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Experimental Onion-Induced Hemolytic Anemia in Dogs

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Abstract. Within one day following a single oral dose of dehydrated onions, dogs were found to have large numbers of Heinz bodies within erythrocytes. The percentage of erythrocytes that contained Heinz bodies increased slightly to a maximum on day 3 and then declined. The turbidity index increased more gradually with a maximal value on day 4. Erythrocytes with hemoglobin contracted to one side of the cell (eccentrocytes) also appeared after onion feeding. Eccentrocytes are believed to result from a direct injury to the erythrocyte membrane. As with Heinz body-containing cells, the percentages of eccentrocytes present declined as anemia developed. The packed cell volume began to decrease one day after onion administration. A mean decrease of 19 percentage points was reached by day 5. The most anemic dogs had evidence of intravascular hemolysis. Reticulocytosis was first observed five days after onion administration. A slight increase in methemoglobin content was measured four hours after onion administration. No significant changes in erythrocyte reduced glutathione concentration were measured. Transient neutrophilia occurred concomitant with the peak reticulocyte response.

Hemolytic anemia that results from the consumption or oral administration of onions has been reported in cattle,[10] sheep,[23] horses,[15] cats,[11] and dogs.[3,6,12,19,21,22] Onion-induced anemia in dogs was first recognized in 1930, during research designed to test the potential value of dietary onions in prevention of niacin deficiency.[19] The hemolytic nature of the anemia was demonstrated in a study the following year.[6] Autoclaved onions and autoclaved onion juice produced effects similar to raw onions.

Several clinical cases of onion-induced hemolytic anemia have been reported in dogs.[3,12,21,22] In contrast to early experimental studies where onions were fed daily as part of the diet,[6,19] severe anemias apparently occurred in clinical cases after a single ingestion of onions. One dog consumed several ounces of dehydrated onions,[3] and a second dog ate raw onions.[22] The remaining dogs were fed table scraps that contained cooked onions.[12,21]

The present study was conducted to evaluate sequential hematologic changes in dogs given a single oral dose of onions. In addition to routine hematologic tests, erythrocyte reduced glutathione, methemoglobin, and Heinz bodies were quantified.

Materials and Methods

Adult, clinically normal mixed-breed dogs were utilized in this study. After a 24-hour fast, six test dogs (five males, one female) were fed 5.5 g/kg body weight minced dehydrated onions (Sauers Dehydrated Minced Onions, C. F. Sauer Co., Richmond, VA) mixed with 0.5 kg of raw ground beef. Three control dogs were fed 0.5 kg raw ground beef with no onions added. The following day, all dogs were placed back on their regular diet (Purina Dog Chow, Ralston Purina Co., St. Louis, MO). Desiccated onions are reported to have less oxidative potential than fresh or cooked onions,[6] but were used in this study because of ease of administration. Blood samples were collected, using ethylenediaminetetraacetic acid as an anticoagulant, at time 0 (before feeding onions and/or ground beef), at two, four, and six hours, and on days 1, 2, 3, 5, 7, and 9.

Parameters measured in all blood samples included packed cell volume (determined by the microhematocrit method), reduced glutathione,[16] reticulocyte count,[18] turbidity index,[8] total and differential leukocyte count,[18] Heinz body count, and eccentrocyte count. Heinz bodies were stained by incubating one part blood with two parts methyl violet stain (1% methyl violet in 0.85% NaCl) for 15 minutes prior to blood film preparation. The Heinz body count was determined as the percentage of cells that had Heinz bodies by examination of 500 erythrocytes. The eccentrocyte count was determined by examining 500 erythrocytes on each Wright-Giemsa-stained blood film and reported as the percent of total erythrocytes that appear as eccentrocytes.[7] Adjusted reticulocyte counts were calculated by multiplying the percentage of reticulocytes counted by the measured packed cell volume divided by 45 (mean normal packed cell volume for dogs). The turbidity index was determined by measuring the difference in absorbance of cyanomethemoglobin solutions (1 to 501 dilution of blood) at 540 nm in 1-cm-pathway cuvettes before and after centrifugation at 1000 g for three minutes, and multiplying the difference times 1000 divided by the packed

cell volume (%).[8] Blood methemoglobin content was determined on all samples up to and including the day 3 sample and expressed as percentage of total hemoglobin.[9]

Statistical analyses of results were done utilizing the Student's *t*-test for comparisons of test group and control group means and the analysis of variance and least significant difference tests for comparisons within a group. All differences mentioned in the text are significant ($p < 0.05$) unless otherwise indicated.

