Effects of propylene glycol–containing diets on acetaminophen-induced methemoglobinemia in cats

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Summary: *Soft-moist cat foods contain 7 to 13% propylene glycol (PG) on a dry-weight basis. These diets induce Heinz body formation in feline RBC. In this study, we evaluated cats on a control diet and on a commercial diet containing 8.3% PG. All cats on the PG diet developed an increase in the number of circulating Heinz bodies. We then administered acetaminophen to cats on each diet to determine whether RBC from cats on PG diets were more susceptible to oxidant stress. Methemoglobin concentrations were significantly greater in cats in PG diets after acetaminophen administration. These data indicate that RBC from cats fed PG diets are more susceptible to oxidative stress.*

The soft and moist character of foods prepared for cat, dog, and human consumption is dependent on the presence of the humectant propylene glycol (PG). Propylene glycol, added to cat food at concentrations of 7 to 13% of dry weight, is also a major source of dietary carbohydrate.[1] One side effect in cats on PG-containing diets is higher than normal numbers of circulating Heinz bodies.[2,3] Because Heinz bodies form as a result of oxidative damage to hemoglobin, it has been hypothesized that PG, or a metabolic by-product of PG, has oxidant properties.[2] Cats are susceptible to hemoglobin oxidation and denaturation, probably because of the presence of 8 reactive sulfhydryl groups per molecule of hemoglobin.[4-7]

In recent studies, PG was fed to cats at the approximate concentration found in PG diets (12% of dry weight).[2] The percentage of Heinz bodies increased approximately 9-fold, but no statistically significant changes were found for PCV or methemoglobin or reticulocyte counts. Red blood cell survival was modestly shortened, and D-lactate concentrations increased. When the cats were given PG at a concentration of 41%, plasma D-lactate concentration was markedly increased. This increase was attributed to the metabolism of PG to methylglyoxal, which is converted to D-lactate.[8]

The purpose of the study reported here was to reproduce the PG-related changes by feeding a commerical semi-moist cat food and to determine whether feeding such a diet would predispose cat RBC to oxidative stress.

Materials and methods

Nine adult cats, weighing 2.6 to 4.7 kg, were conditioned for 4 weeks, vaccinated for panleukemia and respiratory disease viruses, and treated for fleas and internal parasites. All cats appeared healthy on initial examination and throughout the study. Results of 3 weekly CBC and clinical chemical profiles were within reference ranges. All cats tested negative for FeLV.

During the first 8 weeks, all cats were fed a commercial dry cat food diet[a] ad libitum. In weeks 9 to 17, all cats were fed a commercial soft-moist diet[b] ad libitum. The propylene glycol content was determined by a private laboratory to be 8.32% on a dry weight basis by gas chromatography.

In weeks 4 and 13, all cats were given an oral dose of acetaminophen.[c] Five cats were given 80 mg of acetaminophen (low-dose group) and 4 were given 160 mg of acetaminophen (high-dose group). Dosages ranged from 18 to 30 mg/kg of body weight (mean, 23.4 mg/kg) for the low-dose group and 40 to 60 mg/kg (mean, 47.75 mg/kg) for the high-dose group. Jugular blood samples were drawn, with the cats under ketamine sedation, 4 hours after acetaminophen administration. Complete blood counts were determined by use of an automated electronic particle counter.[d] Total WBC count, RBC, mean cell volume (MCV), and mean cell hemoglobin concentration (MCHC) were deter-

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[a]Purina Cat Chow, Ralston Purina Co, Ralston, NC.
[b]Tender Vittles cat food, Ralston Purina Co, Ralston, NC.
[c]Children's Tylenol, McNeil Lab Inc, Fort Washington, Pa.
[d]Coulter S Plus IV, Coulter Electronics, Hialeah, Fla.

