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# Efficacy and toxicity of dl-methionine and methionine hydroxy analogue as urinary acidifiers in cats

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Efficacy and toxicity of  
dl-methionine and methionine hydroxy analogue  
as urinary acidifiers in cats

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by

Joseph William Denhart

A Thesis Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
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Department: Veterinary Pathology  
Major: Veterinary Pathology  
(Veterinary Toxicology)

Signatures have been redacted for privacy

Iowa State University  
Ames, Iowa

1990

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## INTRODUCTION

Acidifying agents are used orally as dosage forms or as feed additives in cats to aid in the prevention of formation in the urine of magnesium ammonium phosphate hexahydrate crystals. These crystals are commonly called struvite. Struvite is the most frequently involved mineral in feline uroliths that occur in feline urologic syndrome (FUS). The crystals are more soluble in acid urine and will dissolve at low urinary pH.

An amino acid, dl-methionine, has been used for many years as an aid in treatment of urolithiasis and as a prevention of further occurrence of struvite crystal formation by the acidification of the urine.

While the hydroxy analogue of methionine (MHA) has been used as a dietary replacement for the amino acid in humans and animals, it has not been used specifically as a urinary acidifier in cats. If calcium methionine hydroxy analogue (CaMHA) can safely be used as a urinary acidifier it may have an advantage when compared to methionine since production of ammonia cannot occur from MHA, and therefore, MHA will not contribute to the body burden of ammonia.

The purpose of this study was to characterize and compare the acute toxicities in cats of dl-methionine and calcium methionine hydroxy analogue at three different doses

given for a seven day treatment period. An untreated control group of animals was included in the study. Confirmation of the effectiveness of the two drugs as urinary acidifiers in cats and an estimate of the lowest effective dose were also objectives.

Alteration of the composition of feline urine by diet in order to prevent FUS should be done without inducing harmful side effects. Therefore, the results of this efficacy and acute toxicity study may be used as a basis for a long term study of CaMHA as a feed additive in the diets of cats as an alternative to dl-methionine.

## LITERATURE REVIEW

### Definition, Causes, and Therapy of FUS

The feline urologic syndrome (FUS) has long been recognized as a disorder of the lower urinary tract of cats. The term feline urologic syndrome is now considered synonymous with feline lower urinary tract disease and represents a variety of disorders.<sup>1</sup> Clinical signs of FUS generally include hematuria, dysuria, and crystalluria. The causes may be single, multiple and interacting, or unrelated. It is, therefore, a multifactorial disease syndrome, which is often recurrent in its nature. The roles of diet, etiological agents and other predisposing factors have not been completely elucidated.<sup>2,3,4</sup> Bacterial infection is rarely an initiating cause of FUS,<sup>5,6</sup> but may be a complication as a result of instrumentation used in treating the clinical condition<sup>7</sup> and may be involved in the recurrence of FUS.<sup>8</sup> A herpes virus has been suggested as a cause of FUS.<sup>9</sup> Others have found after intensive studies that if viruses contribute to FUS their role is minor.<sup>6,10</sup> A nutritionally balanced dry diet was found to be unlikely as a cause of FUS.<sup>11</sup> A review of epidemiological studies that have been conducted on FUS also concluded that no single factor is entirely responsible for all cases.<sup>12</sup> One survey

of households containing cats determined the incidence of FUS in the cat population of the United States to be 0.85%.<sup>13</sup>

Feline urolithiasis, while commonly used as a synonym for FUS, is one of the subsets of causes of lower urinary tract disease.<sup>1,2,3,4</sup> Epidemiological studies of feline uroliths have shown that 88 percent of naturally occurring uroliths were composed of at least 70 percent struvite and that 68 percent were composed of 100 percent struvite. It was also found that 94 percent of urethral plugs were composed primarily of struvite and 4 percent were composed of matrix only. The implication is that only struvite is of great clinical importance because of the infrequency of non-struvite minerals in urethral plugs.<sup>14</sup> A five year update of this study published in 1989 revealed a continuation of the finding that struvite is the principal mineral of uroliths and urethral plugs in cats.<sup>15</sup> Struvite is magnesium ammonium phosphate hexahydrate ( $MgNH_4PO_4 \cdot 6H_2O$ ). Microscopically, the crystals appear in cat urine as flattened prisms with variations in size and dimension (squares or rectangles). Struvite crystals may develop a feathery appearance as they go into solution.<sup>16</sup> It has been suggested that the primary cause of FUS is the presence and effects of struvite calculi or crystals in the feline urinary tract.<sup>17</sup> It has been emphasized that detection of crystalluria is not synonymous with urolithiasis.<sup>16</sup> Even



though FUS has heterogeneous causes, the one major factor in the syndrome is the formation of struvite calculi.<sup>1,2,3,18,19</sup>

Struvite crystals are generally present in larger amounts in alkaline urine.<sup>20,21,22</sup> They are more soluble in acid urine and tend not to develop as urinary pH is reduced to below 6.6.<sup>19,20,23,24</sup> The concentration of phosphate in the urine depends heavily on the pH, since as the pH increases, the phosphate concentration increases. Magnesium concentration increases by a lesser amount for a like pH change than does phosphate. An increase in ammonium ion concentration, which occurs as pH declines, decreases struvite solubility. Therefore, pH plays a dominant role in struvite crystallization.<sup>25</sup> Urinary pH increases postprandially, and it has been postulated that this fluctuation may be a triggering factor in cats which predisposes them to FUS.<sup>26</sup> The postprandial alkaline tide occurred specifically if the cats were fed once daily and not given food free choice throughout the day.<sup>20</sup> Another study demonstrated a postprandial rise in urinary pH occurred four hours subsequent to the resumption of feeding cats after a twenty-hour fast. The rise in pH occurred in cats on a dry diet with and without 1.6% ammonium chloride, which is a commonly used urinary acidifier in cats. The rise in pH did not occur in those cats being fed a moist diet.<sup>27</sup> A study

that compared the effects of periodic and continuous feeding schedules determined that the cats fed periodically had their lowest urinary pH at the times the urinary mineral concentrations were the highest. This finding was thought to be a possible minor advantage of a morning periodic feeding schedule over continuous access to food. This study also examined the effects of diets with excessively high concentrations of magnesium and the relation of food accessibility to the incidence of urethral obstruction and found no difference to be associated with the feeding schedules.<sup>28</sup>

Diet as a primary factor in the etiology of FUS has been considered in recent years to involve only one constituent, dietary magnesium concentrations. The importance of dietary magnesium has been emphasized by several workers.<sup>29,30,31,32,33,34</sup> So called calculolytic diets, those low in magnesium, have been recommend from results of these types of studies for the treatment and prevention of FUS.<sup>18,35,36,37,38,39,40,41</sup> It has been questioned whether the excessively high magnesium levels that were used in the experimental diets to cause urolithiasis are a representative model of naturally occurring cases of FUS.<sup>1,42</sup> The finding that addition of a urinary acidifier to diets high in magnesium content inhibited urolith formation and caused dissolution of those

already formed has helped to put the effects of dietary magnesium in perspective. Concentrations of magnesium in the diet are important only if the urinary pH is in the alkaline range.<sup>23,43</sup> Another study compared cats with experimentally produced urinary disease as a result of feeding high magnesium diets to those cats with natural occurring FUS. It was determined that undefined factors in addition to magnesium were involved in the natural disease.<sup>44</sup> Another study in cats concluded that factors that affected urinary pH were more important than magnesium levels in the diet in the etiology of struvite crystalluria.<sup>45</sup>

Further, it has been demonstrated that the chemical form in which magnesium is added to the diet had a direct influence on urinary pH. For example, magnesium chloride decreased urinary pH while magnesium oxide produced an alkaline, calculogenic urine. It was concluded that FUS can be treated and prevented by urinary acidification, and that urinary pH was of primary importance in its pathogenesis. The formation of struvite uroliths is impossible and dissolution will occur at low urinary pH.<sup>46,47</sup>

Recommendations have been made to use urinary acidifiers as part of the treatment regimen and as an aid in reducing the incidence of feline urologic syndrome and its recurrence.<sup>7,17,18,23,35,37,48,49,50</sup> Caution has been advised against chronic over-acidification which could lead



to potential problems such as acidosis, potassium wasting, osteoporosis, and possible precipitation of non-struvite minerals.<sup>47,51</sup>

### **Effects of Methionine in Animals**

A urinary acidifier that has been found effective is dl-methionine.<sup>7,26,35,52</sup> It has been found effective at a dose of 1000 to 1500 mg/day in preventing the postprandial alkaline urine production,<sup>35,37</sup> and the higher dose was sufficient to dissolve struvite uroliths.<sup>16</sup> The urinary pH was effectively maintained at near 6.0 in one study by mixing dl-methionine in canned cat food that was fed for two hours a day to provide 1.5 grams daily intake.<sup>53</sup> Early workers believed that methionine was not an effective urinary acidifier even though it did prolong the time to urethral plugging in cats fed diets containing excessively high magnesium.<sup>54</sup> The perceived ineffectiveness of methionine as a urinary acidifier was because too low a dose was used in this study. An idea, that the sulfate produced from methionine may displace the phosphate in struvite thus preventing calculi formation, was proposed to account for the effectiveness of methionine in preventing urolithiasis when it was not thought to be a good urinary acidifier.<sup>55</sup> The activity of the components of struvite would be affected by the other ions that bind to them. It has been proposed that



less magnesium, ammonium, and phosphate would be available to precipitate as struvite when other ions are bound to them.<sup>56</sup>

Methionine has been found to be equally effective for acidification of urine as ammonium chloride when the two are compared on the basis of milliequivalents (mEq) of inorganic acid produced by each drug. This study demonstrated that to produce 5 mEq of acid, either hydrochloric acid produced from ammonium chloride or sulfuric acid produced from methionine, daily doses of 130-260 mg/kg of ammonium chloride or 185-370 mg/kg of methionine would be required. This 5 mEq of acid produced was normally found to reduce feline urinary pH by 0.8 units.<sup>52,57</sup>

Methionine loading in man led to an elevated rate of oxidation of the sulfur of methionine which resulted in increased rates of renal excretion of sulfate ion and non-metabolizable acid.<sup>58,59</sup> By varying the dietary content of sulfur the urinary output of acid was made to increase or decrease with the output of sulfate.<sup>58</sup> It has also been demonstrated in cats that dl-methionine decreased the urinary pH in essentially a linear dose response relationship when doses of 500, 1000, & 1500 mg were given.<sup>52</sup> After methionine loading in humans the excretion of net acid in urine rises gradually and levels off after two to three days.<sup>59</sup> It has been demonstrated in cats that stable urinary pH is reached

in approximately two days after dl-methionine treatment.<sup>52</sup> The metabolism of methionine to sulfate yields two equivalents of hydrogen ion per mole of sulfur oxidized.<sup>52,59,60</sup>

Calcium methionine hydroxy analogue has been shown to be effective as an urinary acidifier in cats.<sup>61</sup>

A limited study in three cats on the effects of high doses of methionine (1 g/kg/day) found that increases in methemoglobin and Heinz bodies in erythrocytes occurred. At a dose of 0.5 g/kg/day in three other test cats recovery from the anemia started at seventeen to twenty-four days and was complete after fifty-two days even though methionine continued to be fed and methionine levels in plasma and red blood cells continued to increase. Adaptation to excess methionine intake apparently occurs in cats.<sup>62,63</sup>

Heinz bodies are denatured hemoglobin formed when irreversible oxidation of hemoglobin sulfhydryl groups occurs.<sup>64</sup> Cat red blood cells have at least eight reactive hemoglobin sulfhydryl groups,<sup>65</sup> making them more susceptible to oxidation and Heinz body formation than in other animals.<sup>64</sup> This oxidation process is reversed by a reducing system comprised of metabolically generated NADPH and glutathione present within the red blood cell. When extreme oxidative injury occurs red blood cells may lyse within the circulation. Red blood cells containing Heinz

bodies may be phagocytosed in the spleen or just the Heinz body and closely associated membrane may be removed. This removal is called the pitting function of the spleen.<sup>66</sup> The cat spleen appears to have poor pitting capabilities, which may account for increased numbers of Heinz bodies in this species.<sup>67</sup> Most clinically normal cats have inclusions in their erythrocytes. These Heinz bodies have been considered physiologically normal and not related to any specific disease process.<sup>68</sup> A later study demonstrated a relationship between the presence of Heinz bodies and specific disease processes or metabolic alterations, and that endogenous Heinz bodies may contribute to anemia. There was a strong correlation of the diseases, diabetes mellitus, lymphoma, and hyperthyroidism with Heinz body formation. The suggestion was that the metabolic derangements that each of these conditions cause may provide the chemical initiators for Heinz body formation. Glutathione concentration was decreased in cats with Heinz bodies. Low degrees of oxidative stress to red blood cells may result in increased synthesis of glutathione as a protectant.<sup>69</sup> An early study indicated that formation of methemoglobin is an essential preliminary step in the oxidative degradation of hemoglobin and is also a precursor to the production of Heinz bodies.<sup>70</sup> The results of a later study suggested that the formation of methemoglobin and the production of Heinz bodies



are parallel processes, and methemoglobin is not a precursor of Heinz bodies.<sup>71</sup> Recently, it was found that unknown dietary factors have a role in the development of Heinz bodies in feline erythrocytes. These researchers suggested that the normal incidence of Heinz bodies in erythrocytes of healthy cats is an artifact caused by consumption of certain diets. It was also demonstrated in this study that propylene glycol induced Heinz body formation without an accompanying hemolytic anemia or methemoglobinemia.<sup>72</sup>

Circulating hemoglobin is principally in the form of oxygen-saturated hemoglobin (oxyhemoglobin) and nonoxygenated hemoglobin (reduced or deoxyhemoglobin). The process where hemoglobin reversibly binds oxygen is called oxygenation which may be contrasted to the oxidation of hemoglobin where oxygen is tightly bound and unable to dissociate. The iron of the heme moiety of hemoglobin is oxidized from the bivalent ferrous to the trivalent ferric state, and hemoglobin is transformed into a brown pigment, methemoglobin. Methemoglobin is incapable of being oxygenated and is devoid of any respiratory function.<sup>73</sup> This oxidation occurs stepwise, one iron at a time, and intermediate forms exist between hemoglobin and methemoglobin.<sup>74</sup> Methemoglobin disappears from the circulatory system within a few days after removal of the

causative oxidizing agent. Methemoglobin reductase in normal red blood cells reconverts the methemoglobin to hemoglobin.<sup>75</sup>

A study in rats demonstrated adaptation to high dietary levels of methionine (3%). The metabolic adaptation involved an increased ability of the animal to oxidize both the methyl and carboxyl carbons of methionine to CO<sub>2</sub>. It was also shown that the addition of either glycine or serine to the diets enhanced methionine metabolism only after the adaptation. These supplements were suggested to be beneficial in adapted animals via an enhanced rate of methionine catabolism which resulted in lower blood concentrations of methionine and increased food consumption and growth.<sup>76</sup> This adaptation has been shown not to involve increased choline synthesis as the method used by the rat to oxidatively metabolize the increased dietary methionine.<sup>77</sup>

Methionine has been characterized as one of the most toxic amino acids in a detailed review article.<sup>78</sup> The adverse effects due to excessive dietary concentrations appear to be due to an aberrant metabolism of the methionine methyl group and its ultimate conversion to carbon dioxide. Various pathways may be involved when different cellular concentrations of methionine are present. It was postulated that excess methionine may result in the production of methyl mercaptan (methanethiol) which may be toxic.<sup>79</sup> Methionine,

mercaptans, and increased blood ammonia among many other factors have been implicated as being involved in hepatic encephalopathy in the dog.<sup>80</sup> In healthy livers metabolites of methionine are converted to nontoxic substances.<sup>81,82</sup> In monkeys and dogs with surgically altered blood flow through the liver, plasma and cerebral spinal fluid (CSF) concentrations of methionine, cysteine, and ammonia were increased and signs of encephalopathy were evident. Neurological status improved and a decrease in plasma and CSF concentrations of methionine, cysteine, and taurine occurred when a parenteral solution low in methionine and aromatic amino acids and high in branched-chain amino acids was administered.<sup>83</sup> One passage through a normal liver removes virtually all of the ammonia that is received. Mammals can tolerate burdens of ammonia that are far in excess of those arising from normal metabolism. As previously noted the shunting of ammonia past the liver to the systemic circulation leads to neurological complications which have been reproduced by administration of ammonia or other nitrogenous substances.<sup>84</sup> Utilizing methionine hydroxy analogue as a urinary acidifier may have an advantage compared to methionine in that MHA does not contribute to the body burden of ammonia, especially in those animals with impaired liver function.



A study in young rats had results that were consistent with the hypothesis that volatile sulfur compounds, such as methanethiol and hydrogen sulfide, cause methionine toxicosis. If these compounds are produced more rapidly than they are degraded, serious metabolic alterations may result. The oxidation of 3-methylthiopropionate, a product of the transamination pathway of methionine metabolism to sulfate increased as the rats increased in age. This difference between the ability to produce volatile sulfur compounds and to excrete them as sulfate may explain the toxicity of methionine in young animals.<sup>85</sup> A study in cats fed excessive methionine (1 g/kg) demonstrated increased plasma methionine but not 3-methylthiopropionate.<sup>62,63</sup>

The toxicity of methionine may vary with the species to which it is given. It has been shown that guinea pigs were much more sensitive than rats to the effects of l-methionine. High dietary levels of l-methionine (10 mmol/kg/day) given to guinea pigs led to fatty liver, hypoglycemia, and aminoacidemia which was followed by hypothermia, profound hypoglycemia and death within 60 hours. Acute hepatic ATP deficiency (about 30 per cent of control values) was observed in all methionine-fed guinea pigs. None of the rats in this same study given this dose were ill by twenty-one days. There was no difference between the control and test rats in weight gain or blood sugar concentration.<sup>86</sup> Intramuscular

injections (60 mg/kg) in rabbits of l-methionine, either once or daily for four days, resulted in hyperglycemia approximately 24 hours after the first injection with a return to normal after 72 hours in both groups.<sup>87</sup>

Growing kittens have been found to be more sensitive than young rats to excess methionine. The kittens in this study partially adapted to a 2.5% total daily intake of methionine (equivalent to 0.6 g/kg) after an initial reduction in food intake and weight gain. The kittens had a rectilinear weight gain by the end of the third week, and during the last ten days of this six week experiment the feed efficiency was about the same as that of the control group.<sup>88</sup> Another experiment in kittens fed purified diets demonstrated that purified diet containing 2 percent methionine severely depressed food intake and weight gain of female kittens, and after two months produced a dermatitis resembling feline acne.<sup>89</sup> This report did not include a description of the study or a tabulation of the results which limits its interpretation and value.

