RUMEN FLUID FROM "SUDDEN DEATH," LACTIC ACIDOTIC, AND HEALTHY CATTLE AND ITS TOXIC EFFECT IN MICE

by

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

INTRODUCTION

Approximately two years ago sudden deaths were reported in 1000 to 1500 lb cattle being finished in several Kansas feedlots. Some lots experienced daily losses to this syndrome. The sudden death syndrome (SDS) was first reported in Canada by Niilo and Avery (1963). Losses in Kansas due to SDS have been reported by Anthony (1971a, b) and Turner (1971). The etiology of SDS is unknown. Anthony (1971a) gave four possible causes of SDS: bloat; toxic rumen gases; concentrate-roughage ratio; and infectious agents.

This study was initiated to determine changes from normal in certain phases of rumen function in cattle dying from SDS. Rumen fluid from cattle dying from SDS were analysed for pH, and for concentrations of volatile fatty acids, lactic acid, and histamine. Mice were inoculated with rumen fluid to determine if a toxin is present in rumen fluid of cattle dying from SDS. Similar determinations were made with rumen fluid from cattle fed roughage, from cattle manifesting induced lactic acidosis, and from feedlot cattle maintained on a high concentrate ration. The comparisons were made with cattle on different feeding regimens to understand better rumen function in feedlot cattle in hopes of gaining insight into the etiology of SDS. In this study SDS cattle are apparently healthy cattle in feedlots dying suddenly and without sign of sickness or exhibiting lesions indicative of other specific disease conditions.

REVIEW OF LITERATURE

Sudden Death Syndrome

The sudden death syndrome (SDS) in feedlot cattle was first reported in Canada by Niilo and Avery (1963). They associated SDS with Clostridium perfringens enterotoxemia. Batty et al. (1964) reported sudden deaths in heavily fed cattle due to Clostridium oedematiens (novyi). Niilo et al. (1967) reported that "sudden death" was one of the most common diagnoses made in cattle feedlots of western Canada. Approximately 42% of deaths in four feedlots in an 8 month period were due to SDS. Anthony (1971a) reported death losses in a number of major feedlots in Kansas. The cattle weighed from 1000 to 1150 lb. Some feedlots were said to have experienced daily losses from SDS. Turner (1971) also reported losses to SDS in Kansas feedlots.

Clinical signs have not been described because of the sudden demise of apparently healthy animals. Dead cattle have been found near the feed bunks, watering devices, and within the group of cattle in the pen. The usual history has been that pen riders observe the cattle as normal, only to return an hour or so later and find a dead animal (Anthony, 1971b). Evidence of struggling prior to death has been completely absent (Anthony, 1971a; Turner, 1971).

The most consistent necropsy lesion observed was hemorrhage and edema in the tissues located near the thoracic inlet and extending forward along the trachea (Anthony, 1971b; Turner, 1971). Turner (1971) also reported a layer of gelatinous edema between the tracheal mucosa and the cartilage rings and submucosa. These animals seem to bloat rapidly following death (Anthony, 1971b; Niilo, 1967; Turner, 1971). Gas formation has been

reported in the affected tissues (Anthony, 1971b). Histopathologic studies have revealed only congested blood vessels in the peritracheal tissues and areas of hemorrhage. Major organs did not reveal change and the heart was negative for pathologic lesions (Anthony, 1971b).

Different workers have incriminated various causes. Anthony (1971a) gave four possible causes of SDS: bloat, absorption of toxic rumen gases, concentrate:roughage ratio, and infectious agents. Niilo and Avery (1963) speculated that SDS and enterotoxemia were the same condition. But later work by Niilo et al. (1963) showed that intravenous administration of culture filtrates from Clostridium perfringens produced peracute deaths with either central nervous system, respiratory or generalized signs depending on which toxin type was administered. Later Niilo et al. (1967) eliminated Clostridium perfringens as a cause of SDS and concluded that Clostridium perfringens is not the causative agent in feedlot deaths. Batty et al. (1964) incriminated Clostridium oedematiens (novyi) as a cause of SDS. Niilo et al. (1969) conducted experiments with Clostridium novyi in cattle and concluded that Clostridium novyi infections were likely to show clinical signs and pathognomic lesions indicating localization of the clostridia rather than sudden death. Turner (1971) reports Clostridium novyi, sordelli and septicum as the probably etiologic agents of the disease due to the success of a multivalent two dose vaccine program. Anthony (1971a) reported such vaccination procedures did not control the problem. The role of clostridial organisms in SDS is confusing with no consistent patterns. The problem is further complicated because some clostridial organisms are normally present and are known to proliferate after death. No definite conclusions can be drawn concerning the role of clostridial organisms in SDS.