mined. Hemoglobin concentration was determined by use of Drabkin reagent, on lysates that were centrifuged at 6,000 × g to eliminate turbidity. A microhematocrit centrifuge was used to determine PCV. Total plasma protein was determined by use of a refractometer. Blood smears were prepared, and differential leukocyte counts were determined by counting 200 cells. New methylene blue-stained smears were used to quantitate punctate and aggregate reticulocytes.[9] Heinz bodies were quantified by counting 500 RBC on smears stained with brilliant cresyl green.[10] Methemoglobin was determined by measuring the absorption of RBC lysates at 630 n*M* before and after addition of cyanide.[11] The change in absorbance was detected by use of a recording spectrophotometer.[e]

Alanine transaminase, alkaline phosphatase, amylase activities, and total bilirubin, total protein, and albumin concentrations were determined by use of an automated batch-type clinical chemical analyzer.[f] Serum urea nitrogen, creatinine, sodium, potassium, chloride, glucose, and total calcium concentrations, anion gap, and total carbon dioxide content were analyzed on an automated 8-channel sequential analyzer.[g]

Data were analyzed by analysis of variance, using the Duncan multiple-range test. Results of weekly sample analysis for each cat while on the control diet were pooled for comparison with results for the cats while on PG diets.

Results

Clinical effects—All cats remained alert and active throughout the study. After high-dose acetaminophen administration, transient cyanosis was detected in 2 of 4 cats on control diets and 3 of 4 cats on the PG diet. Cyanosis was not observed in the low-dose group.

[e]DU-7 recording spectrophotometer, Beckman Inc, Brea Calif.

[f]Altaire Chemistry Analyzer, Electronucleonics, Inc, Fairfax, NJ.

[g]Astra 8, Beckman Inc, Brea, Calif.

Table 1—Hematologic alterations associated with feeding propylene glycol-containing diets to 9 cats

Variables	Control diet	Propylene-glycol diet
PCV (%)	33.1 ± 0.5*	31.1 ± 0.4†
RBC count (×10^6 μl)	7.0 ± 0.2	6.8 ± 0.2
Hemoglobin (g/dl)	11.3 ± 0.2	10.7 ± 0.2‡
Mean cell volume (fl)	47.1 ± 0.5	46.2 ± 0.4†
Mean cell hemoglobin concentration (g/dl)	34.2 ± 0.1	33.9 ± 0.2†
Total leukocyte count (×10^3 μl)	7.7 ± 0.4	6.8 ± 0.6†
Heinz bodies (%)	2.6 ± 0.8	14.2 ± 1.0‡
Total reticulocytes (%)	9.5 ± 0.9	9.4 ± 1.1
Methemoglobin (% total hemoglobin)	1.8 ± 0.1	2.3 ± 0.6

*Values represent mean ± SE of all observations. †Values are significantly different ($P < 0.05$) as determined by analysis of variance. ‡Values are significantly different ($P < 0.01$) as determined by analysis of variance.

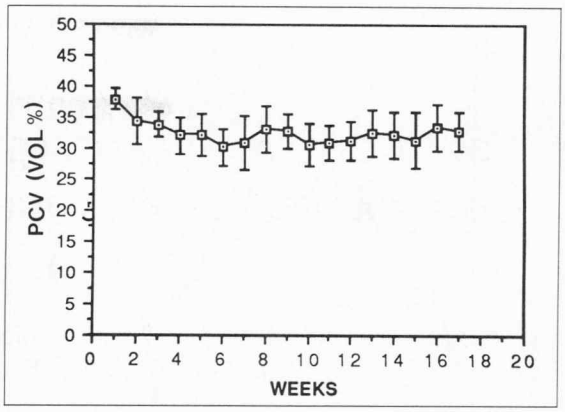

Figure 1—Serial changes in PCV (mean ± 1 SD) in cats on a control diet (weeks 1 to 8) and a propylene glycol-containing diet (weeks 9 to 16).