A combination urinary acidifier containing ammonium chloride and dl-methionine was fed at the rate of 0.45 grams of each drug to 3 kittens with a starting weight of approximately one pound. Death occurred in the 3 kittens at eleven (1) and fourteen (2) days preceded by sudden ataxia, depression, and coma. The plasma ammonia of one of the



kittens prior to death was reported to be 1334  $\mu\text{g}/\text{mL}$ . Severe weight loss had occurred during the feeding period. Two control kittens from the same litter gained weight normally. Two other toxicoses in an eight and a nine week old kitten consuming this same combination of urinary acidifiers in a clinical situation was also described.<sup>90</sup>

A report describing a clinical case of Scotty cramp in a two-year old Scottish terrier was felt to have been induced by ingestion of approximately 9 grams of dl-methionine three hours prior to the onset.<sup>91</sup> The dog's weight was not given in this report.

Methionine also has an effect on cardiac muscle. A study using l-methionine perfusion of isolated hearts demonstrated that l-methionine produced a biphasic effect in contractile function, initially negative inotropism followed by positive inotropism, which was associated with changes in membrane calcium transport due to phospholipid N-methylation.<sup>92</sup> Another study demonstrated that both phospholipid N-methylation and force development of cardiac contraction showed a dose-related dependence on methionine.<sup>93</sup> Whether excessive doses of methionine could produce detrimental effects on cardiac muscle in cats is unknown.

Methionine has been shown to be required for maximum antibody response in broiler chicks, and the amount required was higher than that necessary for maximum growth.<sup>94</sup>

Methionine loading (2.5 mmol/day) in weanling rats has led to a reduction in rate of food intake and body growth and to an extracellular hypochloremic acidosis subject to renal compensation. Balances of calcium and non-metabolizable base remained at normal values despite a decrease in whole blood "base excess." It was concluded that this acidosis was possibly due, not to retention of  $H^+$  released by ionization of endogenous sulfuric acid, but to the accumulation of organic acid metabolites of methionine with  $pK_a$  values high enough to permit efficient renal conservation but low enough to cause the mild extracellular acidosis.<sup>95</sup> Another study in adult rats demonstrated a relationship between the level of sulfur amino acids in the diet and the excretion of calcium in the urine. Acid stress from the catabolism of the sulfur-containing amino acids can inhibit renal tubular reabsorption of calcium as can the complexing of the calcium with sulfate in the urine.<sup>96</sup>

Methionine administration in humans caused an acidosis which was associated with a moderate increase in immunoreactive parathyroid hormone (iPTH) and in urinary calcium excretion. No observations were made during the first six days of the administration, but by day seven the increases were fully established. No significant changes occurred in serum total calcium, ionized calcium, magnesium, phosphorus or in urinary phosphorus excretion. The results

obtained in another chronic treatment with ammonium chloride indicated that chronic acidosis elevated the iPTH mainly by producing hypercalciuria, and acidosis itself is not a primary stimulus to iPTH secretion.<sup>97</sup>

Another study in man demonstrated that administration of ammonium chloride produced mild metabolic acidosis without detectable changes in serum parathyroid hormone concentrations. Total serum calcium and magnesium concentrations decreased. A marked rise in daily urinary net acid and calcium excretion occurred.<sup>98</sup>

The long term use of urinary acidifiers in cats may affect the skeletal system if chronic metabolic acidosis develops. Bone dysfunction, which occurs most commonly in the young, has been associated with chronic metabolic acidosis. There is a delay in growth, presumably as a result of consumption of bone bicarbonate to buffer the excess hydrogen ion. Acidemia also causes an increased release of calcium phosphate from bone, with a reduced tubular reabsorption of these ions. This results in hypercalciuria and hyperphosphaturia directly proportional to the decrease in plasma bicarbonate concentration.<sup>99</sup>

Studies in the dog have suggested that in metabolic acidosis two separate phases of bone buffering occur. The first phase is thought to be a rapid, dynamic equilibrium that occurs within hours between the bicarbonate and hydrogen



ion of the extracellular fluid and the carbonate of the bone. This may be a surface phenomenon which is not dependent upon calcium mobilization since no significant mobilization of calcium or phosphorus is noted during this phase. The second phase is a more long-term mobilization of carbonate from the bone that occurs over a period of days which is evident by mobilization of calcium and phosphorus associated with a lowering of extracellular bicarbonate.<sup>100</sup>

The oxidation of sulfur amino acids, such as methionine, coupled with ureagenesis tends to cause metabolic acidosis. Urea formation tends to decrease as severe acidosis occurs, and ammonia concentration tends to increase. If the ammonium ion produced is not properly eliminated, hyperammonemia could result.<sup>101</sup> The shift in nitrogen excretion from urea to ammonium in acidosis has been interpreted as a gain in bicarbonate since the latter is utilized in urea formation. Therefore, when waste nitrogen is excreted as ammonium, no bicarbonate is utilized and new bicarbonate, generated by the carbon skeleton, helps to maintain hydrogen ion homeostasis.<sup>102</sup> The traditional role ascribed to ammonia formation in the kidneys, which is to be utilized for the trapping of excess hydrogen ions for their excretion as ammonium, has been challenged by some scientists who concluded that the major reason for pumping protons is to trap ammonia. These researchers also believe that urea

synthesis is important more for utilization of bicarbonate and in blood pH regulation. Urea synthesis decreases during acidosis so that bicarbonate may increase. They further discuss that ammonium ion in the urine does not represent excretion of acid.<sup>103,104,105</sup> This view has been challenged with the contention that the net effect of ureagenesis on acid-base balance should be nil since two ammonium ions are consumed for each bicarbonate ion.<sup>106,107</sup>

The sulfuric acid produced from the oxidation of sulfur-containing amino acids, such as methionine, is initially buffered in the extracellular fluid by bicarbonate. The carbon dioxide produced by this reaction is excreted by the lungs. Although the bicarbonate minimizes the increase in the hydrogen ion concentration, the excess hydrogen ions must be excreted by the kidney to prevent progressive depletion of bicarbonate and the other body buffers and severe metabolic acidemia.

The kidney contributes to acid-base balance by regulating hydrogen ion excretion so that the plasma bicarbonate concentration remains within appropriate limits. The reabsorption of bicarbonate and the formation of titratable acidity and ammonium ion all occur by hydrogen ion secretion from the tubular cell into the lumen.

Since the urinary concentration of free hydrogen ion is extremely low, the net quantity of hydrogen ion excreted in the urine is equal to the amount excreted as titratable acidity and ammonium ion minus any hydrogen ion loss to the body because of urinary bicarbonate loss.

Because of its favorable pKa (6.8) and relatively high concentration, the bulk of the urinary buffering is performed by monobasic phosphate. This process is referred to as titratable acidity since it is measured by the amount of NaOH that must be added to a 24-hour urine collection to titrate the urine pH back to pH 7.4, physiological pH.

Since phosphate excretion does not increase markedly in the presence of an acid load, the ability to enhance net hydrogen ion excretion by increased formation of titratable acidity is limited.

Ammonia ( $\text{NH}_3$ ) is a base which can combine with hydrogen ion to form ammonium ( $\text{NH}_4^+$ ). The pKa of the  $\text{NH}_3/\text{NH}_4^+$  system is 9.3 which makes this an ineffective buffer. Instead ammonia acts by passive, nonionic diffusion to allow hydrogen ions secreted into the lumen to be trapped as ammonium ions. Virtually all the ammonia in urine exists as ammonium. It is the continued diffusion of ammonia out of the cells that allows the  $\text{NH}_3/\text{NH}_4^+$  system to act as an effective "buffer" even though its pKa is so far from urine pH. The ability to augment ammonia production and ammonium excretion is the main



adaptive response by the kidney to an acid load. Net acid excretion may be dramatically increased in metabolic acidosis. This is mostly due to enhanced ammonium excretion since titratable acidity is generally limited by the amount of phosphate in the urine.<sup>108</sup>

During acute acidosis, there is an increase in ammonia excretion that can be related simply to a change in urinary pH, without invoking a change in the actual rate of total ammonia production. During chronic acidosis, ammonia excretion is greater at any given pH compared to the controls. This adaptive increase in ammonia excretion is explained by an accelerated rate of ammonia production by the renal tubule.<sup>109</sup>

The reactions which produce ammonia in renal tissue involve glutamine as the major precursor of the ammonia by giving up both of its nitrogens as ammonia in sequential reactions. Intracellular changes resulting from acidosis account for increased tubular production of ammonia. The excretion of ammonia by mammals is primarily concerned with urinary excretion of non-volatile acid, not with elimination of waste nitrogen.<sup>110</sup>

Abnormalities of acid-base status can shift potassium into or out of cells and thereby alter plasma potassium concentration. In metabolic acidosis, a large proportion of the excess hydrogen ions are buffered intracellularly and as

hydrogen ions enter cells, potassium and sodium leave to maintain electroneutrality. The infusion of organic acids, such as lactic acid or keto acids, into animals caused a smaller increase in plasma potassium concentration than did mineral acids.<sup>111</sup>

The feeding of the free acid of MHA (MHA-FA) and l-MET to chicks resulted in an acid-load that was manifested by a slight metabolic acidosis, which was greater in those chicks fed MHA-FA than those fed l-MET.<sup>112,113</sup>

Mature rats fed dl-methionine at a rate of 4.8% of their diet for a twenty day period demonstrated a depletion of fat stores and hypertrophy of the kidneys.<sup>114</sup> Excessive methionine, 2.5% and 4.5% of the total dietary intake, administered to rats for thirty days resulted in minor histopathological changes in the pancreas, gastrointestinal tract, salivary glands, kidneys, spleen, thymus, thyroid, and adrenal glands. It was also demonstrated in this study that the kidney weights in relationship to body weights were increased. Most of the changes could be attributed to the decrease in food intake, especially with the 4.5% methionine diet.<sup>115</sup> In addition, glycine and/or arginine added to the diets seemed to result in less extensive alterations histopathologically than when the methionine was supplemented alone.<sup>116</sup>



### Metabolism of Methionine

Methionine is metabolized by a variety of reactions and pathways with many intermediates and products. Along with the toxicity problems of excess methionine many metabolites serve functions that are essential for survival of the organism. The sulfur amino acids have important catalytic roles in the active sites of many enzymes. Methionine is metabolized primarily to S-adenosylmethionine (AdoMet), a sulfonium compound that mediates most biochemical methylation reactions. It has been estimated that methyl transfer reactions as a group consume about 95% of the AdoMet formed. Homocysteine occupies a branch point in methionine metabolism. Approximately one-half of the homocysteine is irreversibly converted by trans-sulfuration to cysteine, alpha-ketobutyrate and ammonia. The remainder is remethylated to methionine.<sup>117</sup> Methionine alone can provide all the needed body sulfur compounds with the exception of the two vitamins containing sulfur, biotin and thiamine. Generally, methionine in the diet at twice the required level is well tolerated, but at a three-fold or above level toxicosis often results.<sup>118</sup>

The other metabolic pathway that has been described for methionine degradation, the transamination-decarboxylation pathway, is independent of AdoMet formation.<sup>119</sup> The trans-sulfuration pathway has been characterized as anabolic

metabolism in that diverse methylation products, such as phosphatidylcholine, epinephrine, and sarcosine are synthesized. The transamination pathway has been characterized as catabolic metabolism whereby excess dietary methionine is degraded for elimination.<sup>120</sup> It has been suggested that the transamination pathway operates when the capabilities of the trans-sulfuration pathway has been exceeded or impaired, and the intermediate, 3-methylthiopropionate, in this pathway may be the source of methionine toxicosis.<sup>121</sup> This pathway is known to exist in the rat, monkey, pig, sheep, and probably other mammals.<sup>122</sup> Adaptive increases in methionine oxidation have been demonstrated in both pathways in response to high levels of dietary methionine.<sup>123</sup>

#### **Methionine hydroxy analogue**

A study in weanling and adult cats demonstrated that methionine is an indispensable amino acid, and that calcium MHA was not utilized well as a source of methionine when the cats were fed a crystalline amino acid diet. MHA was shown to be well utilized as a source of methionine when the diet was a semi-purified one providing amino acids from intact protein in the form of soybean meal.<sup>124</sup>

The total sulfur amino acid requirement of growing kittens is approximately 0.9 percent of the diet with the minimal methionine contribution being between 0.3 and 0.45

percent.<sup>125,126</sup> When the diet of cats consists of a high amount of animal protein the substantial amounts of sulfur amino acids present (methionine, cystine) are oxidized to sulfate and contribute to urinary acidification. Vegetable protein and low-protein diets tend to be low in sulfur amino acids and high in potassium and magnesium resulting in more alkaline urine and struvite precipitation.<sup>7</sup>

Methionine hydroxy analogue has also been found to be effective in replacing the l-methionine requirement in the diet of growing dogs.<sup>127</sup>

Complete substitution or replacement of dl-methionine in the diet of rats by CaMHA has been made without effect on growth or feed efficiency. Also noted was an increase in urea clearance in the groups fed the hydroxy analogues.<sup>128</sup>

An experiment in rats, studying the improvement of the protein value of soya, included supplementation of the soya protein with l-MET or dl-MHA. Both MET and MHA were maximally efficient in elevating the biological value of the soya protein at a 1 percent concentration. When the supplementation exceeded 18 percent, similar toxic effects of reduced food intake and deterioration of general health were noted with both MHA and MET. The toxic effects were characterized to be similar but were less drastic with MHA than with MET. This difference in degree of severity was accounted for by the absence of the dilution of the



assimilable protein nitrogen fraction when MHA was used instead of MET. The toxicoses of the amino acid or hydroxy analogue excesses caused anorexia resulting in tissue breakdown which resulted in increased urinary nitrogen, and thus an apparent decrease of biological value of the protein as the amount of MET and MHA were increased in the diet.<sup>129</sup>

The conversion of MHA into MET was shown to be accomplished via a ketomethionine intermediate involving an enzyme-dependent oxidation reaction. The ultimate conversion of the ketomethionine to l-methionine was accomplished through a transamination reaction involving various donor amino acids in the chick<sup>130,131</sup> and the rat.<sup>132</sup> The rat study demonstrated that kidney contained the greatest amount of activity per gram of tissue, but the liver, because of its greater size, played a predominant role in converting MHA into MET in the intact animal. The chick studies also demonstrated that the renal threshold for dl-MHA was equivalent to that for l-MET, and that they both appeared to be reabsorbed against concentration gradients. Dietary equivalence of MHA and MET was shown in both studies. Later studies revealed stereospecific different enzymes involved in the oxidation of the d- and l- isomers of MHA to ketomethionine prior to the transamination reaction.<sup>133</sup> The transamination reaction of ketomethionine to l-MET is

reversible and may proceed to the transamination degradation pathway from the ketomethionine as was discussed earlier.

A study in chicks fed a crystalline amino acid diet demonstrated that the calcium salt of ketoMET was less efficacious than dl-MET, but more efficacious than CaMHA. The implication was that conversion is not 100 percent efficient. MHA was characterized as considerably less toxic than an equimolar quantity of MET.<sup>134</sup>

Another study showed that CaMHA was slightly inferior to dl-MET in feed conversion efficiency in layer hens. The free acid form of MHA (MHA-FA) in diets caused distinctly inferior performance of hens to that of birds fed dl-MET.<sup>135</sup> In attempts to characterize and define the reasons for inferior performance of MHA-FA, excretion studies determined that l-MET was the major excretion product of both MHA-FA and l-MET, and that MHA-FA is not actively excreted by the avian kidney.<sup>112,113</sup>

Another comparison of the metabolism of MHA and MET demonstrated no differences in the oxidation of the l-MET, dl-MET, and dl-MHA in young growing day old broilers, but the amount of dl-MHA in the excreta was significantly greater compared to the other two sources.<sup>136</sup>

A study in three week old pigs showed that the methionine requirement can be satisfied with either l-MET, dl-MET, or dl-MHA at a level equal to 0.51 percent of the

dietary solids in a liquid feeding system. It was apparent that MHA was converted to MET since the plasma MET concentration increased significantly with increment increases in concentration of dietary MHA. This test demonstrated that a decrease in plasma urea occurred when MHA or MET was added to a diet deficient in sulfur-bearing amino acids. It was determined that the amount of dietary MET required to support maximum growth is greater than that which is required for maintaining nitrogen balance since MET is an amino acid that has many functions other than protein synthesis. The safety margin was narrow between optimum growth and toxicosis.<sup>137</sup>

CaMHA used as the methionine source in rainbow trout resulted in lower growth rates than when the source was either l-MET or dl-MET. Higher dietary levels of CaMHA were required to prevent cataracts in the fish than MET.<sup>138</sup>

A study of kidney fibroblast growth found that dl-MHA could be utilized as a source of MET, but the efficiency was dependent on the presence of a basal amount of MET. Higher concentrations of MHA than MET were required. l-MET had a lower margin of safety compared to dl-MHA.<sup>139</sup>

In vitro studies in rats have demonstrated that jejunal uptake of MHA is mediated by a Na<sup>+</sup>-independent carrier system associated with lactate transport and is distinct from the methionine uptake mechanism which is Na<sup>+</sup>-dependent. MHA was



found to be more slowly absorbed than methionine. It was postulated that this difference could explain the lower biological utilization of MHA as the sole source of methionine in purified crystalline amino acid diets.<sup>140</sup> However, this is contrasted to the finding in chickens that passive diffusion is the mechanism of MHA absorption. In this study the uptake of CaMHA was less than half that of dl-methionine.<sup>141</sup> Other in vitro studies in chicks found that MHA uptake was linear in relation to concentration and methionine was absorbed by both concentration and energy dependent processes. The results indicated that MHA as a substitute for methionine would not be limited by absorption.<sup>142</sup> These absorption differences may be due partially to different experimental designs in these studies. It is not known whether absorption of increased levels of MHA or methionine is a factor in their comparative performance in cats.

