

Clinical and clinicopathologic changes in cows with endotoxin-induced mastitis treated with small volumes of isotonic or hypertonic sodium chloride administered intravenously

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Summary

We characterized the clinicopathologic manifestations of experimentally induced endotoxin-induced mastitis. Responses to hypertonic fluid therapy also were assessed. Eight cows received 1 mg of endotoxin by intramammary infusion in the left forequarter. Four hours after endotoxin administration, cows received 0.9% NaCl, 5 ml/kg of body weight ($n = 4$) or 7.5% NaCl, 5 ml/kg ($n = 4$) IV. Endotoxin-infused cows had expanded plasma volume, hyponatremia, transient hyperchloremia and hypophosphatemia, increased serum glucose concentration, and decreased serum activities of liver- and muscle-specific enzymes. Calculated plasma volume increased at 6 hours in cows receiving hypertonic NaCl, and at 12, 24, and 48 hours after endotoxin infusion in both groups. Concurrent observations of decreased serum protein concentration, erythrocyte count, and hematocrit supported observations of increased plasma volume. Relative plasma volume was greater in cows receiving hypertonic NaCl (124.3%) than in cows receiving isotonic NaCl (106.6%) at 6 hours after endotoxin infusion. Cattle receiving hyper-

tonic NaCl had increased voluntary water intake after IV fluid administration. Increased water consumption was not accompanied by increased body weight, indicating probable occurrence of offsetting body water loss. Serum sodium concentration in cows receiving hypertonic NaCl was increased 2 hours after fluid administration, but the magnitude of the change was minimal (< 4 mmol/L) and transient, indicating rapid equilibration with either interstitial or intracellular spaces. Serum sodium concentration was decreased in cows receiving isotonic NaCl at 12, 24, and 48 hours after endotoxin administration, compared with concentration prior to endotoxin administration, indicating selective loss of sodium.

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Intramammary infections caused by gram-negative bacteria are noteworthy because of the acute course and high fatality rate.¹⁻⁴ Although many clinicians have observed that fluid therapy is beneficial in cases of gram-negative bacterial mastitis,^{1,3} adult cattle rarely receive IV fluid therapy because of the difficulty in delivering volumes sufficient to improve systemic circulation. Treatment is particularly problematic when cattle are treated in an on-farm setting. The volumes of fluid required, 40 to 60 L/d,^{1,5} and the time, difficulty, and expense of treatment limit use of fluid therapy in mature cattle.

Oral or enteral fluid administration is often advocated as a practical alternative to IV administration. Advantages of orally administered fluids include ease and speed of delivery, and low cost. Unfortunately, orally administered fluids have minimal benefit if the animal has poor gastrointestinal tract absorption, impaired circulation, or severe dehydration. In acute gram-negative bacterial mastitis, gastrointestinal tract

motility is impaired.^{6,7} Furthermore, decreased cardiac performance is frequently observed in experimental models of gram-negative sepsis.⁸⁻¹¹ Consequently, orally administered fluids probably have limited efficacy for treatment of peracute gram-negative bacterial mastitis.

Small volumes of hypertonic solutions can be transported easily and administered rapidly.¹² To the authors' knowledge, the effects of hypertonic fluid therapy for treatment of gram-negative bacterial mastitis have neither been studied or reported. Similar modes of treatment have been used successfully in shock models involving human beings, laboratory animals, and livestock neonates.¹³⁻¹⁷ Results of preliminary studies indicate that hypertonic solutions may be administered to cattle with endotoxin-induced mastitis without causing marked changes in CSF sodium concentration or osmolality, or clinical signs of salt poisoning.¹⁸ Hypertonic fluids have been advocated for use in cattle with acute mastitis, dehydration, heat stroke, and neonatal diarrhea^a; however, results of controlled studies have not indicated efficacy of this treatment in adult cattle. The author of a review article specifically recommended against the use of hypertonic fluids in many of the disease conditions for which use of hypertonic fluids has been advocated in the popular press.¹²

The primary goal of the study reported here was to characterize the metabolic and physiologic responses to IV administered isotonic (IS) or hypertonic NaCl (HS) solutions in an experimental model of gram-negative endotoxin-induced mastitis of dairy cattle. The second goal was to characterize the clinicopathologic changes associated with experimental endotoxin-induced mastitis. Although intramammary endotoxin infusion models frequently have been used to study the pathogenesis of acute-gram negative bacterial mastitis, published serum biochemical and physiologic characterizations of the experimentally induced disease are cursory and incomplete. Most studies have limited analyses to measurements of intramammary inflammation, hematologic changes, selected acute-phase reactant, proteins, or inflammatory mediators.^{6,7,19-31} To our knowledge, studies evaluating serial serum protein concentrations, RBC count, and plasma volume changes, measured either by dye dilution or changes in hematologic indices, have not been performed in adult cattle with endotoxin-induced or gram-negative bacterial mastitis. Improved characterization of endotoxin-induced mastitis with an emphasis on results of routine clinicopathologic tests should improve understanding of systemic manifestations of intramammary inflammation. This improved understanding will assist clinicians in interpreting clinicopathologic data, and may permit more rational design of treatment regimens.

Materials and Methods

Sample population and experimentally induced mastitis model—Study subjects ($n = 8$) were clinically normal, lactating Holstein cows in their sec-

^a Hines JA. 7% Saline IV, a new approach to an old problem (abstr) in Proceedings. Am Assoc Bovine Pract 1991;24:142.

ond or greater lactation. Each cow received 1 mg of endotoxin derived from *Escherichia coli* O111:B4 by phenol-chloroform-ether extraction^b dissolved in 5 ml of pyrogen-free isotonic sodium chloride solution by intramammary infusion in the left forequarter. This dose of endotoxin is consistent with previous studies.¹⁸⁻¹⁹ Milk samples from all challenge-exposed quarters had negative results (using a 5-point scale: negative, trace, 1, 2, and 3) of the California mastitis test and were free of bacterial pathogens at 72 and 24 hours prior to challenge exposure.

