

Response of healthy dogs to infusions of human serum albumin

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Objective—To evaluate the clinical and immunologic response in healthy dogs to infusions of human serum albumin (HSA).

Animals—9 healthy purpose-bred mixed-breed dogs.

Procedures—Each dog was administered a 25% HSA solution once or twice. Various physical examination and laboratory variables were serially evaluated. Antibody against HSA was assayed before and after infusion by use of an ELISA. Intradermal testing was also conducted. A repeated-measures ANOVA or Friedman repeated-measures ANOVA on ranks was used to compare results for the variables.

Results—Adverse clinical reactions were observed after the first or second infusion in 3 dogs. Anaphylactoid reactions were observed in 1 of 9 dogs during the first infusion and in 2 of 2 dogs administered a second infusion. Two dogs developed severe edema and urticaria 6 or 7 days after an initial infusion. All dogs developed anti-HSA antibodies. Positive responses for ID tests were observed in 8 of 9 dogs. Short-term increases were detected in blood protein, total bilirubin, and calcium concentrations after HSA infusion. Serum cholesterol concentrations and platelet counts decreased after HSA infusion.

Conclusions and Clinical Relevance—Administration of HSA resulted in profound reactions in 2 of 9 dogs administered a single infusion and in 2 of 2 dogs administered a second infusion. This indicates that there is risk of life-threatening adverse reactions to HSA infusion in healthy dogs. (*Am J Vet Res* 2007;68:657–663)

Hypoalbuminemia is a common complication of a number of disease processes in dogs,¹ and it is associated with a worsened prognosis for recovery from several diseases.^{2–5} Although treatment of underlying disease is crucial to the treatment of hypoalbuminemia, it is not always possible to immediately identify or eliminate the causes of low albumin concentrations.

Synthetic colloids, such as hydroxyethylstarch, can improve intravascular COP, but they cannot provide the multiple other vital functions of albumin.^{6,7} Therefore, albumin is often administered for supportive care. Canine plasma typically contains 25 to 30 g of albumin/L.⁸ Thus, to adequately replete albumin in a severely hypoalbuminemic dog, large volumes of canine plasma would be required. This poses problems related to cost, availability, and the potential for volume overload. Currently, there are no commercially available concentrated canine albumin products. However, pharmaceutical-

ABBREVIATIONS	
COP	Colloid osmotic pressure
HSA	Human serum albumin
BSA	Bovine serum albumin
OD	Optical density

grade concentrated HSA is commercially available, and veterinarians sometimes administer concentrated HSA to critically ill dogs.^{8,a,b}

We are aware of only retrospective and anecdotal reports^{8,a,b} on the safety of HSA infusion in ill dogs. Although albumin molecules are somewhat conserved, there are distinct differences among albumin molecules found in various species.^c Because HSA is a xenoprotein for dogs (ie, a protein foreign to the species), it is reasonable to assume that it can elicit an immunologic response that may have adverse consequences after initial or repeated exposure. In fact, mild to marked type III hypersensitivity reactions have been described after infusions of BSA in dogs.^d We hypothesized that infusion of a commercial pharmaceutical-grade HSA product to healthy dogs would lead to development of specific antibodies and that clinically relevant adverse reactions would be observed after repeat albumin infusion.

Materials and Methods

Animals—Nine healthy sexually intact purpose-bred adult mixed-breed dogs (6 females and 3 males) with serum albumin concentrations within the reference range were enrolled in the study. Dogs were ob-

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tained from several sources. They were of similar size (mean weight, 20.3 kg; range, 18.5 to 25.8 kg) and judged to be in good health on the basis of results of physical examination and a preenrollment CBC, serum biochemical analysis, and urinalysis. Dogs were housed in a routine manner in animal facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were reviewed and approved by the University of Missouri Animal Care and Use Committee.

