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THE EFFECT OF ACETAMINOPHEN TOXICITY ON SELECTED BLOOD BIOCHEMICAL PARAMETERS IN THE CAT

by

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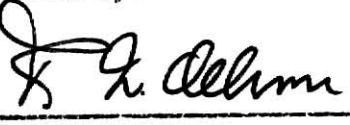

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INTRODUCTION

Acetaminophen (APAP) is an aspirin substitute that has become increasingly important the last 20 years (1). With increased usage of APAP there has been a rise in the number of incidences of APAP poisoning in cats (2-8). Signs of APAP toxicity in cats include anorexia, depression, cyanosis, vomiting, facial edema, and dark-colored urine (4-10). Dypsnea (6,7,11), icterus (4,6), and increased or depressed body temperature (4) have also been associated with this toxicity in cats. Unfortunately little is known as to why the cat shows these signs and appears more sensitive to APAP than other species.

To study the toxicity of APAP in cats, we examined the concentration of methemoglobin and reduced blood glutathione at various APAP dosages. In addition, the two major enzymes in methemoglobin reduction and glutathione reduction were studied. NADH methemoglobin reductase is the major cellular enzyme responsible for reducing methemoglobin; NADPH glutathione reductase is the major enzyme in the reduction of oxidized glutathione. These two systems were examined in adult cats to see if they were significantly impaired in APAP toxicity. Our observations will assist in understanding the mechanism of APAP poisoning in cats, and will help direct efforts to apply an efficient treatment for poisoned individuals.

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THE EFFECT OF ACETAMINOPHEN ON METHEMOGLOBIN AND BLOOD GLUTATHIONE PARAMETERS IN THE CAT

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SUMMARY

Acetaminophen (APAP) was given orally to six mature male and female cats single progressive doses of 20 (low), 60 (medium), and 120 mg APAP/kg body wt (high), each dose given three weeks apart. Methemoglobin, reduced blood glutathione (GSH) and APAP blood concentrations were measured for eight days to study the changes in the concentrations of these compounds due to APAP exposure. NADH methemoglobin reductase and NADPH glutathione reductase, responsible for the reduction of methemoglobin and oxidized glutathione (GSSG), respectively, were also examined for changes in activity. A statistically significant increase in methemoglobin formation occurred following the medium and high doses. The mean methemoglobin concentration was 46% after the high dose, approximately twice as much as the methemoglobin reductase concentration after the medium dose. The activity of NADH methemoglobin reductase at the high dose was statistically, significantly decreased from the activity following the low APAP dose. In addition, a dose-related trend indicated decreased NADH methemoglobin reductase activity as the APAP dosage increased. There was no statistically significant change in the concentration of red blood cell GSH at the low APAP dose. The medium dose produced a statistically significant decrease in blood GSH within 4 h of dosing. At the high dose, GSH was statistically decreased the first 24 h of dosing. The GSH concentration returned to normal by 192 h (day 8). The activity of NADPH glutathione reductase was not significantly altered by the low or medium APAP

dose, however it was statistically decreased in activity at the high dose. This suggests a drug-related decrease in glutathione reductase activity at high APAP dosages.

INTRODUCTION

Acetaminophen (APAP) has become increasingly popular in the last 20 years as an aspirin substitute (1). With increased usage there has been a rise in the number of APAP poisonings in cats resulting in adverse effects at lower APAP mg/kg dosages than in other animal species and man (2-9). Unfortunately, little is known as to why cats react so adversely. Detoxication of APAP is impaired in cats (10,11) and may play a role in the inability of cats to handle APAP.However, an early clinicl sign of APAP posioning in cats is cyanosis (apparently due to methemoglobin formation), which develops at relatively low APAP dosages (5,11,12). Reduced liver glutathione (GSH) in man has been identified as the target molecule in APAP posioning (13,14).

In severe APAP toxicosis liver GSH may decrease to about 20% of normal (14,15). When hepatic GSH levels are low, reactive APAP metabolites combine with nucleophilic components of the liver causing cell death (13-15). Concentrations of GSH in other tissues during APAP toxicity have not been studied. In a preliminary study with cats (Nash, S.; Savides M.; and Oeme F.; unpublished data, 1982) and in studies in dogs (16), changes in concentrations of methemoglobin and GSH in red blood cells occurred following APAP administration. Enzymatic mechanisms in the reduction of methemoglobin and

maintenance of GSH therefore became important to examine during the course of APAP toxicity.

NADH methemoglobin reductase is the major enzyme in the reduction of methemoglobin to hemoglobin (17). High levels of methemoglobinemia have been associated with a congenital deficiency of this enzyme (18,19). Activity of this enzyme could be impaired in APAP toxicity, thus causing methemoglobinemia. Several drugs can increase the rate of methemoglobin formation (20).

NADPH glutathione reductase is responsible for the reduction of oxidized glutathione (GSSG) (21,22) and is an important pathway by which the red blood cell may rid itself of GSSG (23). GSH in the red blood cell protects the cell (24) and its proteins (25) from oxidative damage. In addition, GSH may protect hemoglobin from oxidation, although it is not known what role it specifically plays in cats (12). GSH is oxidized in the cell by oxidants such as hydrogen peroxide (26,27). The reduction of GSH is important in maintaining adequate concentrations of red blood cell GSH. Glutathione reductase is not a limiting enzyme in glutathione reduction, according to Paniker et al. (28), but low levels of GSH may develop when glutathione reductase activity is greatly decreased (21).

A study was performed to examine methemoglobin, blood GSH, and two enzyme systems associated with the maintenance of these two molecules in cats receiving various dosages of APAP. Measurements and activity of these entities in red blod cells were monitored for eight days.

MATERIALS AND METHODS

Animals and Dosages Used

Six mature domestic shorthair cats, three of each sex and weighing an average of 2.7 ± 0.5 kg, were used. Each cat received single progressive oral doses of APAP, each approximately 3 weeks apart. Prior to dosing, the cats were fasted for 12 h. Administration of APAP to fasted cats was done between 7:30 and 8:30 AM. The first dosage given was 20 mg APAP/kg body wt (low dose), the next dosage was 60 mg APAP/kg body wt (medium dose), and the third dosage was 120 mg APAP/kg body wt (high dose). Before the next higher APAP dose was given, all physiological and specific biochemical parameters had returned to normal.

Blood Collection

Blood was obtained from the jugular vein using heparin as anticoagulant. Two to three baseline samples were taken prior to dosing with APAP. Samples were collected 0, 1, 2, 4, 8, 12, 24, 48, 96, and 192 h (day 8) after APAP administration. The cats were bled between 7:30 and 9:30 AM for the 0, 24, 48, 96, and 192 h blood samples. The blood was stored at 4 C. All assays were completed within 18 days.

Assay Methodology

Concentrations of APAP in plasma were measured using the method of Lo and Bye (29), the solvent system of Knox and Jurand (30), and a

Perkin-Elmer Series 2 HPLC, M-1 Integrator, LC-15 detector with Brownlee Labs MPLC Guard Column and Altex Ultrasphere IP, 5 µm, 5 cm x 4.6 mm ID column. The limit of detection was 1 µg/ml.

A Hitachi Perkin-Elmer Model 124 Double-Beam Grating Spectrophotometer and Varian Model A-25 recorder were used to assay blood samples for methemoglobin and GSH and to record methemoglobin reductase and glutathione reductase activity. The methemoglobin determination was done using a modification of the method described by Henry (31). The ammonium hydroxide addition step was omitted since sulfhemoglobin was not determined. Blood GSH was determined by the method of Beutler et al. (32).

Preparation of the hemolysate for the enzyme assays was by the method described by Beutler (33). The whole blood in saline (1:10) mixture was centrifuged at 2700 rpm for 10 min. NADH methemoglobin reductase was assayed using the method of Board (34). The difference in absorbance of the blank was greater when the hemolysate was omitted than when potassium ferricyanide was omitted; therefore, potassium ferricyanide was used to start the reaction after the cuvette was incubated for 10 min. NADPH glutathione reductase activity was measured without the addition of FAD using the method described by Beutler (35).

Statistical Analysis

Data were analyzed using the SAS ^{R}-MATRIX. Significance was tested at the 0.05 level using a multivariate profile analysis (36)

and analysis of variance. Pretrial values of the measured parameters were compared using analysis of variance for 0 h of each APAP dosing with no statistically significant differences found, indicating that all cats had normal levels of each parameter at the beginning of each exposure period. Results of the three APAP dosages were also compared with each other using analysis of variance. A multivariate profile analysis was used to compare the samples within a specific APAP dosage group.

RESULTS

Acetaminophen

Following oral administration, blood concentrations of APAP peaked at all dosages at 1 h, and were dose-related in peak concentration and duration of persistance in blood (Table 1).

Methemoglobin

Methemoglobin formation was not statistically significant at the low dose. Formation of methemoglobin was statistically significant following the medium and high APAP doses (Figures 1 and 2). More methemoglobin was produced at the high dose--about 46%--than at the medium dose which peaked at 22% (Table 1). Methemoglobin levels in the blood remained high for longer periods of time following the high APAP dose with 10% still present at 24 h. The methemoglobin peaked at 4 h at the medium dose compared to a plateau between 4 and 12 h at

the high dose. A statistically significant difference in methemoglobin concentration was found between dosages at all time periods except 0 h and at 192 h (day eight) (Table 1). Methemoglobin concentrations at the high dose were statisticaly increased from 1 h to 48 h compared to concentrations at the medium dose and the low dose. The methemoglobin concentrations at the medium dose were higher than the low dose from 1 h to 8 h.

Statistically significant differences in NADH methemoglobin reductase activity were found between the low and high doses at 12 h through 96 h (Table 1). However, there was also a dose-related trend for decreased methemoglobin reductase activity as the dose and time-after dosing increased.