Anthony (1971b) reported that studies at one feedlot showed no correlation between death and feed consumption. Turner (1971) noted that cattle appear to be most susceptible to SDS when they are fed a finishing ration equal to or exceeding 80% concentrate and 20% roughage.

Bloat has been considered as a possible cause of SDS (Anthony, 1971a). Mills (1970) described the pathogenesis and lesions in bloat. The reported lesions were: compressed lungs; congestion and hemorrhage in the cervical esophagus; pale and blanched thoracic esophagus; pale and blanched lymph nodes and muscles in the caudal aspect of the cadaver; congestion and hemorrhage in the cranial half. Mucosal petechia and ecchymoses occurred throughout the length of the trachea. The musculature of the cranial part of the animal, especially the cervical and pectoral part was moderately to severely congested and hemorrhagic. Anthony (1971b) and Turner (1971) reported similar lesions in SDS. Mills (1970) eliminates clostridial infections, lightning stroke, or accidental electrocution in the diagnosis of bloat on the basis of redistribution of blood. Because of the similarity of the lesions between death due to bloat and SDS, bloat cannot be conclusively ruled out as a cause of SDS.

Rumen Toxins

One of the four possible causes mentioned by Anthony (1971a) was toxins in the rumen. In 1941 Dougherty investigated the possibility of toxins in the rumen in legume bloat in cattle. High levels of carbon monoxide and hydrogen sulfide gas were found. Later studies by Dougherty (1942) revealed histamine and hydrogen sulfide gas present in the rumen of cattle bloated on legume pasture. Concentrations of these occurring in vivo never reached those necessary to kill the animal in either

investigation. Dougherty and Cello (1949) found a toxic substance in the rumen digesta of sheep and cattle. The substance depressed the blood pressure of dogs and goats, inhibited rumen motility in sheep, stimulated respiration in all intact animals, stimulated motor activity of the lower gut in the intact dog, goat, and sheep, and had variable effects on isolated ileum segment of the rabbit. Digesta from two cows with acute indigestion had a strong depressing effect on the isolated gut, and had a more pronounced depressor effect on blood pressure of the dog than equal amounts of normal digesta. In some instances intravenous administration of digesta from atonic rumens were fatal to dogs. The toxic substance was heat-stable, dialyzable through a cellophane membrane, and not volatile when steam distilled.

Dougherty and Cello (1952) reported additional studies on toxic factors in the rumen fluid of cattle and sheep. Rumen fluid from sheep in acute overeating disease was decidedly more toxic than rumen fluid from the same animal before the induced grain overload. Plasma from acutely sick sheep depressed the blood pressure of dogs whereas plasma from normal sheep did not have this depressor effect. Injection of toxic rumen fluid caused a pronounced leukopenia. The leukopenia coincided with the fall in blood pressure. Dogs became refractory to subsequent intravenous injections of rumen fluid. The physiological reactions were similar to those produced by histamine. Antihistaminics gave a fair degree of protection. Absorption studies indicated that the toxic factor is absorbed from the rumen as well as from the intestinal tract. The toxic factor was not ether-extractable and was not inactivated by either pepsin or trypsin digestion. The factor was stable to change in pH.

Prier (1954) conducted experiments with dogs and sheep on toxic

factors produced by rumen microorganisms. Two blood pressure responses were noted. The blood pressure depressing action reported by Dougherty and Cello (1949) was found to be present in the filtrate from thick, short gram positive spore-forming rods in rumen fluid. An additional factor was also present that caused a marked rise in blood pressure. Pure cultures producing a rise in blood pressure were thin branching gram positive spore-forming rods. Respiratory rate markedly increased immediately following injection. They occurred simultaneously with the initial blood pressure change. In all cases respiration was increased in both depth and rate.