Experimental design—Dogs were administered 50 g (200 mL) of a pharmaceutical-grade 25% HSA solution.^e Infusions were administered IV via a catheter inserted in a cephalic vein. Attitude, rectal temperature, pulse rate and quality, respiration rate, and systolic blood pressure were monitored every 15 minutes for the first hour and then at intervals of 30 minutes until completion of infusion. Initial infusion rate was 0.5 mL/kg/h, and it was increased incrementally to a maximum of 4 mL/kg/h. Typically, the rate was increased from 0.5 to 1 mL/kg/h after the evaluation at 15 minutes, with subsequent increases of 1 mL/kg/h after subsequent evaluations. Infusion was stopped when systolic blood pressure exceeded 180 mm Hg or adverse reactions (assessed subjectively) were detected. When hypertension, tachycardia, or tachypnea developed, infusion was temporarily halted and reinstituted when these variables returned to the respective reference range. When severe adverse reactions were detected (eg, collapse or hypotension), infusion was discontinued and the dog treated by IV administration of physiologic saline (0.9% NaCl) solution at an initial rate of 90 mL/kg/h (which was subsequently adjusted on the basis of clinical response) and diphenhydramine (2 mg/kg, IV).

Originally, we intended to perform identical procedures on all dogs 5 weeks after the initial HSA infusion. Because of serious adverse reactions observed in the first 2 dogs administered a repeat infusion, we opted to abandon the second infusion for the remaining dogs.

Collection of data—Immediately prior to infusion of HSA, a serum sample was obtained for antibody assay and dogs were evaluated by use of physical examination (including systolic blood pressure measurement and gait evaluation), CBC, and serum biochemical analysis and measurement of plasma COP and urine albumin concentration. Physical examination and subjective gait evaluation were conducted on each dog by 1 of 2 investigators (LAC or MEK). Systolic blood pressure was measured by use of a Doppler technique. Serum biochemical analysis, CBC, and urinalysis were conducted in a routine manner by personnel at the Veterinary Medical Diagnostic Laboratory of the University of Missouri. Urine was assayed for albumin content by use of a commercial test kit.^f Plasma COP was measured by use of standard techniques.^{9,g}

Vital signs (ie, rectal temperature, pulse rate and quality, respiratory rate, and blood pressure) and attitude were monitored frequently throughout infusion and at 1, 2, 4, and 8 hours after completion of HSA infusion. Physical examination, including blood pressure measurement and gait evaluation; CBC; and assay of urine albumin concentration were repeated 24, 48, and

72 hours and 7, 14, and 21 days after infusion. Serum biochemical analysis was repeated 24 hours and 7 days after infusion, and measurement of plasma COP was repeated 24 hours after infusion. Serum was obtained for antibody assay 7, 14, and 21 days after infusion. Intradermal testing was conducted on each dog at least 5 weeks after the final administration of HSA.

Antibody determination—Antibodies against HSA were measured in serum samples by use of an indirect ELISA. Two hundred microliters of HSA diluted to a concentration of 4 µg/mL in coating buffer (0.1M NaHCO₃ [pH, 9.6]) or coating buffer alone was added to wells of 96-well plates. The HSA was allowed to bind (incubation at 4°C for 48 hours). Wells were emptied, and 300 µL of blocking buffer (PBS solution with 0.5% non-fat dry milk and 0.05% Tween 20) was then added to each well. Plates were incubated at 20°C for 30 minutes. Serum that had been stored at -80°C was diluted 1:50 in fresh blocking buffer, and 200 µL of this solution was added to HSA-coated and uncoated wells. After incubation at 37°C for 1 hour, plates were washed 3 times by use of PBS solution with 0.05% Tween 20. Then, 200 µL of alkaline phosphatase-labeled secondary antibody^h was added to each well, and plates were incubated at 37°C for 1 hour. Plates were washed 5 times by use of PBS solution with 0.05% Tween 20, and 100 µL of substrate solution (1 mg of phosphatase substrateⁱ/mL of substrate buffer [2mM MgCl₂, 27.5mM NaCO₃, and 22.5mM NaHCO₃]) was added to each well. Plates were then incubated in the dark at 20°C for 1 hour.

The OD of each well was measured at 405 nm, adjusted at 490 nm, and used to calculate the relative concentration of anti-HSA antibody in each serum sample by subtracting the OD of the HSA-coated well from the OD of the uncoated well. Wells coated with HSA but not exposed to serum served as blanks. Serum collected from a dog that had previously received an infusion of HSA and had a strong reaction to HSA for ID tests was used as a positive control sample. Each sample was assayed in duplicate.