Fluid therapy—Cows were assigned at random to either IS ($n = 4$; 0.9% NaCl)^c or HS ($n = 4$; 7.5% NaCl) treatment groups. The HS was prepared by adding 23.4% NaCl^d to 5% NaCl solution.^e Fluids were administered IV via a jugular vein catheter (5 ml/kg of body weight, 200 ml/min) 4 hours after intramammary endotoxin administration. Catheters were placed 12 hours prior to intramammary infusion of endotoxin.

Hematologic and serum biochemical determinations—Blood samples were collected via the jugular vein catheter for hematologic and serum biochemical analyses immediately before challenge exposure and at T3 ($T = \text{time in hours after intramammary administration of endotoxin}$), T6, T12, T24, and T48. Specific analyses included hemoglobin concentration; hematocrit; RBC count; total and differential WBC counts; platelet count; serum activities of L-iditol dehydrogenase (ID), γ -glutamyltransferase (GGT), aspartate transaminase (AST), and creatine kinase (CK); serum concentrations of creatinine, glucose, urea N, Ca, inorganic P, total protein, albumin, Na, K, Cl, and total CO₂, and anion gap. Complete blood count was performed, using an automated cell counter.^f Differential counting of 100 leukocytes was performed on Romanowsky-stained blood smears. Serum concentrations of creatinine, glucose, urea N, inorganic P, total protein, and albumin, and activities of GGT, AST, and CK were determined, using reagent kits.^g Serum ID activity was determined by use of an adaptation of a commercial reagent kit.^h Serum biochemical analyses were performed with the aid of an automated chemistry analyzer.ⁱ The Na, K, Cl, and total CO₂ values were determined by use of an ion-specific electrode method.^j Red blood cell indices, differential leukocyte count, and serum globulin concentration (total protein concentration minus albumin concentration) were calculated. Anion gap was calculated by subtracting the sum of total CO₂ and Cl concentrations from the sum of Na and K concentrations.

^b Endotoxin derived from *Escherichia coli* O111:B4, List Biologics, Campbell, Calif.

^c 0.9% Sodium chloride injection, Baxter Healthcare Corp, Deerfield, Ill.

^d 23.4% Sodium chloride, Walker Drugs, Birmingham, Ala.

^e 5% Sodium chloride injection, Baxter Healthcare Corp, Deerfield, Ill.

^f Serono Baker Diagnostics, Allentown, Pa.

^g Roche Diagnostic Systems Inc, Montclair, NJ.

^h Sigma Chemical Co, St Louis, Mo.

ⁱ Cobas Mira, Roche Diagnostic Systems Inc, Montclair, NJ.

^j Model E4A, Beckman Instruments, Somerset, NJ.

Heart rate, blood pressure, plasma volume, and temperature—Heart rate and systolic, diastolic, and mean arterial blood pressures were measured, using a noninvasive blood pressure monitor^k placed over the coccygeal artery at the base of the tail, at T0, T3, T6, T12, T24, and T48. Rectal temperature was measured at T0, T3, T6, T12, T24, and T48. Relative changes in plasma volume were calculated from hematologic values (hematocrit and hemoglobin concentration), using accepted formulas^{9,32}:

$$\text{percentage of relative plasma volume} =$$

$$\frac{\text{Hgb}_B}{\text{Hgb}_A} \times \frac{1 - \text{Hct}_A \times 10^{-2}}{(1 - \text{Hct}_B \times 10^{-2})} \times 100$$

where Hgb_A = blood hemoglobin concentration at second sample collection; Hgb_B = blood hemoglobin concentration at first sample collection; Hct_A = blood hematocrit at second sample collection; and Hct_B = blood hematocrit at first sample collection.

Milk sample collection and measures of intramammary inflammation—Two milk samples, 1 from the left forequarter and a composite sample containing roughly equal volume from the remaining 3 quarters, were collected at T0, T3, T6, T12, T24, T48, T120, T168, T216, and T288. Milk samples were frozen within 1 hour after collection. Milk albumin concentration was measured by radial immunodiffusion. Samples with no detectable zone of precipitation were assigned a zone diameter of 2.5 mm. Sample albumin concentration was calculated, using an equation derived from assays performed with albumin standards.

Body weight and voluntary water intake—Body weight was measured at T0, T24, and T48. Ad libitum access to water was provided throughout the study, and intake volume was recorded for the following intervals; T0 to T3, T3 to T6, T6 to T9, T9 to T12, T12 to T15, and T15 to T24.

Statistical analysis—Measured dependent variables, including clinicopathologic results, heart rate, arterial blood pressures, body weight, and water intake, were compared at each sample collection interval between groups receiving IS and HS, using ANOVA for repeated measures. Mean values for each dependent variable also were compared with the T0 mean value for the same group, using ANOVA for repeated measures. Relative plasma volume at the 4 posttreatment sample collection intervals, T6, T12, T24, and T48, was compared with relative plasma volume at T0 for the same group and between groups at the 5 postchallenge-exposure sample collections (Table 1). Because observation intervals were of unequal duration, only between-group comparisons were made for interval water consumption. Calculations were performed with the aid of a statistical software package.^l Levels of significance are reported with tabular data.

^k Critikon Inc, Tampa, Fla.

^l BMDP 4V, Univariate and multivariate analysis of variance and covariance, including Repeated Measures, BMDP Statistical Software, Los Angeles, Calif.