Results

Initial packed cell volume ranged form 39 to 55% in test dogs. The change in packed cell volume relative to initial 0 time values is given (fig. 1). Packed cell volumes began to decrease on day 1, with the lowest mean value reached on day 5. Packed cell volumes had not increased significantly by day 9. The magnitude of decreases in packed cell volume varied considerably. Three dogs had maximal decreases of 25 to 30 units, while the remaining 3 dogs had maximal decreases of only 12 to 13 units. The packed cell volume in control dogs was unchanged during the experimental period.

Intravascular hemolysis, observed as red plasma in centrifuged microhematocrit tubes, was present on days 1 and 2, in two of the most severely affected dogs. Although urine was not collected for evaluation in this study, prominent hemoglobinuria was observed in one of these dogs on day 2.

Significant Heinz body formation was observed on day 1, with maximal percentages seen on day 3 (fig. 1). No Heinz bodies were observed in control animals. Heinz bodies varied in appearance from a single large one to several small ones per erythrocyte. Heinz body

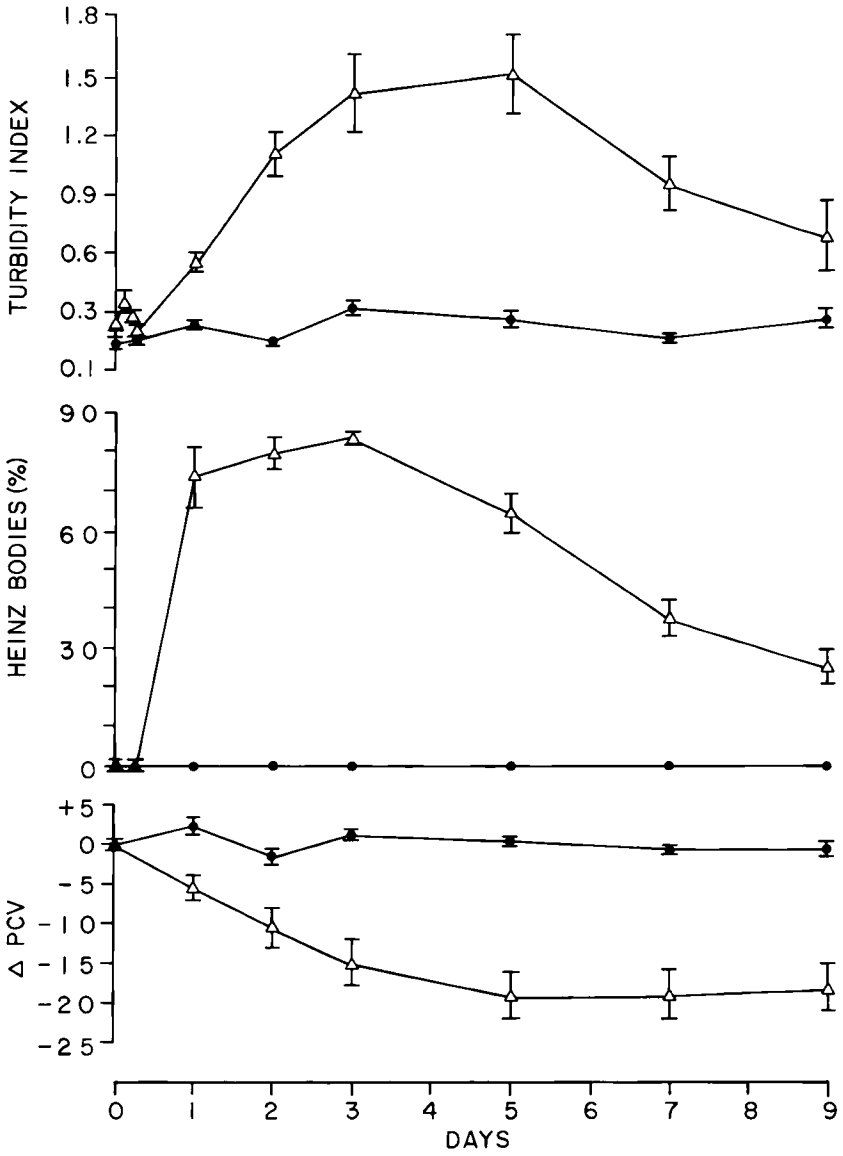

Fig. 1: Mean (± standard error) values for turbidity index, percentage of erythrocytes that contained Heinz bodies, and changes in packed cell volume (Δ PCV) from test dogs (open triangles, n = 6) fed ground beef that contained dehydrated onions and control dogs (closed circles, n = 3) fed only ground beef at time 0. Changes in packed cell volume are given as percentage points above (+) or below (−) the prefeeding (time 0) packed cell volume value.

numbers decreased after day 3, but were still present on the last day examined (day 9).

A significant increase in turbidity index was observed on day 1 (fig. 1). The turbidity index reached a maximum on day 5 and then declined. The mean turbidity index in control dogs remained low throughout the experimental period.