ID testing—Intradermal testing was conducted at least 5 weeks after completion of the final HSA infusion (range, 5 to 10 weeks; mean, 6.1 weeks). Physiologic saline solution was used as a negative control injection, whereas histamine^j diluted in PBS solution to achieve a concentration of 5 µg/mL was used as the positive control injection. A solution was made that comprised 0.1 mL of 25% HSA solution and 0.4 mL of physiologic saline solution. An area over the lateral portion of the thorax was clipped of hair, and 0.05 mL of each solution was administered by ID injection. Wheal reactions were graded 15 and 30 minutes after ID injection by use of a scale of 0 (no response) to 4+ (maximum response), where response to the positive control injection (ie, histamine) was equivalent to a 4+ reaction.

Statistical analysis—Only data from dogs that received an entire HSA infusion were included in statistical analysis. Therefore, data from 1 dog were excluded from statistical analysis for the first infusion, and data from both dogs were excluded from analysis for the second infusion. Summary statistics were re-

ported as mean $\pm$ SEM. Parametric (repeated-measures ANOVA) or nonparametric (Friedman repeated-measures ANOVA on ranks) tests were used to compare clinical and clinicopathologic variables, depending on the normality of distribution of the data. To determine specific differences among time points, post hoc testing was conducted by use of Holms-Sidak (parametric) or Tukey (nonparametric) tests. Plasma COP measured before the start of infusion and 24 hours after infusion were compared by use of a paired *t* test. Significance (values of $P < 0.05$) was determined by use of statistical analysis software.^k

Serum antibody concentrations, results of ID tests, urine albumin concentrations, and nonquantifiable changes during physical examination were reported descriptively.

Results

Clinical reactions—Adverse clinical reactions were observed after initial and second albumin infusions. Eight of 9 dogs were administered the complete infusion volume for the first infusion, whereas infusion was discontinued after administration of only a small amount of HSA in the other dog. Only 2 dogs were administered a second infusion of HSA because of the severity of adverse reactions that developed during that infusion, and neither of those 2 dogs were administered more than a miniscule amount of HSA during the second infusion. When we were able to complete the HSA infusions, mean time to completion was 3.75 hours (range, 3 to 4.5 hours).

The initial infusion was temporarily discontinued in 2 dogs because of hypertension. Infusion was reinstated within 20 minutes in both dogs, and infusion

was completed at a slightly slower final rate (3 mL/kg/h) in both dogs without further incident.

An additional dog developed a shock-like reaction within 10 minutes after receiving an estimated infusion volume of < 1.5 mL of HSA during the initial infusion. That dog became profoundly weak and collapsed; the dog had poor responses to stimuli and pale, muddy-colored mucous membranes. The dog became hypotensive (systolic blood pressure, 80 mm Hg) and tachypneic (respiratory rate, 60 breaths/min). Pulses were weak, but pulse rate remained within the reference range. The dog was treated immediately by IV administration of physiologic saline solution and diphenhydramine (2 mg/kg, IV). Rectal temperature decreased to a low of 37.3°C 30 minutes after the infusion was stopped. During the first 45 minutes of treatment, systolic blood pressure returned to the reference range. Although the dog vomited twice and had diarrhea once during the next several hours, it continued to improve clinically. Vital signs were in the respective reference ranges within 3 hours, and the dog was able to stand and walk. The dog appeared completely normal by the following morning. Because this dog did not complete the initial albumin infusion, results from hematologic and biochemical evaluations after infusion were not included in the statistical analysis. Although the attitude of the dog remained unaffected, the dog developed severe facial edema and urticaria 6 days after the shock-like episode. At that time, the dog was treated with diphenhydramine again, and clinical signs resolved during the subsequent 2 to 3 days.

In a second dog, we were able to complete the initial infusion without incident, but this dog also developed edema and urticaria 7 days after the initial HSA infusion. The dog remained alert and active and had

