, liver, stomach content, small intestine content, cecal content and feces. Samples were analyzed for monensin by the thin layer chromatography and spectrophotometry method as described by Golab (9).

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[3]Philip's Electrical Instruments Inc., 85 McKee Dr., Mahwah, NJ 07430.

[4]Cellsolve, McKesson Chemical Co. Crocker Plaza, One Post Street, San Franscisco, California, 94104.

[5]Lipshaw Trimatic, Lipshaw Manufacturing Corporation, Detroit, Michigan, 48210.

[6]Ames Histotech, Miles Laboratories, Elkhart, Kansas.

CHAPTER IV

RESULTS

Clinical Signs

Horses #1, 2, and 3 received single oral doses of monensin at 25, 5 and 2 mg/kg respectively. Onset of clinical signs occurred at 4 to 6 hours and did not appear to be dose related at the levels administered.

Figure 1. Horse #2 (5 mg/kg monensin per os) with
patchy sweating.

Refusal of grain was the earliest sign observed, however, horses

continued to eat prairie hay until 4-8 hours before death. Intermittent patchy sweating began 5-9 hours after treatment, occured first in the axillary, inguinal and tail head regions and rapidly became more profuse and generalized (see Figure 1).

Intermittent ataxia began 5 hours after dosing in horses given 25 and 2 mg/kg, however the horse given 5 mg/kg did not have ataxia until 17 hours post-treatment. Early ataxia was characterized by swaying of the hindquarters and overextension of the hind limbs when walking. Periods of normal gait intervened between periods of locomotor difficulty. Affected horses fell against the side of the pen and then regained their balance. Ataxia progressed until the horses became recumbent.

While in lateral recumbency, horses would periodically sweat profusely and would look at their sides as if suffering mild abdominal pain. No kicking at the abdomen or attempts at rolling were observed. The horses then unsuccessfully attempted to rise and pawed with their front feet while in lateral recumbency. Horses died approximately 3 hours after becoming terminally recumbent.

Heart rates doubled to 80-90 per minute four to six hours after dosing and remained elevated until death. Respiratory rates remained normal until a few hours before death when the respiratory rate tripled (80 respirations/min.) Intestinal motility was variable throughout the poisoning episode with borborygmus being present in about half of the hourly examinations.

Capillary refill time remained normal until shortly before death when mucous membranes of the mouth became muddy and capillary refill time increased.

The horse that received 25 mg/kg died 31 hours after receiving monensin, the other horses that received single oral doses died 33 hours after dosing.

The following clinical signs were noted in horse #4 that received monensin in the feed at 30 ppm for 30 days. The heart rate, respiratory rate, temperature, color of mucous membranes, capillary refill time, and intestinal motility remained normal throughout the 30 day feeding period. Although appetite was only slightly depressed throughout the trial, horse #4 lost 54 pounds. On days 19 and 29, the horse became ataxic and fell against the side of the pen. Ataxia, primarily in the hindquarters, continued for about 15 minutes. Patchy sweating occurred with each bout of ataxia.

Clinicopathologic Observations – Single Oral Dose

The packed cell volume of all horses that recieved a single oral dose of monensin increased moderately about ten hours after dosing and remained elevated until death (Table I).

The white blood cell count (WBC) reflected a stress response with a mild to moderate leukocytosis due to neutrophilia, lymphocytopenia and eosinopenia. The total white blood cell count (WBC) increased by 29 to 43% nine hours after dosing and remained elevated until death (Table I).

Total bilrubin increased to above normal limits (normal 0.0 – 2.7 mg/100 ml) about 24 hours after dosing and reached a high of 3.3 mg/100 ml, 4.4 mg/100 ml and 2.9 mg/100 ml in horses #1, 2 and 3 respectively. Direct bilirubin increased only slightly and remained within normal limits (0.0 – 0.4 mg/100 ml) throughout the toxic episode.

TABLE I

BLOOD PARAMETERS OF HORSES GIVEN A SINGLE ORAL DOSE OF MONENSIN

	Parameter		Time (hrs)			
			0	9	24	29
Horse #1:	Total WBC[a]	(Cells/mm^3)	9200	11900	11400	9300
(25 mg/kg)	Neutrophils	(Cells/mm^3)	5244	8568	10374	8184
	Lymphocytes	(Cells/mm^3)	3312	2975	570	897
	PCV (%)[b]		36	36	54	46
Horse #2:	Total WBC[a]	(Cells/mm^3)	14900	21300	18200	17300
(5 mg/kg)	Neutrophils	(Cells/mm^3)	6407	16827	15834	14532
	Lymphocytes	(Cells/mm^3)	6854	3195	1638	2076
	PCV (%)[b]	(Cells/mm^3)	34	50	40	41
Horse #3	Total WBC[a]	(Cells/mm^3)	9600	13900	14600	14200
(2 mg/kg)	Neutrophils	(Cells/mm^3)	5856	11954	12410	12780
	Lymphocytes	(Cells/mm^3)	3552	1807	2190	1278
	PCV (%)[b]	(Cells/mm^3)	36	38	38	42

[a] White Blood Cell Count

[b] Packed Cell Volume

Blood urea nitrogen (BUN) increased only slightly reaching above normal levels (normal 10-20 mg/100 ml) in one horse (5 mg/kg monensin per os) with a high of 36 mg/100 ml at 28 hours post dosage.

Serum calcium levels remained unchanged in the horse given 25 mg/kg monensin per os. Horse #2 (5 mg/kg monensin per os) had a slight decrease in serum calcium with a low of 8 mg/100 ml eight hours after dosing (normal 11.2 - 13.6 mg/100 ml). Horse #3 (2 mg/kg monensin per os) had a decrease in serum calcium from 10.3 mg/100 ml to 9.3 mg/100 ml which then returned to 10.4 mg/100 ml late in the toxic episode.

Serum chloride concentrations decreased to slightly below normal (normal 99-109 mg/100 ml) in all horses given single oral doses of monensin with a low of 88 mg/100 ml in horse #3 (25 mg/kg monensin per os) seven hours after dosing. Generally, serum chloride concentrations were maintained in the 90 to 100 mg/100 ml range throughout the experiment.

Serum creatine phosphokinase (CPK) concentrations increased to 200-400 IU/L (normal 2.4 - 23.4 IU/L) in all horses about eight hours after administration of monensin and remained elevated until death.

Serum glucose concentrations increased to 150 - 300 mg/100 ml (normal 60 - 110 - mg/100 ml) in all horses six hours after dosing and remained in this elevated range until death.

Serum lactic dehydrogenase (LDH) concentrations increased slightly to 250 - 500 IU/L (normal 41-104 IU/L) six hours after dosing and remained high until death. The horse that recieved 25 mg/kg monensin per os had a high serum LDH concentration of 1600 IU/L twenty-four hours after dosing.

Serum inorganic phosphorous concentrations were variable with horse #3 (2 mg/kg monensin per os) having a gradual decrease in serum inorganic phosphorous to 1.7 mg/100 ml (normal 3.1 - 5.6 mg/100 ml) while horse #2 (5 mg/kg monensin per os) had a gradual increase in serum phosphorous concentrations from 3.8 mg/100 ml to 5.1 mg/100 ml. Serum inorganic phosphorous concentrations remained within normal limits for horses #1 and #2.

