ENDOTOXIC AND ANAPHYLACTIC-TYPE SHOCK IN STEERS FROM INTRAVENOUS INJECTION OF ESCHERICHIA COLI ENDOTOXIN AND RUMINAL ABSORPTION OF ENDOTOXIN

by

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INTRODUCTION

The large gram-negative bacterial population in the rumen of cattle is a potential source of a large quantity of endotoxin. Rumen bacterial endotoxin has been implicated in certain diet induced diseases of cattle such as lactic acidosis and the sudden death syndrome. Endotoxin or lipopolysaccharide is capable of producing two types of shock: (1) endotoxic shock and (2) anaphylactic shock. Although the exact role of rumen bacterial endotoxin in the pathogenesis of diet induced diseases is not known, it is hypothesized that anaphylactic shock may be the cause of the sudden death syndrome in feedlot cattle. However, a clear disinction between endotoxic and anaphylactic shock due to endotoxin is lacking. Also, evidence regarding the absorption of endotoxin from the rumen of cattle is not available. This study was conducted in two parts: Part I was to distinguish between endotoxic shock and anaphylactic shock in cattle from intravenous injection of endotoxin, and Part II was to determine the ruminal absorption of endotoxin.

PART I

ENDOTOXIC AND ANAPHYLACTIC-TYPE SHOCK IN STEERS FROM INTRAVENOUS INJECTION OF ESCHERICHIA COLI ENDOTOXIN

INTRODUCTION

The large gram-negative bacterial population in the rumen of cattle is a potential source of large quantity of endotoxin (Nagaraja et al., 1978). Rumen bacterial endotoxin has been suggested to play a role in certain diet-induced diseases such as lactic acidosis (Dougherty, 1976) and the sudden death syndrome (Nagaraja et al., 1979). The mechanism of endotoxin participation in the pathogenic process of lactic acidosis and the sudden death syndrome is unknown. Because endotoxin molecule is composed of toxic moiety (lipid) and antigenic moiety (polysaccharide), there are two possible mechanisms of action: (1) endotoxic shock as a result of endotoxemia attributable to the lipid moiety and (2) anaphylactic shock as a result of hypersensitive reaction to the antigenic moiety of the molecule (Kim and Watson, 1966). Intravenous injection of calves with rumen bacterial endotoxin induced signs and pathophysiological changes typical of endotoxin shock. The signs and changes were similar to those observed in calves that received Escherichia coli endotoxin (Nagaraja et al., 1979). Second injection of endotoxin to calves on day 15 caused enhanced clinical response suggestive of anaphylactic-type response. Because a clear distinction between endotoxin shock and anaphylactic-type shock is lacking, this study was initiated. The primary objective was to study clinical signs, hematological changes and pathological changes associated with endotoxin shock (single injection) and anaphylactic shock (repeated injections) in steers.

MATERIALS AND METHODS

Experimental Animals and Diet. The experimental animals were Holstein steers aged 4 to 8 months with body weights ranging from 100 to 225 kg. The steers were fed a diet consisting of 75% alfalfa hay and 25% grain supplement. The grain diet consisted of a mixture of 40.6% sorghum grain, 40.6% corn, 17.5% soybean meal, .5% dicalcium phosphate, .5% salt, and .5% Vitamin A and D

supplement.

Endotoxin. Lyophilized commercial Escherichia coli endotoxin (Lipopolysaccharide B, E. Coli 0111:B4, Difco Laboratories, Detroit, MI) was used in the study. Endotoxin was dissolved in sterile normal saline (.85% sodium chloride) before intravenous administration.

Blood Sample Collection and Schedule. Blood samples were collected from the jugular vein just before endotoxin injection and at 5, 15, 30, 60, 120, 180, 360, 720, and 1440 minutes after injecting endotoxin. Blood samples were collected in the following vacutainer tubes (Becton-Dickinson Co., Rutherford, NJ):

1) tube containing ethylene diaminetetraacetate (EDTA) as an anticoagulant for total and differential leukocyte counts, platelet counts, and for fibrinogen and plasma protein determinations

2) tube containing sodium oxalate and sodium fluoride for plasma glucose determination

3) tube containing no anticoagulant for serum calcium determination

4) tube containing sodium citrate for prothrombin and activated partial thromboplastin time determinations.

Blood Analyses. Total leukocytes in the blood samples were counted in a Coulter counter (Coulter Electronics Inc., Hialeah, FL). Stained blood smears were prepared from each sample and a total of 200 leukocytes counted. In samples with marked leukopenia only 100 or sometimes 50 cells were counted. Platelet counts were made with unopettes (Becton-Dickinson Co.) and a Neubauer counting chamber. Total plasma protein and fibrinogen were measured by the refractive index method with the TS meter (American Optical Co., Buffalo, NY). Serum calcium was determined by SMA 12 Autoanalyzer (Technicon Corp., Tarrytown, NY) and plasma glucose was measured with Abbott

Laboratories Reagent Kit (Abbott Laboratories, Pasedena CA). Prothrombin time (PT) and activated partial thromboplastin time (APTT) determinations were made with a Fibrometer (Becton-Dickinson Co., Buffalo NY). PT determination was made by adding .1 ml citrate plasma sample to .2 ml rabbit brain thromboplastin (Activated thromboplastin, Dade Diagnostics, Inc., Miami, FL). APTT was performed by mixing .02 ml Actin Activated Cephaloplastin reagent (Dade Diagnostics Inc.) with 0.1 ml citrated plasma. At each PT and APTT determination, citrated plasma from a healthy steer was used as a control.

Experiment 1. Endotoxin shock in steers

The objective of this experiment was to elucidate the clinical signs and hematological changes associated with endotoxin shock in steers. Five steers were injected intravenously with 10 µg/kg body weight E. coli endotoxin. Steers were observed for clinical signs and blood samples were collected from three steers at 0, 5, 15, 30, 60, 120, 180, 360, 720, and 1440 minutes after endotoxin injection. Blood samples were analyzed for total and differential leukocyte counts, platelet counts, total protein, fibrinogen, calcium, and glucose. Citrated plasma collected from two steers was used for PT and APTT determinations. Steers that died of endotoxic shock were necropsied. Gross lesions were recorded and tissue slices from the heart, lung, and liver were taken for routine histo-pathological examination. All tissue sections were stained with hematoxylin and eosin.