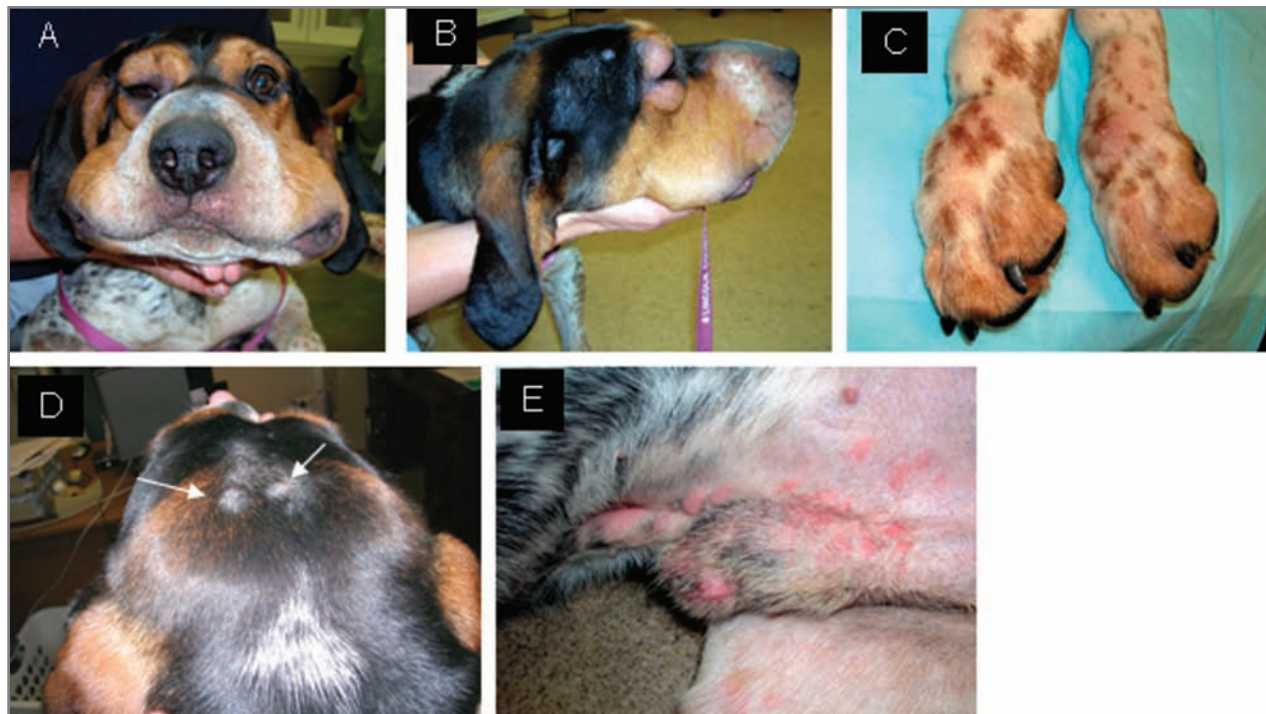


Figure 1—Photographs of a dog 7 days after IV infusion of 50 g of pharmaceutical-grade HSA. Notice the severe edema (A, B, and C) and urticaria (D and E). The dog also was pruritic. In panel D, notice the large urticarial wheals (arrows) on the top of the dog's head.

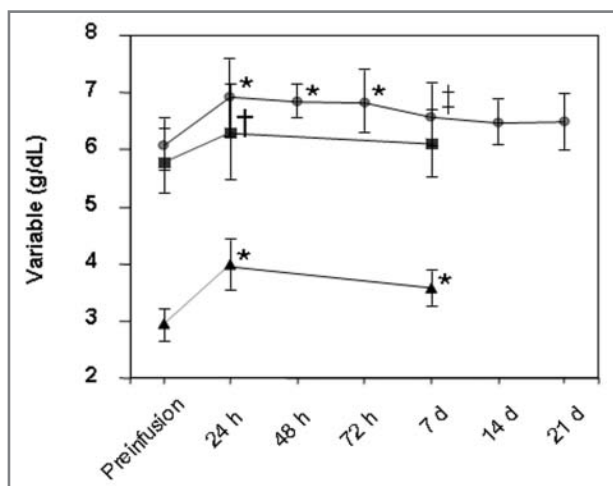


Figure 2—Mean $\pm$ SEM plasma concentrations of total solids (squares) and serum concentrations of total proteins (circles) and albumin (triangles) in 8 dogs before (preinfusion) and at various time points after IV infusion of 50 g of pharmaceutical-grade HSA. *†‡Within a variable, value differs significantly from the preinfusion value (* $P < 0.001$; † $P = 0.006$; ‡ $P = 0.026$).

an apparently normal appetite. Vital signs were within the respective reference ranges. However, the dog developed marked edema in the facial area and distal portions of the limbs. The dog also developed urticaria and was pruritic (Figure 1). The dog was treated initially by IM administration of diphenhydramine (2.16 mg/kg), which was followed by oral administration of diphenhydramine every 12 hours for 3 doses. Edema, urticaria, and the pruritic condition improved during the subsequent 3 days.