Results

Experimental endotoxin-induced mastitis—Intramammary endotoxin infusion caused consistent and uniform clinical signs of disease. Fever (rectal temperature [RT] > 39.4°C) was present in 7 of 8 cows by T3 (mean RT, 40.6°C) after experimental challenge exposure and in all cows by T6 (mean RT, 40.9°C). Fever was not observed in any cow at either T24 (mean RT, 39.0°C) or T48 (mean RT, 39.1°C). Significant differences in RT were not observed between treatment groups. Cows had poor appetite from T3 to T12, and normal appetite was observed in all cows by T24. An attempt was not made to compare subjective measures of health between treatment groups.

Milk from composite control quarters had normal appearance throughout the study. Mean albumin concentration increased significantly ($P < 0.05$) in milk from endotoxin challenge-exposed quarters at T3 (67.15 mg/dl), T6 (45.32 mg/dl), T12 (47.30 mg/dl), and T24 (21.41 mg/dl), compared with T0 concentrations (13.29 mg/dl). Thereafter, milk albumin concentration did not differ from prechallenge-exposure concentration. Significant increases in nonchallenge-exposed composite sample milk albumin concentration were not observed. Albumin concentrations in milk from challenge-exposed quarters exceeded that in milk samples from nonchallenge-exposed quarters at T3, T6, T12, and T24 ($P < 0.05$).

Body weight and voluntary water intake—Mean body weight at T24 (IS, 565 ± 51 kg; HS, 566 ± 19 kg) and T48 (IS, 583 ± 36 kg; HS, 572 ± 22 kg) did not differ significantly from body weight at T0 (IS, 570 ± 30 kg; HS, 565 ± 18 kg) for either group. Additionally, significant change was not observed in mean body weight between groups at T0, T24, and T48. Mean water intake from T3 to T6 was significantly ($P < 0.05$) higher in HS-treated cows (48.75 ± 3.75 L) than in IS-treated (3.75 ± 3.75 L) cows (Fig 1). Water intake did not differ between groups during other observation intervals.

Circulatory responses—Systolic blood pressure was significantly ($P < 0.05$) higher in IS-treated cows (132.0 mm of Hg) than in HS-treated cows (115.3 mm of Hg) at T3; however, this difference was observed prior to treatment at T4 (Table 2). Other significant differences in heart rate, and diastolic, systolic, and mean arterial blood pressures were not observed between IS- and HS-treated cows. Heart rate was significantly ($P < 0.05$) increased for both treatment groups at T3 (IS, 82.0 beats/min; HS 82.0 beats/min), T6 (IS, 84.0 beats/min; HS, 90.8 beats/min), T12 (IS, 78.0 beats/min; HS, 84.3 beats/min), and T24 (IS, 69.8 beats/min; HS, 69.5 beats/min), compared with the T0 heart rate for the same group (IS, 65.3 beats/min; HS, 64.5 beats/min). Heart rate at T48 (IS, 72.3 beats/min; HS, 68.0 beats/min) did not differ significantly from prechallenge-exposure rate. Systolic blood pressure in HS-treated cows decreased significantly ($P < 0.05$) at T3 (115.3 mm Hg) and T48 (98.5 mm Hg), compared with T0 values for the same group (135.5 mm of Hg). Mean arterial blood pressure increased

Table 1—Mean and SEM serum protein, globulin, albumin, and fibrinogen concentration in 8 lactating cows treated IV with either 0.9% (IS, n=4) or 7.5% (HS, n=4) NaCl (5 ml/kg of body weight) 4 hours after intramammary infusion of 1 mg of *Escherichia coli* endotoxin in the left forequarter

Time	Protein (g/dl)				Globulin (g/dl)				Albumin (g/dl)				Fibrinogen (mg/dl)			
	IS		HS		IS		HS		IS		HS		IS		HS	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Prechallenge exposure	8.20	0.32	8.03	0.27	4.38	0.09	4.18	0.11	3.95	0.21	3.98	0.11	428	36	443	17
Postchallenge exposure																
3 H	7.45 ^a	0.37	7.30 ^a	0.14	4.10	0.17	3.85 ^a	0.06	3.58 ^a	0.18	3.70	0.12	420	46	433	17
6 H	7.20 ^b	0.40	6.55 ^b	0.15	3.93	0.20	3.45 ^b	0.05	3.48 ^b	0.19	3.35 ^b	0.06	403 ^a	43	415 ^b	17
12 H	7.03 ^b	0.38	6.75 ^b	0.12	4.25	0.13	4.13	0.06	3.48 ^b	0.19	3.40 ^b	0.12	455	27	525 ^b	32
24 H	7.43 ^b	0.36	7.23 ^b	0.11	4.48	0.14	4.28	0.13	3.63 ^b	0.21	3.55 ^b	0.06	418	27	435	18
48 H	7.85	0.37	7.70	0.19	4.10	0.14	3.98 ^a	0.10	3.55	0.18	3.55 ^b	0.10	453	32	460	15

^aP < 0.05 that the mean differs from the prechallenge-exposure mean for the same treatment group. ^bP < 0.01 that the mean differs from the prechallenge-exposure mean for the same treatment group.

IS = isotonic saline solution; HS = hypertonic saline solution.

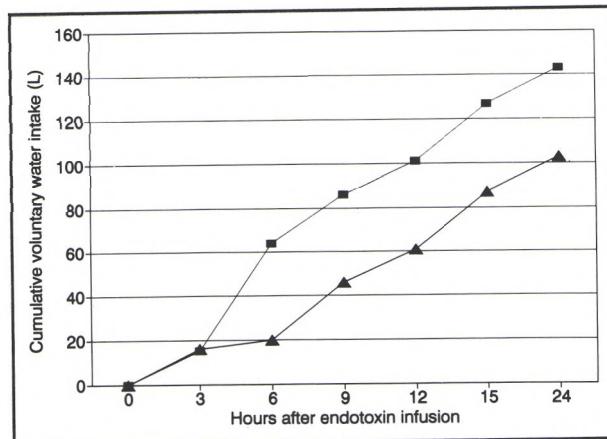


Figure 1—Cumulative voluntary water consumption in 8 lactating cows treated IV with either 0.9% (▲, n=4) or 7.5% (□, n=4) NaCl (5 ml/kg of body weight) 4 hours after intramammary infusion of 1 mg of *Escherichia coli* endotoxin in the left forequarter.

significantly ($P < 0.05$) in IS-treated cows at T3 (94.5 mm of Hg), compared with T0 values (79.0 mm of Hg) for the same group. All other comparisons of postchallenge-exposure heart rate and diastolic, systolic, and mean arterial blood pressures with prechallenge-exposure values for the same group were not significant ($P > 0.05$).