Glutathione

There was no statistically significant change in blood GSH at the low APAP dosage. A statistically significant difference occurred in the first 4 h following the medium dose (Figure 1). There was a slight decrease in mean GSH concentration, falling from 75.31 mg GSH/dl RBC at 0 h to 64.01 mg GSH/dl RBC at 2 h (Table 1). At the high dosage a dramatic decrease in GSH was seen within the first 24 h, and then a slow rise in levels occurred over the next seven days (Figure 2). Mean GSH concentration at 0 h was 77.42 mg GSH/dl RBC (Table 1). It rapidly fell to 17.25 mg GSH/dl RBC at 8 h, and reached the lowest level at 24 h when mean GSH concentration was 13.97 mg GSH/dl RBC. By 192 h (day 8), mean GSH concentration was

68.86 mg GSH/dl RBC (Table 1). A statistically significant difference was seen in GSH concentrations when the high dosage results were compared with the two lower dosages from 4 h through 96 h following APAP dosing.

When the results following the three dosages were compared with each other, no statistically significant difference in NADPH glutathione reductase activity was found except at 12 h and at 96 h when the activity following the high dose was statistically decreased from the low dose (Table 1). NADPH glutathione reductase activity at the high dose was statistically decreased when 2, 8, 24, and 96 h concentrations were compared (Figure 2).

DISCUSSION

Acetaminophen toxicity in the cat is an increasingly important problem; from 1978 to 1981, APAP accounted for 44% of the drugs or medications ingested by cats that produced concern (2). Acetaminophen is more toxic to cats than aspirin (37,38).

Statistically significant differences were found at different APAP dosages for each of the four parameters. It can not be concluded that the differences found were all real and due to the various dosages of APAP used, although the changes in methemoglobin and GSH concentrations were dramatic, dose-related, and highly significant. The cats received progressive APAP dosages, and although all parameters were normal at the start of each dosage period, the possibility of an additive effect of APAP on the cats must be considered.

None, however, has been reported. The cats were also dosed in groups of two at different times (two of the six cats were dosed each week), but environmental conditions were controlled to minimize any day-to-day variations. No statistically significant differences were found in the parameters measured at 0 h or 192 h (day 8), the first and last evaluation of each dosage period.

Methemoglobin formation appeared to be dose-related. After the 20 mg APAP/kg dose, no significant change occurred through the 192 h (day 8) evaluation period (Table 1). After the 60 mg APAP/kg dose an approximately 10-fold increase in methemoglobin occurred at 2 h and 4 h. With the doubling of the APAP dose from 60 mg APAP/kg to 120 mg APAP/kg, the concentration of methemoglobin approximately doubled from 1 h to 4 h. After 8 h, methemoglobin concentrations rapidly returned to normal levels after the 60 mg APAP/kg dose; methemoglobin concentrations after the 120 mg APAP/kg dose remained elevated through 24 h. Although methemoglobinemia was significantly elevated after 60 mg APAP/kg and 120 mg APAP/kg, only a moderate decrease in NADH methemoglobin reductase activity occurred at these dosages (Table 1). It is possible that methemoglobin was formed at a rate faster than it could be reduced, as suggested by Letchworth et al. (20). The iron in hemoglobin could have been oxidized by either APAP or its metabolites, as suggested by Finco et al. (5). NADH is necessary for reduction of methemoglobin in the red blood cell. This entity was not measured in the present study, but it could be oxidized in a manner similar to hemoglobin, making it unavailable for

the availability of glucose may also be affected by toxic amounts of APAP.

The decrease of GSH at the 60 mg APAP/kg dose was slight compared to the 5-fold decrease in mean GSH concentration that occurred after 120 mg APAP/kg dose from 0 h to 24 h. Whether or not blood GSH and hepatic GSH follow the identical pattern of decline will require future study. A suggestion of decreased NADPH glutathione reductase activity following the 120 mg APAP/kg dose was seen (Table 1). Further studies are required to examine this response. Glutathione reductase enzyme activity also appeared to increase at 192 h (day 8), but the significance of this response is not known. Red blood cell GSH, like hemoglobin, may be oxidized by APAP and its reactive metabolites (12). While GSSG concentrations were not measured in the present study, it was assumed that GSH was lost as GSSG. Future studies need to be done to confirm whether the total amount of GSH plus GSSG remains constant or if it changes in APAP toxicity. It is possible that some of the blood GSH is binding to reactive metabolites as GSH does in the liver (13-15).

The availability of NADPH may also be important in APAP toxicity. If APAP and its reactive metabolites are oxidizing molecules such as hemoglobin and GSH in the cell, it is possible that NADPH is also oxidized. Another source of NADPH deficiency may be enzymes from the hexose monophosphate pathway, such as glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. These enzymes may be impaired so that little to no

NADPH is regenerated during APAP toxicity. Decreased enzyme activity with slow reduction of oxidized GSH would result.

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Table 1. Mean ± standard deviation for selected blood parameters from 6 cats receiving 3 single doses of acetaminophen, each 3 weeks apart.

TIME (hrs)	[APAP] (µg/ml)	MetHb (g MetHb/g total Hb)	MetHb-R (IU/g Hb)	GSH (mg GSH/dl RBC)	GSSG-R (IU/g Hb)
20 mg APAP/kg					
0	0.00 ± 0.00	0.011 ± 0.008	10.31 ± 2.92	65.97 ± 8.52	20.38 ± 7.05
1	9.38 ± 3.94	0.023 ± 0.011	14.07 ± 7.21	61.90 ± 7.98	20.57 ± 5.47
2	4.48 ± 3.04	0.020 ± 0.018	11.05 ± 3.94	61.58 ± 9.04	21.28 ± 7.11
4	0.31 ± 0.77	0.014 ± 0.006	11.26 ± 2.25	65.45 ± 12.51	21.95 ± 6.14
8	0.00 ± 0.00	0.007 ± 0.003	9.73 ± 3.22	67.99 ± 7.68	21.84 ± 7.32
12	0.00 ± 0.00	0.013 ± 0.003	11.61 ± 4.94	66.31 ± 10.25	22.42 ± 7.30
24	0.00 ± 0.00	0.014 ± 0.011	9.05 ± 3.72	68.70 ± 8.75	21.10 ± 7.04
48	0.00 ± 0.00	0.011 ± 0.007	9.92 ± 2.87	63.67 ± 13.39	18.87 ± 5.55
96	0.00 ± 0.00	0.019 ± 0.005	12.03 ± 3.21	64.40 ± 11.73	22.90 ± 7.80
192	0.00 ± 0.00	0.017 ± 0.009	12.05 ± 6.42	69.13 ± 12.71	24.54 ± 8.68
60 mg APAP/kg					
0	0.00 ± 0.00	0.010 ± 0.005	8.51 ± 1.95	75.31 ± 7.01	22.25 ± 4.10
1	37.30 ± 6.02	0.105 ± 0.043[a]	9.05 ± 1.64	69.91 ± 6.16	22.71 ± 2.99
2	28.14 ± 6.33	0.174 ± 0.060[a]	9.11 ± 2.47	64.01 ± 4.00	24.77 ± 8.46
4	18.04 ± 7.97	0.217 ± 0.070[a]	8.56 ± 2.33	65.21 ± 4.68	21.07 ± 6.29
8	5.69 ± 5.67	0.097 ± 0.077[a]	7.97 ± 2.20	71.51 ± 9.81	19.77 ± 3.44
12	1.47 ± 2.03	0.033 ± 0.023	8.44 ± 0.77	64.81 ± 15.16	21.50 ± 6.39
24	0.00 ± 0.00	0.014 ± 0.002	7.22 ± 1.30	64.83 ± 5.93	18.90 ± 4.73
48	0.00 ± 0.00	0.008 ± 0.003	9.04 ± 0.98	70.62 ± 11.81	19.85 ± 5.09
96	0.00 ± 0.00	0.008 ± 0.004[a]	9.08 ± 0.26	69.72 ± 6.70	21.20 ± 5.17
192	0.00 ± 0.00	0.013 ± 0.004	8.55 ± 1.29	74.93 ± 9.66	22.44 ± 6.56
120 mg APAP/kg					
0	0.00 ± 0.00	0.016 ± 0.005	10.59 ± 5.28	77.42 ± 11.91	20.17 ± 7.30
1	90.69 ± 3.56	0.250 ± 0.069[a,b]	8.44 ± 5.89	71.71 ± 14.30	20.62 ± 3.48
2	75.86 ± 5.31	0.392 ± 0.067[a,b]	8.68 ± 5.56	63.73 ± 13.22	19.28 ± 3.75
4	58.70 ± 6.37	0.447 ± 0.066[a,b]	9.96 ± 5.45	45.57 ± 16.08[a,b]	18.70 ± 4.11
8	33.05 ± 8.67	0.451 ± 0.087[a,b]	10.35 ± 6.71	17.25 ± 22.04[a,b]	19.32 ± 6.29
12	18.42 ± 9.67	0.455 ± 0.109[a,b]	7.22 ± 1.83[a]	15.13 ± 23.76[a,b]	16.47 ± 6.04[a]
24	0.00 ± 0.00	0.100 ± 0.065[a,b]	6.82 ± 1.99[a]	13.97 ± 19.30[a,b]	15.91 ± 5.63
48	0.00 ± 0.00	0.022 ± 0.008[a,b]	6.46 ± 2.23[a]	23.35 ± 18.53[a,b]	15.96 ± 5.70
96	0.00 ± 0.00	0.018 ± 0.008	6.09 ± 3.37[a]	43.34 ± 17.10[a,b]	16.71 ± 5.23[a]
192	0.00 ± 0.00	0.016 ± 0.006	8.21 ± 3.23	68.86 ± 18.48	28.51 ± 8.96

[a] Statistically significant difference from the 20 mg APAP/kg dose, $P < 0.05$

[b] Statistically significant difference from the 60 mg APAP/kg dose, $P < 0.05$

Figure 1. Mean blood concentrations (± std. dev.) of acetaminophen (APAP), methemoglobin (MetHb), NADH methemoglobin reductase (MetHb-R), reduced glutathione (GSH) and NADPH glutathione reductase (GSSG-R) in 6 cats that received 60 mg APAP/kg of a single oral dose. The unit of measurement for APAP is ug/ml, for MetHb is g MetHb/g total Hb, for MetHb-R is IU/g Hb, for GSH is mg GSH/dl RBC, and for GSSG-R is IU/g Hb.