Neither of the dogs administered a second infusion of HSA had an adverse reaction to the first infusion (during the infusion or the 5-week period thereafter). However, both had an anaphylactic response during the second infusion nearly identical to that seen in the aforementioned dog. In both dogs, the adverse reaction developed soon after the second HSA infusion was initiated (total infused volume was < 1.5 mL). Both dogs became weak; collapsed; had a poor response to stimuli; and had pale, muddy-colored mucous membranes. Each dog also became severely hypotensive (systolic blood pressure could not be immediately registered in 1 dog and was 40 mm Hg in the other dog), hypothermic, and tachypneic. Pulses were weak in both dogs, but pulse rate remained within the reference range. Both dogs responded well to treatment with IV administration of physiologic saline solution and diphenhydramine. Both dogs vomited and had diarrhea during the subsequent few hours, but vital signs returned to within the respective reference ranges. Both dogs appeared clinically normal again within 1 day, and neither developed facial edema or urticaria at any point after infusion. Because of the severity of the adverse reaction observed in both the dogs administered a second HSA infusion, it was decided that we should not administer a second infusion to the remaining dogs.

Except for the 3 aforementioned anaphylactic episodes and 2 episodes of urticaria and edema, important changes were not detected during physical examination. Although there were day-to-day variations, there

was no significant alteration in systolic blood pressure, rectal temperature, pulse rate, or respiratory rate in the days after HSA infusion. None of the dogs developed a change in gait during the course of the study. In 1 dog, a grade II/VI systolic left basilar murmur was ausculted only after HSA infusion. This murmur persisted and was determined via echocardiography to have been caused by mild pulmonic stenosis; we believe the murmur was not detected by investigators during the preinfusion examination.

Clinicopathologic evaluation—Significant clinicopathologic changes were identified after the initial infusion of HSA. Mean $\pm$ SEM platelet count was decreased significantly ($P = 0.005$) from the preinfusion value ($331 \times 10^3 \pm 94 \times 10^3$ platelets/ μ L) at 7 days after infusion ($252 \times 10^3 \pm 101 \times 10^3$ platelets/ μ L). Plasma concentration of total solids was increased at all time points from 24 hours after infusion through 7 days after infusion (Figure 2). There were no significant alterations in hemoglobin concentration, total number of WBCs, or neutrophil counts. Serum albumin concentration was increased significantly from the preinfusion value (2.95 ± 0.28 g/L) at 24 hours (4.00 ± 0.44 g/L; $P = 0.009$) and 7 days (3.58 ± 0.31 g/L; $P = 0.01$) after infusion. Serum total protein concentration was increased significantly ($P = 0.017$) at 24 hours after infusion (before infusion, 5.80 ± 0.54 g/L; 24 hours, 6.30 ± 0.85 g/L). Total bilirubin concentration was increased significantly from the preinfusion value (0.20 ± 0.08 mg/dL) at 24 hours (0.65 ± 0.30 mg/dL; $P < 0.001$) and 7 days (0.46 ± 0.07 mg/dL; $P = 0.004$) after infusion. Serum calcium concentration was significantly ($P < 0.001$) increased from the preinfusion value (9.80 ± 0.55 mg/dL) at 24 hours after infusion (10.43 ± 0.38 mg/dL). Serum globulin concentration was significantly ($P = 0.008$) decreased at 24 hours after infusion (before infusion, 2.83 ± 0.58 g/L; 24 hours, 2.30 ± 0.69 g/L). Serum cholesterol concentration was significantly ($P < 0.001$) decreased from the preinfusion value (129.2 ± 20.6 mg/dL) at 24 hours (110.0 ± 18.9 mg/dL) and 7 days (100.2 ± 23.2 mg/dL) after infusion. No significant alterations were identified in serum urea nitrogen, creatinine, or glucose concentrations; amylase, alkaline phosphatase, or alanine transaminase activities; total carbon dioxide content; or sodium, potassium, chloride, or phosphorous concentrations. Plasma COP (reference range, 18 to 24 mm Hg) was significantly ($P < 0.001$) increased from the preinfusion value (21.9 ± 2.1 mm Hg) at 24 hours after infusion (29.8 ± 2.1 mm Hg).