Serum potassium concentrations remained within normal limits (2.4 - 4.7 meq/1) for all horses recieving single oral doses of monensin.

Serum total protein concentrations gradually increased throughout the toxic episode but only exceeded normal limits (5.7 - 7.9 mg/100 ml) in one horse (25 mg/kg monensin per os) when it reached a serum concentration of 8.6 mg/100 ml approximately eighteen hours after dosing.

Serum aspartate aminotransferase (formerly glutamic-oxalacetic transaminase SGOT) concentrations increased slightly above normal (normal 58.0 - 94.0 IU/L) levels and remained in the 100 - 200 IU/L range.

Serum sodium concentrations generally remained within normal for all horses throughout the experiment.

Clinicopathologic Observations
Thirty Day Feeding

Complete blood counts and serum chemistries were analyzed at three day intervals throughout the thirty-day feeding trial.

The packed cell volume varied from 28% to 37% throughout the experiment. The white blood cell count ranged from 10,500 WBCs to 16,000 WBCs/mm^3. Total bilirubin and blood urea nitrogen remained

within normal limits. Serum calcium ranged from low normal (normal 11.2 - 13.6 mEq/L) to slightly below normal with a low of 9.7 mEq/L on day 26. Serum chloride ranged from low normal (normal 99–109 mEq/L) to below normal with a low of 93 mEq on day 3. Serum CPK ranged from 33 IU/L to 120 IU/L (normal 2.4 - 23.4 IU/L) with the highest value recorded on day 1 taken before the experimental feed was introduced. Serum glucose concentrations generally remained within normal lmits. Serum LDH concentrations ranged from 270 IU/L on day 1 (before treatment) to 90 IU/L on day 6 but were generally above normal limits (normal 41 - 104 IU/L). Serum inorganic phosphorous, potassium and protein remained normal throughout the experiment. Serum aspartate aminotransferase (AST) concentrations ranged from normal (58 - 94 IU/L) to a high of 160 IU/L) on day 24. The pretreatment AST concentration was also elevated (160 IU/L).

Electrocardiographic Observations

Electrocardiograms were taken prior to monensin administration and again during clinical episodes.

Horses given single oral doses of monensin had prolonged atrial and ventricular depolarization and repolarization when compaired to predose electrocardiograms. The prolonged intervals were, however, within normal limits. Increased S wave amplitude was also noted.

Horse #3 (2 mg/kg monensin per os) had an abscence of P and increased T waves after monensin administration.

Horse #4 (30 day feeding trial) had prolonged atrial and ventricular depolarization with slightly prolongated repolarization.

Necropsy

Horses number 1, 2, and 3 died thirty-one to thirty-three hours after dosing. Lesions in the myocardium were similar in all three horses on gross necropsy examination. There was a diffuse pale streaking throughout the ventricular myocardium and the myocardium tore easily. The myocardium had increased firability. Other changes observed on gross necropsy examination, not consistently seen in all three horses, included: accentuated hepatic lobular pattern (horse 1), pulmonary congestion and edema with froth in the air passages (horse 2), shrunken, firm spleen (horse 1, 2) and ecchymotic hemorrhage in the left caudal lung lobe (horse 3). No changes were noted in horse number 4 (given 30 ppm monensin in the feed for 30 days) on gross necropsy examination.

Histopathology

Histopathologic changes were noted in the heart and liver of all horses given monensin. Changes seen consistently in the myocardia of horses given monensin were: swollen and shrunken myocardial fibers with dense acidophilic cytoplasm, loss of longitudinal and cross striations, granulation of the sarcoplasm of affected cells, foci of myocytic necrosis, and intracytoplasmic vacuolization with minimal-to-moderate inflammatory reaction. These changes were evenly scattered and involved approximately 5% of myocytes. In addition to the above described changes, horse #3 (2 mg/kg) had neutrophilic foci associated with myonecrosis (Figure 2). Although horse 4 was given low doses of monensin in the feed for thirty days, the changes noted in the myo-cardium on histopathologic examination are characteristic of an acute

degenerative process. These changes probably occurred during the bout of ataxia and sweating on day 29.

Changes noted on microscopic examination in the liver of horses 1, 2, and 3 were similar and consisted of central and midzonal vacuolization of hepatocytes (fatty change). Hepatopathy in horse 4 (given 30 ppm monensin in the feed for 30 days) consisted of hepatocytic swelling and vacuolization with irregular granulation and reticulation of hepatocytic cytoplasm. There was mild bile duct proliferation.

Swollen and shrunken myocytes with increased eosinophilic staining and loss of cross striations were present in diaphragmatic muscles of horses 1, 2 and 3. These changes were less frequent and less severe than those noted in the myocardium.

Other histopathologic findings noted in some but not all experimental horses include: necrotic cellular debris in germinal centers of lymph nodes and in splenic Malpighian corpuscles (horses 2 and 3), focal pulmonary alveolar and septal edema (horses 2 and 3), and mild changes in the kidneys (horses 1 and 2) consisting of occassional dilated collecting tubules containing pink proteinaceous material and a few erythrocytes, congestion of glomerular capillaries and vacuolization of proximal tubular epithelial cells. In addition, horse 2 had nephrosis of approximately five percent of the distal tubules. Horses number 3 and 4 had numerous eosinophils in the submusosa of the cecum and colon.

Ultrastructural Changes

Ultrastructural changes were noted in the myocardium of all experimental horses. Lesions were not observed in the tissues of control horses. In experimental horses cardiac mitochondria were
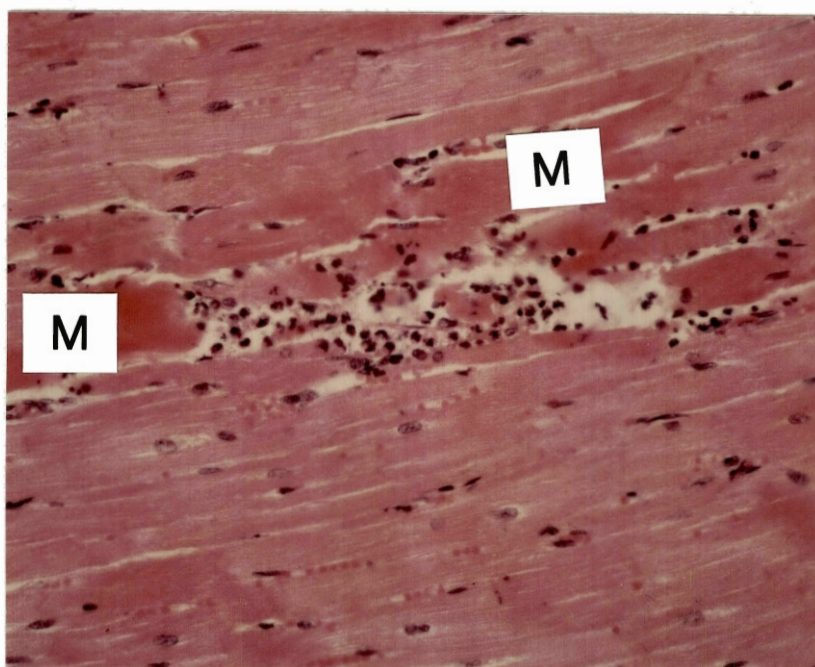
Figure 2. Light micrograph of left ventricle myo-
cardium from experimental horse #3 (2 mg/kg mon-
ensin per os) with degenerative myocytes (M),
densely acidophilic cytoplasm, myonecrosis and
inflammatory cell infiltrate.

frequently enlarged 2 to 5 times normal size (Figure 4). Cristae of enlarged mitochondria were often fragmented. There was decrease in the electron density of the matrix of mitochondria. Vesicles were frequently found between cristae and in some cases lipids internally replaced degenerated cristae and ground substance (Figure 5). Fragmentation of myofibrils was noted in the myocardia of experimental horses with occasional associated disruption of myofibrillar organization (Figure 5).