Experiment 2. Endotoxin induced anaphylactic-type shock in steers

The objective of this experiment was to determine the ability of endotoxin to induce anaphylactic-type shock in steers. To determine an injection sequence and proper endotoxin dosage required to induce anaphylactic-type response, nine steers divided into groups of 5 and 4 each (groups 1 and 2) were used in the study. Steers in both groups were intravenously injected with 10 µg E. coli

endotoxin/kg body weight on days 1 and 4. Steers in group 1 were injected with a challenging dose (10 µg/kg body weight) on day 5. Steers in group 2 received their challenging dose on day 7 with two steers receiving 10 µg and the other two receiving 100 µg/kg body weight. All steers were observed for clinical signs after each endotoxin injection. After endotoxin injection on day 5 or 7, blood samples were collected. Sampling schedule and blood analyses were as before. Two steers that received challenge dose on day 5 were euthanitized 24 hours after the endotoxin administration. Gross lesions were noted and tissue samples collected from brain, heart, lung, liver, kidneys, adrenal, and small intestine for routine histopathological examination.

Experiment 3. Effect of sodium meclofenamate on Endotoxic and Anaphylactic-type shock in steers

The objective of this experiment was to pre-treat steers with sodium meclofenamate in order to distinguish between endotoxic and anaphylactic-type shock. Sodium meclofenamate has been shown to prevent systemic experimental anaphylaxis in cattle (Eyre et al., 1973). Seven steers were used in the experiment. Four steers were sensitized with endotoxin injection (10 µg/kg body weight) on days 1 and 4, and three remained unsensitized. On day 5 all steers were intravenously injected with 2 mg sodium meclofenamate/kg body weight (Parke-Davis and Co., Detroit, MI) 5 minutes before endotoxin (10 µg/kg body weight) administration. (All steers were observed for clinical signs after endotoxin injection.) Blood samples were collected and analyzed as before. One steer from each group was euthanitized 24 hours after endotoxin injection and necropsied. Tissue samples were taken as before for histopathological examination.

RESULTS

Experiment 1. Endotoxin shock in steers

Clinical signs. Among the five steers injected with 10 µg E. coli endotoxin/kg body weight, two died within six hours. Both showed severe respiratory distress with blood and foamy discharge from the mouth and nostrils. Steers that survived showed increased respiratory rate within 3 to 5 minutes after the endotoxin injection. The increased rate gradually led to dyspnea with marked abdominal respiration with occasional grunting. The phase of respiratory distress lasted for several hours. Salivation was profuse. Steers usually urinated within 8 to 12 minutes after injection. Some steers had diarrhea as early as 20 minutes but usually after 1 hour following endotoxin administration. Ruminal distension with gas was evident within 2 hours. Steers were anorectic and depressed. Full recovery was observed within 24 to 48 hours.

Blood changes. Blood changes in steers exhibiting endotoxin shock are shown in tables 1 to 3. The leukocyte response was typical of endotoxemia. There was a marked leukopenia within 180 minutes after endotoxin administration followed by leucocytosis with a shift to the left. Leukopenia was due to decrease in both neutrophils as well as lymphocytes. However leukocytosis appeared to be due entirely to increase in neutrophils (table 1). Platelets decreased significantly in 12 to 24 hours after endotoxin injection. Fibrinogen concentration decreased. Decrease in plasma protein after endotoxin injection may be reflective of fibrinogen decrease. Serum calcium also decreased. There was slight hyperglycemia followed by hypoglycemia in 12 to 24 hours (table 2). PT increased following endotoxin injection, however, changes in APTT appeared to be inconsistent (table 3).

Pathological changes. Gross lesions in two steers that died of endotoxin shock were confined to the respiratory system. Trachea was filled with foamy and blood

TABLE 1. LEUCOCYTE RESPONSE IN STEERS TO INTRAVENOUS INJECTION OF ESCHERICHIA COLI ENDOTOXIN[a]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	10433 ± 3525[b]	48	4899	4994	308	123	30
5	7700 ± 3593	0	4205	3342	20	71	59
15	5400 ± 2022	0	2393	2905	13	65	13
30	3233 ± 231	0	472	2685	12	51	10
60	2200 ± 346	0	163	1930	11	89	0
120	1767 ± 404	0	187	1423	0	133	17
180	1467 ± 252	5	169	1132	0	145	12
360	2200 ± 854	110	559	1335	10	172	10
720	8933 ± 4754	218	3706	2355	129	84	0
1440	20367 ± 6688	1339	14052	4432	580	0	0

[a]Endotoxin dose was 10 μg/kg body weight.

[b]Mean ± standard deviation of three steers.

TABLE 2. HEMATOLOGICAL CHANGES IN STEERS INJECTED INTRAVENOUSLY WITH ESCHERICHIA COLI ENDOTOXIN[a]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)	Plasma protein (g/dl)	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	699±165[b]	433±252	7.6±.6	9.5±.6	67±11
5	523±272	433±252	7.8±.4	9.5±.6	67±10
15	520±224	533±321	7.7±.6	9.2±.5	79±15
30	542±205	500±100	7.4±.5	8.9±.6	103±25
60	530±195	333±153	6.9±.5	8.6±.2	77±28
120	508±227	400±200	6.8±.7	8.0±.6	71±25
180	338±113	333±115	6.7±.5	7.6±.5	46±13
360	230± 74	333±153	6.5±.7	7.1±.6	45± 2
720	149[c]	267± 58	6.7±.6	7.2±.9	47± 7
1440	128±101	367± 58	7.0±.6	7.1±.5	48± 6

[a]Endotoxin dose was 10 µg/kg body weight.

[b]Mean ± standard deviation of three steers.

[c]Mean of two steers.

TABLE 3. PROTHROMBIN AND PARTIAL THROMBOPLASTIN TIME IN STEERS INJECTED INTRAVENOUSLY WITH ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Prothrombin time (sec)	Partial thromboplastin time (sec)
0	14.5(14.1)[c]	40.3(39.7)
5	14.6	39.8
15	14.9	38.7
30	14.9	47.9
60	15.7	39.0
120	16.0(14.3)	39.3
180	19.3(15.3)	42.9(44.3)
360	20.1(15.1)	26.8(46.4)
720	-	-
1440	21.2(15.5)	48.4(40.9)

[a]Endotoxin dose was 10 μg/kg body weight.

[b]Mean of two steers.