Another transport system for MHA is involved in the growth in tissue culture of porcine kidney fibroblasts. Although the d- and l- isomers of MHA are oxidized by different enzymes, the conversion rates to the alpha keto methionine are similar. However, the d- isomer of MHA promoted the growth of the fibroblasts while the l-MHA did not when each was used as the sole source of methionine. It

appeared that a stereospecific transport system was present which permitted d-MHA to be moved into the cell but not l-MHA.<sup>143</sup>

MHA, because it does not add to the dietary nitrogen content, has been used in protein evaluation trials to replace MET in "protein-free" diets to effectively reduce the time required for stabilization of the endogenous urinary nitrogen output in young rats.<sup>144</sup>

In adult humans in renal failure, where maintenance, not growth, is the goal, the use of keto and hydroxy acids in replacement of amino acids makes the goal more attainable.<sup>145</sup> Nitrogen-free analogues of essential amino acids given as supplements to a low protein diet in humans have been shown to be nitrogen sparing. This treatment regime for chronic renal disease is only useful when dietary protein is restricted. When the protein is high in a diet these amino acid analogues are rapidly degraded.<sup>146,147</sup>

Model systems to assess bioequivalence of different methionine sources have included nonpurified diets, purified diets, crystalline amino acid diets, and cell cultures. Therefore, the many studies comparing MHA and MET have contradictory results attributable to differences in basal methionine concentrations, whether cystine is in the diet and at what concentration and ratio to methionine, the age and species of animal tested, and how much choline or lecithin is



provided in the diet. The toxicity of methionine has been found to be at concentrations slightly above those concentrations which allow a maximum feed gain response.<sup>139</sup>

## MATERIALS AND METHODS

### Test Drugs

The test drugs were manufactured and provided by Vet-A-Mix Animal Health, Inc., 604 West Thomas Avenue, Shenandoah, Iowa.<sup>a</sup> The dosage forms were manufactured under the Current Good Manufacturing Practice (GMP) regulations.<sup>148</sup> The two drugs that were used in this study, dl-methionine<sup>b</sup> and calcium methionine hydroxy analogue<sup>c</sup> were formulated into compressed tablets in a palatable base. Dosages were formulated based on the theoretical amount of sulfuric acid produced by the metabolism of each compound.<sup>149</sup> Each MET tablet contained 75 mg of methionine which is equal to one milliequivalent of sulfuric acid produced. Each MHA tablet contained 85 mg of calcium methionine hydroxy analogue which is equal to one milliequivalent of sulfuric acid produced.

The chemical formula for calcium methionine hydroxy analogue is  $[\text{CH}_3\text{S}(\text{CH}_2)_2\text{CHOHCOO}]_2\text{Ca}$ , and for dl-methionine the chemical formula is  $\text{CH}_3\text{S}(\text{CH}_2)_2\text{CHNH}_2\text{COOH}$ .

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<sup>a</sup>Sponsor of the research study

<sup>b</sup>Methio-Form<sup>TM</sup>, Vet-A-Mix Animal Health, Inc., Shenandoah, IA.

<sup>c</sup>Investigational New Animal Drug #6460, Vet-A-Mix Animal Health, Inc., Shenandoah, IA.

### Test Animals

The test system was 42 healthy mature cats which were at least 6 months of age. There were equal numbers of males and females. The weight range was 2.5 to 5.3 kilograms. The cats were random-source animals which had been acquired and conditioned (which included vaccination, worming, and an isolation period to assure health) by Laboratory Animal Resources, Iowa State University, Ames, Iowa. During the study they were housed in individual stainless steel cages with water and food available ad libitum. Each cat was permanently identified by a tattoo on the inner surface of the pinna of the ear.

### Test Diet

Purina Cat Chow<sup>d</sup> was chosen as the test diet because it did not contain any added dl-methionine. One manufacturing lot number of food was used for the entire study. The food was provided free choice to the cats, instead of providing the food for a limited time period or on a meal basis, so that individual eating habits would not affect intake. Analysis of the food was conducted for presence of free methionine by the sponsor of the study and none was detected.

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<sup>d</sup>Lot #3D107R, Ralston Purina Company, St. Louis, MO.

### Test Groups

The study was divided into 3 replications of 14 cats each, which were randomly assigned to 7 treatment groups with one male and one female in each group per replication. The two treatments, MHA and MET, were given at 3 different levels (low, medium, and high). The control group had no treatment applied. The low dose was approximately the normal dose of dl-methionine that is recommended to be given daily in a clinical situation, and the medium dose was considered the high end of the normal dose range.<sup>e</sup> Equivalent amounts of the two test drugs were given at each level. The result was 7 test groups, each having a total 6 cats, 3 of which were females and 3 of which were males, with an overall total of 42 cats. The test groups are summarized below.

<u>Groups</u>	<u>Treatment</u>	<u>Level</u>	<u>Dose (mEq/kg)</u>
1	CONTROL	none	0
2	MHA	low	2.5
3	MHA	medium	5.0
4	MHA	high	10.0
5	MET	low	2.5
6	MET	medium	5.0
7	MET	high	10.0

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<sup>e</sup>1988-89 Product Catalog, Vet-A-Mix Animal Health, Inc., Shenandoah, IA.



### Test Design

The study was conducted to comply with current Good Laboratory Practices (GLP).<sup>150</sup>

The testing procedures were begun after the cats had been acclimated to the basal diet and housing for 14 days. The test medication was administered once a day at the same time each morning and was given for 7 days. The cats were observed for 7 days after the dosing was stopped.

### Sample Collection

Sample collection was started after the 14 day acclimation period and was made 5 times: two times prior to treatment to establish baselines, two times during the actual dosing, and one time seven days after the last dose was given. The last sample collection was made to determine whether the parameters examined had returned to normal by the end of the trial. The sample collection times are summarized as follows:

#### Sample Time

1. -3 day (3 days prior to the 1st dose)
2. 0 day (just prior to the 1st dose)
3. +3 day (24 hours after the 3rd dose)
4. +7 day (24 hours after the 7th dose)
5. +14 day (7 days after last dose)

**Samples, urine**

The cats were monitored for the entire 24 hour urine collection period to prevent fecal contamination and to assure fresh urine samples. During each of the collection periods, urine was collected from each cat by allowing it to urinate through the grates of its cage into the collecting tray beneath. Prior to the start of the collection period, the cage grates and trays beneath were thoroughly washed and rinsed several times with distilled water, dried and replaced. This was to provide uncontaminated samples. After each time the cats defecated or urinated, the trays were rewashed, rinsed and dried. The samples were drawn from the trays with syringes, the volumes determined, and the urine placed in glass containers containing toluene as a preservative. They were kept in an ice water bath until analyses were done. At the end of each collection period distilled water was sprayed through the floor grates into three different cage trays. This water was collected from the trays in a similar manner as the urine. These water samples were found to contain only an occasional trace of ammonia, thereby insuring that the ammonia found came from the urine.

**Samples, jugular blood**

A total of 6 mL of blood was drawn from each cat at each collection. Glass syringes and needles were filled with heparin, which was expelled prior to collecting blood for blood gas analyses and methemoglobin determinations. Sterile blood collecting vacuum tubes<sup>f</sup> containing potassium EDTA as an anticoagulant were used to collect blood for the hematology and ammonia determinations. Untreated sterile blood collecting vacuum tubes<sup>f</sup> were utilized to collect blood samples to determine electrolytes, urea nitrogen, creatinine, liver enzymes, and glucose values. All samples were placed in an ice water bath as soon as they were collected and kept there until analysis. The samples for blood gas measurements were submitted for analyses as soon as every four samples were collected or approximately every 30 minutes. The serum samples were submitted at the end of the blood collection time (1.5-2 hours). The procedures to determine blood ammonia concentrations were begun after the final blood sample was drawn. The hematologic tests were conducted approximately 2.5-3.5 hours after the last sample was taken.

**Samples, necropsy**

A complete necropsy was performed on 6 cats in the first replication: 2 control cats from group one, 2 high dose MHA

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<sup>f</sup>Sherwood Medical, St. Louis, MO.

cats from group four, and 2 high dose MET cats from group seven. A complete necropsy was performed on 10 cats in the second replication: 2 control cats from group one, 2 medium MHA cats from group three, 2 high MHA cats from group four, 2 medium MET cats from group six, and 2 high MET cats from group seven. Two of the cats died in the first replication, one from group four and one from group seven. Necropsies were performed on these two animals approximately one hour and three hours, respectively, after their deaths. Euthanasia was accomplished by lethal injection<sup>9</sup> into a jugular vein of each of the remaining cats, and they were necropsied within 30 minutes of the time of death. Tissues were observed grossly and sections of the tissues were fixed in buffered-neutral formalin for histopathologic examination. The tissues collected included: liver, kidney, spleen, adrenal, brain, duodenum, pancreas, heart, and urinary bladder.

### **Test Parameters**

#### **Food intake**

The food was fed ad libitum. The cats were fed twice daily as the bowls did not have enough capacity for the 24 hour needs for some of the larger cats. The 24 hour feed consumption for the entire length of study was determined by

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<sup>9</sup>Sleepaway, Ft. Dodge Laboratory, Ft. Dodge, IA.



weighing the food that was not consumed each morning and calculating the intake for the previous day. The food was weighed on a triple beam balance<sup>h</sup> that was routinely validated and calibrated by using class M-2 traceable weights.<sup>i</sup>

### **Body weight**

The cats were weighed at each sampling day just prior to blood collection. The animals were weighed on a small platform scale<sup>j</sup> that was validated to be accurate prior to each replication.

### **General observations**

The test subjects were observed twice daily before and after the dosing period. During the urine collections, the cats were monitored continuously for 24 hours. On the other treatment days the animals were checked frequently during each day.

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<sup>h</sup>Ohaus Scale Corporation, Florham Park, NJ.

<sup>i</sup>Denver Instrument Company, Arvada, CO.

<sup>j</sup>Model 2020, Toledo Scale Company, Toledo, OH.

## Urine

The parameters that were measured in the urine included the following:

pH  
Titratable acidity to pH 7.4 and 8.4  
Ammonia  
Electrolytes: Magnesium, Phosphorus, Calcium  
Volume  
Creatinine  
Urea nitrogen  
Specific gravity

Urine volume was determined as each voided sample was collected during the 24 hour collection period. After the collection period approximately 4 mL was submitted for measurement of magnesium, phosphorus, calcium, creatinine, and urea nitrogen on an automated instrument<sup>k</sup> by standard methods of the Clinical Pathology Laboratory of the College of Veterinary Medicine at Iowa State University. A 20 mL sample was transferred from beneath the toluene preservative and allowed to come to room temperature. The pH was determined with a pH meter.<sup>l</sup> The pH electrode was rinsed

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<sup>k</sup>Abbott Spectrum, Dallas, TX.

<sup>l</sup>Model #16087, Markson pH Meter, Phoenix, AZ.

with distilled water and calibrated with a 7.0 pH standard buffer between each sample. The sample was titrated to two end points, 7.4 and 8.4, with 0.075N NaOH, and the titratable acidity in the twenty-four sample was calculated.

The ammonia was determined on a 0.5 mL sample from the initial specimen by the Modified Conway Method.<sup>151</sup> This microdiffusion method of ammonia determination involved the gaseous diffusion of ammonia from the outer chamber of the Conway dish after the sample was mixed and incubated with a releasing buffer. The ammonia that diffused into the inner trapping and indicator chamber was quantitated by titrating with dilute sulfuric acid from a micropipette. The specific gravity of the urine was measured with a total solids meter<sup>m</sup> according to the standard methods of the Clinical Pathology Laboratory, College of Veterinary Medicine, Iowa State University.

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<sup>m</sup>American Optical, Buffalo, NY.

**Blood**

The measurements made either on blood, serum, or plasma were:

pH	Ammonia
Bicarbonate	Electrolytes
Red blood cell count	Sodium
Packed cell volume	Potassium
Hemoglobin	Chloride
Urea nitrogen	Calcium
Creatinine	Phosphorus
Alkaline phosphatase	Magnesium
Alanine aminotransferase	Heinz body formation
Glucose	Methemoglobin

The blood pH and bicarbonate concentrations were determined on a blood gas machine.<sup>n</sup> An automated hematologic analyzer<sup>o</sup> was used to determine the red cell count, packed cell volume, and hemoglobin. The Heinz body counts were made from blood smears that had been stained with new methylene blue supravital stain.<sup>65,152</sup> The remaining measurements, with the exception of methemoglobin and

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<sup>n</sup>Radiometer, ABL 3, Copenhagen, Denmark.

<sup>o</sup>Coulter Electronics, Inc., Hialeah, FL.



ammonia, were made on an automated instrument<sup>P</sup>. The preceding procedures were all performed according to the standard methods of the Clinical Pathology Laboratory, College of Veterinary Medicine, Iowa State University. The Heinz bodies in 1000 red blood cells were counted under oil emersion at 1000X. Methemoglobin levels were determined<sup>Q</sup> from fourteen cats from the third replication at time four (7 days after treatment) by the classic method.<sup>153</sup> The ammonia nitrogen concentration of the blood was determined by the Modified Conway Method<sup>151</sup> similar to the procedure performed on the urine.

### Histopathology

Tissues were processed,<sup>R</sup> embedded in paraffin blocks, and sectioned to a thickness of five microns and stained with hematoxylin and eosin. All tissues collected were examined from the animals necropsied in the first replication. Only the liver and kidney were examined histologically in the second replication. The liver and kidney in the second replication in addition to hematoxylin and eosin were also

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<sup>P</sup>Abbott Spectrum, Dallas, TX.

<sup>Q</sup>Procedure No. 8268, Mayo Medical Laboratories, Rochester, MN.

<sup>R</sup>Histomatic MVP, Fisher Scientific, Pittsburgh, PA.

stained with PAS (Periodic Acid-Schiff) with diastase digestion of glycogen to confirm the presence of glycogen.<sup>154</sup>

### Test Statistics

Statistical analyses were performed<sup>8</sup> using all test parameters as variables. Collection times one and two were merged into one baseline sample time for purpose of analyses. Initially, the effects of replication, treatment, sex, replication\*treatment, and sex\*treatment were examined at each sample time. Replication\*treatment was used as the error term to compare treatments using the analysis of variance (ANOVA) procedures. Further analyses were conducted on variables that demonstrated significant differences among treatment groups at  $P < 0.05$  level. These additional analyses consisted of comparing the control group to the other six treatment groups and the three MHA groups to the three MET groups. Also, linear and quadratic models were applied to the three MHA groups and to the three MET groups.

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<sup>8</sup>General Linear Models Procedure, Statistical Analysis Systems, SAS, Inc., Cary, NC.

## RESULTS AND DISCUSSION

The results and discussion are divided into two main sections; those test measurements which did not demonstrate differences among the treatment groups, and those test values which did demonstrate differences among the treatment groups. There are a few minor exceptions to this general subdivision with some of the parameters measured. The exceptions primarily involved situations where statistical differences could not be related to treatments or could not be related to values outside those considered to be normal.

In general, the baseline sample time, which is pre-treatment collection Time 1 and Time 2 averaged into one value, shows no significant differences among groups, as would be expected, since no treatments were applied at these times. The collection Time 5, which was seven days after the last treatment, also showed no significant differences between groups for most measurements. Specific instances in which differences between groups persisted into the recovery period are referred to when the individual variables are discussed.

### Parameters with Changes Presumed Not to be Treatment Related

The test parameters that appeared not to be altered by the treatments included the following:

Body weight

Urine        Calcium, Creatinine, Specific gravity.

Blood        Red blood cell count, Packed cell volume,  
Hemoglobin, Methemoglobin, Alkaline  
phosphatase, Alanine aminotransferase,  
Glucose, Sodium, Chloride, Calcium,  
Magnesium, Ammonia, pH, pCO<sub>2</sub>.

Necropsy

Histopathology

The numerical mean values for these parameters are listed in Tables 1-16 with a short discussion preceding each one. The standard error of means, F-value, and probability of significance are given at each time period.

#### Body weight

The average body weight among groups was not significantly affected at any of the sample times. This occurred even though food consumption was significantly reduced. The short treatment period for this test did not allow time for the effects of treatments to be manifested in weight loss. The cats in this test were mature instead of growing cats which also helps to account for no effect on



body weight over the period of the test. The results of these comparisons are provided in Table 1.

Table 1. Body weight means in kilograms, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline	Time	Time	Time
		Time 1, 2	3	4	5
1	None-Control	3.67	3.66	3.66	3.72
2	Low MHA	3.89	3.87	3.89	4.00
3	Med MHA	3.72	3.77	3.73	3.87
4	High MHA	3.87	3.79	3.70	3.63
5	Low MET	3.66	3.67	3.78	3.91
6	Med MET	4.27	4.14	4.12	4.17
7	High MET	3.97	3.87	3.73	3.90
Standard Error of Means		0.55	0.55	0.56	0.56
F-Value		0.74	0.43	0.37	0.44
Pr > F		0.6276	0.8474	0.8817	0.8398

### Urinary calcium

Hypercalciuria did not occur in any of the groups during the treatment phase of the study. There was no correlation between the metabolic acidosis that some of the treatment groups developed and calcium excretion. The increased calcium excretion that can occur with acute and chronic

metabolic acidosis, which was described in the literature review, was not evident in this study. Apparently, the seven day treatment period did not result in loss of calcium from the skeleton to provide carbonate for buffering purposes of the metabolic acidosis that occurred. The increases of calcium excretion that were noted in some groups during treatment were not significant as can be seen in Table 2.