Hepatocytes of experimental horses had mitochondria that were occasionally moderately to markedly enlarged (2 to 4 times normal size) with decreased matrix density and fragmentation of cristae. There was a slight to moderate increase in the number of peroxisomes in experimental horse hepatocyes (Figures 8,9). Changes in the mitochondria of hepatocytes were most evident in the horse that received monensin daily for 30 days. Lipid droplets were present in the hepatic cytoplasm of horse #2 (5 mg/kg monensin).

Significant ultrastructural changes were not noted in the kidney and diaphragm of experimental horses.
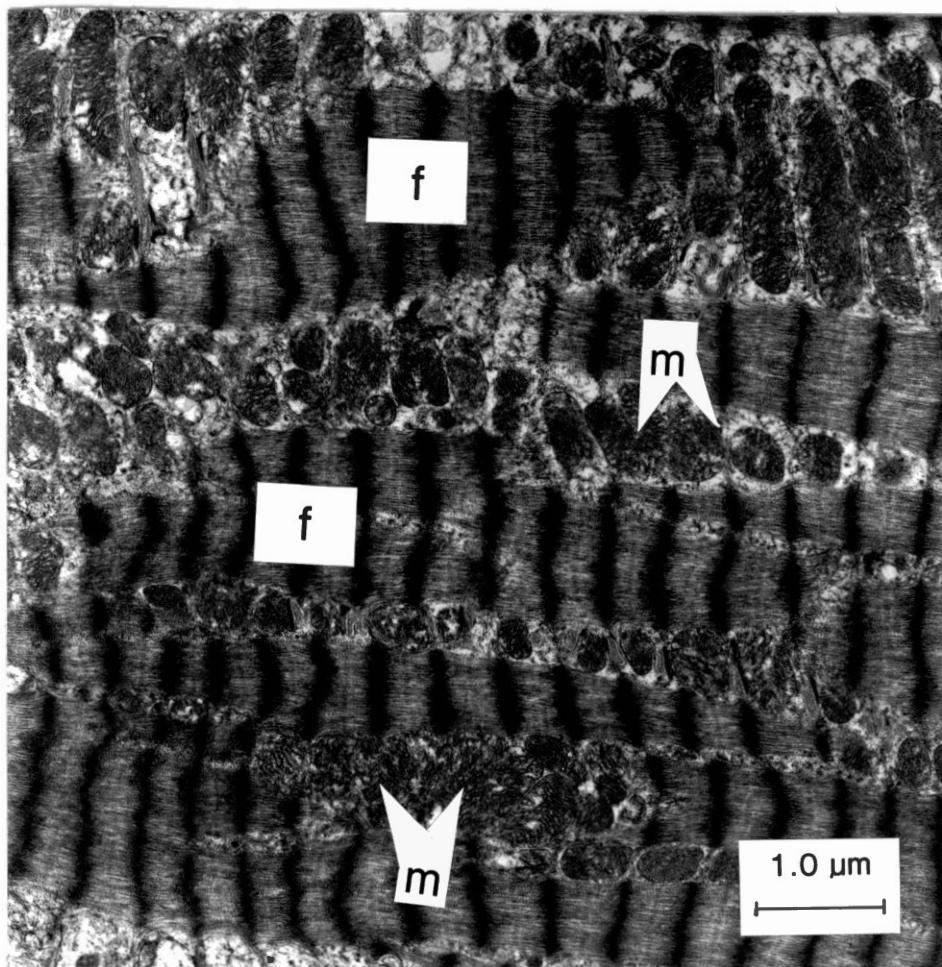
Figure 3. Electron micrograph of a cardiac myocyte from a
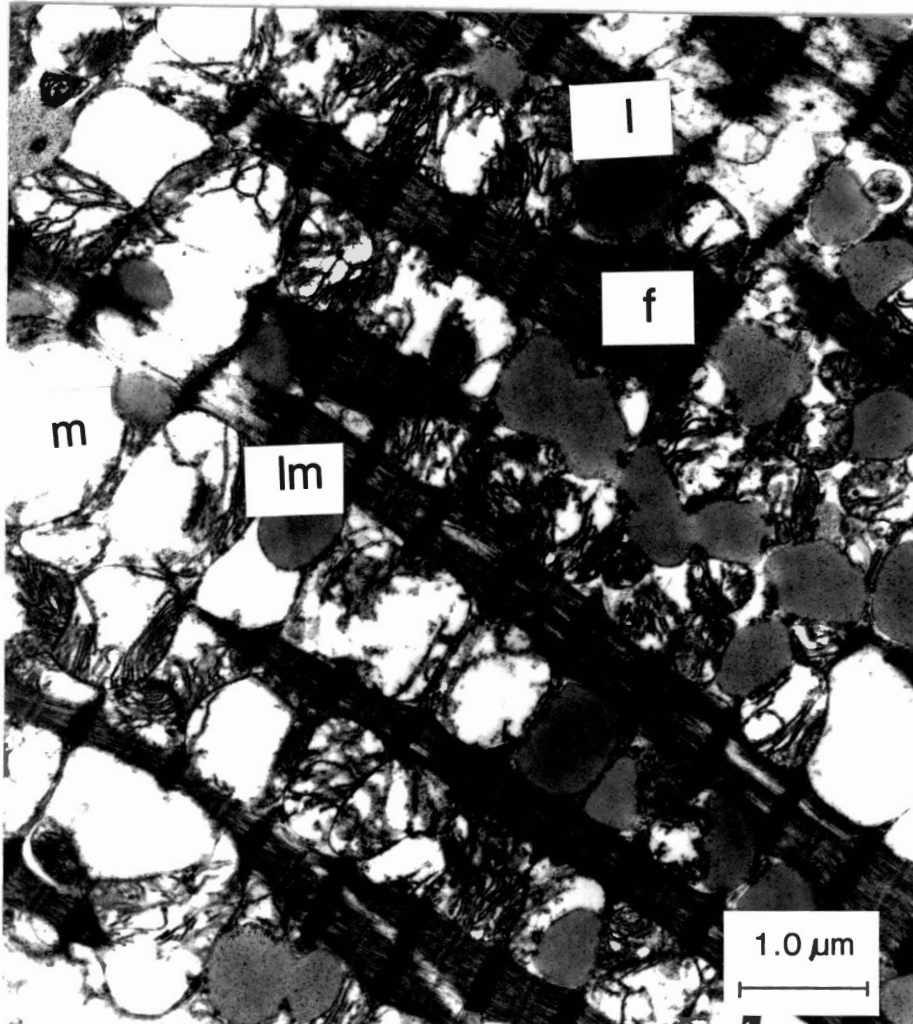   control horse with mitochondria (M) and myofibrils (f)
   being evident.