[c]Numbers in parentheses are control values.

tinged fluid. Lungs were edematous with numerous petechial and ecchymotic hemorrhages particularly in the cranioventral region of the lung lobes. Histologically, lung showed hemorrhage, edema, emphysema and infiltration with polymorphonuclear cells (Figure 1).

Experiment 2. Endotoxin-induced Anaphylactic-type shock in steers

Clinical signs. Clinical signs observed in steers after endotoxin administration (days 1 and 4) were similar to the signs shown in steers in the previous experiment. However steers after endotoxin injection on days 5 and 7 reacted differently. Steers collapsed within seconds of intaruminal endotoxin injection with signs of severe respiratory distress. Steers remained laterally recumbent for several hours. During recumbency steers exhibited other signs of endotoxin shock such as salivation cyanotic mucus membrane, diarrhea, and abdominal distension. No difference was seen in clinical signs between steers receiving challenge dose on days 5 and 7. Signs in steers that received 100 µg/kg body weight were slightly more pronounced than the steers that recieved 10 µg/kg body weight.

Blood changes.Leukocyte response and changes in blood constituents in endotoxin sensitized steers challenged with endotoxin on days 5 and 7 are shown in tables 4 to 10. Leukocyte response in sensitized steers were similar to that of unsensitized steers described in experiment 1. There was leukopenia followed by leukocytosis. Because there was initial leukocytosis (result of the previous injection) leukopenia appeared to be less marked in steers challenged on day 5 compared to the steers challenged on day 7 (tables 4, 7, and 9). Changes in neutrophils and lymphocytes were similar to that of unsensitized steers. Thrombocypenia was evident in steers challenged on day 5 or 7 with 10 µg/kg body weight, but absent in steers challenged with 100 µg/kg body weight. Fibrinogen and plasma protein concentrations remained normal for steers challenged on day 5, but those

TABLE 4. LEUKOCYTE RESPONSE IN ENDOTOXIN SENSITIZED STEERS TO INTRAVENOUS CHALLENGE OF ESCHERICHIA COLI ENDOTOXIN[a]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	15433 ± 2470[b]	2195	6423	5926	602	213	48
5	10267 ± 1266	525	2183	7383	54	103	19
15	6233 ± 3349	77	1750	4157	29	105	101
30	3533 ± 1041	11	237	2761	29	154	9
60	3233 ± 461	9	161	3094	30	151	0
120	2967 ± 208	29	197	2496	87	148	10
180	3167 ± 757	104	339	2506	73	137	8
360	8067 ± 2532	1334	2390	3033	87	197	0
720	17467 ± 3092	1778	8019	6636	372	566	96
1440	19633 ± 4038	843	8373	6577	359	146	0

[a]Steers were sensitized with E. coli endotoxin (10 μg/kg body weight) on days 1 and 4 and challenged on day 5 (10 μg/kg body weight).

[b]Mean ± standard deviation of three steers.

TABLE 5. HEMATOLOGICAL CHANGES IN ENDOTOXIN SENSITIZED STEERS CHALLENGED INTRAVENOUSLY WITH ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)	Plasma protein (g/dl)	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	782±1032	467±115	6.9±.6	9.4±.5	68±14
5	680± 839	467± 58	7.0±.4	9.4±.3	85±37
15	642± 804	400±100	6.9±.4	9.3±.5	80±32
30	545± 636	433±115	6.6±.4	9.0±.4	75±33
60	481± 653	500±100	6.6±.6	9.1±.4	66±30
120	513± 601	600±173	6.7±.4	8.6±.7	65± 9
180	482± 567	433±115	6.5±.5	8.7±.7	64±12
360	519± 557	500±100	6.7±.4	8.7±.2	56± 7
720	525± 412	500±100	6.7±.5	8.8±.2	59±11
1440	528± 377	567± 58	6.8±.3	8.8±.2	64± 8

[a]Steers were sensitized with E. coli endotoxin (10 µg/kg body weight) on days 1 and 4 and challenged on day 5 (10 µg/kg body weight).

[b]Mean ± standard deviation of three steers.

TABLE 6. PROTHROMBIN AND PARTIAL THROMBOPLASTIN TIME IN ENDOTOXIN SENSITIZED STEERS CHALLENGED INTRAVENOUSLY WITH ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Prothrombin time (sec)	Partial thromboplastin time (sec)
0	16.0(14.6)[c]	43.8(36.6)
5	13.6	48.2
15	13.9	47.8
30	13.8	48.4
60	15.2	45.6
120	16.5(14.3)	43.4
180	16.7(16.0)	41.4(39.2)
360	16.8(15.8)	47.1(38.9)
720	-	-
1440	16.1(15.5)	43.0(40.7)

[a]Steers were sensitized with E. coli endotoxin (10 µg/kg body weight) on days 1 and 4 and challenged on day 5 (10 µg/kg body weight).

[b]Mean ± standard deviation of three steers.

[c]Numbers in parentheses are control values.

TABLE 7. LEUKOCYTE RESPONSE IN ENDOTOXIN SENSITIZED STEERS TO INTRAVENOUS CHALLENGE OF ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	8350	0	1668	6139	382	162	0
5	4850	0	1181	3496	148	26	0
15	5450	0	1227	4114	28	83	0
30	3700	0	315	3349	19	19	0
60	3800	54	438	3072	218	36	0
120	2100	0	309	1740	51	0	0
180	2200	44	176	1980	0	0	0
360	2700	27	1188	1215	162	108	0
720	13700	3288	6165	3425	0	411	0
1440	20500	205	15888	3690	410	205	102

[a]Steers were sensitized with E. coli endotoxin (10 μg/kg body weight) on days 1 and 4 and challenged on day 7 (10 μg/kg body weight).

[b]Mean of two steers.

TABLE 8. HEMATOLOGICAL CHANGES IN ENDOTOXIN SENSITIZED STEERS CHALLENGED INTRAVENOUSLY WITH ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)	Plasma protein (g/dl)	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	720	-[c]	-	9.6	75
5	520	-	-	9.9	109
15	493	-	-	9.2	171
30	473	-	-	9.2	198
60	430	-	-	9.3	144
120	548	-	-	8.9	117
180	503	-	-	9.1	45
360	455	-	-	8.6	43
720	323	-	-	8.4	62
1440	383	-	-	9.2	62

[a]Steers were sensitized with E. coli endotoxin (10 µg/kg body weight) on days 1 and 4 and challenged on day 7 (10 µg/kg body weight).