Table 2. Urinary calcium means in mg/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	0.292	0.217	0.300	0.240
2	Low MHA	0.258	0.467	0.350	0.200
3	Med MHA	0.292	0.650	0.367	0.300
4	High MHA	0.225	0.300	0.567	0.275
5	Low MET	0.308	0.500	0.350	0.300
6	Med MET	0.258	0.533	0.417	0.200
7	High MET	0.325	0.500	0.425	0.200
Standard Error of Means		0.032	0.102	0.099	0.032
F-Value		1.16	2.07	0.73	1.76
Pr > F		0.3871	0.1333	0.6347	0.2149

**Urinary creatinine**

The urinary creatinine excretion values were not significantly altered except seven days after the last treatment at Time 5. The differences at Time 5 could not be related to any particular treatment group. The values are tabulated in Table 3.

Table 3. Urinary creatinine means in mg/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	318	317	316	297
2	Low MHA	341	358	342	340
3	Med MHA	310	288	285	305
4	High MHA	292	236	272	256
5	Low MET	348	319	279	278
6	Med MET	328	292	328	337
7	High MET	316	254	257	330
Standard Error of Means		22	24	20	12
F-Value		0.73	2.93	2.36	6.16
Pr > F		0.6325	0.0536	0.0964	0.0038

**Urine specific gravity**

The mean specific gravity of the urine was generally high for all groups at all collection times. The range was

from 1.051 to 1.061. The cat is capable of producing very concentrated urine as the animals in this study demonstrated. The significant difference among groups at Time 3 was not considered to be a clinically significant finding since the values were not outside normal ranges, the change did not persist at Time 4, and the change could not be linked to any particular treatment. The specific gravity values are given in Table 4.

Table 4. Urine specific gravity means, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	1.056	1.051	1.055	1.052
2	Low MHA	1.058	1.059	1.063	1.059
3	Med MHA	1.060	1.059	1.063	1.061
4	High MHA	1.056	1.048	1.056	1.054
5	Low MET	1.059	1.058	1.058	1.061
6	Med MET	1.059	1.053	1.062	1.059
7	High MET	1.059	1.053	1.057	1.061
Standard Error of Means		0.002	0.002	0.003	0.003
F-Value		0.92	4.73	1.09	1.54
Pr > F		0.5139	0.0107	0.4224	0.2478



**Red blood cell count**

The red blood cell counts demonstrate that no effects can be attributed to treatments applied for the seven day period as far as any erythrocyte destruction is concerned. Time 5 approached significance,  $P < 0.0510$ . Group 7, high MET treatment, showed the greatest reduction in RBC count compared to the other groups at this time. The possibility that a clinical anemia could have developed with continued treatment exists, but the RBC count in Group 7 was still well within normal values. The values may be compared in Table 5.

Table 5. Red blood cell count means in million/ $\mu$ l, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	8.480	7.690	8.003	8.080
2	Low MHA	6.691	6.375	8.018	7.515
3	Med MHA	7.896	7.403	7.527	7.532
4	High MHA	7.886	6.432	7.457	6.752
5	Low MET	7.434	7.373	7.362	7.713
6	Med MET	7.311	6.987	7.225	7.340
7	High MET	7.253	7.490	7.816	6.236
Standard Error of Means		0.606	0.782	0.414	0.369
F-Value		0.84	.045	0.58	2.98
Pr > F		0.5610	0.8316	0.7418	0.0510

**Packed cell volume**

This hematologic parameter was not significantly altered at any of the sample times by any treatments. Again, Group 7 had the lowest PCV which is consistent with the lower erythrocyte count in this group at this collection time. The PCV was at the low end of the normal range at this collection. Packed cell volumes are given in Table 6.

Table 6. Packed cell volume means in %, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	37.8	35.2	35.5	37.3
2	Low MHA	39.4	35.2	34.0	33.7
3	Med MHA	35.4	33.8	33.0	34.2
4	High MHA	35.6	36.2	34.3	32.4
5	Low MET	36.5	33.2	33.0	35.3
6	Med MET	33.1	32.7	33.3	34.5
7	High MET	37.2	34.8	36.0	30.4
Standard Error of Means		1.96	1.77	1.73	1.67
F-Value		0.94	0.50	0.42	1.83
Pr > F		0.991	0.7995	0.8543	0.1760

Hemoglobin

Hemoglobin values were not altered. The inter-relationships of the three hematologic parameters, PCV, RBC count, and hemoglobin, as measured by mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), and mean cell hemoglobin (MCH) were also calculated and each were found not to be different among groups. The hematologic parameters measured give no evidence of a hemolytic anemia occurring as a result of the treatments. The hemoglobin values are compared in Table 7.

Table 7. Hemoglobin means in g/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline	Time	Time	Time
		Time 1, 2	3	4	5
1	None-Control	12.5	11.5	12.0	12.2
2	Low MHA	12.9	11.6	11.4	11.1
3	Med MHA	11.7	11.1	11.2	11.3
4	High MHA	11.7	12.0	12.2	10.8
5	Low MET	12.1	10.8	11.0	11.6
6	Med MET	10.8	10.6	11.0	11.2
7	High MET	12.3	11.4	12.8	10.4
Standard	Error of Means	0.75	0.68	0.72	0.60
F-Value		0.77	0.56	0.82	0.99
Pr > F		0.6085	0.7572	0.5743	0.4744

### Methemoglobin

A problem in the laboratory prevented conducting this test at all collection times. The only time values were determined was on the last replication after fourteen cats had been given the treatments for seven days. The average methemoglobin percent of total hemoglobin for two cats by group did not demonstrate apparent differences between groups (Group 1=1.05%, Group 2=1.05%, Group 3=1.05%, Group 4=0.95%, Group 5=1.2%, Group 6=1.05%, Group 7=1.4%). These values are not outside the normal range.

The laboratory conducting this test also reported sulfhemoglobin as a percent of hemoglobin for each group (Group 1=0.65%, Group 2=1.45%, Group 3=2.15%, Group 4=2.80%, Group 5=1.50%, Group 6=2.75%, Group 7=3.90%). An interesting observation in this limited sampling was that the sulfhemoglobin concentration tended to increase as the dose of either drug was increased. Normal sulfhemoglobin levels are considered to be below 1.0 percent in humans by the laboratory that conducted the tests. This test has not been routinely conducted in cats so reference values are not known, but there is no reason to believe that the 1.0 percent value should be exceeded in normal cats. Further trials would need to be performed to determine if the sulfur of MHA and MET was a contributing factor in the increased sulfhemoglobin concentrations found in the treated animals in this study.



**Liver enzymes**

Alkaline phosphatase (ALP) and alanine aminotransferase (ALT) values were not significantly altered by the treatments. There was not any apparent effect on the liver by either of the two drugs using lack of change in the concentration of either of these two enzymes as the criteria. The values may be compared in Table 8 and Table 9, respectively, for each enzyme.

Table 8. Alkaline phosphatase means in IU/L, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline	Time	Time	Time
		Time 1, 2	3	4	5
1	None-Control	30.8	28.7	24.5	32.3
2	Low MHA	18.5	17.8	20.0	21.2
3	Med MHA	35.8	38.7	41.3	49.2
4	High MHA	27.9	31.7	34.3	37.2
5	Low MET	23.9	21.8	26.7	26.5
6	Med MET	33.3	27.8	26.7	31.2
7	High MET	22.3	21.5	26.6	21.4
Standard Error of Means		5.1	5.4	6.4	7.0
F-Value		1.50	1.69	1.23	1.91
Pr > F		0.2576	0.2077	0.3584	0.1610

Table 9. Alanine aminotransferase means in IU/L, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline	Time	Time	Time
		Time 1, 2	3	4	5
1	None-Control	84.3	84.3	89.3	123.3
2	Low MHA	73.0	78.3	109.5	95.0
3	Med MHA	178.3	109.5	159.5	168.8
4	High MHA	145.0	156.3	280.7	142.6
5	Low MET	73.1	73.3	81.3	73.5
6	Med MET	74.3	71.5	61.5	60.5
7	High MET	93.8	83.2	76.6	79.2
Standard Error of Means		28.8	25.9	65.4	30.7
F-Value		2.10	1.37	1.34	1.63
Pr > F		0.1285	0.3008	0.3131	0.2224

### Serum electrolytes

Four of the six serum electrolytes measured; sodium, chloride, calcium, and magnesium, were found not to be significantly changed by the treatments. Tables 10 through 13 provide the comparison of the concentration of these four electrolytes at various collection times of the study.

The variation in sodium serum concentration was slight in this study, both between groups and within groups, as seen in Table 10.

Table 10. Serum sodium concentration means in mEq/L, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline	Time	Time	Time
		Time 1, 2	3	4	5
1	None-Control	156.5	155.0	154.7	154.7
2	Low MHA	155.3	155.0	153.5	153.8
3	Med MHA	156.9	154.8	154.8	154.4
4	High MHA	155.0	154.5	153.3	152.8
5	Low MET	157.0	154.2	153.8	154.8
6	Med MET	155.8	154.0	154.3	154.0
7	High MET	155.9	153.5	153.4	154.8
Standard Error of Means		0.72	0.70	0.72	0.70
F-Value		1.11	0.66	0.68	0.99
Pr > F		0.4122	0.6798	0.6692	0.4731

The mean chloride values in Table 11 demonstrate that the serum chloride concentration varied little among groups. The chloride concentration did not vary inversely with the change in bicarbonate concentration (Table 35). The non-hyperchloremic metabolic acidosis that occurred in this study is discussed later.

Table 11. Serum chloride concentration means in mEq/L, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	123.8	122.5	123.7	123.7
2	Low MHA	124.1	123.7	123.8	123.5
3	Med MHA	124.3	123.3	124.5	122.8
4	High MHA	123.4	124.3	124.5	122.0
5	Low MET	124.3	122.5	122.2	122.2
6	Med MET	123.8	123.7	124.2	121.8
7	High MET	124.0	123.0	124.0	123.0
Standard Error of Means		0.78	0.56	0.77	0.87
F-Value		0.15	1.42	1.08	0.70
Pr > F		0.9849	0.2852	0.4253	0.6534



Tables 12 and 13 demonstrate that serum calcium or magnesium concentrations were not affected by a seven day treatment with the urinary acidifiers used in this study.

Table 12. Serum calcium concentration means in mg/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	9.7	9.5	9.8	9.5
2	Low MHA	9.4	9.4	9.2	9.1
3	Med MHA	9.8	9.5	9.6	9.7
4	High MHA	9.8	10.0	10.7	9.2
5	Low MET	10.0	9.6	9.7	9.6
6	Med MET	9.6	9.7	9.7	9.7
7	High MET	9.8	9.5	9.5	9.3
Standard Error of Means		0.24	0.26	0.57	0.30
F-Value		0.52	0.67	0.63	0.57
Pr > F		0.7802	0.6769	0.7063	0.7502

Table 13. Serum Magnesium concentration means in mEq/L, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	1.9	2.0	2.1	1.8
2	Low MHA	1.9	2.0	2.0	1.9
3	Med MHA	1.9	1.9	2.0	1.9
4	High MHA	1.9	1.8	2.1	1.9
5	Low MET	1.8	1.8	2.0	1.9
6	Med MET	1.9	1.9	2.0	1.9
7	High MET	1.8	1.8	1.9	1.9
Standard Error of Means		0.06	0.06	0.09	0.04
F-Value		0.55	2.55	0.36	1.09
Pr > F		0.7624	0.0791	0.8891	0.4222

### Blood ammonia

The ammonia concentration in the blood was not significantly changed by the treatments. The amino group that methionine contains and the hydroxy analogue lacks did not have a significant effect on blood ammonia as seen in Table 14. Unless liver metabolism is altered or venous

shunting is present, the ammonia levels in the blood, as measured in this study, would not be expected to be affected by the use of either of these two drugs, even at high doses.

Table 14. Blood Ammonia means in mg/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline	Time	Time	Time
		Time 1, 2	3	4	Time 5
1	None-Control	0.39	0.22	0.59	0.38
2	Low MHA	0.36	0.28	0.42	0.41
3	Med MHA	0.34	0.14	0.35	0.31
4	High MHA	0.31	0.33	0.32	0.31
5	Low MET	0.38	0.35	0.41	0.36
6	Med MET	0.37	0.27	0.45	0.39
7	High MET	0.27	0.27	0.26	0.34
Standard Error of Means		0.04	0.06	0.07	0.06
F-Value		1.00	1.00	2.32	0.37
Pr > F		0.4707	0.4671	0.1008	0.8839

### Blood pH

Blood pH did not change significantly among treatments, although significance was approached ( $P < 0.0675$ ) at Time 4. The pH ranged from a low of 7.182 to a high of 7.295 during the study. After three days of treatment the high MET group

had a mean pH of 7.204 (n = 6). Seven days of treatment were required for the high MHA group to reach an average pH of 7.203 (n = 6). After seven days the high MET group's pH had dropped further to a mean pH of 7.182 (n = 5) while the control group's blood pH was 7.280 (n = 6). The cats' adaption to the metabolic acidosis that developed is evident by minimal change in blood pH that may be seen for all groups except, possibly, Group 4 and Group 7, which are the high dose groups of each drug, in Table 15.

Table 15. Blood pH means in pH units, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	7.255	7.291	7.280	7.292
2	Low MHA	7.248	7.274	7.273	7.267
3	Med MHA	7.270	7.259	7.260	7.275
4	High MHA	7.246	7.225	7.203	7.294
5	Low MET	7.245	7.242	7.295	7.284
6	Med MET	7.292	7.243	7.244	7.267
7	High MET	7.239	7.204	7.182	7.259
Standard Error of Means		0.016	0.024	0.024	0.015
F-Value		1.35	1.56	2.70	1.24
Pr > F		0.3106	0.2422	0.0675	0.3525



Blood pCO<sub>2</sub>

The reduction in blood CO<sub>2</sub> as an adaptive measure to compensate the metabolic acidosis is evident in the treated groups. However, this decrease was not statistically significant among groups at any treatment time. The largest decrease was in the high MET group. Table 16 provides a comparison of pCO<sub>2</sub> values.

Table 16. pCO<sub>2</sub> means in mm/Hg, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	47.5	43.9	45.8	44.0
2	Low MHA	47.9	44.1	41.9	45.7
3	Med MHA	46.6	44.1	42.3	44.4
4	High MHA	44.8	41.7	40.4	43.2
5	Low MET	47.9	44.7	40.7	48.5
6	Med MET	42.3	41.9	41.4	45.3
7	High MET	46.0	39.3	36.2	44.7
Standard Error of Means		2.51	2.54	2.26	2.28
F-Value		0.66	0.59	1.49	0.57
Pr > F		0.6844	0.7338	0.2614	0.7438

### Necropsy

The complete necropsies performed on the six cats in the first replication and on the ten cats in the second replication revealed no gross abnormalities in any of the animals. All the animals necropsied were in excellent condition with an abundance of subcutaneous and internal body fat.

### Histopathology

The microscopic examination of the tissues did not demonstrate any differences between the animals receiving the various treatments and the control animals. The special staining techniques employed revealed that the vacuoles present in the tubule lining cells of the kidney and the hepatocytes of the liver contained glycogen in both control and treatment groups.

### **Parameters with Variations Among Treatments**

The test parameters with group mean values that were significantly different and that could be related to the treatment effects included the following:

Daily feed intake

Urine        Phosphorus, Magnesium, Urea nitrogen, Ammonia  
              pH, Titratable acidity.

Blood        Phosphorus, Potassium, Bicarbonate, Heinz body  
              formation.

General     Two deaths, Toxicosis.

Tables containing the mean of each parameter by group at each sample time are listed with a brief discussion of the results preceding each one. In addition to the standard error of means, F-value and probability of significance at each time period, tables of statistical comparisons between control and treatments and between the two drugs are made to further clarify the effects. Selected measurements at specific treatment times comparing the three doses of each drug to each other and to the control group at a given time are presented in graphical form.

#### Daily food intake

Daily food intake was affected by the treatments. Since the twenty-four hour food intake was calculated every day for the entire study, this measurement was divided into three time periods: the first being the fourteen day pre-dosing period, the second being the seven day dosing period, and the third being the seven day post-dosing period. The pre-dosing and post-dosing periods did not show significant differences in average daily food intake among treatment groups. There were significant differences during the treatment period. No satisfactory explanation can be given to explain the decrease in food intake by the non-treated control group during the dosing period. Table 17 provides the average daily food intake for each group during each period.

Table 17. Daily food intake means in grams, standard error of means, F-values, and probabilities of significance for treatment groups during pre-dosing, dosing and post-dosing periods.

Group	Level of Treatment	Periods		
		Pre-Dosing	Dosing	Post-Dosing
1	None-Control	75.6	65.2	68.9
2	Low MHA	80.6	79.2	83.9
3	Med MHA	85.2	78.2	85.9
4	High MHA	82.7	54.5	82.8
5	Low MET	79.1	74.7	89.4
6	Med MET	79.6	54.2	74.5
7	High MET	78.8	39.4	66.1
Standard Error of Means		6.7	2.1	5.8
F-Value		0.20	5.15	1.98
Pr > F		0.9686	0.0078	0.1483

During the seven day dosing period, the average daily food consumption of the MHA and MET groups were significantly different ( $P < 0.0193$ ) from each other but not from the control group ( $P < 0.8045$ ). The decline in food intake was linear with respect to increase in dose with both drugs (MHA,  $P < 0.0209$  and MET,  $P < 0.0026$ ). The statistical comparisons of mean daily food intake are given in Table 18.