Direct measurement of plasma volume was not performed. Plasma volume was determined relative to prechallenge-exposure plasma volume for the same cow. Significant differences in relative plasma volume were not observed between IS- and HS-treated cows. However, the difference in plasma volume between IS ($109.63 \pm 0.94\%$)- and HS ($124.33 \pm 6.71\%$)-treated cows at T6 was marginally significant ($P = 0.0731$). Additionally, significant increases in plasma volume were observed earlier (T6) in HS-treated cows ($124.33 \pm 6.71\%$) than in IS-treated cows (T12). However, IS- and HS-treated cows had significantly ($P < 0.05$) increased plasma volume at T12 (IS, $111.78 \pm 2.75\%$; HS, $116.88 \pm 4.68\%$) and T24 (IS, $111.93 \pm 3.83\%$; HS, $113.25 \pm 2.38\%$; Fig 2).

Hematologic results—Differences attributable to treatment group were not observed in any of the measured hematologic variables (Table 2). A be-

tween-group difference in monocyte concentration was observed at T0, prior to the intramammary infusion of endotoxin or fluid therapy. Hematocrit was decreased at T12 and T24 in IS-treated cows, and at T6, T12, and T24 in HS-treated cows, compared with prechallenge-exposure values for the same group. The IS- and HS-treated cows had decreased RBC count at T6, T12, and T24, compared with prechallenge-exposure values for the same group. Compared with prechallenge-exposure values, hemoglobin concentration was decreased at T12 and T24 in IS-treated cows and at T6, T12, and T24 in HS-treated cows.

Decreased mature neutrophil count was observed in IS- and HS-treated cows at T3 and T6 and in HS-treated cows at T24. Band neutrophil count was increased in HS-treated cows at T24. Lymphocyte count was significantly ($P < 0.05$) decreased in IS-treated cows at T3 and T6 and in HS-treated cows at T6, T12, and T24. Monocyte count was decreased in HS-treated cows at T3, T12, and T24. Increased eosinophil count was observed in IS-treated cows at T48 (Table 3).

Serum biochemical results—Serum Na, Cl, K, Ca, inorganic P, and total CO₂ concentrations, and anion gap were determined for IS- and HS-treated cows (Table 1). Serum Na and Cl concentrations were significantly ($P < 0.05$) higher in HS-treated cows at T6 (2 hours after fluid administration). Between-group differences in serum K, Ca, inorganic P, and total CO₂ concentrations were not significant at any sample collection period.

Serum Cl concentration in IS- and HS-treated cows was significantly ($P < 0.05$) increased at T6, compared with T0. Thereafter, serum Cl concentration was significantly ($P < 0.05$) decreased in IS-treated cows at T12 and T48 and in HS-treated cows at T12. In HS-treated cows, the increased Na concentration at T6 decreased at T12, T24, and T48 and did not differ significantly from prechallenge-exposure concentration. Compared with T0 concentration, serum Na concentration in IS-treated cows was significantly ($P < 0.05$) decreased at T12, T24, and T48, but was not decreased at T3 and T6. Significant differences in serum K concentration were not observed between treatment groups at any sample collection interval. Additionally, serum K concentration at T3, T6,

Table 2—Mean and SEM hematologic values in 8 lactating cows treated IV with either 0.9% (IS, n=4) or 7.5% (HS, n=4) NaCl (5 ml/kg) 4 hours after intramammary infusion of 1 mg of *E coli* endotoxin in the left forequarter

Time	Hematocrit (%)				RBC ($\times 10^6/\mu\text{l}$)				Hemoglobin (g/dl)				Segmented neutrophils (cells/ μl)			
	IS		HS		IS		HS		IS		HS		IS		HS	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Prechallenge exposure	30.5	1.5	31.5	2.4	6.60	0.55	6.84	0.54	9.78	0.37	10.23	0.82	3,924	614	3,898	502
Postchallenge exposure																
3 H	32.0	1.8	32.5	3.0	6.91	0.57	7.06	0.62	10.25	0.56	10.45	0.82	733 ^b	263	1,399 ^b	431
6 H	28.6	1.4	27.8 ^b	2.6	6.16 ^a	0.53	5.99 ^b	0.61	9.15	0.39	8.75 ^b	0.69	1,355 ^a	192	1,095 ^b	139
12 H	28.0 ^a	1.5	28.5 ^b	2.1	6.11 ^a	0.54	6.21 ^b	0.48	9.08 ^a	0.38	9.18 ^b	0.64	1,645	347	1,800	1152
24 H	27.8 ^b	1.4	28.5 ^b	2.3	6.04 ^b	0.44	6.22 ^b	0.48	9.10 ^a	0.36	9.45 ^a	0.67	3,289	729	1,806 ^b	601
48 H	28.5	2.2	29.8	2.0	6.25	0.59	6.51	0.51	9.45	0.61	9.83	0.68	4,229	456	3,971	605
	Band neutrophils (cells/ μl)				Lymphocytes (cells/ μl)				Monocytes (cells/ μl)				Eosinophils (cells/ μl)			
	IS		HS		IS		HS		IS		HS		IS		HS	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Prechallenge exposure	0	0	0	0	4,403	532	6,581	36	36 ^c	23	258	85	342	217	488	184
Postchallenge exposure																
3 H	45	32	0	0	2,624 ^b	371	5,613	2,518	0	0	36 ^a	36	273	188	427	127
6 H	0	0	0	0	2,521 ^a	529	4,096 ^a	2,138	66	41	57	47	83	54	53	23
12 H	415	119	368	234	2,915	930	3,150 ^a	684	22	22	0 ^b	0	178	100	117	67
24 H	111	37	216 ^b	72	3,430	625	5,072 ^a	2,528	97	28	134 ^a	58	524	295	473	152
48 H	0	0	23	23	3,975	356	6,275	2,333	119	51	167	69	753 ^b	340	714	203

See Table 1 for key. ^cP < 0.05 that the mean differs between treatment groups at the specified sample collection time.