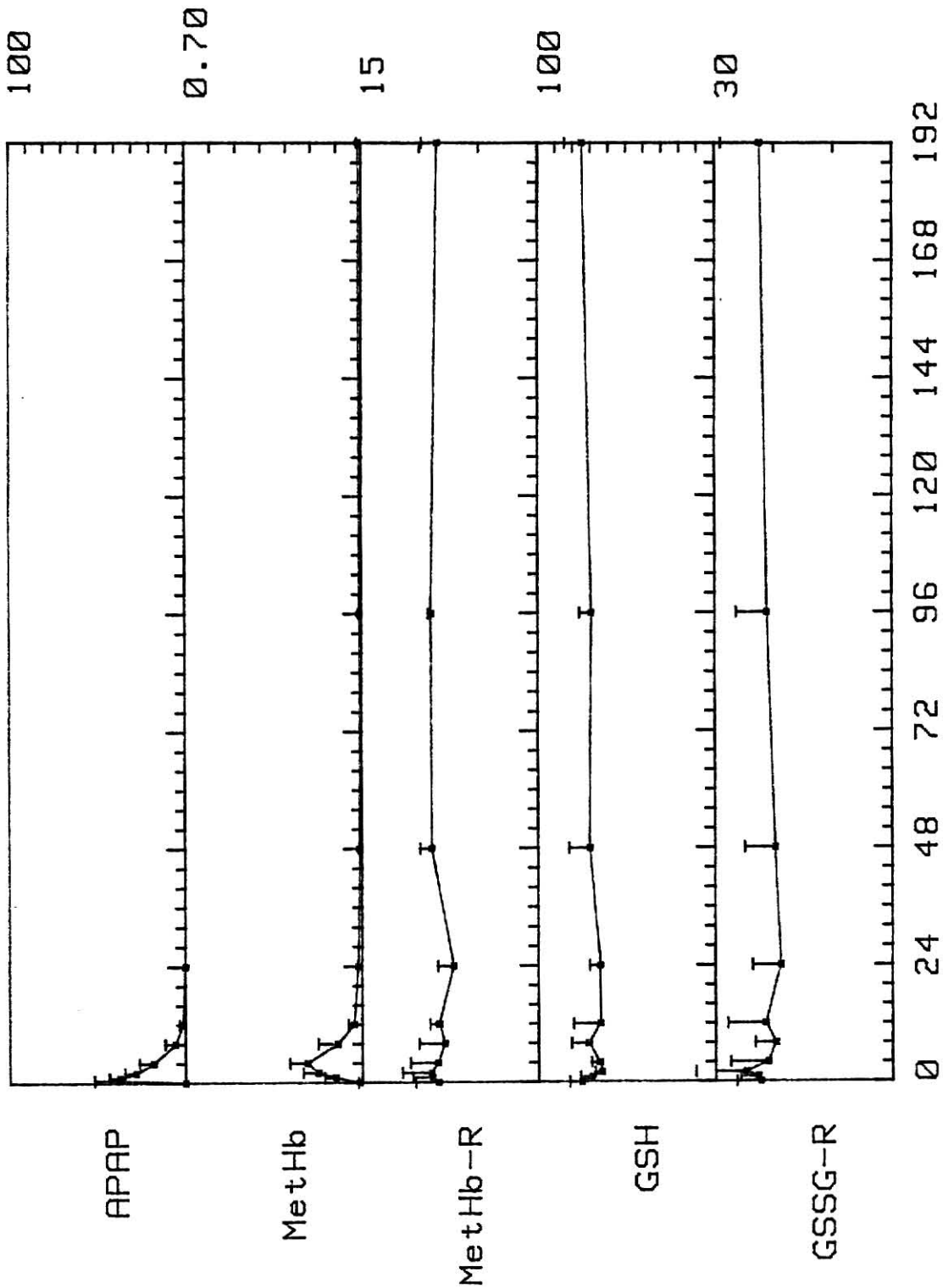
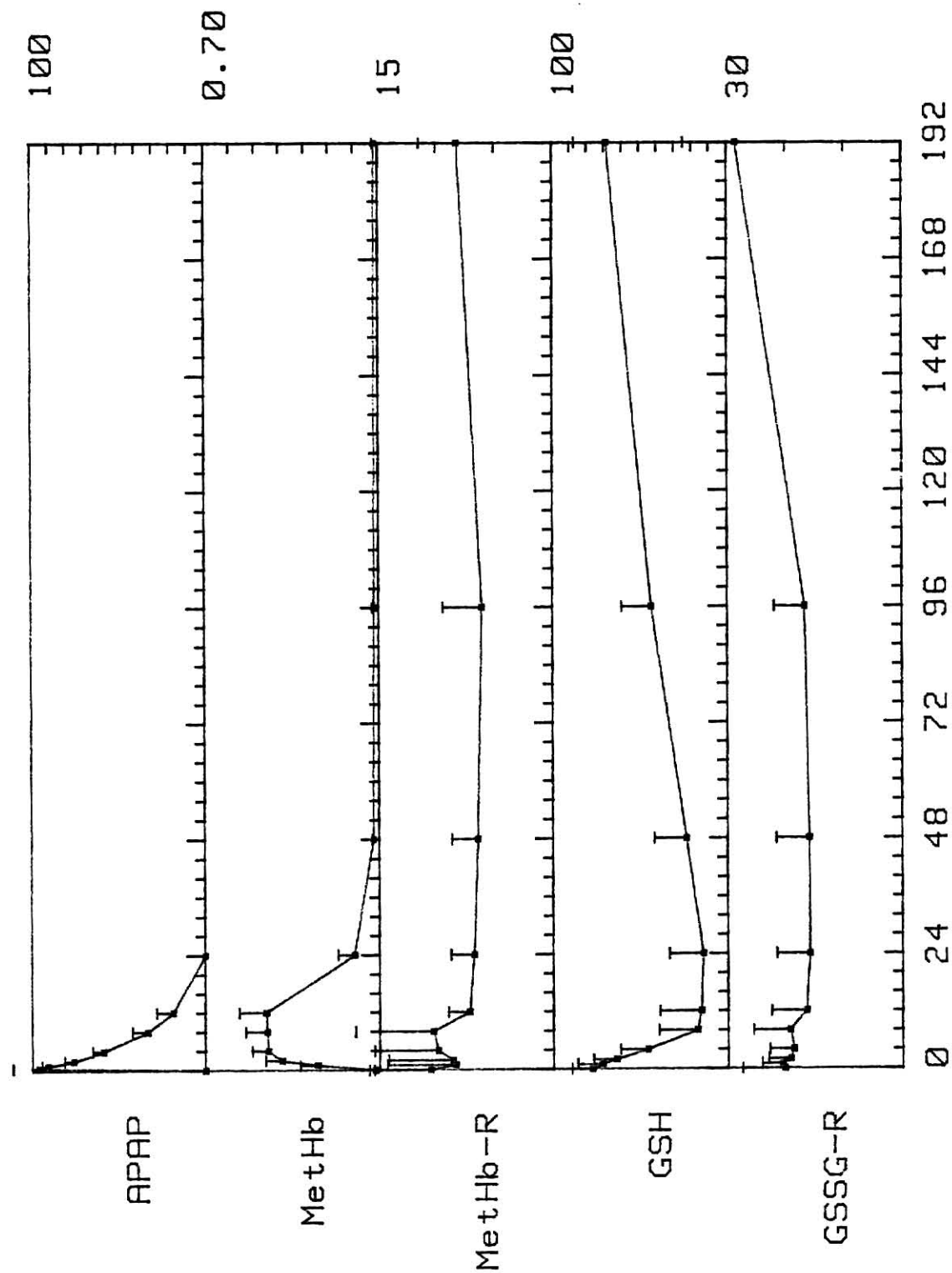

APAP
MetHb
MetHb-R
GSH
GSSG-R
100
0.70
15
100
30
0
24
48
72
96
120
144
168
192
TIME (HRS)

Figure 2. Mean blood concentrations (± std. dev.) of acetaminophen (APAP), methemoglobin (MetHb), NADH methemoglobin reductase (MetHb-R), reduced glutathione (GSH) and NADPH glutathione reductase (GSSG-R) in 6 cats that received 120 mg APAP/kg of a single oral dose. The unit of measurement for APAP is ug/ml, for MetHb is g MetHb/g total Hb, for MetHb-R is IU/g Hb, for GSH is mg GSH/dl RBC, and for GSSG-R is IU/g Hb.


100
0.70
15
100
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96
120
144
168
192
TIME (HRS)
APAP
MetHb
MetHb-R
GSH
GSSG-R

APPENDIX I

A Review of Acetaminophen's Effect on Methemoglobin, Glutathione, and Some Related Enzymes

A REVIEW OF ACETAMINOPHEN'S EFFECT ON METHEMOGLOBIN, GLUTATHIONE, AND SOME RELATED ENZYMES

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INTRODUCTION

Acetaminophen toxicity in cats has become an increasing problem. Acetaminophen was first mentioned by von Mehring in 1893, but the drug has had popular usage as an aspirin substitute only in the past 20 years (1). With the increasing popularity of acetaminophen, there has been an increased incidence of acetaminophen poisoning in cats. Unfortunately, little is known about why cats have more sensitivity to acetaminophen than do other species of animals. Acetaminophen has been found more toxic than aspirin to cats (2,3); Kohler (4) noted that although acetaminophen was a more effective analgesic in cats than aspirin, it was also more toxic.

The toxicity of acetaminophen to cats is due, at least in part, to two factors. The first is the inability of the cats to effectively metabolize and excrete acetaminophen. Acetaminophen is primarily excreted in urine as sulfate and glucuronide conjugates when low therapeutic levels of the drug are administered to most mammals (5-7). Cats have a deficiency in glucuronyl transferase (7,8), so the amount of acetaminophen that can combine with glucuronide is limited. A study done by Welch et al. (9) found that cats excreted less then 3% of administered acetaminophen as the glucuronide, while in dogs and man 50-60% of acetaminophen was excreted as the glucuronide. In the same study, Welch et al. noted that sulfate conjugation was only a minor pathway in cats, dogs, and man. Acetaminophen is also metabolized to various reactive metabolites which combine with hepatic glutathione and are excreted in urine as cysteine and mercapturic acid conjugates (5). When

glutathione levels in liver drop to about 20% of normal (5,10), the reactive metabolites begin combining with the nucleophilic cells of the liver causing cell death (5,10,11).