[b]Mean of two steers.

[c]Not determined.

TABLE 9. LEUKOCYTE RESPONSE IN ENDOTOXIN SENSITIZED STEERS TO INTRAVENOUS CHALLENGE OF ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	10600	40	2754	7302	439	67	0
5	4100	53	931	9126	35	156	0
15	4000	0	79	3880	0	41	0
30	3750	0	90	3545	18	98	0
60	3100	17	62	2943	46	17	0
120	2250	0	33	2183	0	34	0
180	1700	17	102	1513	17	51	0
360	3200	128	832	1952	64	192	0
720	7900	1004	3922	2658	135	181	0
1440	16350	3855	7508	4766	222	0	0

[a]Steers were sensitized with E. coli endotoxin (10 μg/kg body weight) on days 1 and 4 and challenged on day 7 (100 μg/kg body weight).

[b]Mean of two steers.

TABLE 10. HEMATOLOGICAL CHANGES IN ENDOTOXIN SENSITIZED STEERS CHALLENGED INTRAVENOUSLY WITH ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)	Plasma protein (g/dl)	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	342	-[c]	-	9.2	70
5	243	-	-	9.6	79
15	210	-	-	9.4	98
30	231	-	-	9.7	120
60	221	-	-	9.1	122
120	248	-	-	8.9	123
180	213	-	-	9.2	27
360	270	-	-	8.4	45
720	-	-	-	8.0	49
1440	213	-	-	8.7	61

[a]Steers were sensitized with E. coli endotoxin (10 µg/kg body weight) on days 1 and 4 and challenged on day 7 (100 µg/kg body weight).

[b]Mean of two steers.

[c]Not determined.

determinations were not made for steers challenged on day 7. Serum calcium also did not decrease in steers challenged on day 5 or 7. Plasma glucose concentration remained normal in steers challenged on day 5, but steers challenged on day 7 showed typical hyperglycemia followed by hypoglycemia.

Pathological changes.Gross lesions in the steers necropsied were confined to the respiratory system. Lungs showed prominent hemorrhages and appeared edematous. Microscopic changes in the lung were similar to the changes in steer that died of endotoxic shock. There was hemorrhage, edema, emphysema and infiltration with polymorphionuclear cells (Figure 2).

Experiment 3. Effect of sodium meclofenamate

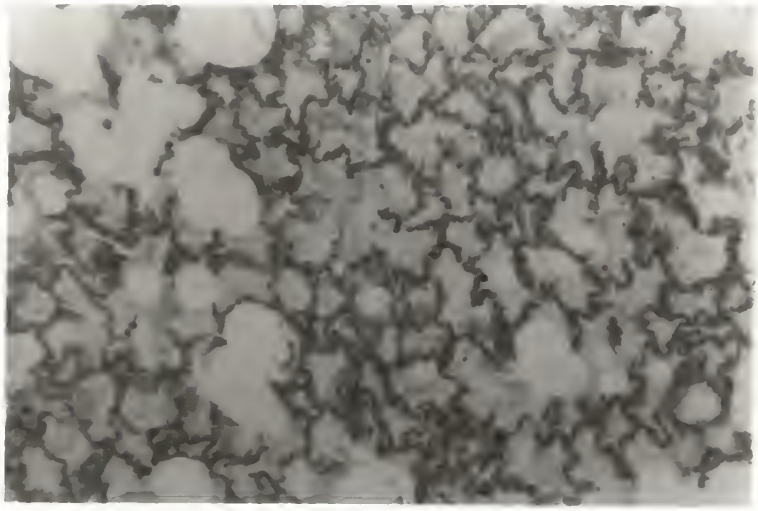
Clinical signs.Sodium meclofenamate injection (2 mg/kg body weight) had no effect on the clinical signs observed in the steers injected with 10 µg endotoxin/kg body weight. Steers showed signs typical of endotoxin shock observed in experiment 1. Steers sensitized with endotoxin on days 1 and 4, and treated with sodium meclofenamate on day 5 did not collapse immediately after endotoxin injection. However, steers exhibited other signs of endotoxin shock.

Blood changes.Leukocyte response to endotoxin injection in steers pretreated with sodium meclofenamate was similar to the untreated steers. There was leukopenia followed by leukocytosis with shift to the left (table 11). Thrombocytopenia was evident but concentration of fibrinogen did not decrease. There was slight reduction in plasma protein and serum calcium. Although initial hyperglycemia was not evident, hypoglycemia was seen in 6 hours after endotoxic injection.(table 12). PT and APTT increased slightly. (table 13).

Endotoxin challenge in sensitized steers pretreated with sodium meclofenamate was similar to the unsensitized steers (table 14). Thrombocytopenia was evident but concentrations of fibrinogen and plasma

Figure 1. Photomicrograph of section of lung of steer (died of endotoxic shock). Note edema, emphysema, hemorrhage and infiltration with polymorphonuclear cells H and E stain (120 X).

Figure 2. Photomicrograph of section of lung of steer (euthanitized 24 h after inducing anaphylactic type shock). Note hemorrhage, edema, emphysema and infiltration with polymorphonuclear cells, H and E stain (120 X).



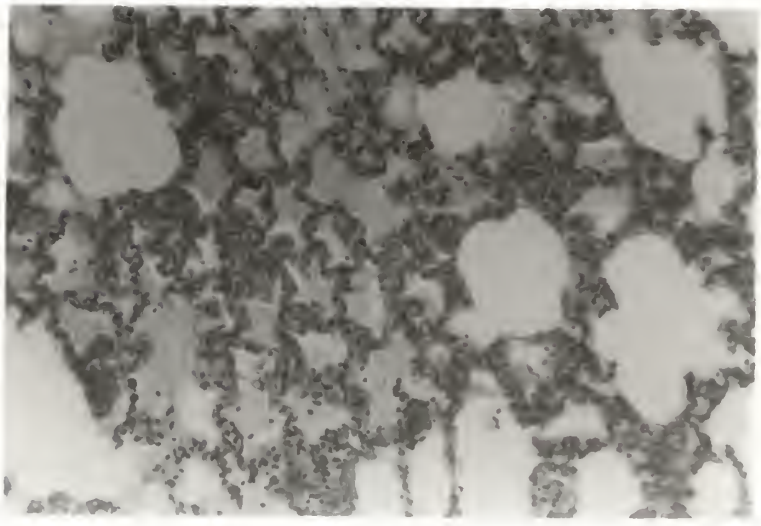


TABLE 11. LEUKOCYTE RESPONSE IN SODIUM MECLOFENAMATE TREATED STEERS TO ESCHERICHIA COLI ENDOTOXIN ADMINISTRATION[a]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	10533 ± 1644[b]	0	3220	6419	594	237	57
5	7467 ± 1498	0	2479	4719	58	137	68
15	6867 ± 1332	0	1579	5065	59	107	53
30	2967 ± 808	0	80	2736	0	143	0
60	2367 ± 723	46	30	2152	0	129	0
120	1800 ± 436	12	148	1624	8	80	30
180	1467 ± 551	3	168	1210	7	72	3
360	2733 ± 1790	104	857	1388	9	348	22
720	13700 ± 7519	917	8643	3698	72	324	0
1440	21633 ± 8598	3402	11716	3104	582	150	0