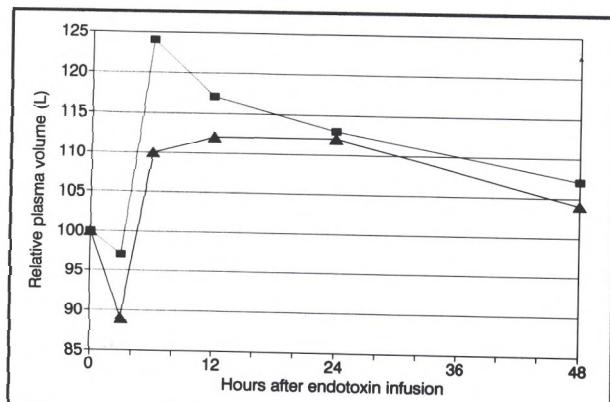


Figure 2—Relative plasma volume in 8 lactating cows treated IV with either 0.9% (▲, n=4) or 7.5% (■, n=4) NaCl (5 ml/kg) 4 hours after intramammary infusion of 1 mg of *E coli* endotoxin in the left forequarter.

T12, T24, and T48 did not differ significantly from T0 concentration for the same group.

Serum Ca concentration was significantly decreased (P-treated) in IS-treated cows at T6, T12, and T24, compared with T0 concentration. Similar decreases were observed in HS-treated cows at T6, T12, T24, and T48. Compared with T0, significant decreases in serum inorganic P concentration were observed in both groups at T6. Serum inorganic P concentration of 1.0 mg/dl was observed at 6 hours after challenge exposure in 1 HS-treated cow. Significant increases over T0 inorganic P concentration were observed at T24 and T48 in IS-treated cows and at T48 in HS-treated cows.

Serum total CO₂ concentration was significantly (P < 0.05) decreased, compared with T0 concentrations, in IS-treated cows at T12 and HS-treated cows

at T6, T12, and T24. Significant between-group difference was not observed at any sample collection time. Significant difference in anion gap was not observed between cows receiving IS and HS solution at any sample collection time. Calculated anion gap was decreased at T3 and T6 in IS-treated and at T6 in HS-treated cows, compared with anion gap at T0 for the same group. Anion gap was significantly (P < 0.05) increased in cows of both treatment groups at T12 and T24.

All postchallenge-exposure serum glucose concentrations were increased, compared with prechallenge-exposure concentrations for IS- and HS-treated cows. Serum glucose concentration was greater in IS-treated cows than in HS-treated cows at T3. Other between-group comparisons of serum glucose concentrations were not statistically significant. Within- and between-group comparisons of serum urea nitrogen concentration were not significant. Serum creatinine concentration in IS- and HS-treated cows were significantly (P < 0.05) decreased at T24 (IS, 0.63 mg/dl; HS, 0.60 mg/dl), compared with T0 (IS, 0.78 mg/dl; HS, 0.75 mg/dl). All other within- and between-group comparisons of serum creatinine concentration did not differ significantly.

Serum concentrations of protein, globulin, albumin, and fibrinogen did not differ significantly between IS- and HS-treated cows at any sample collection period. The IS- and HS-treated cows had decreased serum protein concentration at T3, T6, T12, and T24, compared with T0. Serum globulin concentration in HS-treated cows was decreased at T3, T6, and T48, compared with T0 for the same group. Compared with T0, serum albumin concentration was decreased at T3, T6, T12, and T24, in IS-treated cows and T6, T12, T24, and T48 in HS-treated cows. Serum

Table 3—Mean and SEM serum sodium, chloride, calcium, inorganic phosphorous, total CO₂, and glucose concentrations, and anion gap in 8 lactating cows treated IV with either 0.9% (IS, n=4) or 7.5% (HS, n=4) NaCl (5 mL/kg) 4 hours after intramammary infusion of 1 mg of *E coli* endotoxin in the left forequarter