A second factor contributing to higher acetaminophen toxicity in cats is the pet owner's failure to appreciate the small size of cats in relation to adult humans. In several case histories reported by veterinarians, the client had administered one or more acetaminophen tablets for several hours to several days before bringing the cat to the veterinaran (4,12-17). Cats have been poisoned with two tablets of acetaminophen (each tablet contains 325 mg) given at four-hour intervals (7,14) or from a single 500 mg acetaminophen tablet (13,15). Currently, acetaminophen is not recommended for cats at any dose.

SIGNS

The signs most frequently seen in cats poisoned with acetaminophen were anorexia, depression, cyanosis (apparently due to anoxia from the formation of methemoglobin), vomiting, subnormal or elevated temperature, facial edema, dark blood-colored urine, anemia, icterus, and hemoglobinuria (4,7,14-16,18). Other signs reported were abdominal pain (7,18), hyperventilation (7), dyspnea (4,16,18), convulsions (18), elevated serum glutamic-pyruvic transaminase activity (7), and lighter hair color (14). Hepatic necrosis was absent in cats that were killed or died after receiving an acute toxic dose of actaminophen (7,19). Cats that received acetaminophen orally for 26 weeks had liver necrosis (2).

In Cats

In cats, cyanosis and central nervous system depression are seen

at about two hr after 143 mg acetaminophen/kg was administered (19). Cats that received an initial dose of 325 mg acetaminophen showed signs of cyanosis at four hr (7,14). Vomiting may occur after a second dose of 325 mg acetaminophen given four hr after the first dose (14). Facial edema and salivation were present by eight hr (19), but was not present in all cats (14). At 24 hr, anorexia, depression and weakness, icterus and increased rectal temperature were seen (14). Cyanosis was (14,19) or was not (7) present at this time. Finco et al. (14) reported cyanosis in one cat until day two. Facial edema may be present for as long as three days after administration of a toxic dose of acetaminopen (14,19). According to the study done by Gaunt et al. (19), the edema was noticed in the chin and lips, and progressed toward the neck with time. Anorexia lasted for as long as four to seven days (14). The depression and weakness was present for as long as six days (14). Finco et al. (14) noted elevated rectal temperatures in two cats studied on day one through day five, with one exception; cat 2 had a subnormal temperature on day three. Other signs seen in the cats were poor tissue perfusion, dark-colored urine, and dehydration (14). Finco et al. (14) reported that cats were clinically normal three weeks after a toxic dose of acetaminophen was admnistered.

In Dogs

Acetaminophen toxicity in dogs did not seem to present as much of a problem as in cats. Signs of poisoning in dogs were similar to those in cats, and included cyanosis, paw and facial edema, vomiting, dark-colored urine, gastrointestinal disturbance and coma along with laboratory findings reflecting hepatic centrolobular necrosis,

hemoconcentration, and leukocytosis (20). The first sign seen in the dogs was cyanosis (due to the methemoglobinemia) and occurred about two hr after administraton of 600 mg acetaminophen/kg. Cyanosis was most severe between two and six hr and was gone by 24 hr. Facial and paw edema developed with time. Itching and lacrimation were often seen with the edema (20). Signs of gastrointestinal disturbance were delayed bloody vomitus and melena (20). Dark-colored urine had blood or bilirubin in it (20). One dog was reported icteric.

TREATMENT

Many different treatments have been attempted in cats poisoned with acetaminophen. These include N-acetylcysteine (7,10,15,19), ascorbic acid (3,7,15), steroids (13,15,21), antibiotics (15,21), antihistamines (21), blood transfusions (3), furosemide (15), oxygen (15), and intravenous fluids (21). The current recommended therapy for acetaminophen toxicity in cats includes N-acetylcysteine, fluids, oxygen, furosemide, and ascorbic acid (22). N-acetylcysteine (Mucomyst^{R}) provides sulfhydryl groups for the reactive metabolites of acetaminophen to react with and be safely excreted. It also "spares" liver glutathione (23), which when reduced leads to hepatic failure. N-acetylcysteine has been given at varying dosages and time intervals (7,15,19) with some success in cats. The recommended dosage of N-acetylcysteine in man is a loading dose of 140 mg/kg followed by 70 mg/kg every four hours for a total of 17 doses (23). Booth (10) cited a study by Davis where a similar treatment regimen was used in cats employing intervals of 6 hr. The use of antihistamines in cats for acetaminophen toxicity is not recommended (10).

METHEMOGLOBINEMIA

Methemolobinemia is often associated with acetaminophen poisoning in cats and dogs, but it occurs at lower dosages of acetaminophen in cats. It is of significance to discover the basis for this species difference in the formation of methemoglobin. Factors to be considered include the mechanism of hemoglobin oxidation, other drug-induced methemoglobinemias, possible species sensitivity, and the mechanism of reduction of methemoglobin. Understanding the formation and reaction of methemoglobin (Figure 1) may help explain the sensitivity of cats to acetaminophen and similar compounds.

Methemoglobin (ferrihemoglobin, hemiglobin) is different from hemoglobin in that it contains iron in the ferric (Fe^{+++}) state, while hemoglobin contains iron in the ferrous (Fe^{++}) state. Ferric iron is incapable of carrying oxygen. This means that the oxygen dissociation curve is shifted to the left when methemoglobin is present in significant concentrations (24). However, Jaffe (25) cited several studies that indicate that the curve is shifted only in drug-induced methemoglobinemia and not in hereditary methemoglobinemia. The reason for this difference is not known. This shift of the oxygen dissociation curve means that less oxygen is delivered to the body tissues. Methemoglobin is visually seen as blood that is dark red to chocolate-brown in color, depending on how much methemoglobin is present. The more methemoglobin present, the more brown the blood is.

The concentration of blood methemoglobin depends on the balance between the rates of methemoglobin formation (hemoglobin oxidation)

and reduction (25). Levels of methemoglobin in the red blood cells of healthy mammals are 2% or less of the total hemoglobin (26). In humans, the methemoglobin present in red blood cells is thought to result from autoxidation (27). Red blood cells with varying concentrations of methemoglobin have the same resistance to hemolysis as determined by various concentrations of NaCl solution, according to Jung, as cited by Bodansky (28). In addition, methemoglobin appears to have no effect on the life span of human red blood cells (25).

Methemoglobinemia occurs when oxidizing compounds are administered orally or parenterally and the formation of methemoglobin exceeds the capacity of methemoglobin-reducing systems (29). There are several factors that determine whether methemoglobin formation will exceed reduction. First, one chemical's ability to oxidize hemoglobin does not necessarily mean that other chemical oxidants have the same action on hemoglobin (30). Further, phenylhydrazine and related redox compounds react with molecular oxygen to form oxidant intermediates or free radicals that oxidize cellular molecules such as hemoglobin (31). A study by Harvey and Kaneko (30) supports the theory that oxidation of hemoglobin occurs primarily by oxidant intermediates. Therefore, the capability of an oxidant chemical to form these intermediates is important in methemoglobin formation. Thirdly, methemoglobin is formed after a minimum amount of the oxidant chemical has been given, and this minimum dose is not the same for all animals (32) or all chemicals (28). Finally, there is a maximum production rate of methemoglobin that can not be further increased with the administration of more

chemical; the methemoglobin just remains in the body longer (32).

Methemoglobin is thought produced by three different processes: Autoxidation; the direct action of oxidants; and the interaction of atmospheric oxygen and hydrogen donors (25,28). Autoxidation of hemoglobin may involve intermediates of oxyhemoglobin or reduced hemoglobin (25), and seems dependent on oxygen tension; maximum methemoglobin formation occurs at a low oxygen tension of about 25 mm Hg (28). Methemoglobin may also be formed by the direct action of oxidants, such as ferricyanide, nitrites, quinones, chlorates, and dyes with high oxidation-reduction potentials (25,28). The iron of hemoglobin is directly oxidized to the ferric state by the oxidant. Methemoglobin is also formed by the interaction of atmospheric oxygen and hydrogen donors. Dyes, such as methylene blue, are important in this type of methemoglobin formation (28). The oxidation-reduction potential of methylene blue is lower than that of methemoglobin, but this does not hold true for all dyes (28). There are two theories as to how methylene blue produces methemoglobin (28). The first is that methylene blue interacts with part of the hemoglobin molecule, forming methemoglobin and leucomethylene blue. The leucomethylene blue is reoxidized back to methylene blue by atmospheric oxygen and the cyclic reaction begins with methylene blue interacting with hemoglobin. The second theory is that of Lemberg, Legg and Lockwood, as cited by Bodansky (28) - Autoxidation of a hydrogen donor (for example leucomethylene blue) forms hydrogen peroxide which either directly or indirectly oxidizes the iron of hemoglobin to the ferric state. Other compounds that have contributed to this type of oxidation are aminophenols, phenylhydrazine, phenylhydroxylamine, and

hydrazobenzene (28). Methemoglobin formation with these compounds is similar to that produced by methylene blue (28).

Methemoglobinemia produced by drugs usually returns to normal 24 hr after the drugs were last given (33). Hemolytic anemia may result from chronic administration of drugs such as acetanilid and acetophenetidin that also produce methemoglobinemia (34). The reason for this hemolytic anemia is currently unknown (34). The drugs themselves do not appear to be directly hemolytic, but it is possible that metabolic breakdown products are involved (34). The blood picture rapidly returns to normal when the drug administration ceases (34). Drug induced methemoglobinemia is dependent on many factors, some of which are: Duration of drug in the body; potency of the drug; animal species involved; reduction rates of methemoglobin; activity of methemoglobin reductase (35); route of administration of the drug; types of bacteria in the intestinal tract; metabolism pathways of the drug to its metabolites; excretion rate of metabolites; and rate of intestinal absorption of an orally administered drug (28).

Cats have been widely used to study compounds that produce methemoglobinemia becuase they are the species most susceptible to methemoglobin formation (28,32,36). Cats, dogs, and man form methemoglobin more readily than rats, rabbits, monkeys, and guinea pigs (28,32,36). In a study by Spicer (37), methemoglobin formation occurred faster in rabbits than in cats, but the degree of methemoglobinemia was higher in cats and dogs.