Table 18. Comparisons of daily food intake during the seven day dosing period between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	During Treatment Period	
	F-Value	Pr > F
Control vs. Treatments	0.66	0.8045
MHA vs. MET	7.29	0.0193
Linear MHA	7.05	0.0209
Quadratic MHA	1.97	0.1859
Linear MET	14.44	0.0026
Quadratic MET	0.12	0.7329

The food intake of the low MHA and MET groups were similar. The decline in food intake in those cats receiving the medium MET treatment and the high MHA dose were similar. The high MET dose resulted in somewhat greater decline in food intake. The graph in Figure 1 demonstrates that the linear decline in food intake with respect to dose was faster and greater in the MET treated groups than in the MHA groups.

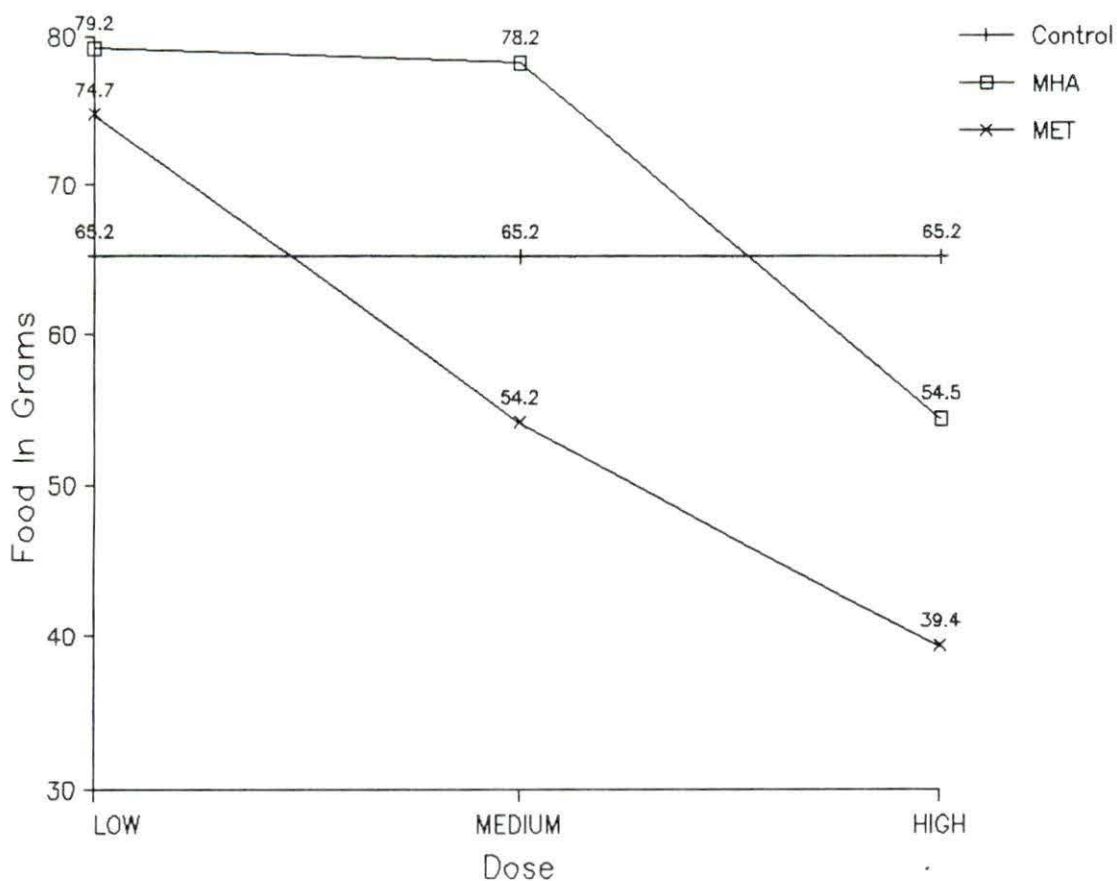


Figure 1. Average daily food intake for control, 3 MHA and 3 MET treatment groups during the seven day dosing period

The reduction in food intake is a prominent toxicological alteration that the high dose of MHA and the medium and high dose of MET affected in this study.

Urinary phosphorus

Table 19 gives the tabulation of urinary phosphorus values and demonstrates that there were significant differences among groups at both Time 3 ( $P < 0.0001$ ) and Time 4 ( $P < 0.0161$ ).

Table 19. Urinary phosphorus means in mg/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	257.0	233.5	242.0	238.3
2	Low MHA	237.0	231.7	212.0	251.3
3	Med MHA	282.0	217.5	214.2	268.8
4	High MHA	239.0	127.5	186.3	256.2
5	Low MET	282.4	280.5	279.0	276.6
6	Med MET	274.8	314.3	323.0	263.9
7	High MET	247.1	284.8	309.2	284.8
Standard Error of Means		20.6	14.9	24.7	14.3
F-Value		0.93	16.82	4.24	1.07
Pr > F		0.5093	0.0001	0.0161	0.4304

Table 20 summarizes the comparisons of groups that were made at Time 3 and Time 4 and provides statistical data that

suggest that the treatment groups were not significantly different from the control group in the amount of phosphorus excreted in the urine. The MHA groups and the MET groups were found to be different from each other at a high degree of significance at both sample Time 3 ( $P < 0.0001$ ) and sample Time 4 ( $P < 0.0006$ ).

Table 20. Comparisons of urinary phosphorus concentrations at Time 3 and 4 between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	Time 3 F-Value	Pr > F	Time 4 F-Value	Pr > F
Control vs. Treatments	0.33	0.5775	0.14	0.7176
MHA vs. MET	68.83	0.0001	21.26	0.0006
Linear MHA	24.40	0.0003	0.54	0.4765
Quadratic MHA	4.31	0.0600	0.25	0.6289
Linear MET	0.04	0.8406	0.21	0.6571
Quadratic MET	3.01	0.1085	1.36	0.2660

MHA demonstrated a significant linear tendency to cause a decline in urinary phosphorus as the dose increased at Time 3 ( $P < 0.0003$ ). This tendency was not present after treatments had been given for seven days. The urinary phosphorus levels tended to be lower in the MHA groups and



higher in the MET groups. The reasons for this require further study. Figure 2 and Figure 3 graphically demonstrate urinary phosphorus levels at Time 3 and Time 4, respectively, for control and treatment groups.

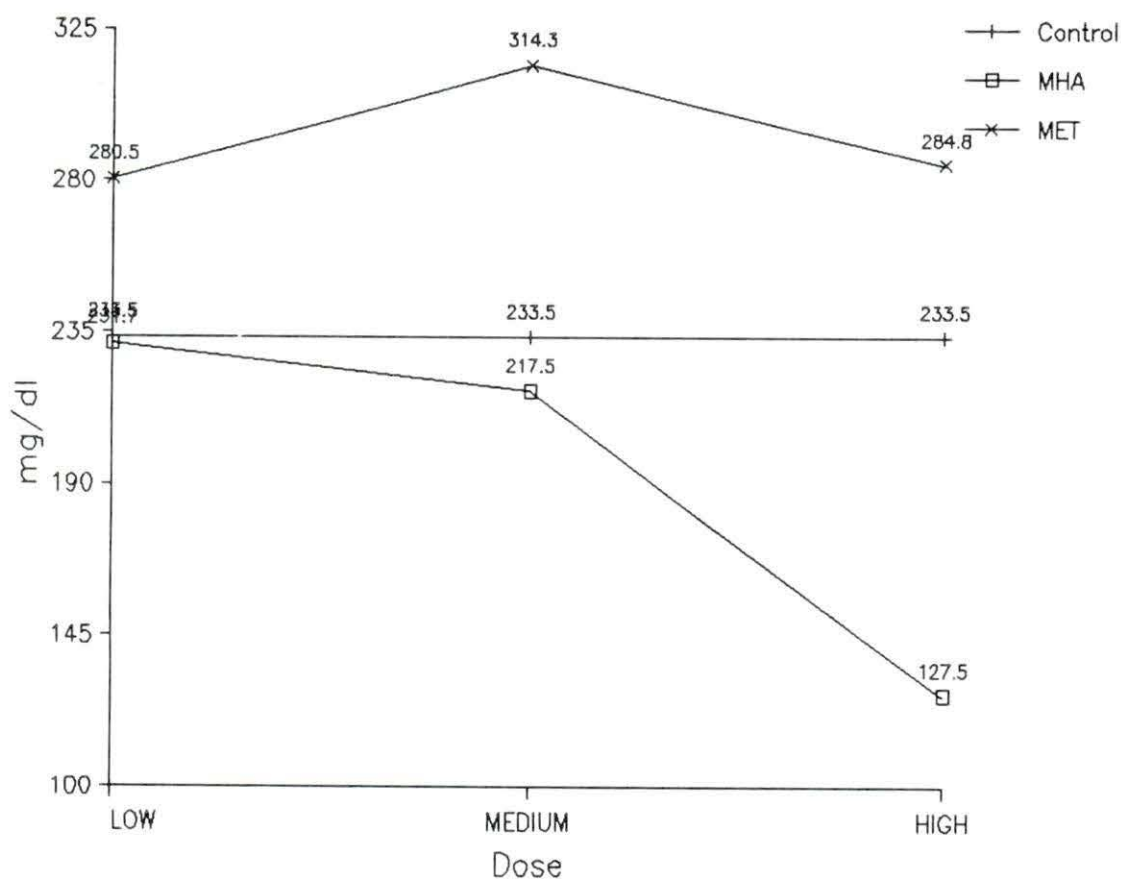


Figure 2. Urinary phosphorus concentrations for control, 3 MHA and 3 MET treatment groups after three days of treatments

The higher phosphorus concentration in the urine of the cats in the MET groups is possibly a reflection of the higher titratable acidity values in these groups. Phosphorus in the form of phosphate is part of a buffer system which aids in the elimination of the hydrogen ion that resulted from the metabolic acidosis of the treatments. The excess hydrogen ion production in the highest dose groups may result in the depletion of the phosphate buffer, accounting for the decrease of phosphorus as the dose increased.

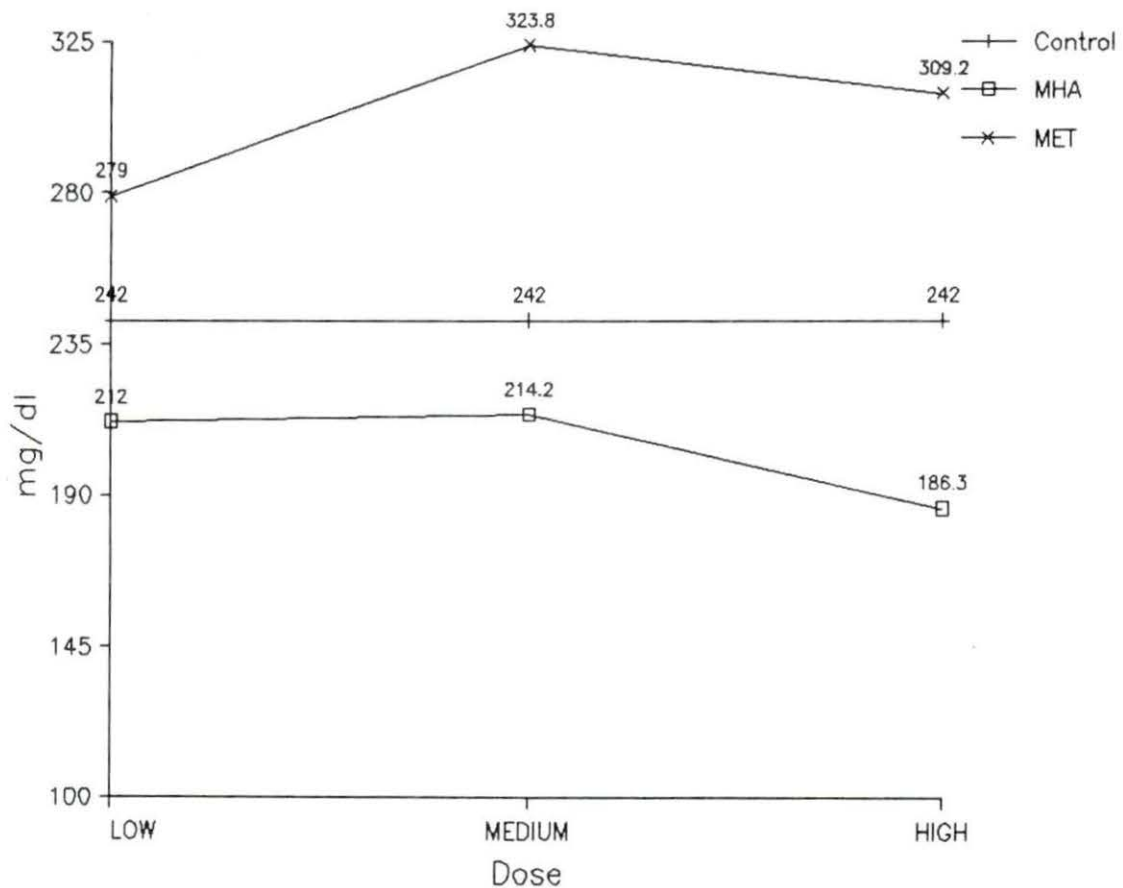


Figure 3. Urinary phosphorus concentrations for control, 3 MHA and 3 MET treatment groups after seven days of treatments

Urinary magnesium

Treatment with either drug at all dosage levels resulted in an increase in urinary magnesium values when compared to controls. Table 21 gives the comparison of the magnesium levels between groups at each sample time. The increase was not strictly dose related.

Table 21. Urinary magnesium means in mEq/L, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	10.9	7.9	10.9	5.5
2	Low MHA	10.2	14.8	15.9	5.8
3	Med MHA	11.7	20.0	19.4	7.7
4	High MHA	10.0	16.4	20.8	8.8
5	Low MET	10.3	22.9	19.4	11.6
6	Med MET	17.1	15.6	18.3	7.4
7	High MET	12.8	11.4	14.1	9.2
Standard Error of Means		2.5	2.0	2.8	2.3
F-Value		1.00	6.09	1.56	0.85
Pr > F		0.4672	0.0040	0.2404	0.5571

There did seem to be a tendency for an increasing MHA dose to cause an increase in magnesium excretion and an increasing MET dose a decreasing trend. A linear model could

be applied to MET at three days but not at seven days. During the dosing period the difference in urinary magnesium levels between the control group and the treatment groups was significant at both Time 3 ( $P < 0.0015$ ) and Time 4 ( $P < 0.0449$ ). The two drugs were not found to be significantly different in their effect on magnesium urinary excretion. The statistical comparisons are found in Table 22.

Table 22. Comparisons of urinary magnesium concentrations at Time 3 and 4 between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	Time 3 F-Value	3 Pr > F	Time 4 F-Value	4 Pr > F
Control vs. Treatments	16.62	0.0015	5.01	0.0449
MHA vs. MET	0.06	0.8062	0.74	0.4051
Linear MHA	0.31	0.5883	1.51	0.2422
Quadratic MHA	3.12	0.1028	0.09	0.7733
Linear MET	16.03	0.0018	2.61	0.1322
Quadratic MET	0.40	0.5391	0.45	0.5155

### Urinary urea nitrogen

The urinary urea nitrogen data are provided in Table 23. Treatments were different only at Time 3. It may be noted that high doses of each drug had a tendency to cause a decrease in urea. This may represent a shift in nitrogen



excretion away from urea and to ammonium which may be associated with the metabolic acidosis that occurred in the high MHA and MET groups. Alternatively, this may represent a decline in urea formation because of decreased food intake, especially at the high doses of the drugs.

Table 23. Urinary urea nitrogen means in mg/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	4709.0	4511.7	4725.8	4779.5
2	Low MHA	4884.1	5175.0	4901.3	5370.2
3	Med MHA	5136.8	4941.7	4920.8	5476.0
4	High MHA	4742.8	3561.7	4492.5	4880.0
5	Low MET	4974.4	4693.3	4861.7	5173.7
6	Med MET	5149.6	4140.8	4650.2	5513.5
7	High MET	4932.2	3759.2	4302.0	5496.4
Standard Error of Means		194.8	195.0	278.2	237.2
F-Value		0.79	9.52	0.62	1.59
Pr > F		0.5978	0.0006	0.7095	0.2329

It is demonstrated in Table 24 that after three days of treatment urinary urea nitrogen concentration demonstrated a linear tendency to decline in response to increased dose of each of the drugs. However, this did not occur after seven

days of treatment, and at none of the sample times, including Time 3, was the control group different from the treatment groups.

Table 24. Comparisons of urinary urea nitrogen concentrations at Time 3 and 4 between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	Time 3 F-Value	3 Pr > F	Time 4 F-Value	4 Pr > F
Control vs. Treatments	0.40	0.5394	0.01	0.9381
MHA vs. MET	5.16	0.0423	0.36	0.5618
Linear MHA	34.22	0.0001	1.08	0.3193
Quadratic MHA	5.76	0.0335	0.43	0.5235
Linear MET	11.77	0.0054	1.26	0.2840
Quadratic MET	0.13	0.7268	0.01	0.9402

The graphs in Figure 4 and Figure 5 depict the urinary urea nitrogen at Time 3 and Time 4, respectively, for the seven groups.