Diet-related changes—The mean weight of cats decreased slightly, from 3.4 ± 0.6 to 3.1 ± 0.5 kg, while on the PG diet. When values for cats on control diets (weeks 1 to 8) were compared with values for the PG diet (weeks 9 to 17), PCV, Hg, MCV, MCHC, and WBC were significantly ($P \leq 0.05$) lower and Heinz bodies were significantly ($P \leq 0.01$) greater in cats on the PG diet (Table 1; Fig 1). However, except for Heinz bodies, the changes were slight and remained within reference ranges. Heinz body numbers increased progressively the first 2 weeks after initiation of the PG diet, decreased the next 2 weeks, and then increased until the last sample collection (Fig 2). Diet-related changes were not observed for total reticulocytes, punctate reticulocytes, or aggregate reticulocytes (Table 1; Fig 3). Although differences were noticed in total serum protein, urea nitrogen, creatinine, total bilirubin, amylase, and creatine kinase values (Table 2), all remained within reference ranges for cats (Table 2).

Acetaminophen-related changes—Four hours after acetaminophen administration, samples differed significantly ($P \leq 0.01$) in methemoglobin concentrations (Fig 4). In the group given 80 mg of

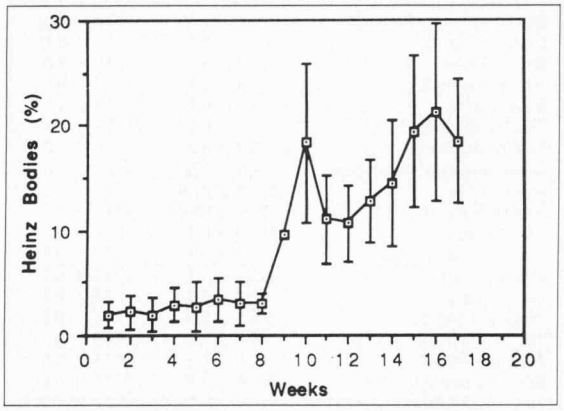

Figure 2—Serial changes in Heinz bodies in cats on a control diet (weeks 1 to 8) and a propylene glycol-containing diet (weeks 9 to 16).

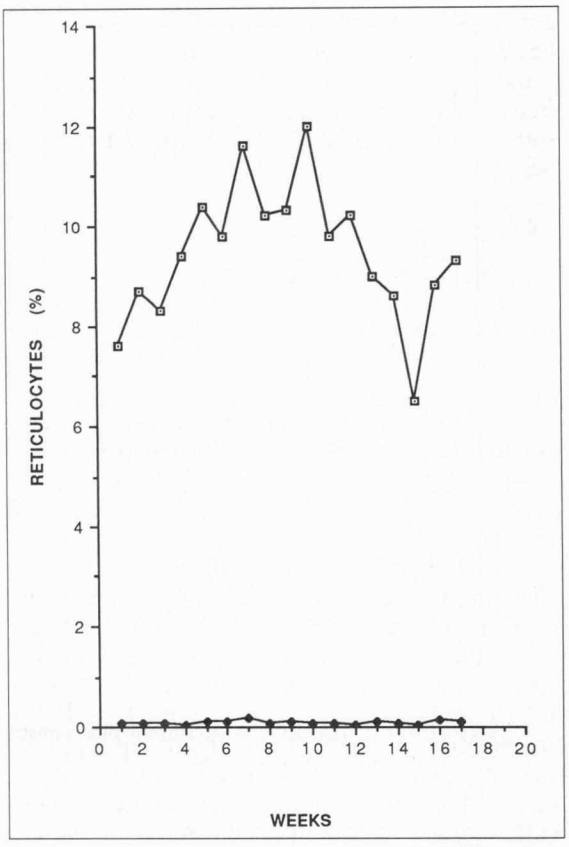

Figure 3—Serial changes in total reticulocytes (□) and aggregate reticulocytes (◆) of cats on a control diet (weeks 1 to 8) and a propylene glycol-containing diet (weeks 9 to 16).