The rate of methemoglobin reduction may be an important factor in explaining why some species develop methemoglobinemia more readily than others (32). The resistance of rodents to methemoglobin

formation has been attributed to a high rate of methemoglobin reductase activity rather than to a hemoglobin that is resistant to oxidation (38,39). Mice and rabbits have high methemoglobin reductase activity (38). Stolk and Smith (38) reported that the hemoglobins of humans, dogs, rabbits, mice, and cats were not different in their reaction to nitrite, but their methemoglobin reductase activities varied. However, the hemoglobin of cats appeared more prone to oxidation than the hemoglobins of dogs, horses, and man (30). The hemoglobin of cats has at least eight reactive sulfhydryl groups (40), which is twice the amount found in other species (30). Because cats have more sulfhydryl groups it may be more difficult to keep them uniformly reduced under oxidant stress (30).

Variations between species in methemoglobin formation may be due to varied metabolism of drugs, such as antipyretics, among species (32). Welch et al. (9) found that cats and dogs excreted higher amounts of aromatic amines than man, who only excreted trace amounts of aromatic amines after acetaminophen administration. The sensitivity of cats to compounds which produce methemoglobin may in part be due to this animal more readily forming reactive metabolites from aromatic amines (38). The ability of amides to produce methemoglobin depends on the type of amines they are hydrolyzed to (41). A correlation between the excretion of aromatic amines and the formation of methemoglobin by acetophenetidin was made; the more diazotizable amine excreted in urine, the higher the level of methemoglobin produced (9). Cats excreted the most diazotizable amine, followed by dogs and man (9).

The total amount of body hemoglobin may also determine the sensitivity of various species to methemoglobin formation. Spicer (37) suggests that because cats have a smaller amount of total body hemoglobin when compared to dogs, more methemoglobin is produced in cats when they are given nitrite on a body weight basis. In other words, the amount of methemoglobin produced in cats and dogs would be about the same if nitrite were given according to the total amount of hemoglobin in each species. Similar results were seen in dogs and cats given aniline; however, cats may still be more sensitive to aniline because of the ability of cats to form a reactive metabolite (37).

Other factors that affect sensitivity to methemoglobin formation are age of the animal (36), ability of the oxidizing drug to permeate the red blood cell membrane (37), detoxication and excretion rates of the drug, and mechanisms outside the red blood cell involved in methemoglobin reduction (39). Unlike nitrite, where resulting methemoglobinemia lasts as long in vitro as in vivo, methemoglobinemia produced by phenylhydroxylamine remains longer in vitro than it does in vivo (39). Smith et al. (39) attributed this difference in the duration of methemoglobinemia resulting from phenylhydroxylamine administration to factors external to red blood cells. It is likely there are also other factors not presently recognized.

SIGNS OF METHEMOGLOBINEMIA

Methemoglobinemia has been described by Smith (35) as a functional anemia. The signs of methemoglobinemia are difficult to isolate because drugs which produce methemoglobinemia also have other

effects on the body (35). The body copes with increased methemoglobin levels by "...increasing cardiac output, increasing tissue blood flow, and increasing respiratory ventilation" (35). However, these mechanisms do not always come into play, even with methemoglobin levels of 30-50% (35). Bodansky (28) reported that darkened mucous membranes was the outstanding sign seen with 60% or less methemoglobin. Methemoglobinemia became lethal at 80-85% (28) when dogs were given p-aminopropiophenone (in propylene glycol) intravenously. Vandenbelt et al. (42) recorded the signs of methemoglobinemia in dogs given p-aminoacetophenone or p-aminopropiophenone orally "...ataxia at 60% methemoglobin, salivation and prostration at 75%, loss of consciousness at 85%, and death at 95%." Prior to death, rapid panting was noticed; death was ascribed to decreased cardiac output (42). In addition to the signs described by Vandenbelt et al., Bodansky (28) reported vomiting at 60-80% methemoglobin. These signs were seen when high drug dosages were given. It is possible that signs of methemoglobinemia differ from those described when other drugs and other species of animals were used.

METHEMOGLOBIN REDUCTION

Methemoglobin reduction is influenced by two major factors. First, reduction of methemoglobin is slowed when body temperature is lowered (43). Second, methemoglobin reduction is dependent on the availability of glucose in red blood cells (28) and proceeds faster when glucose is readily available (33). The biochemical pathway for the reduction of methemoglobin requires NADH as a factor, and NADH is a product of the glycolytic pathway dependent on the utilization of

glucose. If glucose is limited the availability of NADH for methemoglobin reduction will also be limited.

Factors which do not affect methemoglobin reduction are the concentration of methemoglobin in red blood cells (43,44) and the total amount of hemoglobin and methemoglobin present (43).

The reduction of methemoglobin to hemoglobin can be accomplished by at least four mechanisms (45). The first of these is NADH methemoglobin reductase (NADH diaphorase), which is responsible for most of the methemoglobin reduction occurring in red blood cells (25). The reduction of methemoglobin is thought initiated by a rapid transfer of electrons to diaphorase, an enzyme or group of enzymes (25,38). The second part of the reaction involves a slow reduction of the ferric iron of methemoglobin to the ferrous state. As discovered in individuals with hereditary methemoglobinemia, either a deficiency in NADH methemoglobin reductase or in NADH will interfere with this reduction (45). People with a NADH methemoglobin reductase deficiency may have a lowered glutathione reductase activity for reasons not known; but no such correlation has yet been made in dogs (46). Normal levels of reduced glutathione (GSH) were found in humans even though there was a deficiency of methemoglobin reductase (47). There are three possibilites for the deficiency of NADH (45): The enzymes in glycolysis may be inhibited, preventing the formation of NADH; there may be other metabolic reactions using the available NADH; or there could be a lack of NAD.

A second mechanism of methemoglobin reduction is through NADPH methemoglobin reductase (NADPH diaphorase, methylene blue reductase). This reaction requires an unknown carrier that can be replaced with

an artificial electron carrier, such as methylene blue or the autoxidizable dyes (48). NADPH reduces methylene blue (49), the electron carrier; then, leucomethylene blue rapidly and nonenzymatically reduces methemoglobin (45). The reaction catalyzed by NADPH methemoglobin reductase is faster than that involving NADH methemoglobin reductase (25,38). NADPH methemoglobin reductase is considered a reserve system (25) and the survival of red blood cells does not appear to depend it (50). Reduction of methemoglobin by methylene blue is impaired if glucose is not present in the red blood cells of dogs and man (38). The activity of the hexose monophosphate pathway (HMP) and the utilization of glucose in human, cat, dog, and horse red blood cell suspensions is increased by methylene blue (51). Two limitations in the NADPH methemoglobin reductase system which can occur are deficiencies of glucose-6-phosphate dehydrogenase (G-6-PD) or NADPH methemoglobin reductase (50).

The third mechanism for methemoglobin reduction is by ascorbic acid; this is a non-enzymatic reduction unlike the two mechanisms previously mentioned (45). It is thought that ascorbic acid provides a reserve system for methemoglobin reduction in red blood cells (45).

A fourth mechanism of methemoglobin reduction is through reduced glutathione (GSH); this is also a nonenzymatic reduction (44) with GSH also considered a reserve system in red blood cells. GSH and ascorbic acid are thought to reduce methemoglobin when the NADH methemoglobin reductase system is overwhelmed (25). The GSH in the red blood cell protects hemoglobin from oxidation (52-54), although it is not known what specific role GSH plays in protecting hemoglobin in cats. GSH can slow down the production of methemoglobin and

hinder Heinz body formation and in that way protect red blood cells against oxidation (53). Hemoglobin is thus protected from denaturation by GSH (55). Cohen and Hochstein (56) listed the formation of methemoglobin, loss of GSH, and increased osmotic fragility as indicators of red cell damage.

BLOOD GLUTATHIONE

When toxic amounts of acetaminophen have been ingested, liver GSH is reduced. Liver necrosis results when liver GSH concentration is about 20% of normal (7,10). Little is known about GSH levels in other body tissues in acetaminophen toxicity. Of particular concern is red blood cell GSH. Gaunt et al. (19) suggest that blood GSH can be depleted by oxidized hemoglobin or by reactive metabolites of acetaminophen in the blood. To better understand GSH, the actions of GSH in red blood cells, factors in red cells affecting GSH concentrations, and the oxidation and reduction of GSH will be discussed.

The most prevalent sulfhydryl compound in red blood cells is GSH (57,58). GSH is about one-half as abundant in red blood cells of man as is hemoglobin (58). It is a tripeptide of the amino acids glutamate, cysteine, and glycine. The synthesis of GSH occurs in two enzymatic steps. The first step involves a unique gamma-peptide bond between glutamate and cysteine. This step is catalyzed by gamma-glutamylcysteine synthetase. GSH appears resistant to the action of peptidases because of this gamma-peptide bond (57,59). The second step in GSH synthesis involves the addition of glycine to the dipeptide and is catalyzed by the enzyme glutathione synthetase.

Red blood cell GSH concentrations in cats, dogs, horses, and man

are very similar, being about 2 mM (60). The half-life of GSH in red blood cells is about four days (57-59). Smith (61) reported the GSH half-life was different among several species of animals and that it is between two to 14 days. The GSH in human red cells is in a dynamic state (58,62). The ratio of oxidized GSH (GSSG) to GSH is low (55,57,59), with most of the glutathione in red blood cells in the reduced form (GSH). The ratio of GSSG to GSH regulates the rate of the HMP pathway; the more GSSG present in the red blood cell, the faster the metabolic rate of the HMP pathway (54). Maruyama et al. (63) found that GSH content in the liver was influenced by diet. Rats that were fasted for two days had decreased GSH levels. When the rats were refed, GSH levels returned to normal. In addition, fasted rats had increased GSH levels when given diets high in carbohydrate or high in protein, but not high in fat.