Mullenax et al. (1966) found toxic factors in rumen fluid and rumen bacteria that possess some of the properties of classical endotoxin in gram negative bacteria. When injected the first time into cattle or sheep the following physiologic responses were produced: leukopenia in 1 to 4 hr; hyperglycemia in 1 to 3 hr; an immediate increase in arterial blood pressure; a decrease in serum protein; momentary apnea and increased respiratory frequency; a decrease in rumen motility and eructation efficiency. The animals became refractory to subsequent injections. Intraruminal administration of a large quantity of glucose to a sheep to simulate grain engorgement also produced some of these responses. The amount of endotoxin present in the rumen was found to be two to three orders of magnitude higher than the lethal intravenous dose of E. coli endotoxin. The response noted was similar to a histamine reaction. It was proposed that the endotoxins of the gram negative rumen bacteria are absorbed and play a significant role in the pathogenesis of certain diet induced diseases.

Halmagyi (1963) described endotoxin shock in sheep. Circulatory changes consisted mainly of a marked rise in pulmonary arterial and

pulmonary arterial wedge pressure, a fall in cardiac output and in systemic arterial pressure. The respiratory response consisted mainly of a severe fall in lung compliance produced by terminal airway closure. Premedication with antihistamines failed to affect the response. It was noted that injection of endotoxin is followed by a prompt rise in plasma catecholamine level. Halmagyi cited Thomas who has suggested that death in endotoxin shock is caused by excessive adrenergic activity. Tikoff et al. (1966) observed hemodynamic effects of endotoxin in calves which were similar to those observed by Halmagyi.

Braude (1964) reported that acute endotoxic shock produced the following responses: high fever, shock resulting from a drastic fall in blood pressure, diarrhea, hemorrhage into the tissues, and muscular pain.

Rumen Histamine

Dain et al. (1955) reported the occurrence of the toxic amines, histamine and tyramine, in rumen digesta of experimentally overfed sheep. The severity of the grain engorgement was found to be directly correlated with the level of histamine in the rumen fluid. As the acidity of rumen fluid became lower than pH 5, histamine formation increased. Histamine has been found in normal rumen fluid by the following workers: Long (1970), Sanford (1963), Shinozaki (1957), Sjaastad (1967a), Sjaastad and Stormorken (1963), Stormorken and Sjaastad (1962), Van der Horst (1961) and Wrenn et al. (1964). There was no indication of rumen dysfunction caused by histamine in any of these papers. Histamine has been found in forages, especially certain silages. Dietary histamine has been reported by: Fowler (1962), Macpherson (1962), McDonald et al. (1963), Neumark et al. (1964), Okamoto (1964), and Sjaastad and Stormorken (1963). Rodwell (1953) found amino

acid decarboxylases producing histamine from histidine in strains of Lactobacillus isolated from sheep rumen fluid.

The histamine content of various organs of the bovine was first reported by Bolotti (1951). Sanford (1962) and Sjaastad (1967b) reported the distribution of histamine in tissues of the ruminant digestive tract. The abomasum had a higher histamine content than did the rumen, reticulum and omasum (Sanford, 1962 and Sjaastad, 1967b). Lorenz et al. (1970) found similar concentrations of histamine in abomasal mucosa. The concentration of histamine in the fore-stomachs was lower than that in the small intestine (Sjaastad, 1967b).

The physiologic responses to histamine are many and varied. Recently Reite (1972) reviewed the physiology of histamine action. Histamine lowers blood pressure (hypotensive); stimulates heart action (similar to catecholamine action - histamine may cause release of epinephrine); may stimulate or relax vascular smooth muscle; increases capillary permeability; stimulates extravascular smooth muscle; stimulates gastric secretion (probably as a local chemostimulator); stimulates bronchial constriction; initiates classical triple reaction on the skin; and causes general hypersecretion. In ruminants intravenously administered histamine has been shown to inhibit rumen motility (Clark, 1950; Dougherty, 1942b; Duncan, 1954). Intraruminal administration of histamine failed to inhibit rumen motility (Sjaastad, 1967a; Taylor, 1968). Dougherty and Cello (1952) sprayed histamine on the dorsal rumen wall and inhibited rumen motility. Sanford (1958, 1961) inhibited contraction of isolated perfused rumen tissue by the addition of histamine. Alexander et al. (1967) reported the physiologic responses of sheep to intravenous administration of histamine. The respiratory signs were: hyperpnoea; increased pleural pressure;