Various degrees of poikilocytosis was observed on routinely stained blood films after onion consumption. In addition to nipple-like projections, that resulted from membrane-bound Heinz bodies, erythrocytes were frequently observed to have hemoglobin that appeared dense and contracted to one side of the cell and left a pale area (fig. 2). These cells have previously been called eccentrocytes in dogs.[7] The percentage of erythrocytes classified as eccentrocytes increased significantly, being maximal one to two days after onion consumption (fig. 3). Considerable individual variability was observed, with maximal eccentrocyte counts that ranged from 3 to 20%. All dogs had eccentrocytes on days 1 through 7, but two dogs lacked eccentrocytes by day 9. Eccentrocytes were not present in control dogs.

Adjusted reticulocyte counts in dogs fed onions were significantly increased above 0 time and control dog values on days 5 through 9 (fig. 3). Although not statistically significant, dogs with the greatest decreases in packed cell volume tended to have lower reticulocyte responses than did mildly affected dogs.

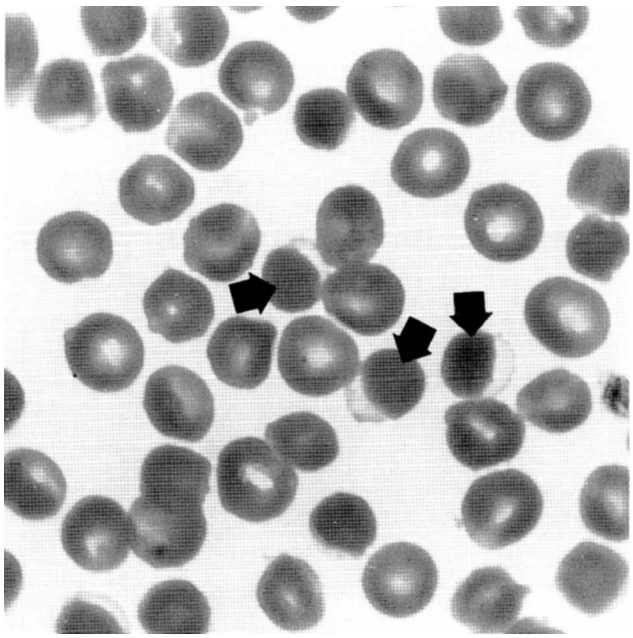

Fig. 2: Eccentrocytes (arrows) in blood film from a dog two days after feeding ground beef that contained dehydrated onions. Wright-Giemsa.

Methemoglobin content in erythrocytes increased slightly (but significantly) from 0.10 ± 0.04% (± SE) at 0 time to a maximum of 1.9 ± 0.3% (not shown) four hours after onions were fed. Methemoglobin content was no longer significantly increased by day 2. Methemoglobin content in control dogs remained low (mean daily values between 0.3% and 0.7%) during the examination period.

Although a substantial decrease in reduced glutathione from 2.9 μmoles/ml erythrocytes to 1.2 μmoles/ml erythrocytes was observed in the most severely affected dog after onion feeding, most test dogs had no decrease in reduced glutathione content. Consequently, no significant differences in erythrocyte reduced glutathione content were found following oral onion administration.