Some of the functions GSH participates in in body cells are conjugation, synthesis of deoxyribonucleotide precursors of DNA, reduction of hydrogen peroxide and organic peroxides and disulfide bonds, and protection of cells from oxidative injury (64). Studies cited by Dimant et al. (62) document the role of GSH in protection of red blood cells from hemolysis when exposed to oxygen, organic mercurial compounds, iodine, or cobra venom. GSH also protects proteins from oxidation by keeping the levels of hydrogen peroxide or organic peroxides low in the cell (57). When oxidants are present GSH is oxidized (65). GSH is oxidized more readily than hemoglobin (54), so that to some extent GSH protects hemoglobin from oxidation (54). "Erythrocyte pentose phosphate pathway (HMP) metabolism was higher in man than in animals in the presence of ascorbate,

suggesting that animal erythrocytes might be less able to protect themselves against peroxides and/or superoxide," according to Harvey and Kaneko (51).

"The concentration of GSH in erythrocytes depends on its rate of synthesis, rate of oxidation, rate of reduction, and rate at which oxidized GSH is eliminated from the cell" (66). GSH levels are controlled by the inhibition of gamma-glutamylcysteine synthetase (58,67), the enzyme involved in the first step of GSH synthesis. As the red blood cell ages, the activity of enzymes needed to synthesize GSH drops (19). In addition to controlling GSH synthesis, red blood cells also appear capable of making and destroying GSH (68).

The rate of oxidation and reduction of GSH has an impact on GSH concentrations in the cell (Figure 2). The presence of oxidants in the cell, along with the availability of enzymes for the oxidation and reduction of GSH, will determine the cellular concentration of GSH at any one time.

Another factor influencing GSH concentration is the transport of GSSG out of the cell. GSSG is transported out of the red blood cell against a concentration gradient (55,57) and can not reenter the cell (55,69). Board (70) suggests that glutathione conjugates may be transported out of the red blood cell by the same mechanism. The transport of GSSG from the cell plays a role in the cellular turnover of GSH (57,61).

GLUTATHIONE PEROXIDASE

Glutathione peroxidase is the enzyme that oxidizes GSH when oxidants are present in the cell, thus protecting the cell from oxidants (71). This enzyme appears responsible for shielding the

breakdown of molecules such as hemoglobin in the presence of hydrogen peroxide (52). Glutathione peroxidase is dependent upon the action of glutathione reductase and the regeneration of NADPH from the HMP pathway to maintain GSH in the cell (71,72). Without GSH, glutathione peroxidase can not function.

Glutathione peroxidase is a selenium-bound enzyme (71,73), but a selenium-independent enzyme has been identified, also (73). If there is selenium deficiency, there will be a glutathione peroxidase deficiency (59,73). The activity of glutathione peroxidase is higher in animal red blood cells than in man (60). Hydrogen peroxide, lipid peroxides, and lipid hydroperoxides are all reduced by glutathione peroxidase (71). It is not currently known which enzyme, (glutathione peroxidase or catalase) is more important in the protection of red blood cells from oxidative damage and in reducing oxidants in red blood cells (71). A present theory is that glutathione peroxidase plays a more important role at the physiological level in eliminating hydrogen peroxide (and other oxidants) from the cell than does catalase (56,72,74). Glutathione peroxidase appears the predominant enzyme in eliminating small quantities of hydrogen peroxide from the cell. Larger quantities of hydrogen peroxide are reduced by catalase. Mills (75) reported that glutathione peroxidase was more effective in protecting hemoglobin from oxidative breakdown when low levels of ascorbic acid were present than was catalase.

Glutathione peroxidase is inhibited by nucleotides, particularly nucleotides with an increasing number of phosphate groups on them (76). Of all nucleotides, the pyrimidine nucleotides had the

greatest inhibiting effect on glutathione peroxidase (76). The presence of some nucleotides in red blood cells regulate glutathione peroxidase activity (76). Glutathione peroxidase has separate sites for catalytic activity and nucleotide binding (76).

The metabolic rate of the HMP pathway is increased by the presence of oxidants in red blood cells (77). This pathway provides the NADPH used in the reduction of GSSG (56,58) and is the only mechanism for glucose oxidation in mature red blood cells (54). It provides all the NADPH in mature red cells (78). The availability of NADPH in cells is important in the reduction of GSH, as the action of glutathione peroxidase is dependent on the availability of GSH. The reduction of GSH is thought an important source of cellular NADP (54). The many actions of GSH in the cell are dependent upon normal enzymatic functioning in oxidizing and reducing GSH. It is therefore not surprising that "the most common disorders of glutathione metabolism involve the mechanisms for oxidation and reduction of glutathione" (59).

GLUTATHIONE REDUCTASE

There are two pathways by which red blood cells may rid themselves of GSSG: reduction of GSSG, and tranportation of GSSG out of the cells (61). The major enzyme responsible for reducing GSSG is glutathione reductase. Adequate levels of GSH have been found in red blood cells even though glutathione reductase activity was only 30-40% of normal (55). It appears that glutathione reductase is not a limiting factor in the reduction of GSSG (77). Other important enzymes in the reduction of GSSG are G-6-PD, hexokinase, and 6-phosphogluconate dehydrogenase (6-PGD) (61). The activity of all

these enzymes, including glutathione reductase, is greater than the rate of GSSG reduction (61). G-6-PD (55,59,79) and 6-PGD (79) are HMP enzymes necessary for the production of NADPH. If there is a deficiency in either one of these two enzymes, the NADPH available will be limited.

Glutathione reductase activity is stimulated by the addition of riboflavin to the diet (80); riboflavin is used to synthesize flavin adenine dinucleotide (FAD). In red blood cells, glutathione reductase has two forms: an active form bound with FAD, and an inactive form that is not bound to FAD (59). Glutathione reductase in red blood cells of dogs and especially of cats is not as saturated with FAD as in horses or man (78). Much of the glutathione reductase in cats is in the inactive form when compared with other animal species (78). The amount of FAD bound to glutathione reductase does not seem to affect the glutathione reductase activity in different animal species (78). Cats have the highest glutathione reductase activity, followed by man, dogs, and horses (78).

NADPH or NADH can be used by glutathione reductase to reduce GSSG (65,81,82), but NADPH is the nicotinamide nucleotide physiologically preferred (59). Francoeur and Denstedt (82) suggested that reduction of GSSG by NADH may need a cofactor for full activation. Another reason NADPH may be preferred over NADH is that NADPH is more effectively bound to the enzyme than is NADH (83).

The action of glutathione reductase is not limited to the reduction of GSSG; dihydrolipoic acid is also reduced by glutathione reductase (83), and has been shown to split the mixed disulfide of hemoglobin and GSH (55). GSSG or a GS-radical can form a mixed

disulfide with the hemoglobin molecule (55). The mixed disulfide may then precipitate in the cell. Jaffe (58) suggests that "...hemoglobin and GSH are sacrificed to early oxidant stress to protect the cell from more serious and irreparable harm," indicating that both molecules are important in red blood cells. The mixed disulfide and active hemoglobin are in a continuous dynamic equilibrium that is influenced by NADPH (55).

The activity of glutathione reductase is increased in G-6-PD deficiency, after the ingestion of flavin mononucleotide (FMN), in in vitro induction of methemoglobin (84), in hepatitis from salicylate poisoning, and in other conditions (85). A deficiency of glutathione reductase activity has also been associated with drug-induced hemolytic anemias (84).

CONCLUSIONS

The increased usage of acetaminophen as an aspirin substitute has created an increased incidence of acetaminophen toxicity in cats. Because cats are deficient in glucuronyl transferase, the detoxication of acetaminophen is impaired. In addition, pet owner's often fail to appreciate the size of cats compared to an adult human. Some of the signs associated with acetaminophen toxicity in cats include anorexia, depression, cyanosis, vomiting, subnormal or elevated temperature, and facial edema. The current recommended therapy for acetaminophen poisoning is symptomatic: N-acetylcysteine, fluids, oxygen, furosemide, and ascorbic acid.

One of the earliest signs of acetaminophen toxicity in cats is cyanosis due to methemoglobinemia. Methemoglobin is incapable of carrying oxygen, so the blood gets dark in color causing body tissues

to appear cyanotic. Methemoglobin formation occurs either by autoxidation, by the direct action of oxidants, or by the interaction of atmospheric oxygen and hydrogen donors. The reduction of methemoglobin occurs primarily by NADH methemoglobin reductase in red blood cells. NADPH methemoglobin reductase, ascorbic acid, and GSH are reserve systems in red blood cells. Some of the factors important in drug-induced methemoglobinemia are the mechanism of hemoglobin oxidation, the mechanism of methemoglobin reduction, the species of animal, the nature of the drug, the formation of active metabolites, and the detoxication and excretion of the drug.

Liver GSH becomes important in acetaminophen detoxication when the glucuronide and sulfate detoxication pathways are reduced. Liver GSH protects the macromolecules of the liver from the reactive metabolites of acetaminophen until GSH levels reach about 20% of normal concentration. The functions of GSH in the body cells are varied, including protection of cells from oxidative injury, reduction of hydrogen peroxide and organic peroxides, synthesis of deoxyribonucleotide precursors of DNA, and conjugation. The concentration of GSH in red blood cells is dependent on several factors, including the rate of synthesis, the rate of oxidation, the rate of reduction, and the rate of GSSG transport out of the cell. GSH is oxidized by oxidants such as hydrogen peroxide in the cell and by the enzyme glutathione peroxidase. Glutathione reductase is the major enzyme responsible for the reduction of GSSG. Either nucleotide, NADPH or NADH, can be used for reduction by glutathione reductase, although NADPH is physiologically preferred.

Acetaminophen toxicity seems to interfere with the mechanisms

involved in maintaining normal concentrations of hemoglobin and blood GSH. The enzymes involved in oxidation and reduction of these two molecules are of primary interest because a change in activity of a particular enzyme can dramatically affect the concentrations of the molecules in question. Because methemoglobinemia is a sign in cats associated with acetaminophen toxicity, the enzyme NADH methemoglobin reductase is of importance for two reasons. The first is that this enzyme is responsible for most of the methemoglobin reduction in red blood cells. Therefore, if this enzyme is impaired the concentration of methemoglobin in red blood cells is increased. The second reason is that specific enzymes have not been associated with methemoglobin formation, so any impairment would most likely be with the enzymatic reduction of methemoglobin. Glutathione, on the other hand, is both enzymatically oxidized and reduced, so there are two enzyme systems to consider. If it is assumed that the GSH in acetaminophen toxicity is lost as GSSG, an impaired activity of glutathione peroxidase would not appear in question. Therefore, the problem may be with NADPH glutathione reductase. If this enzyme were impaired, the concentration of GSH in red blood cells would be low.