Figure 4. Electron micrograph of a cardiac myocyte from
  experimental horse #3 (2 mg/kg per os) with mitochondria
  with enlarged cristae and loss of matrix, lipid vesicles
  (l), and lipid replacement of mitrochondrial matrix (lm),
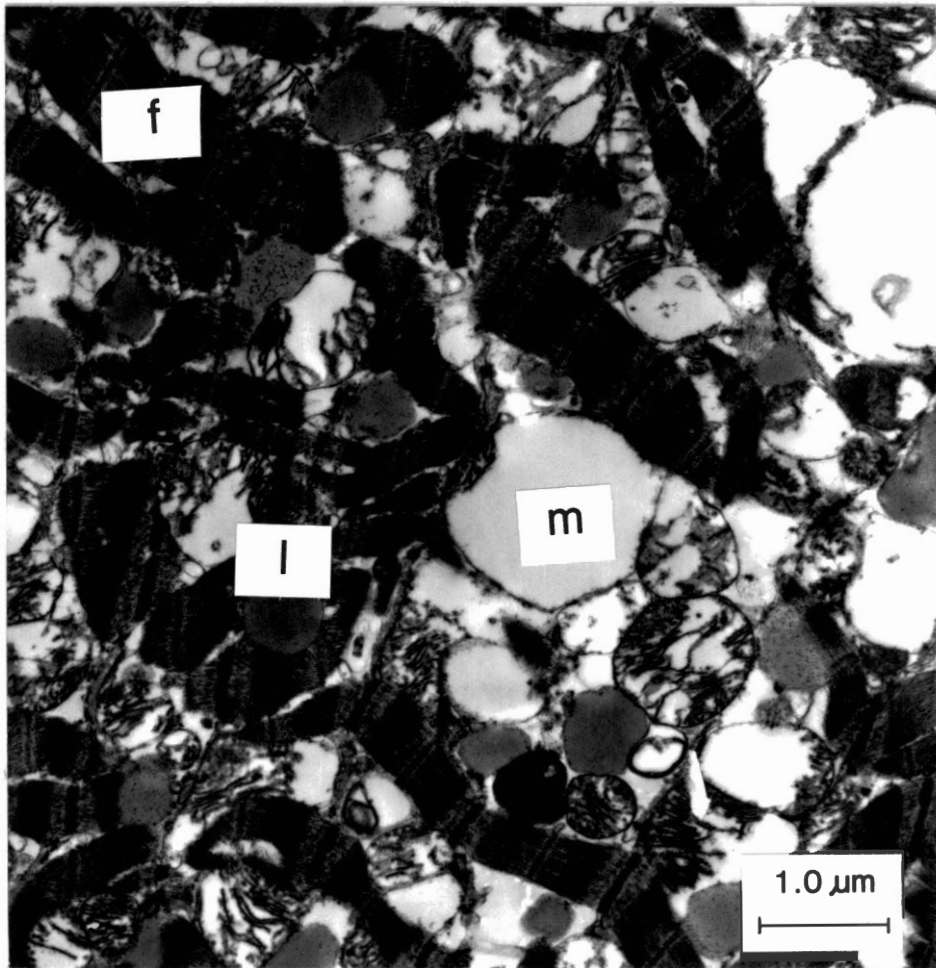 and fragmentation of myofibrils (f).

Figure 5. Electron micrograph of a cardiac myocyte from
   experimental horse #3 (2 mg/kg per os) showing disruption
   of myofibril organization in addition to the changes
   noted in figure 2. Mitochondria (m), myofibril (f),
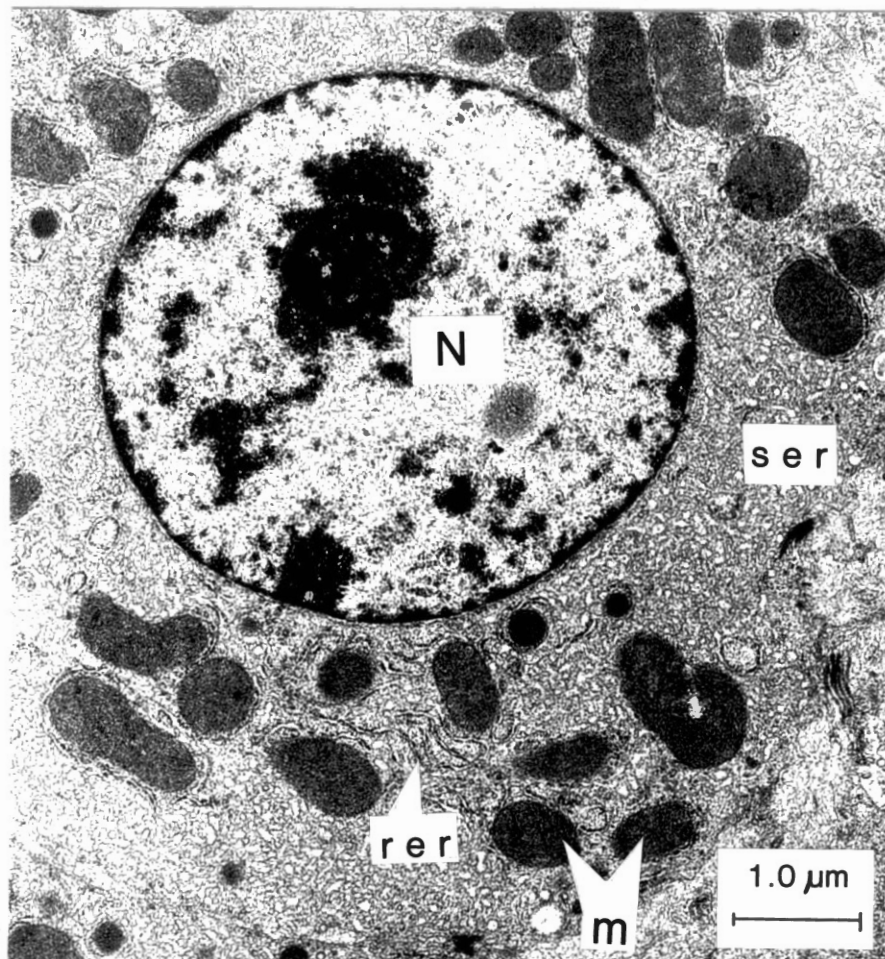   lipid vesicle (l).