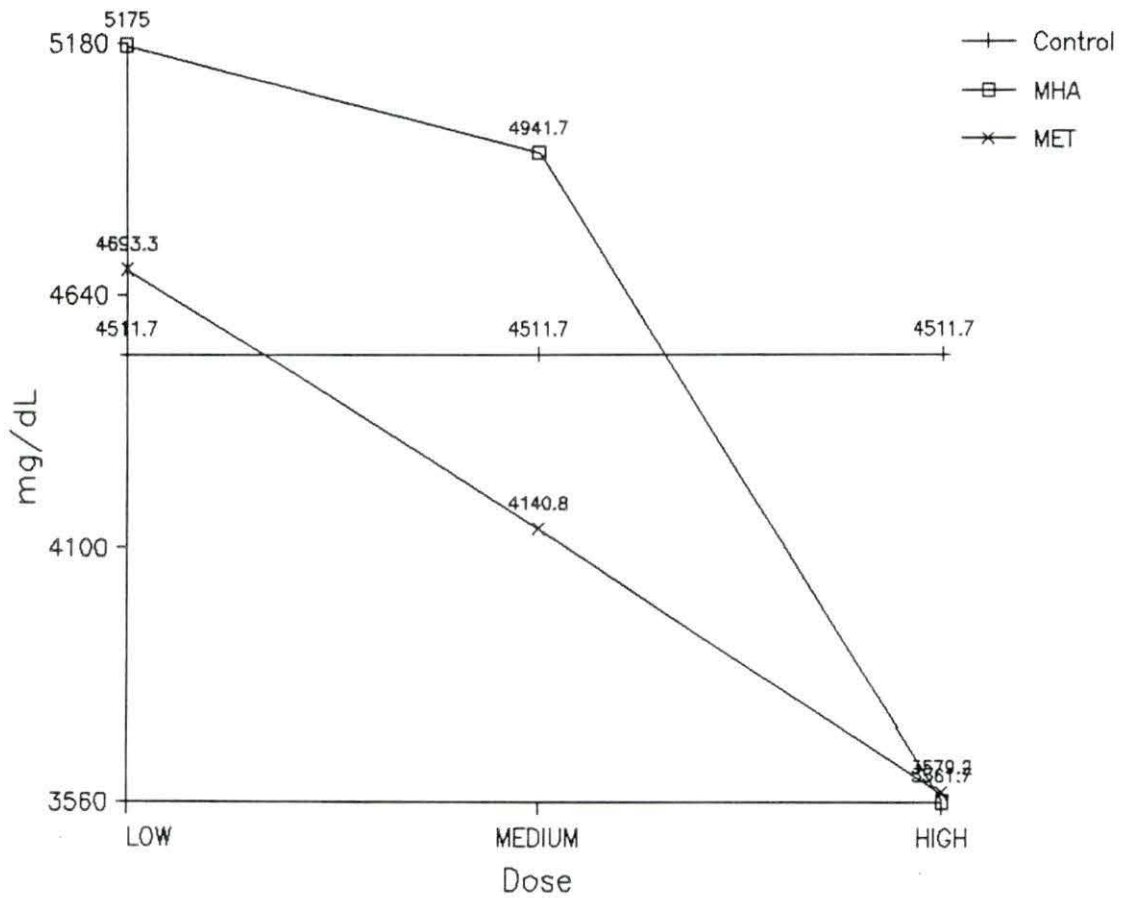


Figure 4. Urinary urea nitrogen concentrations for control, 3 MHA and 3 MET treatment groups after three days of treatments.

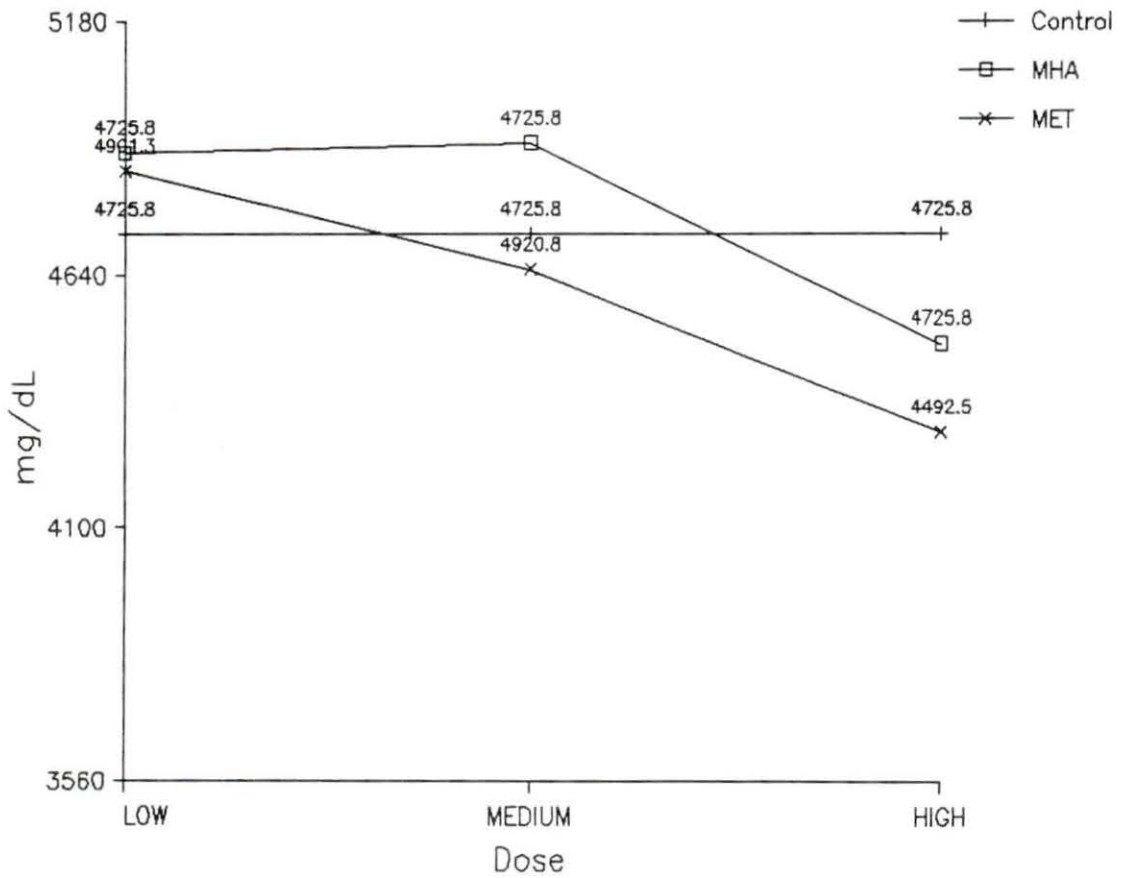


Figure 5. Urinary urea nitrogen concentrations for control, 3 MHA and 3 MET treatment groups after seven days of treatment.



Urinary ammonia

The urinary ammonia concentrations for the study are provided in Table 25. The treatments caused an increase in the concentration of ammonia in the urine when compared to the controls. The ammonia concentrations were higher in the MHA treated cats and lower in the MET groups at all three doses.

Table 25. Urinary ammonia means in mg/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	43.1	41.4	38.8	42.8
2	Low MHA	44.0	48.4	51.3	50.2
3	Med MHA	41.1	49.3	51.1	43.6
4	High MHA	43.9	57.9	59.3	45.6
5	Low MET	43.2	44.4	42.7	39.2
6	Med MET	41.8	47.2	45.6	46.4
7	High MET	42.3	50.6	51.9	41.1
Standard Error of Means		1.3	3.0	3.2	2.9
F-Value		0.70	2.99	4.45	1.57
Pr > F		0.6521	0.0504	0.0135	0.2383

During the dosing period the urinary ammonia levels increased as the dosage level of the two drugs was increased.

This increase was significantly different than the control level at Time 3 ( $P < 0.0260$ ) and at Time 4 ( $P < 0.0061$ ). After seven days of treatment the excretion of ammonia was significantly different ( $P < 0.0232$ ) between the MHA groups and the MET groups. Table 26 gives the comparison.

Table 26. Comparisons of urinary ammonia concentrations at Time 3 and 4 between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	Time 3		Time 4	
	F-Value	Pr > F	F-Value	Pr > F
Control vs. Treatments	6.44	0.0260	11.01	0.0061
MHA vs. MET	3.31	0.0937	6.76	0.0232
Linear MHA	5.02	0.0448	3.09	0.1041
Quadratic MHA	1.07	0.3213	1.13	0.3081
Linear MET	2.09	0.1741	3.93	0.0709
Quadratic MET	0.00	0.9480	0.23	0.6410

The graphs in Figure 6 and Figure 7 depict the urinary ammonia values at Time 3 and Time 4, respectively, for the seven groups.

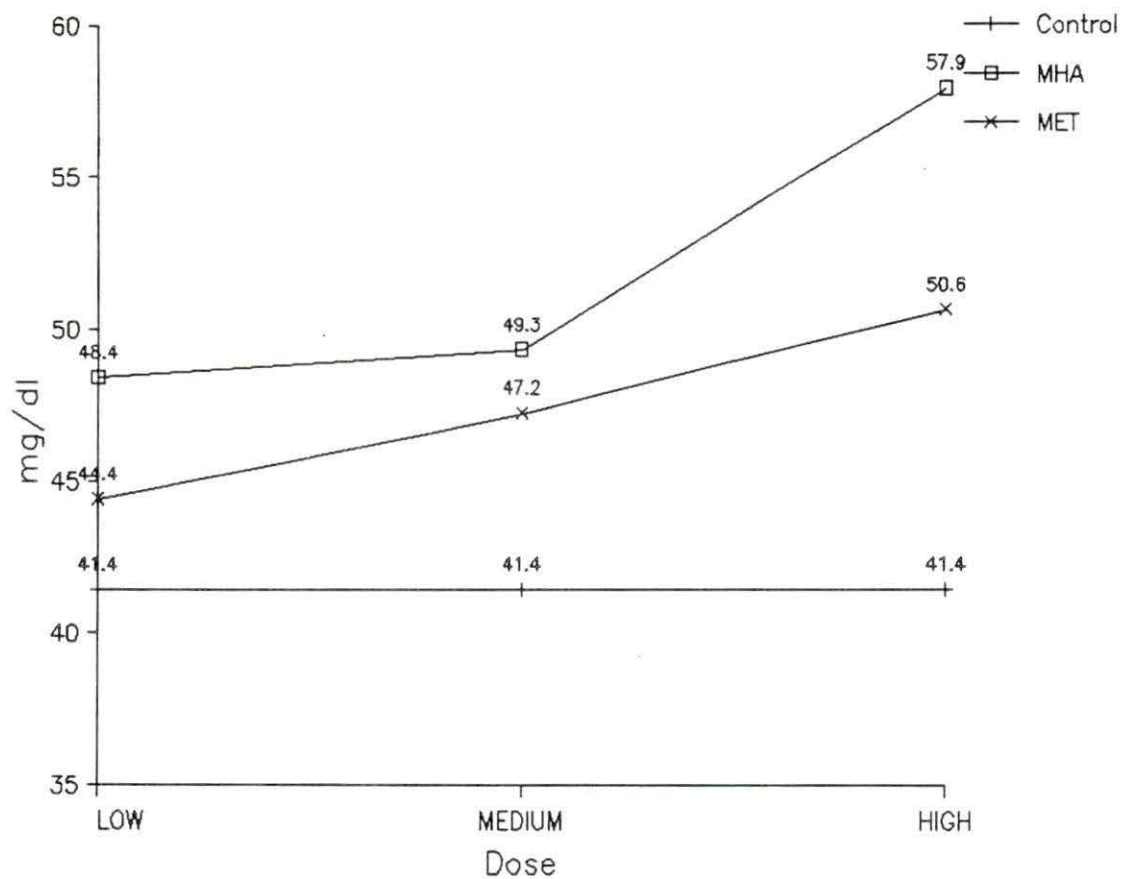


Figure 6. Urinary ammonia concentrations for control, 3 MHA and 3 MET treatment groups after three days of treatments

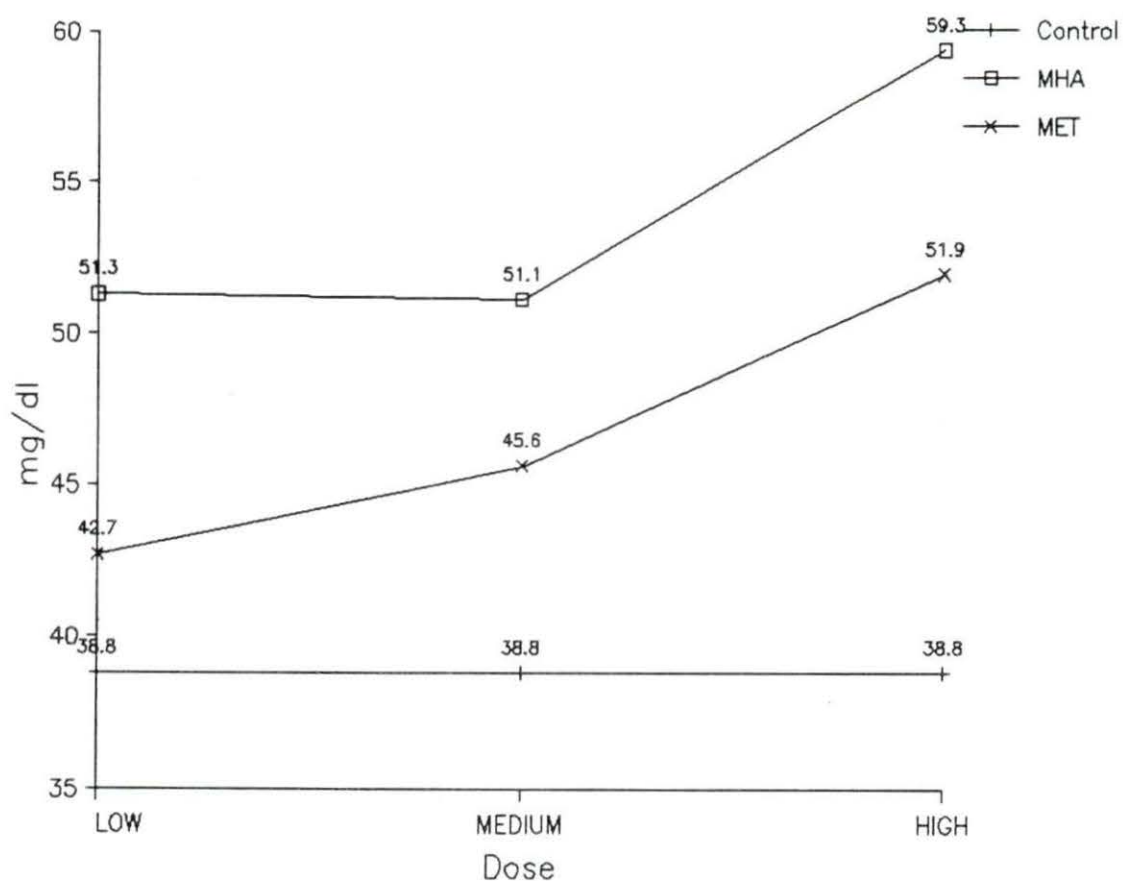


Figure 7. Urinary ammonia concentrations for control, 3 MHA and 3 MET treatment groups after seven days of treatment

The rise in the urinary ammonia concentration associated with the use of the urinary acidifiers MHA and MET may represent a normal compensatory response by the renal tubular epithelial cells in secreting ammonia into the tubular lumen in order to reduce acidity by the subsequent formation of ammonium ion which effectively traps the hydrogen ion for



excretion in the urine.<sup>110</sup> The alternative explanation is that the major reason for excretion of hydrogen ions is to trap ammonia, and that the ammonium ion in the urine does not represent excretion of acid.<sup>105</sup> An explanation to account for increased urinary ammonia in the MHA groups compared to the MET groups cannot be satisfactorily made without further studies. It is interesting to note that titratable acidity values were higher for MET groups and lower for the MHA groups (reversed to the urinary ammonia) indicating that in this study the hydrogen ions may have been disposed in slightly different ways for MHA and MET. The phosphate buffering system appeared to be more involved in eliminating the hydrogen ion in the MET treated cats and the ammonia/ammonium buffering system in hydrogen ion elimination in the MHA treated cats. This will require further study.

### Urinary pH

The urinary pH was decreased following all doses of MHA and MET to below the target pH of 6.6 necessary to prevent struvite formation in the urine. Table 27 demonstrates that steady state appears to have been reached by three days of treatment as the pH at each of the 3 doses of each drug are similar when compared to the pH of the urine after seven days. It should also be emphasized that the lowest dose for

each drug would be adequate to provide the proper urinary pH to preclude struvite precipitation and formation in the urine of cats.

Table 27. Urinary pH means in pH units, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	6.72	6.84	6.68	6.93
2	Low MHA	6.78	6.38	6.39	6.89
3	Med MHA	6.56	6.09	5.99	6.79
4	High MHA	6.59	5.86	5.93	6.65
5	Low MET	6.55	5.98	6.17	6.67
6	Med MET	6.71	5.87	5.92	6.80
7	High MET	6.58	5.82	5.93	6.79
Standard Error of Means		0.11	0.07	0.09	0.14
F-Value		0.66	26.14	11.35	0.46
Pr > F		0.6801	0.0001	0.0002	0.8222

Table 28 provides further comparisons of the groups. After three days or seven days of treatment the pH of the urine of the cats in the control group was significantly different ( $P < 0.0001$ ) than the treatment groups. After three days of dosing the MHA groups and MET groups were statistically different ( $P < 0.0028$ ) from each other as

indicated by the variation in the reduction of urinary pH. Also, MHA caused a significant ( $P < 0.0003$ ) linear decline in urinary pH values at Time 3 as the dose was increased. MET treatments did not demonstrate a dose related decline in pH. The decline in urinary pH was similar at all three doses for the MET treated groups at Time 3. After the treatments had been applied for seven days, the MHA groups were no longer statistically different from the MET groups in lowering the urinary pH. However, the linear model still described the decline of pH with increase in dose ( $P < 0.0027$ ) for the MHA groups.

Table 28. Comparisons of urinary pH at Time 3 and 4 between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	Time 3		Time 4	
	F-Value	Pr > F	F-Value	Pr > F
Control vs. Treatments	115.20	0.0001	44.93	0.0001
MHA vs. MET	13.96	0.0028	1.81	0.2035
Linear MHA	25.16	0.0003	14.14	0.0027
Quadratic MHA	0.08	0.7841	2.62	0.1315
Linear MET	2.36	0.1502	2.92	0.1131
Quadratic MET	0.11	0.7493	1.61	0.2289

The decline of urinary pH is further demonstrated by the graphs in Figure 8 and Figure 9.