Microalbuminuria was detected in only 1 dog, and it was evident before and after HSA infusion. In that dog, the semiquantitative assessment yielded a low positive result before infusion and consistently yielded a medium positive result after infusion.

Immunologic response—Serum antibodies against HSA were not detected in any dog before the initial HSA infusion, but those antibodies developed after infusion. In 1 dog, antibodies could not be assayed because of high nonspecific binding; data for that dog were excluded from further analysis. That dog did not have an adverse reaction to HSA infusion. For the remaining dogs, increased OD values for the ELISA were detected

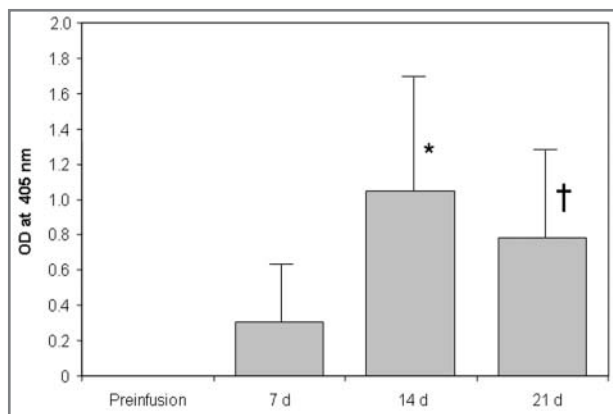


Figure 3—Mean $\pm$ SEM anti-HSA antibody response of 7 dogs before and at various time points after IV infusion of 50 g of pharmaceutical-grade HSA. *†Within a variable, value differs significantly from the preinfusion value (* $P < 0.001$; † $P = 0.005$).

at 7 days after infusion; the OD values were significantly higher at 14 and 21 days after infusion (Figure 3).

Results of ID testing revealed a positive response suggestive of specific anti-HSA IgE production in 8 of 9 dogs. In 3 dogs, response to ID injection of dilute HSA was graded as 4+, with the evaluated wheal and flare actually exceeding the size of that for the histamine control injection. One of these 3 dogs developed the anaphylactic reaction during the initial HSA infusion. In 3 dogs, the response was graded as 2+, whereas the response was graded as 1+ and 3+ in 1 dog each.

Discussion

The hypothesis of the study reported here was that dogs would develop anti-HSA antibodies after infusion of pharmaceutical-grade HSA and that a delayed second exposure would result in antibody-mediated adverse clinical reactions. We were able to verify the development of anti-HSA antibodies in all dogs and an adverse clinical response in both dogs administered a second infusion. In addition, some dogs had an immediate anaphylactoid reaction and delayed adverse reaction to the initial infusion.

Our findings in healthy dogs administered HSA are extremely similar to those described for healthy dogs infused with another xenoalbumin, BSA.⁴ In that study, the investigators administered BSA to 9 dogs that did not have prior exposure to exogenous albumin, and 1 of the dogs immediately developed an urticarial reaction. Two of the dogs were administered a second infusion of BSA 14 days later; 1 of these 2 immediately developed an urticarial reaction, whereas the other dog developed an anaphylactoid reaction. Additionally, 5 dogs administered only a single infusion of BSA in that study developed generalized type III hypersensitivity reactions a mean $\pm$ SD of 15 ± 2.7 days after infusion.

In contrast to our study in which there was a severe adverse reaction to xenoalbumin administration (ie, HSA) in healthy dogs, there are numerous published reports^{2,8,a,b} that suggest that HSA can be used without serious adverse consequences in critically ill dogs. In a retrospective report,⁸ HSA was administered to 64 dogs and 2 cats in a critical care setting. Although 19 of these

animals did not survive until discharge and 5 others died within 18 hours after infusion, the authors attributed these deaths to progression of underlying disease or euthanasia, rather than to an adverse reaction to HSA administration.