Time	Sodium (mmol/L)				Chloride (mmol/L)				Calcium (mmol/L)				Inorganic phosphorous (mg/dL)			
	IS		HS		IS		HS		IS		HS		IS		HS	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Prechallenge exposure	135.0 ^c	1.1	131.8	0.6	104.0	2.3	103.0	0.8	9.28	0.21	9.28	0.21	3.98	0.21	3.73	0.19
Postchallenge exposure																
3 H	132.5	1.2	133.8	1.9	103.5	2.7	104.0	0.4	8.75	0.16	9.03	0.45	3.23	0.19	4.33	0.91
6 H	133.3	0.5	135.5 ^a	1.3	107.8 ^{b,c}	1.4	112.8	1.3	7.73 ^b	0.10	7.30 ^b	0.45	2.35 ^b	0.37	2.08 ^b	0.48
12 H	131.5 ^a	2.5	129.3	0.8	98.0 ^a	1.9	101.3 ^a	1.3	8.35 ^a	0.10	8.05 ^b	0.46	4.75	0.15	3.95	0.52
24 H	130.0 ^b	0.7	130.8	1.3	98.0	1.6	102.3	1.3	8.43 ^b	0.28	8.20 ^b	0.20	4.58 ^a	0.13	4.10	0.32
48 H	130.3 ^a	1.3	131.3	1.0	99.8 ^b	1.0	102.5	1.2	8.43	0.30	8.40 ^b	0.23	5.03 ^a	0.24	4.83 ^a	0.53
	Total CO ₂ (mmol/L)				Anion gap (mmol/L)				Glucose (mg/dL)							
	IS		HS		IS		HS		IS		HS					
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Prechallenge exposure	25.00	1.58	24.00	0.41	5.85	0.85	4.90	4.38	50.8	2.9	47.3	0.6				
Postchallenge exposure																
3 H	25.50	2.33	24.25	0.85	2.88 ^c	0.01	5.75	1.34	62.0 ^{b,c}	1.4	54.3 ^b	2.5				
6 H	23.00	1.08	21.25 ^a	0.48	2.48 ^b	0.95	1.58 ^b	0.49	64.3 ^a	3.7	64.8 ^a	3.7				
12 H	22.00 ^a	0.91	19.75 ^b	1.18	8.78 ^b	0.55	8.23 ^b	0.41	66.0 ^a	5.3	64.3 ^b	2.8				
24 H	24.25	1.44	21.50 ^a	0.65	7.40 ^a	0.65	7.28 ^b	0.22	68.3 ^b	3.3	68.0 ^b	1.9				
48 H	24.50	1.32	23.00	1.32	6.38	0.68	5.75	0.23	61.0 ^b	3.3	58.8 ^a	2.1				

See Tables 1 and 2 for key.

fibrinogen concentration was decreased in IS- and HS-treated cows at T6 and was increased at T12 in HS-treated cows, compared with T0.

Significant difference in serum activities of AST, CK, GGT, or ID was not observed between cows receiving IS and HS at any sample collection time. Serum AST activity was significantly ($P < 0.05$) decreased at T3, T6, T12, T24, and T48, compared with T0 for the same group. Serum CK activity was decreased in IS-treated cows at T24 and in HS-treated cows at T6, T24, and T48, compared with T0 for the same group. Serum GGT activity was significantly ($P < 0.05$) decreased at T3, T6, T12, T24, and T48, compared with T0 for the same group. Serum ID activity was decreased in IS-treated cows at T3 and T12 and in HS-treated cows at T12 and T24, compared with T0 for the same group. The variability and higher serum ID activity in the HS-treated group resulted from high serum activity in an individual cow (T0, 106.5 IU/L; T3, 100.2 IU/L; T6, 106.6 IU/L; T12, 101.5 IU/L; T24, 90.6 IU/L; T48, 66.5 IU/L). This cow likely had preexisting hepatocellular damage. This between-group difference was not statistically significant ($P > 0.05$).

Discussion

The role of endotoxin-induced shock in gram-negative bacterial mastitis is well documented.³¹ Models similar to ours have been used in studies that examined potential therapeutic regimens for gram-negative mastitis.^{23,25,27,29,30} Major differences between study protocols include endotoxin source, dose, and route of administration. The model used in this study mimicked naturally acquired gram-negative bacterial mastitis closely and induced consistent inflammation of the mammary gland and a severe, but

transient, shock syndrome that resolved within 2 days. Systemic shock is recognized by clinical signs of prostration, unresponsiveness, and depression.³³ Endotoxin-induced shock is complex, probably involving cardiogenic, hypovolemic, and to a lesser extent, neurogenic mechanisms.^{7-11,33-37}

Our results substantiated the fact that the cows studied had clinical, hematologic, and biochemical evidence of endotoxemia at the time of fluid therapy (T4). The study was designed in this manner to mimic potential clinical use of HS. Therapeutic intervention will not be undertaken until after recognition of clinical disease.

Three mechanisms have been proposed to explain beneficial clinical responses to rapid IV administration of HS. The first hypothesis proposes that HS causes rapid redistribution of body fluid from the intracellular to intravascular and extracellular fluid compartments, which enhances circulating blood volume and tissue perfusion.^{12,17} The second hypothesis states that hypertonic fluids exert a vagal-mediated inotropic effect on the heart.^{15,16,38,39} Some authors⁴⁰ have proposed that the clinical response to HS is a function of altered peripheral vascular resistance. Probably, all of these hypotheses are true, in part.¹²

Transiently decreased, although not statistically significant ($P > 0.10$), plasma volumes were observed in HS- and IS-treated cows at T3. The reduced plasma volume at T3 was supported by the observed increases in concentrations of globulin, albumin, protein, and hemoglobin, RBC count, and hematocrit. Thereafter, calculated plasma volume was significantly increased in HS-treated cows at T6 and in both groups at T12 and T24. Increased plasma volume was also observed in both groups at T48, but was not

statistically significant. Expanded plasma volume at T6, T12, T24, and T48 was supported by the concurrent observation of decreased concentrations of globulin, albumin, protein, and hemoglobin, RBC count, and hematocrit.

The significant increase in plasma volume observed at T6 in HS-, but not in IS-treated cows, supports the hypothesis that IV administered HS will expand plasma volume. Lack of posttreatment differences in plasma volume between HS- and IS-treated cows may be a function of the small sample sizes. Results of studies in alternative models of endotoxin-induced shock in calves have revealed a rapid, but short-lived expansion of plasma volume after IV administration of hypertonic fluids.⁹ The large magnitude of increase in plasma volume (T6, 9.63%; T12, 11.78%; T24, 11.93%) observed in IS-treated cows and the duration of this response make administration of 5 ml of IS/kg an unlikely cause of the observed response in calculated plasma. Hence, this increase in plasma volume was more likely the result of endotoxemia, rather than therapeutic intervention.

Endotoxin-induced shock is generally described as a physiologic state characterized by decreased circulating plasma volume. Studies in other species have often described hypovolemia, increased hematocrit, or increased serum protein concentration in association with endotoxin-induced shock.^{11,13,41-45} Our results contrast sharply with results of those studies. Expanded plasma volume was a consistent finding in HS- and IS-treated cows.