[a]Sodium meclofenamate injected intravenously (2 mg/kg body weight) five minutes before endotoxin (10 μg/kg body weight) injection.

[b]Mean ± standard deviation of three steers.

TABLE 12. HEMATOLOGICAL CHANGES IN SODIUM MECLOFENAMATE TREATED STEERS TO ESCHERICHIA COLI ENDOTOXIN ADMINISTRATION[a,b]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)	Plasma protein (g/dl)	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	664±172	533±208	7.2±.3	9.7± 0	66± 9
5	655±231	400±115	7.0±.3	9.4±.2	68±10
15	687±209	467±150	7.2±.4	9.6±.4	69±10
30	601±363	500±100	7.1±.4	10.0±.5	70±13
60	552±183	533±252	6.9±.2	9.3±.1	53±22
120	393± 76	500±265	6.6±.2	8.6±.2	65± 9
180	319±153	467±153	6.4±.1	8.2±.2	52± 8
360	278± 11	600[c]	6.4[c]	8.0±.5	44± 9
720	126[c]	467±252	6.4±.1	7.4±.5	40± 7
1440	127± 35	467±208	6.4±.2	7.5±.8	46± 7

[a]Sodium meclofenamate injected intravenously (2 mg/kg body weight) five minutes before endotoxin (10 µg/kg body weight) injection.

[b]Mean ± standard deviation of three steers.

[c]Mean of two steers.

TABLE 13. PROTHROMBIN AND PARTIAL THROMBOPLASTIN TIME IN SODIUM MECLOFENAMATE TREATED STEERS TO ESCHERICHIA COLI ADMINISTRATION[a,b]

Sampling time (min)	Prothrombin time (sec)	Partial thromboplastin time (sec)
0	16.6(15.8)[c]	35.0(38.4)
5	-	-
15	-	-
30	-	-
60	15.9(15.5)	40.6(35.9)
120	16.6(16.1)	39.9(33.9)
180	19.4(16.5)	36.6(34.3)
360	19.7(15.8)	43.9(36.2)
720	-	-
1440	22.4(16.4)	46.5(36.9)

[a]Sodium meclofenamate injected intravenously (2 mg/kg body weight) five minutes before endotoxin injection.

[b]Mean of two steers.

[c]Numbers in parentheses are control values.

TABLE 14. LEUKOCYTE RESPONSE IN SODIUM MECLOFENAMATE TREATED AND ENDOTOXIN SENSITIZED STEERS TO INTRAVENOUS CHALLENGE WITH ESCHERICHIA COLI ENDOTOXIN[a]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	15650 ± 4398[b]	971	8443	5245	717	147	110
5	9600 ± 3030	524	3121	5480	273	71	77
15	6375 ± 3914	104	2248	3869	83	19	53
30	3767 ± 1185	0	104	3480	23	145	15
60	2875 ± 910	21	21	2695	21	90	14
120	2775 ± 866	32	265	2345	50	64	13
180	2725 ± 932	126	319	2042	21	184	33
360	9475 ± 4094	894	3462	4422	133	512	0
720	17150 ± 6348	1898	8222	6287	146	533	33
1440	17650 ± 6981	721	9778	6578	432	141	0

[a]Sodium meclofenamate (2 mg/kg body weight) given intravenously five minutes before endotoxin challenge (10 μg/kg body weight).

[b]Mean ± standard deviation of four steers.

protein did not change. Serum calcium, plasma glucose, PT and APTT also remained unchanged (table 15 and 16).

Pathological changes.Both sensitized and unsensitized steers treated with sodium meclofenamate showed pulmonary edema.

DISCUSSION

Clinical signs and associated pathophysiological changes typical of endotoxemia were seen in steers injected intravenously with Escherichia coli endotoxin (Osborne, 1965; Burrows, 1970, 1971; Musa et al., 1972; Reece and Wahlstrom, 1973; Thomson et al., 1974; Griel et al., 1975). The characteristic changes observed were leukopenia followed by leukocytosis, neutropenia followed by neutrophilia, lymphopenia, thrombocytopenia, lowered serum calcium, and hyperglycemia followed by hypoglycemia. Disseminated intravascular coagulation (DIC), a syndrome of acute, transient coagulation of circulating blood resulting in fibrin thrombi, is considered to be secondary to endotoxemia (Mckay and Shapiro, 1956; Gans and Krivit, 1960; Yoshikawa et al., 1971; Thomson et al., 1974). It is characterized by an initial decrease in fibrinogen level, the presence of soluble fibrin and fibrinogen degredation products, and a clotting factor deficiency. Decrease in fibrinogen level is a feature following intravenous injection of endotoxin in many animal species such as dogs (Gans and Krivit, 1960; From et al., 1975), rats (Muller-Berghaus et al., 1967), baboons (Cavanagh et al., 1970), and cattle (Lumsden et al., 1974). Decrease fibrinogen is the result of increased utilization of the fibrinogen (Yoshikawa et al., 1971). The blood changes in this experiment indicating the occurance of DIC were thrombocytopenia, prolonged prothrombin time, and decrease in fibrinogen concentration.