It seems unlikely that the total dose of HSA administered or the infusion rate explain the adverse reactions seen in the dogs reported here, compared with the effects in clinically affected dogs described in another report.⁸ In that report, the total dose administered varied among patients but ranged from 1.8 to 10.8 mL of 25% HSA/kg (mean, 5.3 mL/kg). All dogs that received a complete infusion in our study received 50 g (200 mL) of 25% HSA. On the basis of the body weights of the dogs in our study, the dose ranged from 7.7 to 10.8 mL/kg (mean, 9.3 mL/kg). Investigators in that other study⁸ described a maximum infusion rate of 4 mL/kg/h for the treatment of hypotensive dogs and constant infusion rates of 0.1 to 1.7 mL/kg/h for other uses. The dogs of the study reported here were administered HSA at an initial rate of 0.5 mL/kg/h with incremental increases to a final rate of 4 mL/kg/h, provided that vital signs remained stable. Because the most severe adverse reactions detected in the dogs of our study were at the slowest delivery rate and after infusion of only miniscule volumes of HSA, it seems unlikely that infusion rate or dose administered could have accounted for these reactions. However, it is likely that our relatively rapid rate of infusion contributed to the 2 episodes of hypertension during infusion in these normovolemic dogs. This was supported by the rapid resolution of hypertension when the HSA infusion was discontinued temporarily and reinstituted at a slower rate.

It also seems unlikely that the specific preparation of HSA used in the study reported here accounted for the adverse events. All commercially available pharmaceutical-grade HSA products are derived from the same basic procedure, Cohn cold ethanol fractionation. The product^c used in our study contained no preservatives, and the stabilizers used in the product were identical to those used in most other products,^{1,m} including the productⁿ used in a retrospective clinical series.⁸ Adverse reactions in our study resulted from infusion of HSA with various lot numbers. When contacted, the manufacturer reported no known adverse reactions in humans administered product from the lots used in our study.

Adverse reactions to albumin infusion could result from nonimmunologic or immunologic mechanisms. True anaphylaxis is a type I hypersensitivity response requiring prior sensitization to the triggering antigen. Clinically identical reactions that are not antibody-mediated events are termed anaphylactoid reactions. Both anaphylactic and anaphylactoid reactions are detected more often with parenteral routes of administration, especially IV injection.¹⁰ Proteins are excellent antigens; therefore, protein-based therapeutics are especially likely to result in anaphylaxis.¹¹ None of these laboratory-raised dogs had prior exposure to HSA, and none had any detectable serum anti-HSA antibodies prior to the initial HSA infusion.

We did not specifically measure anti-HSA IgE concentrations, and serum IgE concentrations are typically low because most of this immunoglobulin is bound to

receptors on mast cells and basophils. Retrospectively, ID testing completed before HSA infusion could have been used to detect background amounts of IgE. Unfortunately, ID tests were not completed until weeks after the initial infusions. By that time, ID test response was positive but serum antibodies (non-class-specific antibodies) had also been identified.

Antibody-mediated anaphylaxis seems less likely than an anaphylactoid response in the dog with only a single exposure to HSA, but antibody-mediated or non-antibody-mediated reactions could explain the severe clinical reactions in both dogs administered a second albumin infusion. In 1 study,¹² investigators postulated that severe adverse reactions in 2 healthy dogs administered HSA were anaphylactoid responses to serum protein aggregates, rather than true anaphylaxis, because neither dog had prior exposure to HSA. In the study reported here, anaphylaxis or an anaphylactoid reaction could not account for the onset of urticaria and edema in 2 dogs 7 days after HSA infusion.

Type III hypersensitivity reactions may also be responsible for adverse reaction to drugs, particularly xenoproteins. Serum sickness is a type III hypersensitivity response in which soluble circulating immune complexes diffuse into vessel walls where they affix and activate complement.¹³ Because antibody formation is not an immediate event, serum sickness is evident 6 to 21 days after exposure to the protein antigen. Deposition of immune complexes and the resulting inflammatory response causes a widespread vasculitis (ie, serum sickness). After subsequent exposures to the inciting antigen, the vasculitis may develop earlier and may be much more profound. Classic physical manifestations of serum sickness include fever, arthralgia, lymphadenopathy, edema, and skin lesions. Two of the dogs in the study reported here developed severe edema and urticarial skin lesions within a time frame compatible with serum sickness, and both dogs had detectable anti-HSA antibodies by 7 days after infusion. In the experience of other investigators,¹⁴ type III hypersensitivity responses have been identified approximately 1 to 2 weeks after administration of pharmaceutical-grade HSA to several healthy dogs. Several of the reactions have been severe, including 2 that were fatal.¹⁴

It remains unclear why adverse reactions of healthy dogs to infusion of HSA were common in the study reported here and have been detected by other investigators¹² but are uncommonly reported in veterinary retrospective reports on use of HSA in a clinical setting. In clinical use, HSA is administered to critically ill dogs. Sick dogs may be immunosuppressed as a result of the underlying disease state or drug treatment. Furthermore, dogs administered HSA are often hypoalbuminemic and may be malnourished; malnutrition impacts immune function in a number of ways.¹⁵ Suppression of immunologically mediated responses to HSA may prevent or blunt the severity of adverse reactions. It is also possible that, in some cases, deterioration of clinical condition or death in extremely sick patients may have been incorrectly attributed to underlying disease, rather than to an adverse drug reaction. This may be especially true when the reaction to infusion was delayed (eg, type III hypersensitivity),

compared with an acute reaction (eg, anaphylactic reaction).