Mean absolute neutrophil numbers increased significantly from 9,890 ± 1,050/μl (± SE) at 0 time to a maximum of 19,840 ± 3,820/μl on day 7 (not shown). This maximal value was also higher than the control group on day 7 (9,540 ± 1,440/μl), but the difference was not statistically significant. Two of the six onion-treated dogs had moderate left shifts associated with their neutrophilias. No significant changes were observed in absolute lymphocyte, monocyte, or eosinophil counts. Low numbers of nucleated erythrocytes (generally less than 6/100 leukocytes) were consistently observed on blood films from test dogs on days 3 through 9. Nucleated erythrocytes were not present in control dog blood samples.

Discussion

Onion ingestion causes oxidative erythrocyte injury as evidenced by the production of Heinz bodies. Heinz bodies are erythrocyte inclusions that form as a consequence of irreversible oxidative denaturation of hemoglobin.[13]

In the present study, the denaturation and precipitation of hemoglobin was measured by determining a turbidity index as well as Heinz body count. Following a single oral dose of desiccated onions, Heinz body numbers reached near-maximal values more rapidly than did turbidity indexes. In a study of oxidant-induced Heinz body formation in cats,[8] Heinz bodies were observed to increase in size for three days after oxidant administration. It is likely that the increase in turbidity index during the first four days in the present study represents a continued aggregation and precipitation of denatured hemoglobin. Changes in Heinz body size could not be accurately measured in these

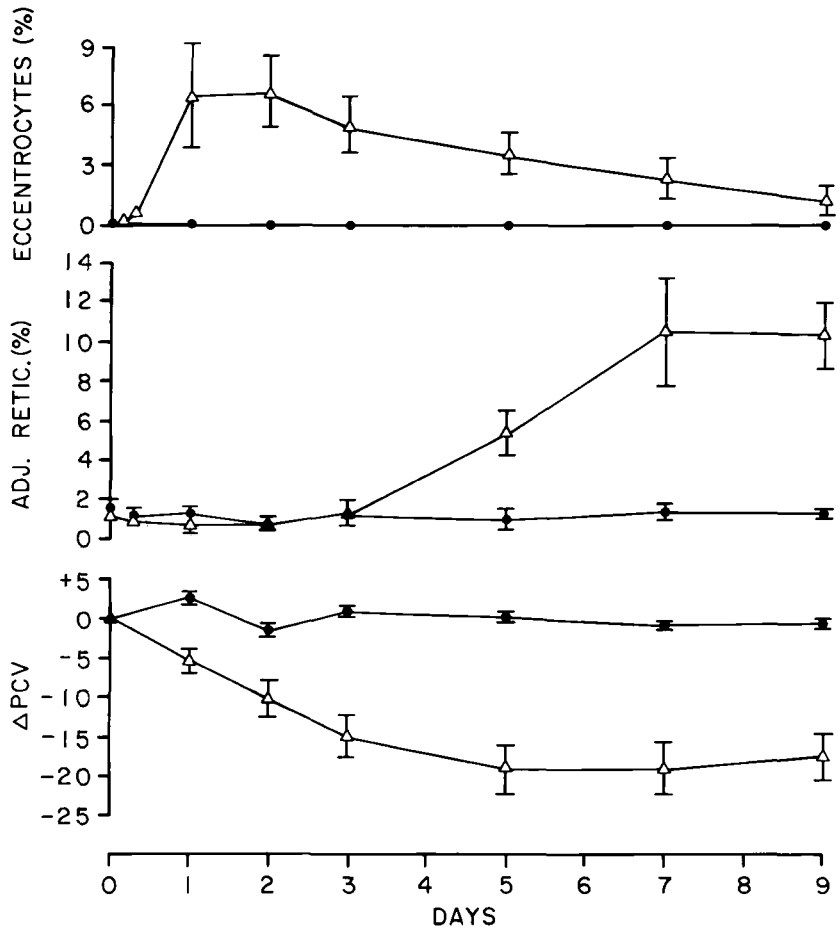

Fig. 3: Mean (± standard error) values for percentage of eccentrocytes, percentage of adjusted reticulocytes (adj. retic.) and percentage points change in packed cell volume (Δ PCV) from test dogs (open triangles, n = 6) fed ground beef that contained dehydrated onions and control dogs (closed circles, n = 3) fed only ground beef at time 0.

dogs, due to the frequent occurrence of multiple, rather than single, Heinz bodies within erythrocytes.