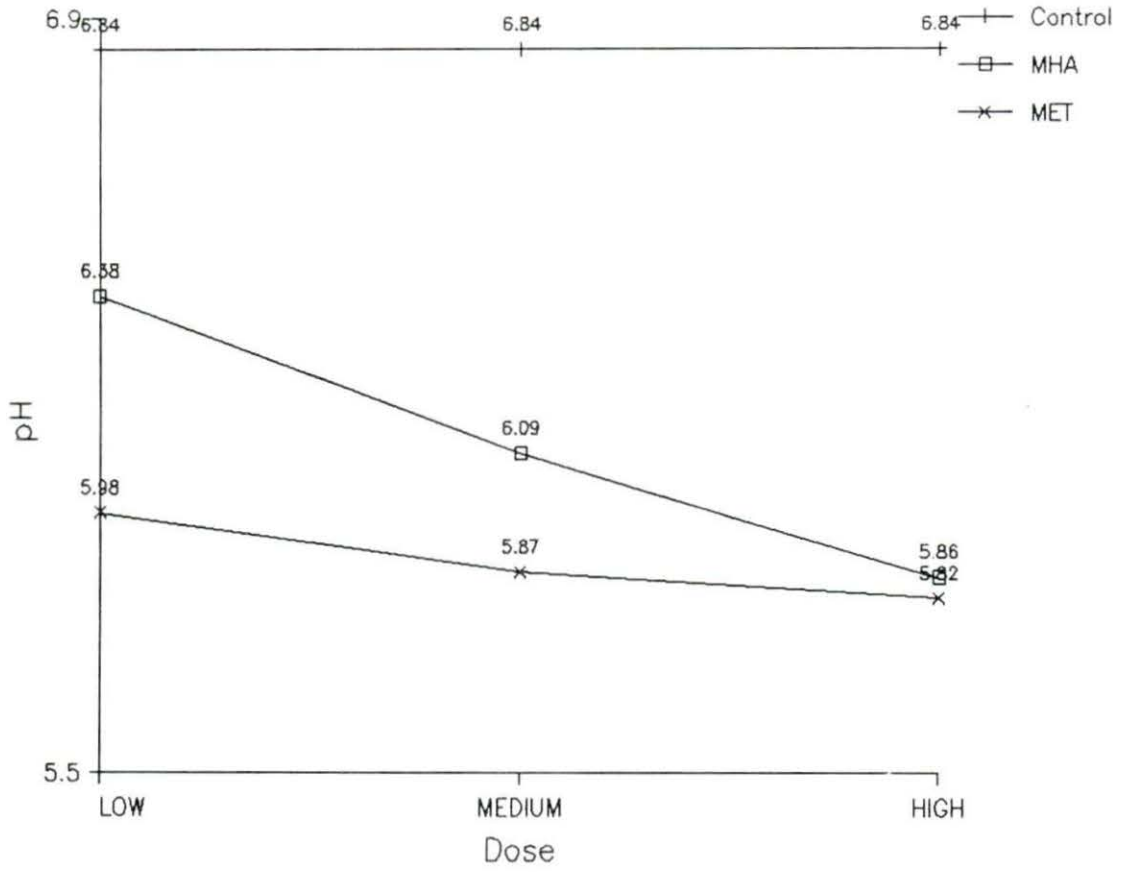


Figure 8. Urinary pH values for control, 3 MHA and 3 MET treatment groups after three days of treatment



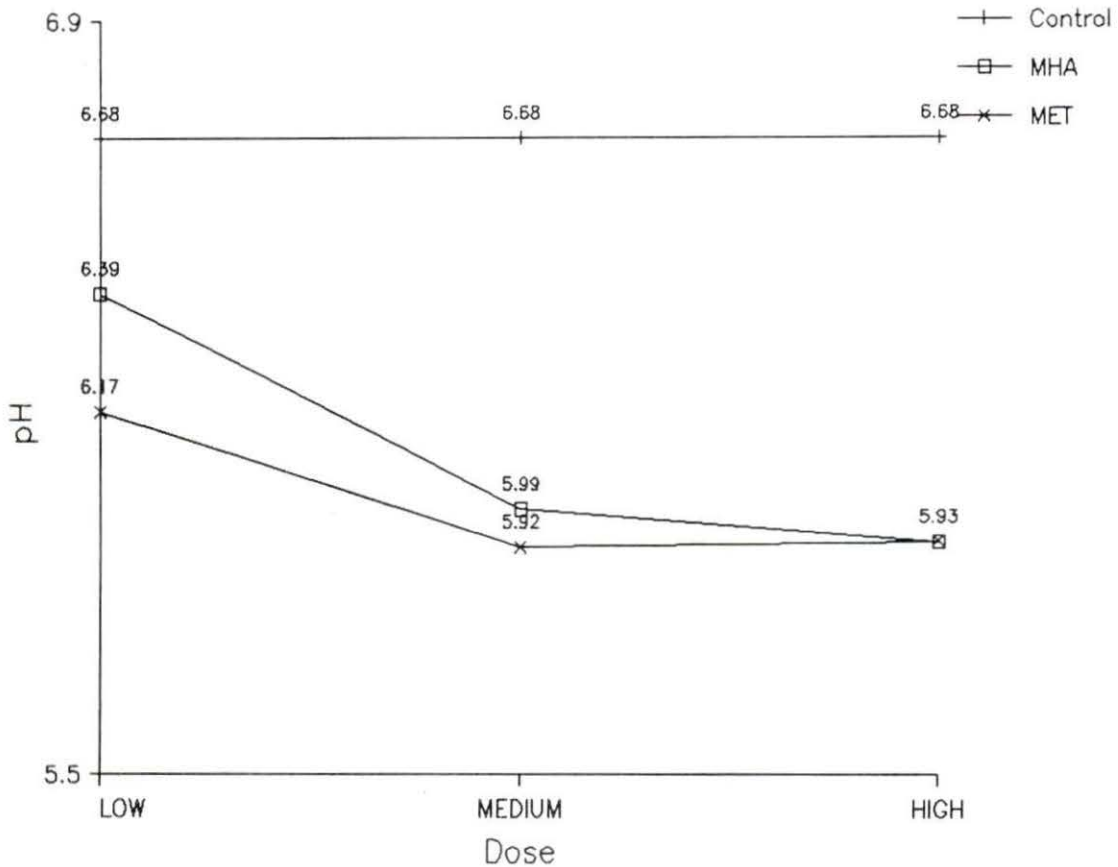


Figure 9. Urinary pH values for control, 3 MHA and 3 MET treatment groups after seven days of treatment

#### Urinary titratable acidity

Twenty-four hour titratable acidity to pH 7.4 was significantly different among groups during treatment at Time 3 ( $P < 0.0001$ ) and Time 4 ( $P < 0.0006$ ). The titratable acidity values to 7.4 were generally lower for the MHA treatment groups and higher for the MET treatment groups. Table 29 provides the data for this measurement.

Table 29. Urinary titratable acidity to pH 7.4 means, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	1.63	1.13	1.47	1.00
2	Low MHA	1.57	2.13	2.25	1.02
3	Med MHA	2.48	2.87	3.42	1.48
4	High MHA	2.36	2.35	3.01	2.12
5	Low MET	1.64	3.87	2.97	1.72
6	Med MET	2.03	5.97	5.02	1.67
7	High MET	1.98	4.38	4.66	1.74
Standard Error of Means		1.41	0.33	0.40	0.43
F-Value		0.80	24.77	9.52	0.73
Pr > F		0.5871	0.0001	0.0006	0.6352

During the dosing period significant differences were demonstrated between the control group and the treatment groups (Time 3,  $P < 0.0001$  and Time 4,  $P < 0.0004$ ) for titratable acidity to pH 7.4. The comparison of titratable acidity the cats in the MHA groups and those in the MET groups revealed a significant difference at both Time 3 ( $P < 0.0001$ ) and Time 4 ( $P < 0.0023$ ).

The titratable acidity of urine is largely dependent on the amount of mono and dibasic phosphate present. The equivalents of base required to titrate a urine specimen to physiologic pH of 7.4 is equal to the excretion of hydrogen ions except for those eliminated as ammonium ion. Table 30 summarizes these statistical comparisons.

Table 30. Comparisons of urinary titratable acidity to pH 7.4 at Time 3 and 4 between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	Time 3		Time 4	
	F-Value	Pr > F	F-Value	Pr > F
Control vs. Treatments	49.06	0.0001	23.05	0.0004
MHA vs. MET	74.25	0.0001	14.90	0.0023
Linear MHA	0.22	0.6461	1.86	0.1982
Quadratic MHA	2.46	0.1427	2.58	0.1340
Linear MET	1.26	0.2834	6.93	0.0219
Quadratic MET	21.37	0.0006	6.23	0.0281

The titratable acidity to pH 7.4 is graphically depicted in Figure 10 and Figure 11 at Time 3 and Time 4, respectively. The graphs demonstrate that the quadratic model ( $P < 0.0006$  at Time 3 and  $P < 0.0281$  at Time 4) best represent the response to increasing MET dosage.

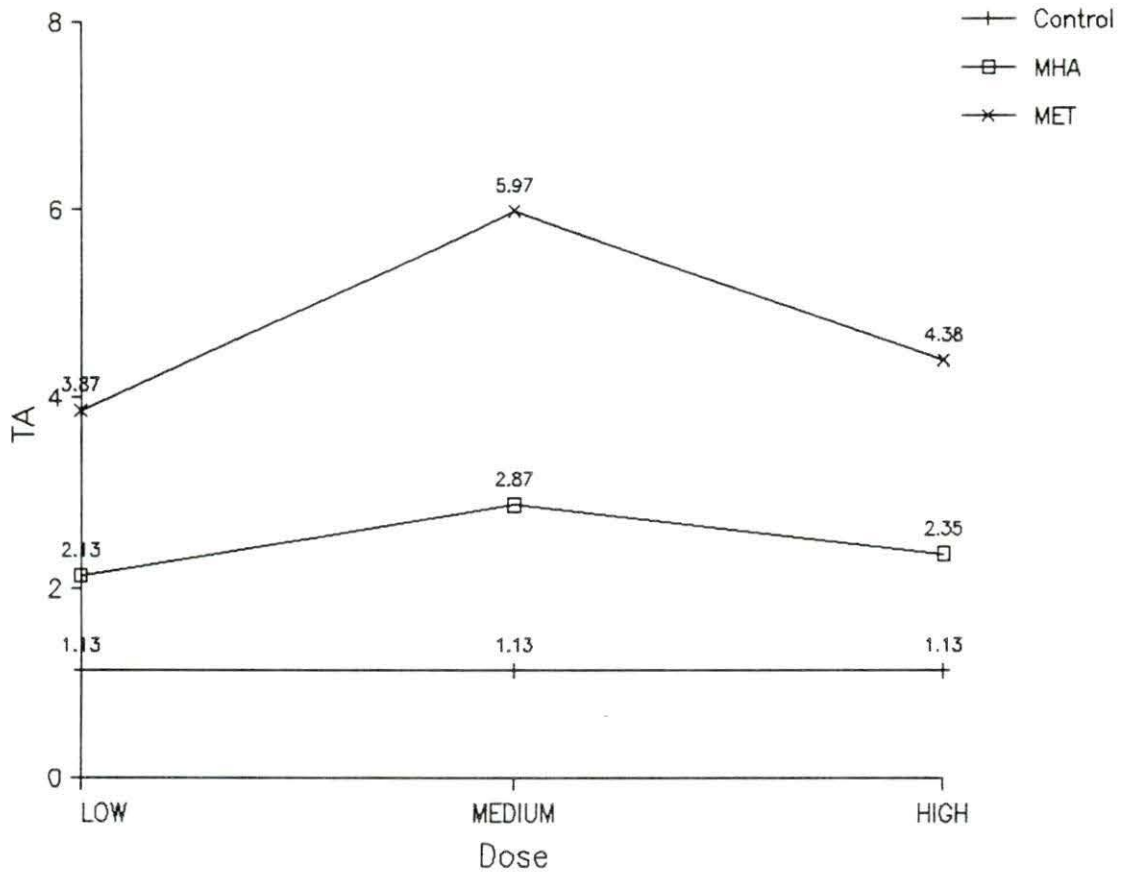


Figure 10. Urinary titratable acidity to pH 7.4 for control, 3 MHA and 3 MET treatment groups after three days of treatments



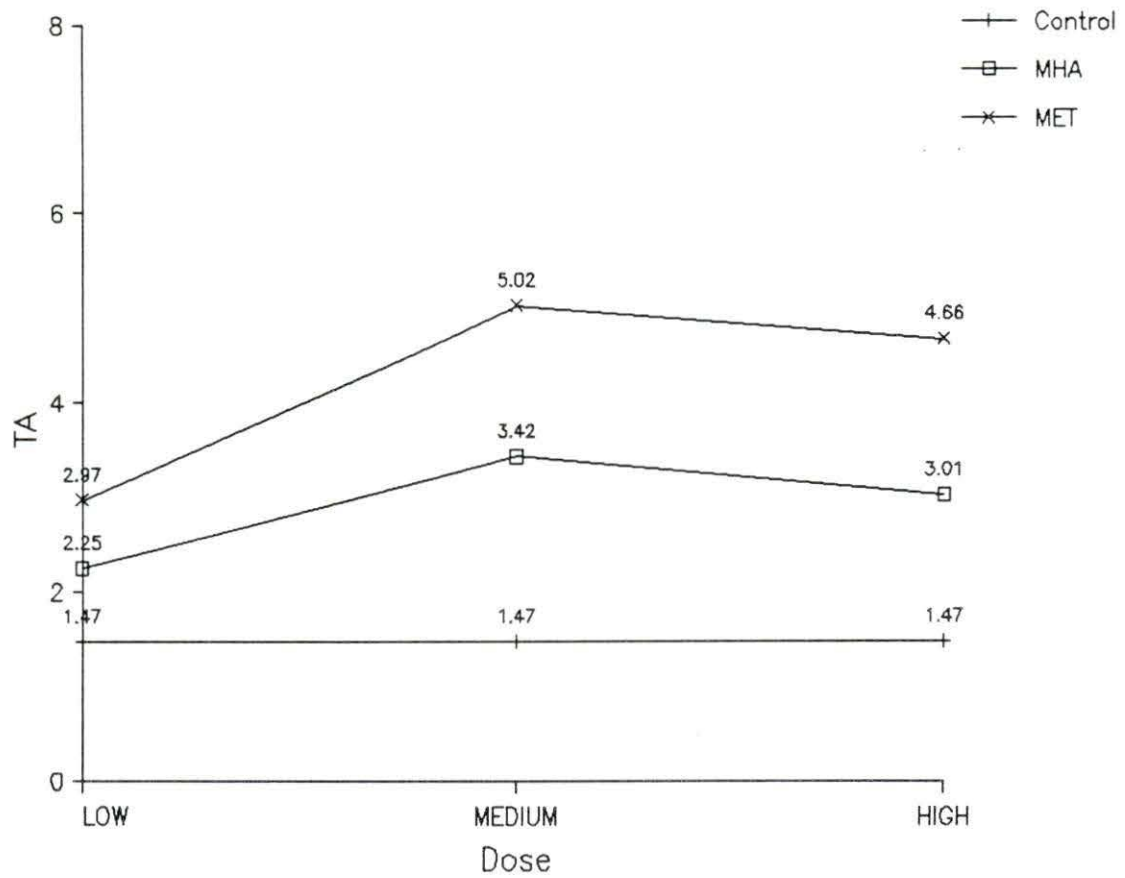


Figure 11. Urinary titratable acidity to pH 7.4 for control, 3 MHA and 3 MET treatment groups after seven days of treatments

Twenty-four hour titratable acidity to pH 8.4 was significantly different among groups during treatment only at Time 3 ( $P < 0.0091$ ) and not at Time 4 ( $P < 0.0850$ ). Again the titratable acidity values to 8.4 were generally lower for the MHA treatment groups and higher for the MET treatment groups. Table 31 provides the data for titratable acidity to pH 8.4.

Table 31. Urinary titratable acidity to pH 8.4 means, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	3.42	2.72	3.23	3.02
2	Low MHA	3.46	4.03	4.13	2.60
3	Med MHA	3.72	4.98	5.88	3.35
4	High MHA	3.95	4.57	3.77	3.40
5	Low MET	3.21	6.17	5.03	3.28
6	Med MET	4.40	4.65	6.75	4.30
7	High MET	4.15	5.83	4.18	3.46
Standard Error of Means		0.47	0.52	0.80	0.58
F-Value		0.84	4.94	2.48	0.79
Pr > F		0.5627	0.0091	0.0850	0.5954

For titratable acidity to pH 8.4 significance between the control group and the treatment groups occurred at Time 3 ( $P < 0.0013$ ) and was approached at Time 4 ( $P < 0.0584$ ). The

comparison of titratable acidity to pH 8.4 between the MHA groups and the MET groups demonstrated significance only at Time 3 ( $P < 0.0322$ ). Table 32 summarizes this information.

Table 32. Comparisons of urinary titratable acidity to pH 8.4, at Time 3 and 4 between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	Time 3 F-Value	3 Pr > F	Time 4 F-Value	4 Pr > F
Control vs. Treatments	17.31	0.0013	4.38	0.0584
MHA vs. MET	5.87	0.0322	1.77	0.2078
Linear MHA	0.53	0.4796	0.11	0.7502
Quadratic MHA	1.17	0.3015	3.93	0.0707
Linear MET	0.21	0.6565	0.09	0.7681
Quadratic MET	4.55	0.0543	3.60	0.0821

The graph in Figure 12 demonstrates the titratable acidity to pH 8.4 at Time 4 after seven days of treatments.