acetaminophen, mean methemoglobin concentrations did not increase above baseline (< 1%) while the cats were on the control diet, but increased to greater than 9% of total hemoglobin when they were on the PG diet. In the group given 160 mg of acetaminophen, the mean methemoglobin concentration increased while the cats were on both

Table 2—Clinical chemical alterations in 9 cats ingesting propylene glycol-containing diets

Variables	Control diet	Propylene-Glycol diet
Urea nitrogen (mg/dl)	21.7 ± 1.1*	20.2 ± 0.6†
Creatinine (mg/dl)	1.2 ± 0.1	1.1 ± 0.1‡
Glucose (mg/dl)	82.3 ± 3.1	83.0 ± 3.0
Total protein (g/dl)	7.3 ± 0.1	7.1 ± 0.1†
Albumin (g/dl)	3.3 ± 0.1	3.4 ± 0.1
Total bilirubin (mg/dl)	0.5 ± 0.1	0.3 ± 0.1†
Alkaline phosphatose (U/L)	28.0 ± 3.9	28.0 ± 4.9
Alanine aminotransaminase (U/L)	64.1 ± 5.5	131.0 ± 6.0
Amylase (U/L)	903.0 ± 47.9	721.0 ± 27.7†
Creatine kinase (U/L)	222.0 ± 59.9	174.0 ± 9.7†
Sodium (meq/L)	157.0 ± 0.7	157.0 ± 0.4
Potassium (meq/L)	4.4 ± 0.1	4.3 ± 0.7
Chloride (meq/L)	123.0 ± 1.1	123.0 ± 0.9
Calcium (mg/dl)	9.6 ± 0.8	9.5 ± 0.2
Phosphorus (mg/dl)	5.5 ± 0.3	6.0 ± 0.3
Total carbon dioxide (meq/L)	17.6 ± 0.9	18.2 ± 0.7
Anion gap (meq/L)	15.5 ± 0.5	15.9 ± 0.6
Osmolality (mosm/L)	308.0 ± 1.6	313.0 ± 0.8

*Values represent mean ± SE of all observations. †Values are significantly different ($P < 0.05$) as determined by analysis of variance. ‡Values are significantly different ($P < 0.01$) as determined by analysis of variance.

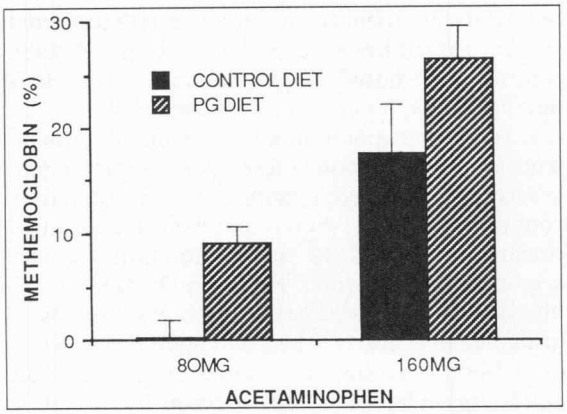

Figure 4—Methemoglobin concentrations 4 hours after administration of 2 doses of acetaminophen to cats while on a control diet and on a propylene glycol-containing diet.

diets, but the magnitude of the increase was statistically greater for the PG diet samples ($P \leq 0.01$). Heinz body counts, 4 hours after acetaminophen administration, for both the low-dose group (12.6 ± 3.6) and the high-dose group (13.2 ± 4.1) were not significantly different from values before acetaminophen administration for both the low-dose group (12.9 ± 3.2) and the high-dose group (12.8 ± 4.1). Additionally, 4 hours after acetaminophen administration, values for PCV, RBC, MCV, and MCHC, and reticulocyte counts were not statistically different when values were compared with those obtained immediately before acetaminophen administration.