Study of the enzymatic mechanisms involved with hemoglobin and GSH should begin to answer some of the questions about the biochemical mechanisms of acetaminophen toxicity in cats and other animals.

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Figure 1. The mechanism of methemoglobin formation and methemoglobin reduction. The ferrous (Fe^{++}) on the hemoglobin molecule is oxidized to the ferric (Fe^{+++}) by oxidizing agents present in the red blood cell, thus forming methemoglobin. Methemoglobin is enzymatically reduced in the cell by methemoglobin reductase and NADH.

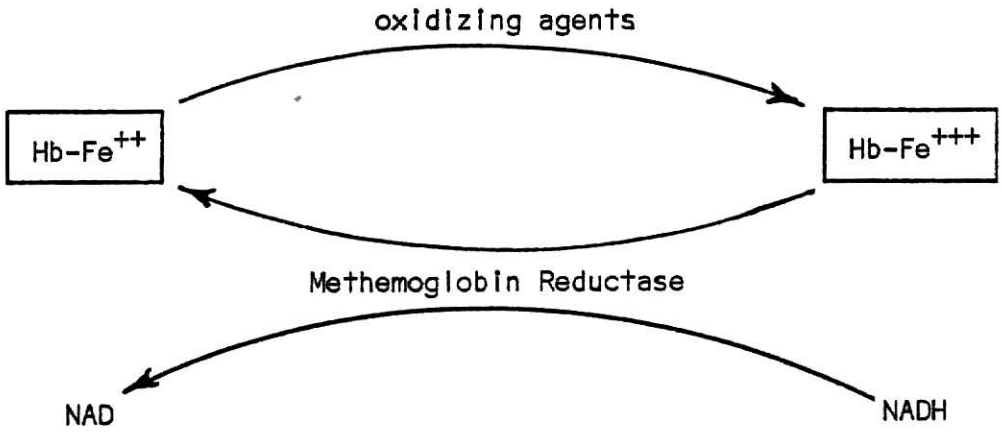
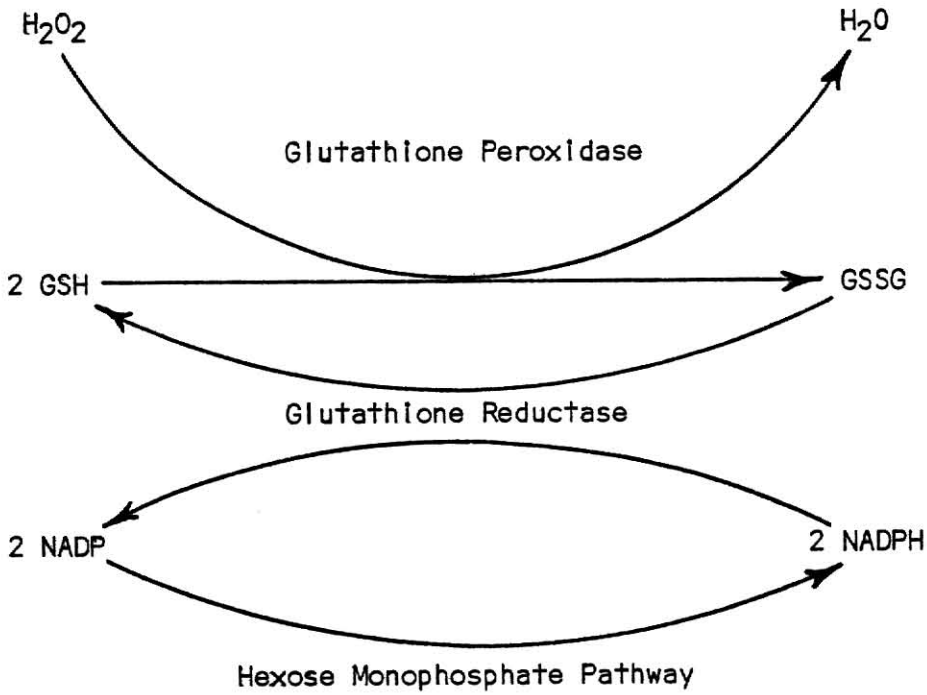

oxidizing agents
Hb-Fe++
Hb-Fe+++
Methemoglobin Reductase
NAD
NADH

Figure 2. The mechanism of glutathione oxidation and reduction. Two molecules of reduced glutathione (GSH) are oxidized to oxidized glutathione (GSSG) by glutathione peroxidase and by oxidizing agents such as hydrogen peroxide present in the red blood cell. Glutathione reductase and NADPH reduce GSSG back to GSH. The availability of NADPH is dependent on normal functioning of the hexose monophosphate pathway.


H_2O_2
H_2O
Glutathione Peroxidase
2 GSH
GSSG
Glutathione Reductase
2 NADP
2 NADPH
Hexose Monophosphate Pathway

APPENDIX II

Individual Animal Data on the Effect of Acetaminophen on Methemoglobin and Blood Glutathione Parameters in the Cat

Each value is the average of two measurements, except for methemoglobin reductase which is the average of two or more measurements.

Cat #1, Sex Male, Age Mature

Date of 20 mg/kg Dose 1/17/83, Weight 3.2 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
-6	0.007	17.81	87.80	10.62
-4	0.012	16.95	77.12	19.56
0	0.000	5.97	78.83	16.82
1	0.007	11.62	67.32	21.49
2	0.013	6.66	69.75	23.33
4	0.012	10.54	79.24	22.97
8	0.009	8.07	77.17	19.46
12	0.015	7.99	78.28	19.20
24	0.007	10.14	74.98	16.21
48	0.022	14.34	84.61	14.07
96	0.013	16.78	85.24	23.97
192	0.014	24.93	87.09	26.68

[a] Time is in hours, except negative time which is in days

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #1, Sex Male, Age Mature

Date of 60 mg/kg Dose 2/7/83, Weight 3.6 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.009	7.09	77.26	26.26
1	0.065	6.35	78.80	21.57
2	0.109	8.29	66.11	25.90
4	0.137	10.59	71.19	22.62
8	0.036	9.25	83.38	20.77
12	0.017	9.71	88.48	22.04
24	0.013	6.16	73.11	22.07
48	0.010	9.05	70.58	19.41
96	0.012	9.17	68.05	22.53
192	0.013	10.40	85.06	21.52

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #1, Sex Male, Age Mature

Date of 120 mg/kg Dose 2/28/83, Weight 3.6 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.011	6.72	85.28	18.42
1	0.252	9.43	78.13	22.30
2	0.395	9.72	73.86	18.59
4	0.343	9.15	58.37	16.74
8	0.315	10.22	12.06	16.84
12	0.405	6.33	2.89	15.08
24	0.153	4.62	6.90	13.74
48	0.024	3.42	23.69	13.78
96	0.028	1.55	51.73	12.22
192	0.025	3.58	78.44	16.92

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #3, Sex Male, Age Mature

Date of 20 mg/kg Dose 1/24/83, Weight 3.2 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
-13	0.011	11.09	74.69	14.02
-11	0.009	9.99	83.96	12.29
-4	0.018	18.51	51.32	13.78
0	0.022	14.27	58.31	14.59
1	0.017	28.38	66.52	15.62
2	0.016	8.95	53.89	11.64
4	0.011	13.12	59.93	13.51
8	0.007	7.64[e]	58.68	13.70
12	0.015	9.34	64.18	12.19
24	0.010	7.89[f]	58.15	15.47
48	0.013	7.46[f]	51.74	15.52
96	0.019	7.37[f]	54.44	16.45
192	0.020	9.32[f]	71.18	18.22

[a] Time is in hours, except negative time which is in days

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

[e] Used 100 µl and 200 µl of hemolysate rather than 50 µl

[f] Used 100 µl of hemolysate rather than 50 µl

Cat #3, Sex Male, Age Mature

Date of 60 mg/kg Dose 2/14/83, Weight 4.1 kg

Time [a]	Methemoglobin [b]	Methemoglobin Reductase [c]	Glutathione [d]	Glutathione Reductase [c]
0	0.004	9.41	87.30	18.26
1	0.164	10.85	73.66	19.09
2	0.266	12.39	61.11	22.13
4	0.331	7.38	67.18	25.56
8	0.212	7.08	67.39	23.06
12	0.049	8.70	60.82	22.03
24	0.011	7.97	62.08	21.62
48	0.007	8.66	63.23	21.85
96	0.010	8.86	62.62	20.93
192	0.016	7.30	84.56	21.91

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #3, Sex Male, Age Mature

Date of 120 mg/kg Dose 3/14/83, Weight 4.5 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.014	13.97	75.01	18.33
1	0.264	6.39	60.31	18.93
2	0.359	6.81	62.53	18.10
4	0.448	10.18	37.16	16.82
8	0.500	6.36	9.76	17.04
12	0.487	6.10	5.32	12.40
24	0.141	5.99	1.68	12.63
48	0.031	7.18	9.18	15.58
96	0.018	4.63	27.16	17.37
192	0.010	8.29	46.56	36.82

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #4, Sex Female, Age Mature

Date of 20 mg/kg Dose 1/24/83, Weight 2.0 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
-13	0.012	15.78	59.22	27.84
-11	0.004	15.22	51.28	27.72
-6	0.017	17.05	73.78	23.91
-4	0.027		59.49	
0	0.009	10.80	72.90	25.96
1	0.040	12.10	67.43	25.17
2	0.053	16.65	69.84	21.70
4	0.023	10.77	81.48	25.72
8	0.006	7.67[e]	77.60	21.27
12	0.014	7.30[e]	79.37	25.82
24	0.028	5.72	82.42	19.30
48	0.007	6.49	61.14	23.96
96	0.027	11.06	69.98	27.89
192	0.005	9.18	70.46	25.36