increased bronchial resistance; bronchoconstriction; dyspnea; pulmonary edema; and dilatation of vessels in the thorax. The circulatory signs were: hypotension followed by hypertension; pulmonary arterial hypertension; and marked hemoconcentration. The post mortem lesions were: hydropericardium; hydrothorax; ascites; generalized petechial hemorrhage; kidney necrosis; and some lung collapse. Turner and Hodgetts (1949-1959) observed extreme hemoconcentration following intravenous administration of histamine to sheep. Histamine had no effect on the isolated perfused sheep spleen, so it was concluded that histamine action was indirect, and was probably by release of catecholamines from the adrenal medulla. Nilsson (1963) reported that histamine increased the secretion of the salivary glands, pancreas, intestinal glands, and sweat glands in cattle.

Neumark et al. (1964) and Neumark and Tadmor (1968) reported an appetite depressing action of histamine in silage especially in combination with formic acid. However, Macpherson (1962), McDonald et al. (1963), and Okamoto et al. (1964) found no correlation between histamine in forages and feed intake.

The question of histamine absorption from the rumen has received considerable attention. Dougherty and Cello (1952) conclude that histamine is absorbed from the rumen because of systemic effects produced after histamine was sprayed on the dorsal rumen wall. Sjaastad (1967c) and Stormorken and Sjaastad (1962) conclude that histamine is absorbed from the rumen because of its rapid disappearance from the rumen. Histamine inactivation was discounted because of very little inactivation in vitro (Sjaastad, 1967c). Shinozaki (1957) infused histamine into an artificially prepared rumen pouch and found little absorption. McDonald (1963) reported that histamine was not detected in blood samples taken at hourly

intervals for four hours after dosing with histamine intraruminally and concluded that there was no absorption from the rumen. Crevasse (1963) reported variable results on histamine absorption from the rumen. After intraruminal administration of histamine two of three cases showed no absorption from the rumen and one case demonstrated absorption based on systemic signs. Dunlop et al. (1965) reported no absorption from the rumen at normal rumen pH. Absorption was noted at pH 9.0 but not when normal values existed. Neumark (1967) and Neumark and Tadmor (1968) reported that histamine is not absorbed from the rumen but that the site of absorption is post ruminal. Their results showed omasal absorption of histamine. They conclude that there is little intestinal absorption of histamine because histamine would have to pass through the acidic abomasum and would be inactivated. Sjaastad and Kay (1966) found virtually no absorption of histamine from the washed-out, tied-off rumen of anesthetized sheep. Taylor (1968) found very poor or no absorption based on lack of increased blood histamine and lack of systemic signs. Dickinson (1969) concluded that histamine is not absorbed from the rumen when no difference in histamine content was found between jugular and rumen vein blood after intraruminal administration of histamine. Dunlop and Stefaniak (1965) and Sjaastad and Kay (1966) reported that histamine is readily absorbed from the intestines in ruminants. Histamine was shown to be absorbed from the intestinal tract of monogastrics by Irvine et al. (1959) and Meakins and Harington (1922).

There are four different pathways by which histamine is inactivated (Reite, 1972). These are: oxidation by diamine oxidase, oxidation by monamine oxidase, methylation by ring nitrogen, and acetylation. In mammals diamine oxidase and histamine-N-methyltransferase are the two quantitatively