Although contradictory results have been observed in cattle studies, the possibility of an expanded plasma volume in cattle with endotoxin-induced shock is not without precedent. Nagaraja et al⁴⁶ observed transient increase in serum protein concentration after IV administration of endotoxin, followed by a decrease that persisted > 24 hours. Several investigators have observed decreases in either hematocrit or serum protein concentration in calves administered endotoxin,^{41,43,46} but none attributed these changes to increased plasma volume. Using dye-dilution methods, Constable et al⁹ observed a short-lived increase in plasma volume between 2 and 3 hours after challenge exposure.

Alternative explanations for decreased serum protein concentration and hematocrit have been proposed. Morris et al⁴¹ suggested that hypoproteinemia and decreased hematocrit in endotoxemic calves might result from loss of protein-rich fluid into the extravascular space that develops simultaneously with intravascular hemolysis, or alternatively, from hemorrhage into tissues as a result of disseminated intravascular coagulation. The effect of endotoxin on vascular permeability is well recognized;³⁶ however, increased permeability is an unlikely cause of the hypoproteinemia we observed. Increased vascular permeability probably results in loss of fluid of similar composition to plasma in the extravascular compartment. Any losses of plasma protein would be accompanied by fluid losses, and serum protein concentration would remain unchanged. For increased vascular permeability to cause hypoproteinemia, fluid lost to the extravascular space would have to be hy-

peroncotic. If decreased serum protein concentration was a function of increased vascular permeability expected in an endotoxic event, disproportionate decreases in low molecular weight serum proteins (albumin) with less pronounced effects in the higher molecular weight globulins should have been observed. Relative concentrations of albumin and globulin remained stable in both groups at T6, when the greatest change in plasma volume was observed.

Evidence of intravascular hemolysis was not observed in this study. Histopathologic changes consistent with disseminated intravascular coagulation have been reported in another study,⁴⁶ but this finding has not been consistent, and gross, extensive hemorrhages are not typical in cattle that are administered endotoxin. Experimental intramammary endotoxin infusion has minimal effects on hemostasis.⁴⁷ Hemolysis was considered to be an unlikely cause of decreased hematocrit, hemoglobin, and RBC count because all 3 variables rapidly increased, concomitant with increased relative plasma volume after T12. This response occurred too soon after endotoxin administration (12 hours) to reflect de novo synthesis of RBC and serum proteins. Regenerative responses to decreased RBC mass typically require 4 to 6 days. These observations, coupled with consistent unidirectional changes in RBC mass and protein concentration, provide supportive evidence that the calculated changes in plasma volume existed.

Dilution methods are preferred by some investigators over calculations based on hematologic indices for monitoring changes in plasma volume.⁴⁸⁻⁵⁰ Evan's blue or ¹³¹I-labeled albumin or, alternatively, ⁵¹Cr-tagged RBC are confined to the blood vascular system.⁴⁸⁻⁵⁰ After equilibration, blood or serum concentration of such markers is determined, permitting calculation of volume of distribution. These methods are not free of problems. Systemic inflammation markedly alters distribution of Evan's blue dye; increased vascular permeability permits low molecular weight albumin with its marker to drift into the interstitial compartment, with subsequent inaccuracies in plasma volume determinations.³⁶ Tissue dye accumulation has been proposed as a marker for capillary permeability.^{32,36} Greenleaf et al³² suggested that either Evan's blue dye or radioactive iodine-labeling techniques are unsuitable for short-term, repetitive calculation of plasma volume, because the long half-life of these markers (Evan's blue, approx 8 hours) creates excess background relative to the injected dose of the tracer compound.³² Repeated measurement is best accomplished by changes in hematologic indices.³² Additionally, albumin or RBC labeling has few, if any, logical advantages over direct measurement. Given the problems anticipated with albumin or RBC labeling and the relative ease of direct albumin and RBC determinations, we decided at the study outset to set the prechallenge-exposure plasma volume at 100% arbitrarily, and to measure proportional changes thereafter. Lack of splenic responsiveness in cattle subjected to stress or disease makes plasma volume determination, based on hematologic indices, more suitable in cattle than in other species.

The consistent increase in plasma volume we

observed does not support the role of systemic hypovolemia in the pathogenesis of gram-negative bacterial mastitis. Manifestations of shock associated with decreased tissue perfusion more likely were caused by either decreased cardiac output or altered vascular resistance. Although not statistically significant, the changes in observed diastolic arterial blood pressure supported the role of vascular capacitance in the pathogenesis of endotoxemic shock. The decreased plasma volume observed at T3 coincided with increased diastolic arterial blood pressure in both IS- and HS-treated cows. Thereafter, diastolic arterial blood pressure was nonsignificantly decreased in both groups. Readily apparent pattern was not observed in the measured systolic arterial blood pressure. Although not statistically significant, decreased diastolic arterial blood pressure still was observed at T6, T12, T24, and T48 in cows of both treatment groups. Decreased mean arterial blood pressure has been observed in calves given endotoxin IV.^{11,44}

Observed decreases in serum total CO₂ concentration probably reflect a mild metabolic acidosis; however, respiratory alkalosis could cause similar changes.⁵¹ Blood gas analyses (partial pressures of PO₂ and PCO₂, pH, and HCO₃⁻ concentration) are required to differentiate metabolic acidosis and respiratory alkalosis.⁵¹ Transient respiratory alkalosis has been reported in some experimental endotoxemia models; however, persistent acid-base derangements are typically characterized as metabolic acidosis.⁷ The continued decreases in serum total CO₂ concentration at T12 and T24 and the lack of clinically overt pulmonary disease support the existence of a metabolic acidosis.⁵¹

Interpretation of acid-base status was complicated by the concurrent decreased serum Na concentration at T12, T24, and T48 in IS- and HS-treated cows. Hyponatremia may be accompanied by decreased serum anion gap, and total CO₂, and Cl concentrations. Decreased total CO₂ concentration, increased anion gap, and low-to-normal Cl concentration observed at T12 and T24 are consistent with titration acidosis.⁵¹ Other studies support the existence of titration-induced metabolic acidosis.^{41,42,46} After endotoxin administration, blood lactate concentration increases, probably caused by decreased tissue perfusion and anaerobic tissue metabolism.