TABLE 15. HEMATOLOGICAL CHANGES IN SODIUM MECLOFENAMATE TREATED AND ENDOTOXIN SENSITIZED STEERS CHALLENGED INTRAVENOUSLY WITH ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)	Plasma protein (g/dl)	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	690±866	600±141	6.9±.2	9.2±.5	62± 8
5	685±779	475± 96	6.9±.2	9.1±.6	63± 7
15	671±859	500±115	6.9±.4	9.1±.6	64± 9
30	224±200	467±231	6.7±.1	9.1±.5	61±11
60	549±645	525±150	6.9±.2	9.0±.6	61±13
120	346±314	500±141	6.5±.2	8.6±.4	59±13
180	280±164	400±183	6.4±.3	8.5±.7	59± 6
360	262±163	500±283	6.3±.2	8.5±.7	52± 5
720	244±191	575± 96	6.8±.2	8.5±.2	51± 7
1440	313±228	450±173	6.5±.2	8.7±.4	61± 9

[a]Sodium meclofenamate (2 mg/kg body weight) given intravenously five minutes before endotoxin challenge (10 µg/kg body weight).

[b]Mean ± standard deviation of four steers.

TABLE 16. PROTHROMBIN AND PARTIAL THROMBOPASTIN TIME IN SODIUM MECLOFENAMATE TREATED AND ENDOTOXIN SENSITIZED STEERS TO ESCHERICHIA COLI ENDOTOXIN ADMINISTRATION[a,b]

Sampling time (min)	Prothrombin time (sec)	Partial thromboplastin time (sec)
0	17.0 (15.5)[c]	40.0 (35.8)
5	-	-
15	-	-
30	-	-
60	-	-
120	-	-
180	18.5 (16.6)	39.4 (36.8)
360	22.5 (16.1)	39.1 (36.6)
720	-	-
1440	18.5 (15.8)	39.2 (37.6)

[a]Sodium meclofenamate (2 mg/kg body weight) given intravenously five minutes before endotoxin challenge (10 µg/kg body weight).

[b]Mean of four steers.

[c]Numbers in parentheses are control values.

Intravenous injection of steers with Escherichia coli endotoxin on days 1 and 4 followed by a challenge dose on day 5 or 7 resulted in clinical signs and associated pathophysiological changes similar to steers receiving only one injection. The main difference was the sudden collapse of sensitized steers into lateral recumbency within seconds of endotoxin administration. This enhanced response suggests an anaphylactic-type reaction (Weil and Spink, 1957).

Sodium meclofenamate has been shown to prevent collapse due to anaphylactic shock (Aitken et al., 1975). Sudden collapse is due to hypotension (Aitken and Sanford, 1969a) resulting from the production of bradykinin during anaphylactic reaction (Eyre and Lewis, 1972). Sodium meclofenamate, a kinin inhibitor (Collier et al., 1968), markedly antagonizes bovine anaphylaxis in vivo (Aitken and Sanford, 1969b; Alexander et al., 1970; Eyre, 1970, 1971). Administration of sodium meclofenamate to sensitized steers prevented the initial reaction (collapse) to the endotoxin challenge. Clinical signs of endotoxemia were not present until several minutes after injection in sensitized and unsensitized steers. Hematological changes and pathological lesions in sensitized steers with anaphylactic-type shock were similar to that of the steers with endotoxin shock. Hematological changes such as the leukocyte response and the various coagulation measurements do occur in cattle with anaphylactic shock due to any antigenic stimulus such as egg albumin or horse serum (Eyre et al., 1973). Therefore, it is difficult to distinguish between endotoxic and anaphylactic-type shock based on blood changes.

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PART II

RUMINAL ABSORPTION OF ENDOTOXIN IN STEERS

INTRODUCTION

Rumen bacterial endotoxin has been suggested to play a role in the pathogenesis of lactic acidosis and the sudden death syndrome in cattle (Dougherty, 1976 and Nagaraja et al., 1979). In order for rumen bacterial endotoxin to participate in the disease process, it must be absorbed into the blood from the gastrointestinal tract. Because of the large pool of endotoxin and the availability of large absorptive area, rumen appears to be the logical site for endotoxin absorption. Also, endotoxin passed into the abomasum or small intestine may presumably get inactivated by acid or enzymes (Nagaraja et al., 1979). Huber et al.(1979) was unable to demonstrate endotoxin absorption from the duodenum of sheep. Lassman (1980) found no radioactivity in lymph and blood samples collected from thoracic duct and portal vein of steers following intraruminal administration of radioactive (chromium 51) endotoxin. It was concluded that normal healthy rumen epithelium is impermeable to endotoxin molecule. The objective of this study was to test absorption of endotoxin from the rumen of cattle with inflamed or damaged rumen epithelium.

MATERIALS AND METHODS

Experimental Animals and Diet. Nine Holstein steers aged 4 to 8 months with body weights ranging from 100 to 225 kg were used in absorption study. All steers were fed twice a day with an all alfalfa hay diet. Steers were divided into groups of three animals each. Steers in group 2 and 3 were rumen fistulated. All steers were sensitized to endotoxin by injecting intravenously Escherichia coli endotoxin (10 µg/kg body weight) on days 1 and 5. The ability of the animal to absorb endotoxin from the rumen was tested on day 7. Steers in group 1 received no change in the diet. Steers in groups 2 and 3 were treated to produce inflammation of the rumen epithelium. Steers in group 2 were induced with lactic acidosis by infusing glucose solution (12.5 g/kg body weight) (Cerulose Dextrose 2001, Corn products Co., Englewood Cliffs, NJ) 12 hours before testing endotoxin absorption. Ruminal pH of glucose infused steers ranged from 4.2 to 4.6 in about 12 hours. The rumens of steers in group 3 were emptied and flushed with ammonia gas to produce severe ruminitis. Infusion of ammonia gas was continued until signs of ammonia toxicity were evident (convulsions and collapse).

Endotoxin absorption was tested by infusing 1 g Escherichia coli endotoxin dissolved in sterile saline into the rumen either through the fistula or injected with long needle (6 inch, 18 guage). Endotoxin absorption was monitored by observing clinical signs and blood analyses for characteristic endotoxin induced changes. Blood samples were collected before, and at 15, 30, 60, 170, 180, 360, 720, and 1440 minutes after intraruminal endotoxin administration. The following determinations were made on the blood samples: total and differential leukocyte counts, platelet counts, and serum calcium and plasma glucose levels. Blood samples from the six rumen-fistulated steers were tested for fibrinogen and plasma protein.

RESULTS

Clinical signs. None of the steers showed any signs suggestive of endotoxin shock following intraruminal administration of E. coli endotoxin. Steers in groups 2 and 3 showed signs of acidosis and ammonia toxicosis, respectively. Steers with lactic acidosis remained dull and anorectic while steers infused with ammonia exhibited convulsions.