Figure 6. Electron micrograph of a hepatocyte from an
untreated horse used as a control. Nucleus (N),
mitochondria (m), smooth endoplasmic reticulum (ser),
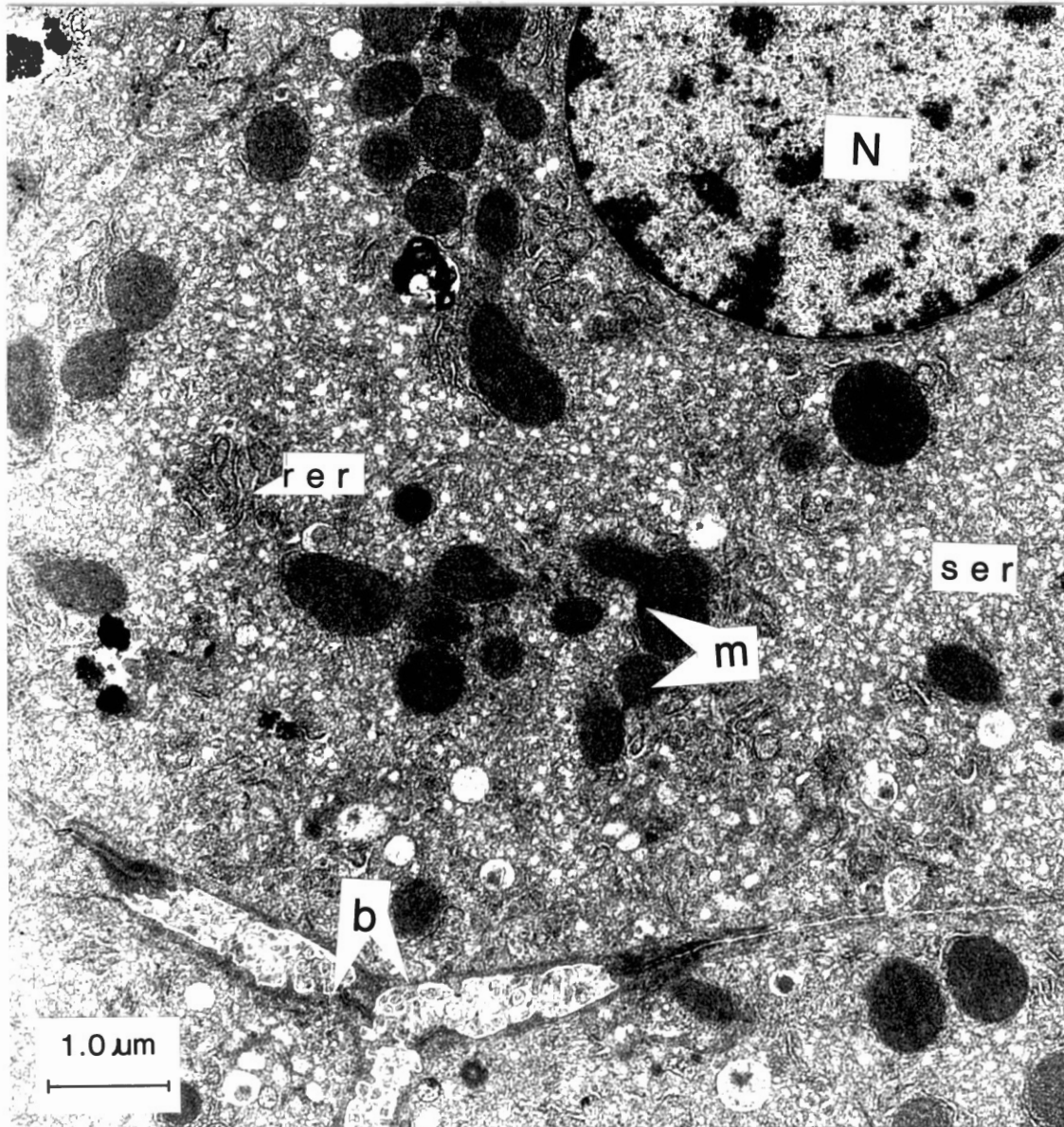rough endoplasmic reticulum (rer).