most important enzymes for the metabolism of histamine (Reite, 1972). Histamine-*N*-methyltransferase has been found in abomasal mucosa by Lorenz (1970). Histaminase or diamine oxidase has been reported in various tissues in the ruminant by Ambrosi (1954), Dickinson (1969), and Sjaastad (1967b, e, f, g, h). Sjaastad (1967e) reported that sheep liver can catabolize great quantities of histamine (178 $\pm$ 57.4 mcg histamine/g wet tissue/hr). At optimal substrate concentrations sheep kidney tissue was found to inactivate 429 $\pm$ 102 mcg histamine/g wet tissue/hr (Sjaastad, 1967f). Sjaastad (1967b) reported little inactivation of histamine by ruminant forestomach tissue but considerable inactivation by intestinal tissue. Sjaastad (1967h) found histamine inactivation in sheep plasma and considerable inactivation in the sheep rumen (Sjaastad, 1967c, g). Sjaastad and Kay (1966) reported that less than 10% of orally administered histamine to sheep reaches the duodenum in a biologically active state. The remaining 90% is probably degraded by the rumen microbes. When histamine is catabolized the imidazole ring always remains intact (Schayer, 1952). Sjaastad and Kay (1970) conducted experiments that suggested that the imidazole ring of dietary histamine is ruptured in the rumen. Sjaastad (1967b, e, g, h) reported that histamine inactivation by digestive tract tissues, liver tissue, kidney tissue, rumen fluid, and sheep plasma all could be inhibited by addition of aminoguanidine. Aminoguanidine has been shown to be a potent inhibitor of diamine oxidase or histaminase (Schuler, 1952). Addition of aminoguanidine to sheep after oral administration of histamine produced toxic signs due to histamine whereas without aminoguanidine there were no signs from the orally administered histamine (Sjaastad, 1967g). Formaldehyde and formic acid alter the metabolism of histamine in sheep (Eliassen and Sjaastad, 1968). Formaldehyde and histamine

together produced toxicity and increased the urinary excretion of biologically active histamine. Formic acid and formaldehyde inhibited inactivation of histamine by liver and kidney tissue. It was concluded that formaldehyde probably inhibits the action of diamine oxidase. This could be of significance since formic acid has been found in fairly high levels in the rumen of animals fed high concentrate rations (Ryan, 1964a). Neumark (1967) and Neumark and Tadmor (1968) reported that the combination of histamine and formic acid in the rumen and omasum caused cessation of rumen motility and depressed feed intake. Studies on the metabolism of ^{14}C-histamine in the goat (Eliassen, 1969), and cow and sheep (Eliassen, 1971) showed that oxidative deamination was the only pathway of any quantitative importance for the degradation of histamine in these species. There was little difference between these three species of ruminants. Most of the injected radioactivity was excreted in the urine within 24 hr indicating that histamine is not retained in the body for a long period of time.

The excretion of histamine in urine was shown by Anrep et al. (1944). It was reported that carnivores and herbivores differ in form and amount of histamine excreted. Carnivores excrete 98-100% in the conjugated form and in large quantities whereas herbivores excrete only very small quantities of free histamine. In contrast to Anrep's results Sjaastad (1970) found that more conjugated than free histamine was excreted in sheep urine. The level of histamine in the urine is dependent on the dietary level (Wrenn et al., 1964). However Sjaastad (1967d, 1970) found very little difference between intake of histamine and urinary excretion. Little histamine was excreted in the feces of sheep; most was via the urine (Sjaastad, 1967d). Sjaastad and Kay (1970) reported that 28% of the

radioactivity after oral administration of ^{14}C-histamine appeared in the exhaled CO_2. This took place after the rupture of the imidazole ring. Sjaastad and Sjaastad (1971) demonstrated rupture of the imidazole ring of histamine by human intestinal contents. Up to 42% of the ^{14}C-histamine was recovered as $^{14}CO_2$. They conclude that microbial degradation of histamine in the monogastric intestinal tract and rumen are similar.

The different compartments of the forestomachs differ in their sensitivity to histamine. Neumark (1967) and Neumark and Tadmor (1968) showed that the most sensitive areas are the esophageal groove, omasum, omasal groove, and abomasum. They reported that the omasum may be the most sensitive. Histamine was infused into the omasum and rumen motility ceased. Sanford (1958, 1961) conducted experiments that showed the omasum and abomasum to be more sensitive to histamine than the rumen and reticulum. Staniszewska-Borkowska (1969) observed abomasal ulcers when histamine was infused into the abomasum of goats. Sjaastad (1967c) reported that when histamine was administered orally, the histamine concentration was also high in the abomasum. Histamine remained at detectable levels for a longer period in the abomasum than in the rumen. This is probably because histamine is not absorbed in an acidic medium (Dunlop et al., 1965). Histamine does not affect either the type or severity of lesions in rumenitis (Ahrens, 1967). Taylor (1968) reported that histamine has no effect on the rumen papillae.