In the study reported here, HSA was administered to normoalbuminemic dogs, which prompted the concern that adverse reactions may have been related to hyperalbuminemia. All immediate reactions developed after only minuscule amounts of albumin were administered. Delayed reactions were detected in 1 dog that received only a small amount of HSA and in an additional dog that received the complete HSA infusion. At the time of the adverse reaction 1 week after infusion, the serum albumin concentration in the latter dog (3.2 g/dL) was within the reference range (reference range, 2.9 to 4.0 g/dL), which effectively eliminated the concern that the adverse reactions were attributable to hyperalbuminemia.

Significant alterations of laboratory variables were evident after infusion of a complete 50-g dose of HSA to the dogs. Although only 2 dogs had obvious edema after infusion, decreased platelet counts 7 days after infusion may have been related to subclinical vasculitis with resultant platelet consumption. Plasma COP was significantly increased, a finding that was not surprising given that an important use of concentrated albumin is to provide colloid support.⁷ Similar to findings in the aforementioned retrospective study,⁸ serum albumin and total protein concentrations and plasma concentrations of total solids all increased after administration of concentrated HSA to the dogs of our study. We detected a decrease in serum globulin concentrations 24 hours after infusion, but this decrease was not significantly different by 7 days after infusion. Because globulin concentrations decreased quickly and were returning to preinfusion values 7 days after infusion, whereas albumin concentrations were still significantly increased at that time, we believe dilution of globulin related to increased osmotic pressure and a resultant increase in intravascular volume was a more likely explanation than diminished globulin production or loss of globulins. Globulin content was actually slightly increased 7 days after infusion in the 2 dogs that had edema and urticaria compatible with type III hypersensitivity responses.

Concentrations of calcium, which binds to albumin, were increased 24 hours after infusion, probably as a response to the increase in albumin as a carrier. Serum bilirubin also strongly binds serum albumin, and the mild increases in bilirubin concentrations in these dogs were not accompanied by increases in hepatic enzyme activity and were unlikely to be of clinical importance. Serum cholesterol concentration was decreased at 24 hours and 7 days after infusion. Although the initial decrease could have been related to a dilutional effect, the continued decrease at 7 days after infusion may also have been related to diminished cholesterol production.

In the study reported here, we detected life-threatening adverse reactions to infusion of pharmaceutical-grade HSA in healthy dogs during both initial and second administrations. Although there have been adverse reactions to HSA infusion in people, such reactions are extremely rare.^{16,17} The pharmaceutical-grade product from any manufacturer contains only a concentrate of

albumin derived from pooled human venous plasma with extremely minute amounts of stabilizing molecules. It appears likely that the adverse reactions in our study were related to the small but real difference in albumin molecules between species, rather than to some other ingredient in the product. Currently, the use of HSA in the treatment of critically ill people is controversial, with many studies and large meta-analyses failing to substantiate an improvement in survival in patients treated with HSA.^{4,7,18,19} Although HSA does provide oncotic support, it is questionable whether it provides dogs with any of the other vital functions of native canine albumin, especially given the differences in albumin among species.^{8,a,b} To our knowledge, there have been no controlled studies that provide evidence for a benefit in survival for dogs receiving HSA. Future studies should be designed in a prospective manner to determine safety of HSA administration to critically ill dogs and to try to determine whether HSA offers any benefit in survival over the use of other colloids. Unless a therapeutic benefit can be documented, other colloids may be safer alternatives to HSA for provision of oncotic support in hypoalbuminemic dogs. We believe that repeat administration of HSA infusion should be avoided altogether.

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- j. Histamine base, Center Laboratories, Port Washington, NY.
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- l. Buminate 25%, Baxter Biosciences, Deerfield, Ill.
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