Figure 7. Electron micrograph of a hepatocyte from an untreated horse used as a control. Nucleus (N), mitochondria (m), smooth endoplasmic reticulum (ser), rough endoplasmic reticulum (rer), bile caniliculus (b).
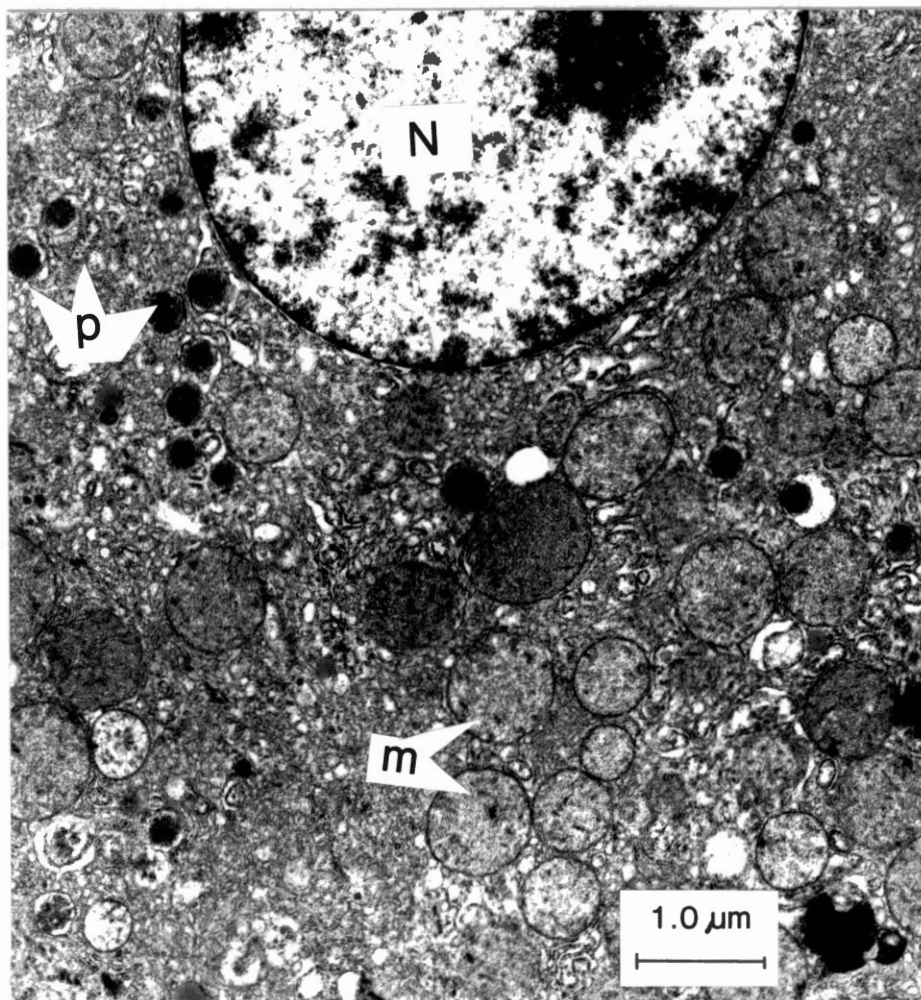
Figure 8. Electron micrograph of a hepatocyte from horse #3 (2 mg/kg monensin per os) with decreased electron density of mitochondria matricies (m) and indistinct cristae, and peroxisomes (p).
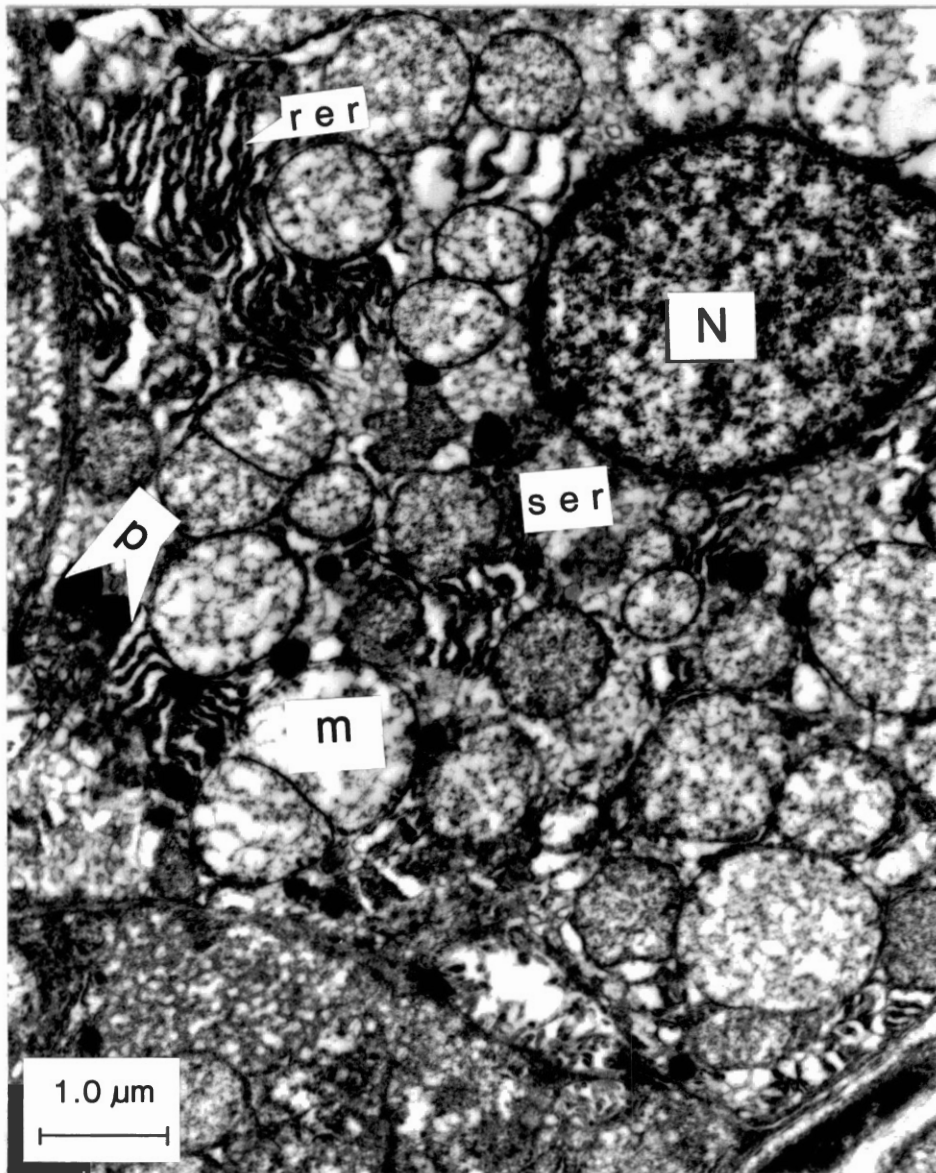
Figure 9. Electron micrograph of a hepatocyte from horse
#4 given monensin in the feed at 30 ppm for 30 days.
Note enlarged mitochondria (m) with indistinct cristae,
and peroxisomes (p). N (nucleus), smooth endoplasmic
reticulum (ser), rough endoplasmic reticulum (rer),
biliary caniliculus (b).
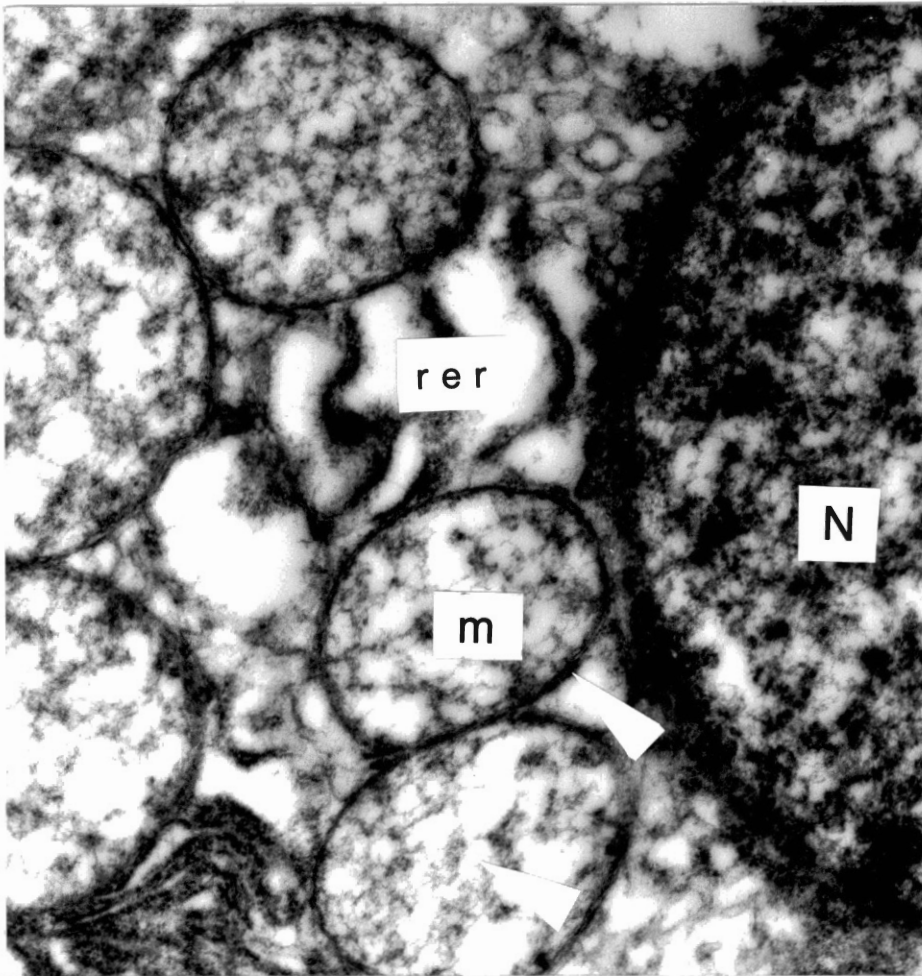
Figure 10. Electron micrograph of same hepatocyte as
  Figure 9 with greater mignification showing bilaminar
  membrane (arrows) of mitochondria (m), fragmentation of
  cristae (arrow heads), rough endoplasmic reticulum
  (rer), nucleus (N).

Toxicology

The following tissues and samples were analyzed for monensin with the following results:

In horse #1 (25 mg/kg) no monensin was detected in urine, kidney, liver or in contents of small intestine. Contents of the stomach contained 0.8 ppm monensin and contents of cecum contained 1.7 ppm monensin.

Monensin was not detected in contents of the stomach and cecum from horse #2 (5 mg/kg).

Monensin was not detected in contents of cecum from horses #3 (2 mg/kg) and #4 (30 ppm in feed for 30 days).

CHAPTER V

DISCUSSION

Monensin is an ionophoric antibiotic that is effective as a feed additive in cattle and as a coccidiostat in poultry. Monensin is highly toxic to most mammals and is especially toxic to horses (53) (54) (55) (56) (57) (58) (59). As an ionophore it has the ability to penetrate biologic membranes and to alter ion concentrations thus adversely affect intercellular and intracellular ionic gradients (60) (61) (62) (63) (64) (65).