[a] Time is in hours, except negative time which is in days

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

[e] Used 50 µl and 100 µl of hemolysate

Cat #4, Sex Female, Age Mature

Date of 60 mg/kg Dose 2/14/83, Weight 2.7 kg

Time[a]	Methemoglobin[b]	Methemoglobin Reductase[c]	Glutathione[d]	Glutathione Reductase[c]
0	0.004	7.22	77.22	26.57
1	0.114	8.90	69.73	27.06
2	0.160	9.49	69.31	39.43
4	0.179	9.42	66.35	29.64
8	0.023	6.02	79.24	22.63
12	0.008	8.59	67.03	31.88
24	0.013	6.03	69.62	21.52
48	0.005	8.46	93.61	27.10
96	0.003	9.06	78.17	29.23
192	0.008	7.36	69.21	34.19

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #4, Sex Female, Age Mature

Date of 120 mg/kg Dose 3/14/83, Weight 3.9 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.012	14.17	70.21	34.76
1	0.299	4.62	68.68	26.14
2	0.431	4.98	55.23	25.67
4	0.488	4.72	38.59	26.98
8	0.411	5.94	12.25	31.16
12	0.499	5.49	11.53	27.90
24	0.028	5.57	15.02	25.61
48	0.014	5.61	33.82	25.66
96	0.022	3.71	59.30	23.19
192	0.012	5.33	68.19	34.46

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #5, Sex Female, Age Mature

Date of 20 mg/kg Dose 1/17/83, Weight 2.3 kg

Time[a]	Methemoglobin[b]	Methemoglobin Reductase[c]	Glutathione[d]	Glutathione Reductase[c]
-6	0.017	11.50	62.05	25.05
-4	0.002	15.57	62.55	19.99
0	0.010	12.34	62.08	16.18
1	0.023	13.64	48.83	19.88
2	0.007	15.12	65.27	24.70
4	0.014	13.59	49.84	22.50
8	0.008	15.69	63.96	24.44
12	0.013	20.75	58.93	26.25
24	0.006	5.92	62.52	22.25
48	0.012	9.00	75.24	21.49
96	0.022	10.38	63.49	26.64
192	0.030	8.36	76.53	29.61

[a] Time is in hours, except negative time which is in days

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #5, Sex Female, Age Mature

Date of 60 mg/kg Dose 2/7/83, Weight 2.3 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.013	7.79	70.62	20.72
1	0.110	9.24	69.84	22.92
2	0.180	8.63	57.98	25.03
4	0.183	5.70	65.48	16.32
8	0.034	5.92	75.93	17.51
12	0.021	8.00	73.49	19.53
24	0.015	6.40	66.45	15.09
48	0.007	8.03	69.98	19.06
96	0.008	9.47	73.85	18.52
192	0.009	8.22	77.87	18.77

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #5, Sex Female, Age Mature

Date of 120 mg/kg Dose 2/28/83, Weight 2.3 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.019	4.82	69.41	16.62
1	0.299	4.92	62.90	17.54
2	0.447	4.86	50.73	16.41
4	0.472	5.26	31.85	16.98
8	0.488	7.78	3.58	15.60
12	0.457	6.39	1.53	13.18
24	0.097	6.30	5.68	12.50
48	0.020	5.65	11.16	8.86
96	0.020	8.87	37.25	12.04
192	0.019	9.59	60.30	30.42

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #6, Sex Male, Age Mature

Date of 20 mg/kg Dose 1/31/83, Weight 2.7 kg

Time[a]	Methemoglobin[b]	Methemoglobin Reductase[c]	Glutathione[d]	Glutathione Reductase[c]
-20	0.015	19.26	60.42	12.66
-18	0.007	16.32	80.15	13.93
-13	0.012	21.50	79.67	12.48
0	0.020	10.06	66.41	16.55
1	0.030	9.88	66.27	13.46
2	0.024	8.80	63.25	14.88
4	0.020	7.39	64.55	16.53
8	0.002	8.10	65.73	17.39
12	0.012	11.39	62.48	18.25
24	0.006	8.84	66.68	18.75
48	0.004	11.45	57.18	12.49
96	0.014	12.88	57.48	10.81
192	0.010	8.80	57.04	11.36

[a] Time is in hours, except negative time which is in days

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #6, Sex Male, Age Mature

Date of 60 mg/kg Dose 2/21/83, Weight 4.1 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.016	7.45	71.93	17.17
1	0.130	8.36	60.57	20.43
2	0.215	5.10	65.62	13.32
4	0.267	6.67	57.03	12.22
8	0.132	7.86	56.78	14.00
12	0.029	7.42	45.97	11.92
24	0.017	7.38	59.98	10.98
48	0.004	9.18	62.08	11.38
96	0.012	8.73	61.66	13.55
192	0.018	8.20	59.90	14.61

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #6, Sex Male, Age Mature

Date of 120 mg/kg Dose 3/21/83, Weight 3.6 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.017	6.32	66.86	14.57
1	0.272	5.35	62.48	16.96
2	0.446	6.25	55.03	15.45
4	0.527	10.57	35.16	16.41
8	0.565	8.13	4.27	13.95
12	0.606	8.98	6.37	12.03
24	0.167	8.60	2.39	11.20
48	0.031	6.78	7.30	13.37
96	0.018	7.31	21.84	12.69
192	0.017	10.12	59.78	17.60

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #8, Sex Female, Age Mature

Date of 20 mg/kg Dose 1/31/83, Weight 2.7 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
-5	0.009	25.02	64.08	33.65
-4	0.023	16.11	71.11	43.90
-3	0.005	11.51	58.07	38.22
0	0.007	8.42	57.27	32.15
1	0.021	8.80	55.05	27.78
2	0.005	10.11	47.50	31.42
4	0.005	12.15	57.65	30.46
8	0.011	11.23	64.81	34.83
12	0.011	12.91	54.59	32.82
24	0.028	15.81	67.45	34.59
48	0.005	10.77	52.10	25.66
96	0.016	13.73	55.77	31.63
192	0.023	11.70	52.45	36.03

[a] Time is in hours, except negative time which is in days

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #8, Sex Female, Age Mature

Date of 60 mg/kg Dose 2/21/83, Weight 1.8 kg

Time[a]	Methemoglobin[b]	Methemoglobin Reductase[c]	Glutathione[d]	Glutathione Reductase[c]
0	0.013	12.09	67.50	24.50
1	0.048	10.59	66.86	25.16
2	0.114	10.75	63.91	22.79
4	0.202	11.60	64.02	20.07
8	0.143	11.67	66.34	20.62
12	0.071	8.22	53.07	21.62
24	0.014	9.37	57.72	22.14
48	0.012	10.84	64.26	20.29
96	0.002	9.20	73.95	22.45
192	0.015	9.84	72.95	23.64

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

Cat #8, Sex Female, Age Mature

Date of 120 mg/kg Dose 3/21/83, Weight 2.5 kg

Time[a]	Methemoglobin[b]	Methemoglobin[c] Reductase	Glutathione[d]	Glutathione[c] Reductase
0	0.023	17.56	97.76	18.31
1	0.115	19.92	97.78	21.84
2	0.274	19.45	85.01	21.48
4	0.401	19.85	72.27	18.27
8	0.427	23.69	61.58	21.30
12	0.278	10.04	63.11	18.20
24	0.016	9.85	52.15	19.78
48	0.013	10.14	54.97	18.51
96	0.003	10.47	62.73	22.72
192	0.012	12.33	99.87	34.82

[a] Time is in hours

[b] Units of measurement = g Met Hb-100 ml^{-1} /g total Hb-100 ml^{-1}

[c] Units of measurement = IU/g Hb

[d] Units of measurement = mg GSH/dl RBC

THE EFFECT OF ACETAMINOPHEN TOXICITY ON SELECTED BLOOD BIOCHEMICAL PARAMETERS IN THE CAT

by

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Acetaminophen (APAP) is an analgesic that has become a popular aspirin substitute because it is not associated with stomach irritation and can be used by aspirin sensitive individuals. The toxicity of APAP varies with the animal speicies exposed, cats being the most sensitive. Information is limited as to why cats are so sensitive to APAP and why methemoglobin formation is so prominent in cats poisoned by APAP. This study investigates some selected blood biochemical parameters that would influence the development of methemoglobin and the loss of blood reduced glutathione (GSH) in cats exposed to APAP.

Six adult cats were each given single progressive doses of APAP--20 mg APAP/kg of body weight (low), 60 mg APAP/kg of body weight (medium), or 120 mg APAP/kg of body weight (high). Each dose was given three weeks apart. Blood was collected at 0, 1, 2, 4, 8, 12, 24, 48, 96, and 192 hr (day 8) after each dosing. Methemoglobin and blood GSH were measured to document the increase in methemoglobin and the decrease in GSH at each dosage. The reduction systems, NADH methemoglobin reductase and NADPH glutathione reductase, were studied to determine any impairment during APAP toxicity.

Methemoglobin formation was significant at the medium and the high dosages, but not at the low dosage. The mean peak concentration of methemoglobin at the high dosage was 46%, about twice that developed at the medium dosage. The methemoglobin concentration plateaued between 4 and 12 hr at the high dosage, but peaked at 4 hr at the medium APAP dosage. Appreciable levels of methemoglobin were still present at 24 hr following the high APAP dose. The activity of

NADH methemoglobin reductase at the high dose was statistically, significantly decreased from the activity following the low APAP dose. A dose-related trend for decreased NADH methemoglobin reductase activity was seen as the APAP dose increased. A significant decrease in blood GSH occurred within the first 4 hr at the medium dosage. At the high APAP dosage, the decrease in blood GSH was most dramatic the first 8 hr after dosing and continued to decrease through 24 hr, when blood GSH concentrations were lowest. By 192 hr (day 8), blood GSH concentrations were nearly normal. No significant difference in NADPH glutathione reductase activity occurred at the low or medium dosages, although a significant decrease in activity occurred after the high APAP.

This study showed that cats responded to APAP in a dose-related fashion by increased methemoglobin formation and decreased blood GSH concentrations. An indirect or limited relationship of APAP on the NADH methemoglobin reductase and the NADPH glutathione reductase enzyme systems was suggested by the observations at the high APAP dosage. Further study of the relationship between APAP exposure and the biochemistry of tissues is necessary to explain this unique toxic response to APAP in cats.