In addition to Heinz body formation, other potential forms of oxidant-induced damage to erythrocytes include oxidation of membrane lipid and sulfhydryl groups and methemoglobin formation. Eccentrocytes are believed to form as a result of direct oxidative injury to the erythrocyte membranes.[7] They have previously been reported to occur in dogs after the subcutaneous injection of acetylphenylhydrazine.[7] Eccentrocytes are erythrocytes with hemoglobin that appears dense and contracted to one side of the cell and leaves a pale area that still contains a small amount of hemoglobin. Similar abnormal erythrocytes, recognized in people given a variety of oxidant drugs,[7] have also been described as pyknocytes[2] and erythrocyte hemighosts.[1] Eccentrocytes are not spherocytes, although they were mistakenly classified as such in a case of methylene blue-induced hemolytic anemia in a dog.[17] The observation of eccentrocytes on blood films from clinical cases should prompt a search for Heinz bodies if they have not already been recognized.

Eccentrocytes are more rigid and less deformable than normal erythrocytes. In experimental studies of acetylphenylhydrazine-induced hemolytic anemia in dogs, erythrocyte destruction was more clearly related to eccentrocyte formation than to Heinz body formation.[7]

Most drugs that induce Heinz body formation also induce methemoglobin formation.[13] Although increased methemoglobin content was measured in erythrocytes following onion administration, the increase was small and of no clinical significance.

Reduced glutathione is a sulfhydryl-containing tripeptide that is considered to be important in the protection of erythrocytes against oxidative injury. It functions as a substrate for both the glutathione peroxidase enzyme and for an additional uncharacterized protein(s) that protects against lipid peroxidation.[4] Although a dramatic decrease in erythrocyte-reduced glutathione was measured in the most severely affected dog, no change was observed in most other dogs given onions. Erythrocytes can reduce oxidized glutathione, and some oxidant drugs actually increase glutathione

synthesis in erythrocytes[14]; consequently, glutathione concentration may be normal or even increased in association with oxidant stress.

Allyl propyl disulfide has been considered to be the main constituent in onion oil responsible for the oxidative damage to erythrocytes.[5] This compound has not been studied experimentally, but a closely related compound, di-n-propyl disulfide, has been shown to have a hemolytic effect in dogs similar to onion administration.[5,24] Onions, garlic and *Brassica* spp are also reported to contain a rare amino acid S-methylcysteine sulphoxide.[20] This compound is relatively inactive as an oxidant, but can be converted to the oxidant dimethyl disulfide by rumen microorganisms in ruminant animals.[20] It is not known whether this amino acid can be metabolized to a substance with oxidative potential in dogs.

Dogs appear to vary considerably in susceptibility to orally administered onions. Three dogs had dramatic decreases in packed cell volume and three were mildly affected. Anemia developed gradually over several days. Consequently, in the absence of substantial methemoglobinemia, clinical signs of onion toxicity may not be recognized until a number of days after onion consumption. Delayed hemolysis after oxidant drug exposure has previously been recognized in animals and man.[8] The only early clinical sign in the present study was the occurrence of diarrhea for one to two days after oral onion administration.

The reticulocyte response to the anemias in this study was dramatic. The peak response occurred several days after substantial decreases in packed cell volume had occurred. This delayed response is expected inasmuch as several days are required for increased reticulocyte production to occur in bone marrow.[18] Based on the decreasing Heinz body counts and increased reticulocyte counts, the plateau of packed cell volume between days 5 and 9 probably results from new erythrocyte production matched with continued destruction of oxidant injured cells.

The only significant leukocyte change was a neutrophilia. Mean neutrophil numbers were highest during days when mean reticulocyte counts were highest. Neutrophilias with left shifts have been reported in two clinical cases of onion-induced hemolytic anemia in dogs.[3,21]

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