The clinical signs observed in three horses given single oral doses of monensin were similar to those previously described in the literature (1) (34) (35) (37) (38).

The horse that received monensin in the feed at 30 ppm for 30 days, had signs similar to those of acute monensin toxicosis on two occasions. This is the first report of clinical signs, other than partial anorexia, associated with this low dose (0.3 mg/kg per os daily).

An oral LD_{50} of 2-3 mg/kg was estimated for monensin in horses from early studies at Eli Lilly Research Laboratories (39). This study was based on ten horses given various levels of monensin in the feed ranging from 33 ppm to 331 ppm. Because of the cost of horses, statistically significant numbers of animals were not tested in order to precisely determine the oral LD_{50} of monensin. It should be noted from this study that at least some horses have significant clinical signs and morpho-

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logic changes when fed approximately the same concentration of monensin that is recommended for cattle (33 ppm).

Clinicopathologic changes were nonspecific and generally mild-to-moderate. Horses that received single oral doses of monensin had leukograms that were indicative of stress of mild intensity.

Only one horse had a mild increase in blood urea nitrogen (BUN) above normal values. Matsuoka (34) considered a BUN of 21.5 mg/100 ml significant and correlated this concentration of BUN with the toxic tubular nephritis that was observed by histopathologic examinations of tissues from horses dosed with monensin. Amend et al. (40) noted that BUN concentrations may be elevated to twice normal in severely affected animals. Based on our study and those noted above, it is the authors opinion that horses suffering from monensin toxicosis will have a normal-to-slightly elevated BUN.

Serum calcium concentrations decreased mildly in two of the three horses given single oral doses of monensin. This was in agreement with the findings of Amend (40) who noted that serum calcium levels initially decline by 1 - 2 mg/dl following monensin ingestion.

Serum creatine phosphokinase (CPK) concentrations increased significantly in all horses given single oral doses of monensin. In studies of CPK isoenzymes by Amend et al. (40), CPK isoenzyme elevations were determined to be the result of skeletal muscular involvement with little contribution of cardiac or brain isoenzymes. Their findings were somewhat surprising because the lesions in the heart in the present study were more severe and the heart is believed to be the primary target organ of monensin toxicosis in the horse. Myopathy of skeletal musculature was present in the study at hand, however, the lesions were

microscopically less severe than those observed in the hearts of affected

horses.

Serum lactic dehydrogenase (LDH) concentrations also increased significantly in horses that received single oral doses of monensin. Isoenzyme studies of LDH by Amend et al. (40) had substantial increases in those fractions associated with cardiac muscle and erythrocytes with little disturbance of the LDH-isoenzymes arising from the liver or skeletal muscle.

Serum potassium concentrations remained within normal limits in our study in contrast to that of Amend et al.(40) who described a transient hypokalemia with serum potassium declining by 1 - 2 mEq/liter in the first 24 hours after dosing.

A gradual increase in serum total protein was observed throughout the toxic episode and this correlated well with the mild-to-moderate increase in packed cell volume (PCV) (see table I). This was believed to be due to hemoconcentration. Amend et al.(40) stated that ponies poisoned with monensin suffered a peracute crisis of hypovolemic shock that was secondary to toxic nephrosis. In the study at hand, changes in BUN, PCV, serum total protein concentration and microscopic evaluation of the kidneys suggest mild to moderate hemodynamic changes and mild degenerative changes in the kidneys with occasional nephrosis. It is doubtful that the renal changes indicate nephrosis of significant proportions.

Serum aspartate aminotransaminase (AST) increased mildly. Because this enzyme is located in the mitochondria and cytoplasm of cardiac and skeletal myocytes as well as in hepatocytes, one might expect a more

dramatic increase in AST as was noted in studies by Amend et al. (40). It should be noted that Amend et al.(40) used ponies rather than horses as were used in this study and the dosages used by Amend et al.(40) were not stated in the literature.

In the present study, electrocardiographic changes due to intoxication of horses by monensin were interpreted by veterinary cardiologist Gerard Rubin (41) as being due to right heart strain or electrolyte abnormality. Amend (40) and Whitlock (1) described "S-T" segment depression as being indicative of myocardial injury. Francene Mallone (42) and Amend (40) noted "T"-wave depression suggestive of hypokalemia. "T"-wave depression was not noted in our study but an increased amplitude of the "T"-wave was observed in horse #3 (2 mg/kg monensin/os). This was suggestive of hyperkalemia. This apparent conflict in results might be explained by the results of studies by Smith et al. (43) wherein small doses of monensin resulted in increased intracellular potassium concentrations in 3T3 fibroblast cell cultures while higher doses of monensin caused a net loss of potassium from the cells.

Most of the dramatic and easily observed changes at gross necropsy examination were nonspecific and suggestive of agonal death. Changes noted in the heart of horses that received single oral doses of monensin were subtle and diffuse but were nevertheless suggestive of significant myocardial degeneration. The myocardium of affected horses had a diffuse pale streaking throughout, was excessively friable, and could be easily torn. The changes in color were similar to those that develop soon after death in otherwise normal animals as a result of postmortem autolysis.

The most remarkable change noted on histopathologic examination of tissues from affected horses was diffuse myocardial degeneration. The degeneration was easily detected because of the presence of shrunken myocytes with dense acidophilic cytoplasm, loss of longitudinal and cross striations, loss of Cohnheim's fields, granulation and vacuolization of sarcoplasm with minimal-to-moderate inflammatory reaction. These diffuse cardiac changes were considered to be nonspecific because they can be observed in other conditions such as cobalt induced cardiomyopathy (45), isoproterenol-induced myocardial necrosis and Cassia occidentalis toxicosis. (46). Cardiomyopathy was not noted in initial studies by Matsuoka (39) who considered toxic hepatitis and toxic tubular nephritis to be the primary morphologic changes resulting from monensin poisoning in horses. In subsequent studies by others myocardial degeneration has been observed (37) (40) (1).

Changes noted in the livers of horses given single oral doses of monensin were mild and nonspecific vacuolization of hepatocytes. Changes noted in the horse given monensin in the diet for 30 days were also mild and consisted of mild granulation of hepatocyte cytoplasm with mild hyperplasia of bile ducts. These changes in the liver were in contrast to the findings of others who have reported severe liver degeneration and necrosis in horses suffering from monensin poisoning (34) (37). However, Amend et al. (40) concluded that the toxic effects of monensin in the liver were minimal.

Only mild changes were noted microscopically in the kidneys of the two horses that were given the largest doses of monensin. Matsuoka (39) and Amend et al. (40) implied that renal impairment was of major

importance in monensin poisoning of horses and ponies respectively. It is my opinion that renal changes are mild and are only a contributory factor in monensin poisoning in horses.