Observed decrease in serum Ca concentration was consistent with results of previous endotoxin challenge-exposure studies in ruminants.^{7,52} This apparent endotoxin-induced hypocalcemia should be interpreted cautiously. Decreased serum albumin concentration was consistently observed, along with probable metabolic acidosis. Hypoalbuminemia and acidosis may increase the proportion of ionized Ca concentration without affecting total serum Ca concentration.^{53,54} Observed decreases in serum Ca concentration may reflect either an endotoxin-induced effect or, be the result of host homeostatic responses to an increased proportion of ionized Ca concentration. Most studies, this one included, measured total serum Ca concentration. The importance of ionized Ca concentration was emphasized in another study when clinical signs of parturient paresis

and low serum ionized Ca concentration were observed in a cow with marked hypercalcemia.⁵⁴

The transient hypophosphatemia observed at T6 has been observed in at least 1 previous study of endotoxemia in calves.⁴⁶ Serum inorganic P concentration approached or was below the threshold at which intravascular hemolysis is reported to occur. We did not observe hemoglobinemia and hematuria; however, more sensitive indicators of hemolysis were not used. Serum inorganic P concentration equaled or exceeded baseline concentration at all subsequent sample collection times, so any RBC stresses may have been of inadequate duration to cause hemolysis.

Infusion of hypertonic NaCl solution was expected to cause sudden and profound changes in serum electrolyte composition. Tyler et al⁴⁸ calculated that administration of 7.5% NaCl (5 ml/kg) would cause an increase in serum Na concentration of 20 mmol/L, unless offset by redistribution or excretion. Hypernatremia of this magnitude was not observed in this study. Although, serum Na concentration was increased at T6 (2 hours after treatment) in HS-treated cows, the magnitude of the change was minimal (< 4 mmol/L) and transient. Minimal increase in serum Na concentration in HS-treated cows indicates a rapid equilibration with extravascular fluid compartments. This hypothesis is supported by the increased plasma volume observed in HS-treated cows at T6 and T12.

Significantly ($P < 0.05$) decreased serum Na concentration in IS-treated cows at T12, T24, and T48 was observed at T12 and T24, compared with T6 concentration. Additionally, serum Na concentration was decreased in HS-treated cows at T12, T24, and T48, compared with either prechallenge-exposure or T6 concentration. These results indicated selective loss of Na or expanded Na space, resulting in dilution of serum Na concentration. The expanded plasma volume preceded, rather than coincided, with decreased serum Na concentration, thereby reducing the likelihood that decreased serum Na concentration was a dilutional effect. An anecdotal observation made during this study was that administration of HS was followed by profound increase in urine volume. Decreased serum Na concentration has been observed in experimental endotoxemia models and was linked to increased production of atrial natriuretic factor.⁵⁵ Further studies are needed in which fractional excretion of electrolytes and serum concentrations of hormones that regulate fluid and electrolyte balance are measured in cattle with endotoxin-induced disease.

Intravenous endotoxin infusion generally causes marked increases in serum activities of liver- and muscle-specific enzymes.⁷ Our results—unchanged or decreased serum enzyme activities—were consistent with results of a study, using an intramammary endotoxin infusion.⁷ This disparity in results highlights the differences between IV and intramammary endotoxin challenge exposures. Pathophysiologic mechanisms probably differ markedly between these models. Our results indicate that intramammary endotoxin challenge exposure may induce a local disease process with systemic manifestations of mediator-induced shock. Serum enzyme activities have

not been characterized well in experimentally induced or naturally acquired gram-negative bacterial mastitis. Further documentation of clinical cases is necessary to determine whether systemic manifestations of shock in naturally acquired coliform mastitis result from local or systemic endotoxemia. Bacteremia is probably an infrequent event in coliform mastitis,⁵⁶ and only 1 study successfully detected endotoxin systemically in cattle with coliform mastitis.³¹

Azotemia, increased serum urea N or creatinine concentration, was lacking in our study. This contrasts sharply with published reviews,^{7,33} and may relate to our previous observation of increased plasma volume maintaining an adequate glomerular filtration.

Although transient hyperglycemia followed by a persistent hypoglycemia has been observed in cattle after endotoxin-challenge exposure,⁴⁶ hypoglycemia was not observed in this study. Serum glucose concentration remained increased, relative to baseline concentration, at all subsequent sample collections. Isotonic (5%) dextrose solution has been recommended for intravenous administration in cases of coliform mastitis.⁵ Increased serum glucose and decreased serum Na and Cl concentrations observed in our study, favor administration of NaCl-based solutions with minimal glucose supplementation, unless indicated by serum biochemical results. Excess glucose supplementation under conditions of preexisting hyperglycemia could cause osmotic diuresis, resulting in increased renal water and electrolyte loss. Addition of bicarbonate or bicarbonate equivalent to IV administered fluids may be of benefit; however, the observed decreases in serum total CO₂ concentration were mild and cannot be definitively attributed to metabolic acidosis. Further studies are needed to determine the effect of preexisting endotoxemia on host responses to administration of HS.

Our study had several unique experimental design features: experimental subjects were conscious (neither anesthetized nor sedated) mature, lactating dairy cattle; endotoxin challenge exposure was administered by intramammary, rather than IV infusion; observations were extended to 48 hours after experimental challenge exposure. These design features were chosen in an attempt to mimic naturally acquired coliform mastitis.

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