Blood changes. Leukocyte counts in all 3 groups of steers remained in the normal range. Leukopenia followed by leukocytosis suggestive of endotoxemia was absent. (tables 1, 3, and 5). Platelet counts, fibrinogen, plasma protein, and serum calcium concentrations remained unchanged in all steers following intraruminal administration of endotoxin. Steers with induced acidosis and ammonia toxicity showed initial hyperglycemia which returned to normal in 24 hours. Hypoglycemia suggestive of endotoxemia was not evident.

DISCUSSION

Endotoxin absorption from the gastrointestinal tract has been a very controversial topic. Several researchers have shown that endotoxins are absorbed in normal and in disease conditions as well as under in vitro conditions (Ravin et al., 1960; Ravin and Fine, 1962; Kocsar et al., 1969; Gans and Metsumoto, 1974 a,b; Nolan, 1975; Nolan et al., 1977)

In cattle with adequate roughage intake, rumen epithelium is normal and without lesions. The rumen epithelium of cattle on high grain rations can undergo extensive degeneration. For example, keratinization, parakeratosis, necrosis, and infiltration of hair may result in tunnels through which rather large molecules and even bacteria may pass (Fell et al., 1968, 1972; McGavin and Morrill, 1976; Szemeredy and Raul, 1976). Lassman (1980) tried to induce inflammation of rumen epithelium by feeding a high grain ration. However, although papillae were

TABLE 1. LEUKOCYTE RESPONSE IN HAY-FED STEERS TO INTRARUMINAL ADMINISTRATION OF ESCHERICHIA COLI ENDOTOXIN[a]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	12367 ± 3050[c]	51	6779	4783	733	21	0
5[c]	-	-	-	-	-	-	-
15	12467 ± 2084	95	6959	4877	335	34	43
30	12733 ± 2350	0	6784	5343	463	143	0
60	12150[d]	161	7035	4710	243	0	0
120	9533 ± 3150	277	4967	3781	476	32	0
180	18267 ± 3479	143	12683	4853	522	33	33
360	21800[d]	0	13739	7300	708	54	0
720	16733 ± 1858	90	10600	6178	535	177	220
1440	13533 ± 1305	0	6190	5761	520	313	67

[a]Endotoxin dose was 1 g/steer, administered on day 7.

[b]Mean ± standard deviation of three steers sensitized to Escherichia coli endotoxin by injecting endotoxin (10 μg/kg body weight) intravenously on days 1 and 4.

[c]Not sampled.

[d]Mean of two steers.

TABLE 2. HEMATOLOGICAL CHANGES IN HAY-FED STEERS TO INTRARUMINAL ADMINISTRATION OF ESCHERICHIA COLI ENDOTOXIN[a]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)[d]	Plasma protein (g/dl)[d]	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	603 ± 206[b]	-	-	$9.6 \pm .1$	71 ± 7
5[c]	-	-	-	-	-
15	625 ± 274	-	-	$9.3 \pm .4$	69 ± 9
30	607 ± 267	-	-	$9.4 \pm .4$	70 ± 6
60	698[e]	-	-	$9.3 \pm .6$	67 ± 7
120	667 ± 348	-	-	$9.8 \pm .7$	66 ± 16
180	643 ± 328	-	-	$10.1 \pm .2$	68 ± 12
360	740[f]	-	-	$9.8 \pm .5$	65[e]
720	755[e]	-	-	$9.9 \pm .4$	68 ± 16
1440	760 ± 325	-	-	$9.6 \pm .3$	66 ± 14

[a]Endotoxin dose was 1 g/steer, administered on day 7.

[b]Mean $\pm$ standard deviation of three steers sensitized to Escherichia coli endotoxin by injecting endotoxin (10 µg/kg body weight) intravenously on days 1 and 4.

[c]Not sampled.

[d]Not determined.

[e]Mean for two steers.

[f]Value for one steer.

TABLE 3. LEUKOCYTE RESPONSE IN STEERS WITH INDUCED LACTIC ACIDOSIS TO INTRARUMINAL ADMINISTRATION OF ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	13500 ± 10054[c]	709	7767	4937	163	0	0
5[d]	-	-	-	-	-	-	-
15	13233 ± 9128	468	8116	4574	255	98	0
30	13200 ± 9271	579	7870	4535	237	31	22
60	15733 ± 12768	724	9926	4980	200	0	0
120	16633 ± 13134	1663	10398	4132	420	18	0
180	15967 ± 12583	1119	9865	4827	352	0	0
360	13400 ± 9103	718	7948	4042	535	227	0
720	10633 ± 6201	497	5924	3993	225	28	20
1440	9066 ± 2159	73	4736	3949	288	31	23

[a]Acidosis was induced with glucose.

[b]Endotoxin dose was 1 g/steer, administered on day 7.

[c]Mean ± standard deviation of three steers sensitized to Escherica coli endotoxin by injecting endotoxin (10 μg/kg body weight) intravenously on days 1 and 4.

[d]Not sampled.

TABLE 4. HEMATOLOGICAL CHANGES IN STEERS WITH INDUCED LACTIC ACIDOSIS TO INTRARUMINAL ADMINISTRATION OF ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)	Plasma protein (g/dl)	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	847 ± 353[c]	567 ± 58	8.4 ± 1.7	7.6 ± 3.2	103 ± 31
5[d]	-	-	-	-	-
15	925 ± 351	533 ± 58	8.3 ± 1.7	7.8 ± 3.0	90 ± 27
30	748 ± 153	600 ± 100	8.3 ± 1.8	9.3 ± 1.0	84 ± 21
60	872 ± 240	633 ± 153	8.1 ± 1.9	8.5 ± 1.6	78 ± 16
120	793 ± 215	567 ± 153	7.9 ± 1.6	7.7 ± 3.2	68 ± 18
180	732 ± 219	533 ± 115	7.8 ± 1.5	$9.9 \pm .8$	76 ± 25
360	736 ± 236	633 ± 153	7.8 ± 1.3	$9.3 \pm .1$	85 ± 48
720	-	567 ± 153	$7.6 \pm .8$	8.4 ± 1.0	65 ± 28
1440	965 ± 363	600 ± 100	$7.6 \pm .5$	8.2 ± 1.6	56 ± 15

[a]Acidosis was induced with glucose.