Significant ultrastructural changes were noted in the myocardia of all experimental horses and consisted of enlargement of mitochondria with fragmentation of cristae, and fragmentation and disruption of myofibrillar organization. In studies of ultrastructural changes in ponies poisoned with monensin, Mollenhauer et al. (36) noted similar changes in mitochondria but found no change in myofibrils. Mitochondria are among the most sensitive of cellular organelles and frequently have the changes noted above when cells undergo degenerative processes. Myofibrillar degeneration is also a nonspecific change characteristic of diffuse myocardial degeneration. Initial changes at the centers of experimentally induced ischemic infarcts were mitochondrial swelling and dilatation of tubules of the sarcoplasmic reticulum with no change in myofibrils (44). Changes in the ultrastructure of the myocardium that were similar to those found in this study have been reported from a variety of cardiotoxins including cobalt poisoning (45), thiamine deficiency, alcoholism, and Cassia occidentalis toxicosis (46), reserpine, digitalis, and poisoning resulting from the antimalarial drug plasmocid (47).

It is interesting to note that degenerative changes were seen at the ultrastructural level in all cardiac myocytes examined from treated horses while light microscopic changes were noted in only approximately 5% of myocytes. Cardiac myocytes examined by electron microscopy were not selected on the basis of having degenerative changes at the light microscopic level. This suggests that many, if not most, cardiac

myocytes were undergoing ultrastructurally visible degenerative changes at the time of death.

Ultrastructural changes were observed in the livers of all experimental horses. Abnormalities were not observed in livers of control animals. The most consistent changes were enlargement of mitochondria with decreased matrix density and cristae distortion. An increase in peroxisomes was also observed. Mollenhauer et al. (36) found that the most obvious changes in hepatocytes of ponies poisoned with monensin were increased smooth endoplasmic reticulum and decreased rough endoplasmic reticulum. Increased numbers of peroxisomes were also observed by Mollenhauer et al.(36). Peroxisomes are small membrane-bound dense bodies that originate from the endoplasmic reticulum and contain catalase and urate oxidase enzymes. It has been proposed that peroxisomal enzymes oxidize hydrogen peroxide formed during cellular injury and thus protect the cell from further injury (47). The exact function of peroxisomal enzymes is unknown although they have been associated with metabolism of lipids, cholesterol and steroids, and gluconeogenesis. Most changes noted in hepatocytes of affected horses involved distortion of membranes of mitochondria with infrequent rupture of membranes. Membrane distortion without rupture was interpreted as a reversible change.

Residues of monensin were found only in stomach contents and in cecal content of horse #1 (25 mg/kg monensin/os). The analysis was performed by the official AOAC method of Golab (9). This method is colorimetric and is qualitative for monensin in feeds. Although this method has been shown to identify as little as 1 ug. of monensin, it has not been validated for levels of monensin below 90 grams per ton. The

most precise method of quantitation of monensin requires microbiologic plate or turbidimetric assays (48) (10) (8) (49) (50) (51) (52). Tissue analysis and analysis of gastrointestinal contents from horses with suspected monensin poisoning would be of value although there is a high potential for false negatives. The presence of any monensin in such samples would indicate exposure and would strongly support a diagnosis of monensin toxicosis that was based only on history, feed analysis, and histopathologic evaluation.

Studies of metabolites of monensin in rats and steers suggest that monensin is well absorbed in rats (95% metabolized) and steers (50-60% metabolized) (29) (30). Quantitatively, monensin and it's metabolites are found primarily in the feces. Bile contains the next greatest concentration of monensin followed by liver. Ninety-seven percent of the monensin detectable in the liver and bile is in the form of metabolites of monensin. Neither radioactively labeled monensin nor its metabolites have been found in other tissues. It was believed that these findings suggest that monensin is well absorbed in mammals (especially monogastrics), and that most of the monensin that is absorbed is removed from the circulation by the liver ("first pass effect"). Monensin thus is absorbed, metabolized, and secreted into the intestine via the biliary system. Studies of metabolism wherein toxic doses of monensin were used have not been reported for any species. It is possible that toxic doses of monensin overload the ability of the liver to remove monensin from the splanchnic venous system thus allowing access to the heart and general circulation. The extreme sensitivity of the myocardium to monensin was demonstrated by studies of Saini et al. (24) who found maximum dilatation of coronary arteries in dogs after

intravenous injection of only 5 ug/kg monensin. Doses of 25 to 100 ug/kg monensin by the intravenous route produced increased blood pressure, cardiac output and cardiac contractility. If the "first pass effect" is the protective mechanism that prevents ingested monensin from reaching the general circulation, it is interesting to speculate that inhaled monensin might gain access to the general circulation via the pulmonary circulation and have an adverse effect on the myocardium of persons who handle monensin daily such as employees of feed mill operators. It is also interesting to note that the Food and Drug Administration (FDA) has classified monensin as a class-I feed additive which requires only minimal handling precautions.

The tissue distribution, rate and extent of metabolism and excretion of radioactively labeled monensin in rats, steers, and chickens, suggests that, at pharmacologically active doses monensin does not accumulate to a significant degree in tissues. Thus, toxicosis as a result of "cumulative effect" from chronic ingestion of small amounts of monensin does not appear to be likely. It is likely, however, that repeated episodes of mild monensin poisoning could lead to scarring of the myocardium and eventual congestive heart failure. Single nonfatal episodes of monensin poisoning could also result in subsequent heart failure if the extent of myocardial damage was such that the remaining cardiac tissue could not compensate.

Treatment for monensin toxicosis aimed at eliminating monensin from the gastrointestinal tract (gastric lavage, activated charcoal, mineral oil) and supportive cardiovascular therapy that includes fluids in moderation should be used. Horses that survive an acute episode of monensin toxicosis should be rested in a stall for 6 to 8 weeks to curtail cardiac activity.

CHAPTER VI

SUMMARY

In this study horses were given varied toxic doses of monensin and
were monitored noting clinical signs, clinical chemistry, hematology,
electrocardiographic changes, gross necropsy, histopathology, ultra-
structural changes and toxicologic analyses. Clinicopathologic findings
included: leukograms indicative of stress, mild decreases in serum
calcium concentrations, increases in serum creatine phosphokinase
concentrations, increases in lactic dehydrogenase concentrations,
increases in serum total protein, packed cell volumes, and mild in-
creases in serum aspartate aminotransaminase concentrations. Electro-
cardiographic changes indicated right heart stress or electrolyte
abnormality. On gross necropsy examination changes were seen only in
the heart. Diffuse pale streaking throughout the myocardium was found
and the myocardium was excessively friable. The principle organs that
had light microscopic lesions were the hearts and livers. There was
diffuse necrosis of the myocardia and hepatocytes were mildly
vacuolated. Changes seen with electronmicroscopy in the hearts were
enlargement of mitochondria with fragmentation of cristae, and
disruption and fragmentation of myofibrils. Changes found in
examination of the ultrastructure of hepatocytes were enlargement of
mitochondria with distortion of cristae, and increased numbers of
peroxisomes. Toxicologic analyses of various tissues and contents of

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the gastrointestinal tracts were performed. Residues of monensin were found only in the gastrointestinal tract of one horse.

The results of the study at hand were compared to those of similar studies found in the literature. Included were findings from the literature on studies of mechanisms of action of monensin in cells of mammals. From information derived from the results and information found in the literature I have speculated on the toxicodynamics of monensin poisoning in horses. I have tried to present the results of this study in proper perspective to provide the reader with an accurate and thorough understanding of monensin poisoning in horses and to aid in making an accurate diagnosis of monensin poisoning in horses.

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