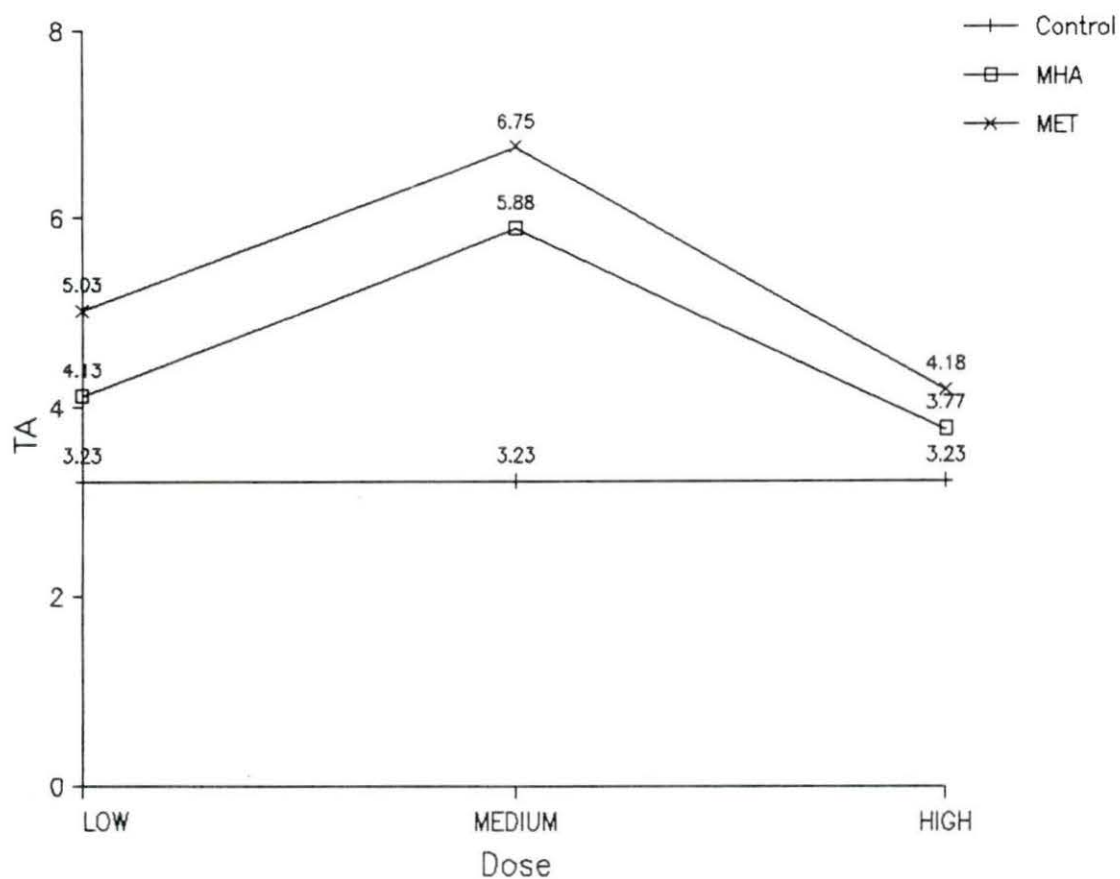


Figure 12. Urinary titratable acidity to pH 8.4 for control, 3 MHA and 3 MET treatment groups after seven days of treatments



**Serum phosphorus**

Table 33 provides the comparison of the phosphate concentrations of the serum. Only at Time 4 is there a significant difference among groups ( $P < 0.0245$ ). The most apparent difference was the decrease in phosphorus concentration at Time 4 for the high MET group.

Table 33. Serum phosphorus concentration means in mg/dl, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	6.6	6.0	5.8	6.0
2	Low MHA	6.3	6.2	5.9	6.1
3	Med MHA	6.5	6.6	6.2	6.4
4	High MHA	6.5	6.3	5.7	6.2
5	Low MET	6.6	6.1	6.6	6.4
6	Med MET	5.5	5.5	5.5	5.8
7	High MET	6.2	5.6	4.7	6.1
Standard Error of Means		0.34	0.24	0.29	0.38
F-Value		1.31	2.53	3.75	0.32
Pr > F		0.3238	0.0811	0.0245	0.9116

**Serum potassium**

Serum potassium concentrations were significantly different among groups only after the treatments had been

given for seven days at Time 4 ( $P < 0.0436$ ). The decrease is most evident in the high MHA group and the high MET group. This finding is somewhat paradoxical as metabolic acidosis is usually associated with hyperkalemia. Urinary potassium was not measured so it is not known whether increased excretion of potassium also occurred in these groups. Table 34 provides the serum potassium data.

Table 34. Serum potassium concentration means in mEq/L, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	5.1	4.8	4.9	5.1
2	Low MHA	5.0	4.9	4.9	5.0
3	Med MHA	5.0	4.9	5.0	5.2
4	High MHA	5.1	4.8	4.4	5.6
5	Low MET	5.3	4.9	5.0	5.0
6	Med MET	5.0	5.0	4.8	5.5
7	High MET	5.0	4.3	4.0	5.5
Standard Error of Means		0.15	0.19	0.20	0.15
F-Value		0.69	1.74	3.13	2.55
Pr > F		0.6595	0.1961	0.0436	0.0789

**Blood bicarbonate**

The blood bicarbonate concentrations were lowered by the treatments. Table 35 is a tabulation of the bicarbonate levels for each group at each collection period. The difference was highly significant at Time 3 ( $P < 0.0152$ ) and at Time 4 ( $P < 0.0001$ ).

Table 35. Blood bicarbonate concentrations means in mEq/L, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline			
		Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	20.4	20.6	21.0	20.6
2	Low MHA	20.2	19.8	18.8	21.2
3	Med MHA	20.6	19.1	18.4	20.2
4	High MHA	18.6	16.6	15.3	20.4
5	Low MET	20.1	18.7	19.3	20.4
6	Med MET	19.8	17.6	17.4	20.1
7	High MET	19.2	15.0	13.1	19.3
Standard	Error of Means	0.68	0.93	0.70	0.86
F-Value		1.05	4.30	12.69	0.22
Pr > F		0.4434	0.0152	0.0001	0.9629

The decline in bicarbonate blood levels was greater as the dose of each of the drugs increased. This decline fit a

linear model for each drug at Time 3 and Time 4  
 (MHA  $P < 0.0336$  and  $P < 0.0039$ , MET  $P < 0.0152$  and  $P < 0.0002$ ).  
 The two drugs were not found to be statistically different  
 from each other in their reduction of blood bicarbonate.  
 Table 36 gives the comparison during the treatment period.

Table 36. Comparisons of blood bicarbonate concentrations at Time 3 and 4 between control group and all treatment groups, between MHA and MET groups, and for linear and quadratic models for MHA and MET

Comparisons	Time 3		Time 4	
	F-Value	Pr > F	F-Value	Pr > F
Control vs. Treatments	7.69	0.0169	25.17	0.0003
MHA vs. MET	3.28	0.0954	1.30	0.2759
Linear MHA	5.76	0.0336	12.67	0.0039
Quadratic MHA	0.69	0.4237	2.29	0.1564
Linear MET	8.01	0.0152	26.47	0.0002
Quadratic MET	0.41	0.5357	0.84	0.3782

Figure 13 and Figure 14 graphically display the bicarbonate levels in the blood at collection Time 3 and Time 4, respectively. It may be noted that a drop in bicarbonate concentration was, in general, greater and faster for the MET groups when compared to the MHA groups.



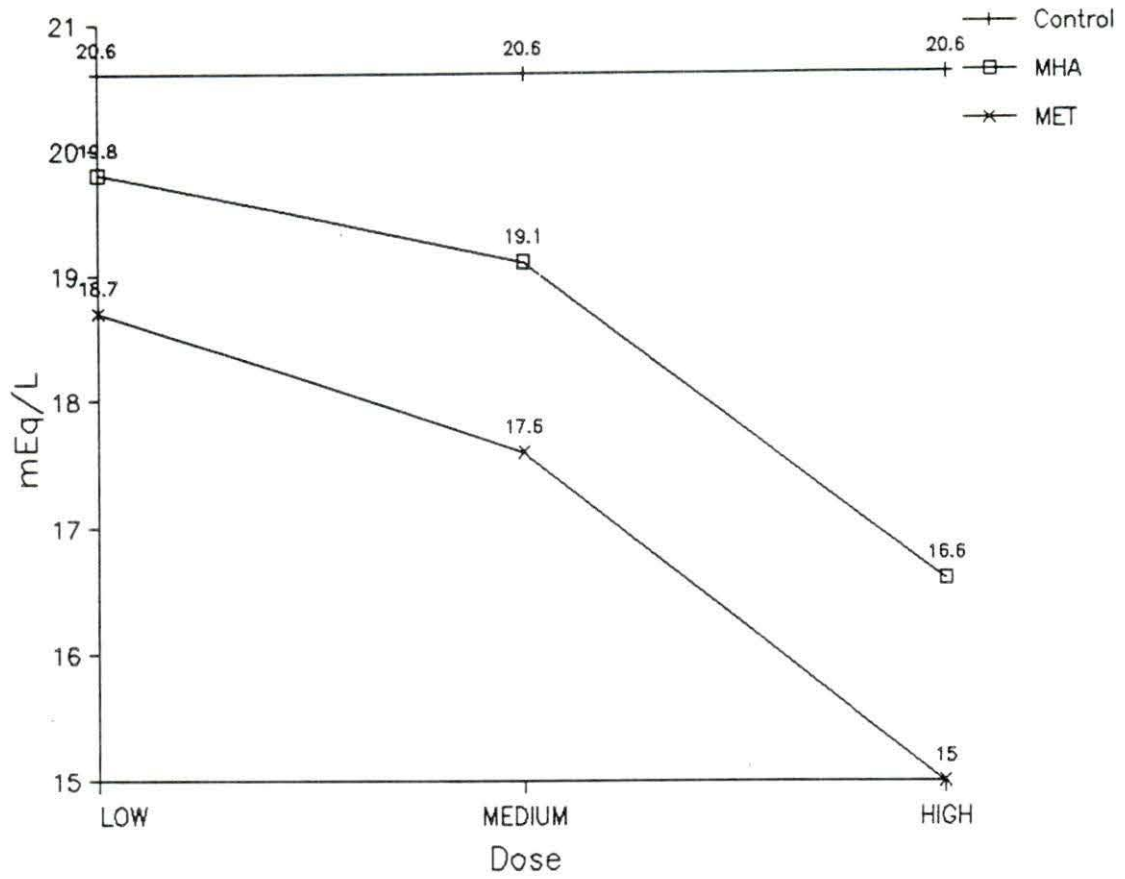


Figure 13. Blood bicarbonate concentrations for control, 3 MHA and 3 MET treatment groups after three days of treatment

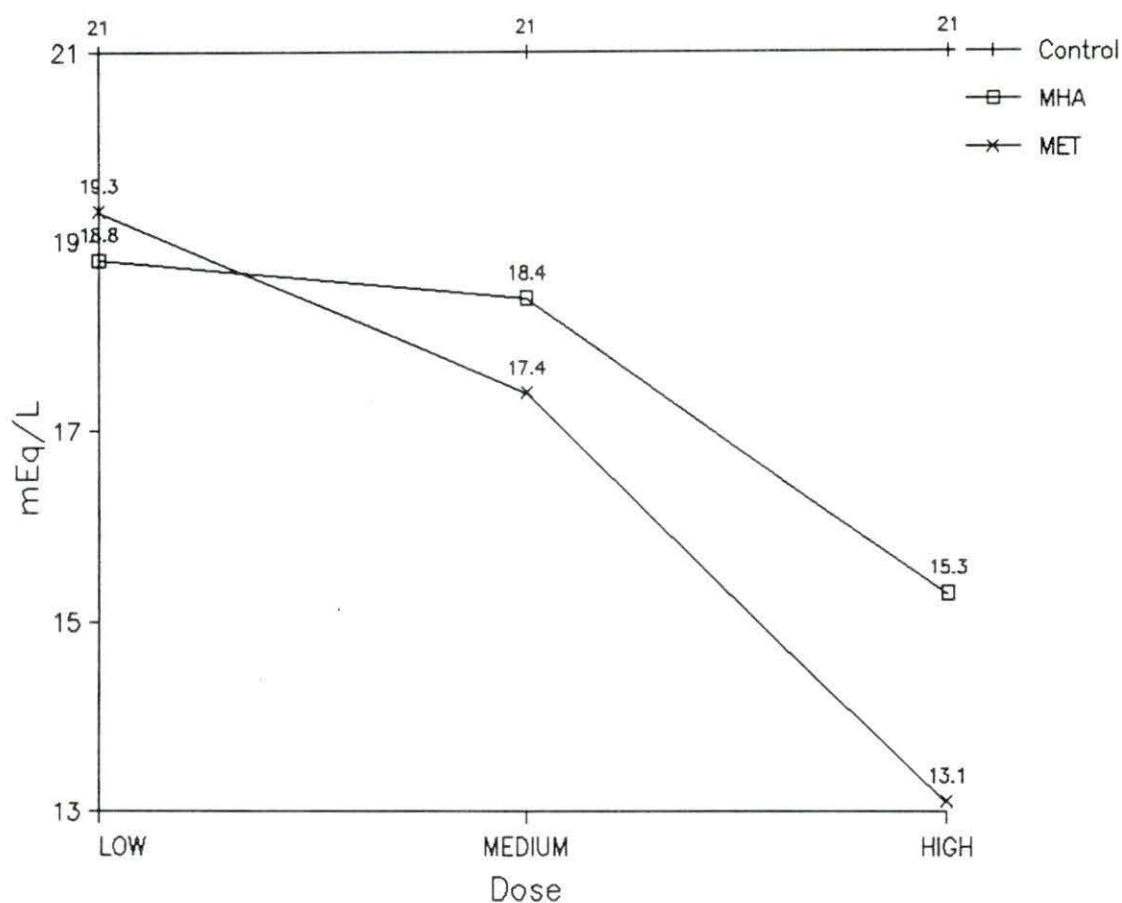


Figure 14. Blood bicarbonate concentrations for control, 3 MHA and 3 MET treatment groups after seven days of treatment

The metabolic acidosis that developed was especially severe at the high doses of each drug. As discussed earlier the compensatory mechanisms were being overwhelmed at the high doses. The anion gap was calculated and was determined to be normal for all groups.

**Heinz body formation**

Not until the animals had been on the treatments for seven days did Heinz bodies appear in the red blood cells of the cats in increased numbers. The two drugs caused oxidation of hemoglobin, and the resulting increases in Heinz body formation were evident at the medium MHA and medium MET doses and the high MHA and high MET doses. The average number of Heinz bodies per 1000 red blood cells for each group at each sample time may be seen in Table 37. The decline of Heinz bodies back to the pre-dosing values for the cats in this test was not complete by seven days (Time 5) after the last treatments. As noted earlier no clinical anemia resulted in the cats in this study because of the treatments. The Heinz bodies or the RBCs containing them were apparently being removed from the circulation without any resulting anemia.

Table 37. Heinz bodies per 1000 RBC means, standard error of means, F-values, and probabilities of significance for treatment groups prior to treatment (baseline), during treatment (time 3 and 4), and after treatment (time 5)

Group	Level of Treatment	Baseline Time 1, 2	Time 3	Time 4	Time 5
1	None-Control	2.17	2.17	2.83	0.33
2	Low MHA	0.10	0.50	0.83	0.33
3	Med MHA	0.50	1.00	83.00	16.67
4	High MHA	0.50	3.67	474.17	54.40
5	Low MET	0.50	0.00	1.33	0.17
6	Med MET	0.33	0.83	23.33	9.00
7	High MET	0.25	3.33	606.00	281.00
Standard Error of Means		0.47	1.37	67.55	55.64
F-Value		2.21	1.07	13.52	3.10
Pr > F		0.1145	0.4333	0.0001	0.0450

### General observations

Two cats in the first replication died. They exhibited extreme listlessness, rapid shallow respiration, vomiting, dehydration, ataxia, and complete inappetence before they died. One, a female, was in the high dose MHA group, and the other, a male, was in the high dose MET group. The apparent cause of the deaths was the effects of the acute metabolic acidosis.

Several other cats in each of the high dose groups displayed toxicological signs of extreme inactivity, rapid



shallow breathing, dilated pupils, occasional ataxia and reduced or no appetite. Recovery was rapid as soon as the treatments were stopped.

A few cats in the medium MET groups exhibited an observed reduction in their activity, but the most apparent clinical sign was reduced food intake. The cats in the medium MHA group did not exhibit obvious clinical symptoms resulting from the treatments.

The urine of all the treated cats changed from a cloudy appearance to clear by the third or fourth day of treatment and returned to the original state after the treatments were stopped. The odor of the urine in the room in which the cats were housed developed a less pungent, offensive odor during treatment.

### CONCLUSIONS

It was demonstrated in this study that methionine and methionine hydroxy analogue effectively lowered the urinary pH. The steady state urinary pH was reached in all treatment groups by the third day after the treatments were initiated. Although monitoring the urinary pH of individual cats is desirable in a clinical setting, it was found in this study that a single daily dose of 2.5 mEq/kg, which was the low dose, of either drug would maintain a urinary pH at 6.6 or below. This is the target pH necessary to prevent struvite crystal formation in the urine of cats. The potential side effects of continuous use of either of the two drugs would presumably be lessened, since the low dose of each drug was sufficient to accomplish the needed reduction in urinary pH.

The comparison of the side effects of the two drugs revealed that many of the parameters examined in this study were unaltered by either drug during a seven day treatment period. Before it can be determined if these measurements will be influenced by chronic use of these drugs, they will need to be monitored in a long-term study.

The toxicosis caused by the two drugs is prominent at the high dose of each. The animals in the medium MET group also exhibited side effects. The cats in the medium MHA group did not seem to be affected as greatly as their

counterparts in the MET group. The side effects noted were consistent with clinical manifestations of metabolic acidosis. The inactivity that was observed and the reduction in daily food intake were the most dramatic clinical changes. The elimination of the excess hydrogen ion in the urine appeared to be by slightly different urinary buffering mechanisms for the two drugs. Whether this would remain a consistent difference with prolonged treatment with the two drugs will require further study.

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