Discussion

We evaluated physical, hematologic, and blood chemical changes associated with feeding a commercial, soft-moist cat food containing 8.3% PG, to determine whether the diet predisposed cat RBC to oxidant stress. Our studies confirm an increase in Heinz body numbers within the first week of feeding PG diets.[h] Although Heinz body numbers remained high throughout the feeding trial, numbers decreased at 4 and 9 weeks. This cyclic decrease was reported in another study and may be attributable to periodic destruction of Heinz body-containing RBC.[2]

The predisposition of cat RBC to form Heinz bodies has been attributed to the presence of 8 external sulfhydral groups on the hemoglobin molecule.[4,5] The spontaneous or induced oxidation of these exposed, and therefore reactive, sulfhydral groups can overwhelm the antioxidant capacity of RBC. Resulting oxidation of these sulfhydral groups induces exposure of internal sulfhydral groups through alteration of the tertiary structure of the

[h]Quast JF, Humiston CG, Wade CE, et al. Results of a toxicology study in cats fed diets containing propylene glycol for up to 3 months in *Proceedings*. 19th Annu Meet Soc Tox 1980;A26.

molecule. Further oxidation of internal sulfhydral groups causes irreversible alteration in the globin chain and subsequent precipitation as Heinz bodies.

Clinically apparent hemolytic disease was not observed in cats on PG diets. Although PCV significantly decreased, the change in the means was only 2%, and hematologic values from all cats remained within reference ranges. The hemolytic effects of PG diets appear to be dose-dependent. In cats fed 12% PG, the mean decrease in PCV was 3.6% whereas the decrease in PCV was 7.3 in cats fed 41% PG diets.[2]

The lack of clinically apparent hemolytic disease in cats with 10 to 20% Heinz body-containing RBC is attributable to the unique ultrastructure of the cat spleen.[12] Large pores in splenic pulp venules permit RBC to enter without deforming, thus preventing the cat spleen from effectively sequestering Heinz body-containing RBC.[12] This is supported by studies indicating that the disappearance of Heinz bodies from the blood is no faster in cats with spleens than in splenectomized cats.[13] Conversely, canine RBC containing Heinz bodies are rapidly sequestered and Heinz bodies are "pitted out" as they pass through small interendothelial slits.[12] This process is associated with hemolysis and shortening of RBC life-span. Therefore, mild to moderate oxidative injury to cat RBC results in only slight shortening of RBC life-span and can be readily compensated for by accelerated erythropoiesis. Additionally, the stimulation of erythropoiesis may be below the threshold needed to produce reticulocytosis.[2]

The marked differences in methemoglobin concentrations indicate that the PG diets predispose cat RBC to oxidant injury. A role of ketamine in increasing the methemoglobin concentration cannot be eliminated; however, ketamine-induced methemoglobinemia occurs sporadically.[14] Therefore, the consistent changes in all cats in this study strongly suggest a diet-related change.

Sources of additive oxidant stress are numerous, being both endogenous and exogenous. In a retrospective study, cats with diabetes mellitus, lymphoma, and hyperthyroidism accounted for 40% of cats with increased numbers of Heinz bodies in a hospital population.[15] These data suggest that these diseases induce oxidant stress. Sources of exogenous oxidants include acetaminophen,[7,8] phenazopyridine HCl,[16] methylene blue,[6] benzocaine,[17,18] menadione,[19] and ingestion of onions,[20] and vegetables of the *Brassica* genus, including cabbage, cauliflower, kale, and rape.[21] Additionally, any disease process that suppresses erythropoiesis may predispose cats to anemia because of the inability of the bone marrow to respond to the decreased RBC life-span. Many causes of depressed erythropoiesis in cats are encountered in clinical medicine. The most common are the anemia of inflammatory diseases[22] and FeLV infection.[23] As a result of the numerous causes of additive oxidant injury to cat RBC and the frequent suppression of erythropoiesis, the consumption of PG diets may pose a threat to feline health.

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