[b]Endotoxin dose was 1 g/steer administered on day 7.

[c]Mean $\pm$ standard deviation of three steers sensitized to Eschrichia coli endotoxin by injecting endotoxin (10 µg/kg body weight) intravenously on days 1 and 4.

[d]Not determined.

TABLE 5. LEUKOCYTE RESPONSE IN STEERS WITH SEVERE, INDUCED RUMENITIS TO INTRARUMINAL ADMINISTRATION OF ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Total WBC/μl	Differential counts in absolute numbers/μl					
		Neutrophils		Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		Band	Seg				
0	12413 ± 501[c]	123	6593	5510	240	117	0
5[d]	-	-	-	-	-	-	-
15	12133 ± 808	77	7220	4537	160	203	0
30	12600 ± 1277	220	7703	4490	157	120	43
60	14900 ± 1825	197	9557	4793	347	143	53
120	20500 ± 2551	880	14250	5090	413	57	57
180	21633 ± 4727	1363	14700	4770	917	157	53
360	20333 ± 6691	1230	13557	4433	1217	130	70
720	16433 ± 6730	733	11270	3970	413	157	0
1440	14667 ± 6253	1167	8560	4487	447	100	0

[a]Severe rumenitis was induced by infusing NH_3 gas.

[b]Endotoxin dose was 1 g/steer, administered on day 7.

[c]Mean ± standard deviation of three steers sensitized to Escherichia coli endotoxin by injecting endotoxin (10 μg/kg body weight) intravenously on days 1 and 4.

[d]Not sampled.

TABLE 6. HEMATOLOGICAL CHANGES IN STEERS WITH SEVERE, INDUCED RUMENITIS TO INTRARUMINAL ADMINISTRATION OF ESCHERICHIA COLI ENDOTOXIN[a,b]

Sampling time (min)	Platelet count ($x10^3/\mu l$)	Fibrinogen (mg/dl)	Plasma protein (g/dl)	Serum calcium (mg/dl)	Plasma glucose (mg/dl)
0	713±211[c]	567±153	7.5±.5	8.3±.1	182±114
5[d]	-	-	-	-	-
15	689±182	467± 58	7.2±.2	8.5±.7	177±112
30	710±259	467±115	7.2±.4	8.7±.3	170±113
60	658±199	433±153	7.2±.3	8.7±.4	141± 84
120	585±118	467±153	7.2±.4	8.8±.6	113± 59
180	612± 94	400±265	7.2±.3	8.7±.3	95± 46
360	669[e]	533±115	7.2±.2	8.8±.6	62± 19
720	-	467±153	7.2±.6	8.8±.5	65± 26
1440	645±264	500± 0	7.1±.7	8.6±.3	73± 34

[a]Severe rumenitis was induced by infusing NH_3 gas.

[b]Endotoxin dose was 1 g/steer, administered on day 7.

[c]Mean ± standard deviation of three steers sensitized to Escherichia coli endotoxin by injecting endotoxin (10 µg/kg body weight) intravenously on days 1 and 4.

[d]Not sampled.

[e]Mean for two steers.

darkened and some were keratinized, no lesions or misshappen papillae were found. In this experiment, inflammation was produced in one group of steers by inducing lactic acidosis until the rumen pH dropped to 4.2 to 4.6 and remained for at least two hours, and in another group, severe inflammation was produced by infusing ammonia gas. Also, steers were sensitized by intravenously injecting endotoxin on days 1 and 4 in order to enhance their sensitivity to endotoxin. However, clinical signs and pathophysiological changes suggesting endotoxemic or anaphylactic shock, were not seen. Evidently, the rumen epithelium is not permeable to endotoxin. If endotoxin does participate in the sudden death syndrome, it must be absorbed lower in the digestive tract, possibly in the duodenum, cranial to the common bile duct. This needs to be investigated.

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ENDOTOXIC AND ANAPHYLACTIC-TYPE SHOCK IN STEERS FROM INTRAVENOUS INJECTION OF ESCHERICHIA COLI ENDOTOXIN AND RUMINAL ABSORPTION OF ENDOTOXIN

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ABSTRACT

Endotoxin from rumen gram-negative bacteria has been suspected to be involved in certain diet induced diseases of cattle such as lactic acidosis and the sudden death syndrome. Endotoxin participation in the disease process may be either by endotoxin shock as a result of endotoxemia or an anaphylactic-type response as a result of hypersensitive reaction to the antigenic moiety of the endotoxin.

Holstein steers injected with Escherichia coli endotoxin were used as an experimental model to distinguish between endotoxic shock and anaphylactic-type shock. Endotoxin shock was induced by single intravenous injection of steers with Escherichia coli endotoxin (10 µg/kg body weight). Anaphylactic type shock was induced by sensitizing the steers with the intravenous injection of endotoxin on days 1 and 4 and injecting challenging dose on day 5 or 7.

Clinical signs observed: respiratory distress, salivation, urination, and diarrhea, were similar in endotoxic and anaphylactic shock. The only consistent difference was that the sensitized steers collapsed into recumbency within seconds of intravenous injection of challenging dose on day 5 or 7. Steers with endotoxic shock reacted several minutes after the endotoxin administration. Administration of sodium meclofenamate (2 mg/kg body weight) to sensitized steers prevented the initial reaction (collapse) to the endotoxin challenge. Blood changes in steers with anaphylactic-type shock were similar to that of the steers with endotoxin shock. There was leukopenia followed by leukocytosis, thrombocytopenia, decrease in fibrinogen and plasma protein, increase in prothrombin time, and hyperglycemia followed by hypoglycemia. Pathological changes were similar in both endotoxic and anaphylactic-type shock. Lesions were confined to the respiratory system with lungs being hemorrhagic and edematous.

Ruminal absorption of endotoxin was tested in steers by administering Escherichia coli endotoxin intraruminally. In order to increase the suseptibility of steers to endotoxin, they were sensitized with 10 μg endotoxin/kg body weight given intravenously on days 1 and 4. Also lactic acidosis and ammonia toxicity was induced in steers in an attempt to inflame rumen epithelium and facilitate endotoxin absorption. Endotoxin absorption was monitored by observing clinical signs and measuring blood changes indicative of endotoxemia. None of the steers exhibited clinical signs or had blood changes suggestive of ruminal absorption of endotoxin. It appears that ruminal epithelium is impermeable to endotoxin.