Cattle on roughage have very little accumulation of histamine in the rumen (Ahrens, 1967; Long, 1970; Sanford, 1963; Sjaastad and Stormorken, 1963; Van der Horst, 1961). This is probably due to a lack of a suitable rumen flora to decarboxylate histidine to form histamine (Krogh, 1963; Rodwell, 1953) and because of rapid inactivation of histamine in the rumen

when roughage is fed (Sjaastad, 1967c, g; and Sjaastad and Kay, 1966, 1970). High ruminal concentrations of histamine have been reported in lactic acidosis (Ahrens, 1967; Dain et al., 1955; Dunlop, 1967; Turner and Hodgetts, 1949-1959) and in ruminants maintained on high concentrate rations or very fermentable legumes (Maclean, 1966; Rodwell, 1953; Shinozaki, 1957). Table 1 shows the rumen histamine values reported by various workers.

The toxicity of histamine in cattle is not well understood. The animal has a great capacity to metabolize histamine as reported by Eliassen (1971), Sjaastad (1967b, c, e, f, g, h), and Sjaastad and Kay (1966, 1970). Dain et al. (1955) found a positive correlation between the degree of sickness in lactic acidosis and rumen histamine concentrations. In contrast to this Broberg (1960) reported that histamine plays no role in lactic acidosis. Histamine has been reported to cause laminitis in cattle by Maclean (1966) and Nilsson (1963). Desliens (1958) reported the lethal dose of histamine for cattle to be 0.06 mg/kg. The lethal intraperitoneal dose is 18 mg/kg (Barnes and Eltherington, 1966). The lethal intravenous dose in dogs is 30 mg/kg and in monkeys 50 mg/kg (Barnes and Eltherington, 1966). Comparatively cattle were the most sensitive animals studied. The lethal dose for cattle was approximately one thousand times less than for guinea pigs, dogs, and monkeys and ten thousand times less than that for mice. In studies on rumen motility, Dougherty (1942) gave a total of 2-5 mg intravenously. For a 500 kg animal the doses ranged from 0.004 - 0.010 mg/kg. Duncan (1954) observed rumen stasis in 30 kg sheep when 0.1 - 0.5 mg of histamine was given intravenously. This dosage is equivalent to 0.0033 - 0.0167 mg/kg. Clark (1950) produced rumen stasis with doses of 1-2 mg given intravenously

TABLE 1. RUMEN HISTAMINE VALUES REPORTED BY VARIOUS INVESTIGATORS

Animal	Treatment and/or diet	Histamine concentration mcg/ml	Rumen pH	Signs	Investigator
Sheep	silage	135.0	...	none	Sjaastad (1967a)
	hay	0.2			
Sheep and goats	legume pasture	2.9-5.6	>5	none	Shinozaki (1957)
Cattle	legume pasture	0.25	...	bloat	Dougherty (1942)
Cattle and sheep	roughage and concentrate (8% CP)	0.498	...	none	Long and Little (1970)
	roughage and concentrate (16% CP)	1.374	...	none	
Sheep	Glucose added to rumen fluid *in vitro*	7.8	5.0	....	Sanford (1963)
Sheep	wheat added to rumen fluid *in vitro*	1.7	3.5-4.0	....	Rodwell (1953)
Cattle	roughage experimentally engorged with wheat	0.057	6.7	none	Ahrens (1967)
		7.10	4.0	lactic acidosis	
Cattle	roughage experimentally engorged with wheat	0.20	...	none	Dunlop (1967)
		7.10	...	lactic acidosis	
Sheep	experimentally engorged with wheat	3.3	4.2	lactic acidosis	Turner and Hodgetts (1949-1959)
Sheep	experimentally engorged with wheat	20-70	4.0	lactic acidosis	Dain *et al.* (1955)

(equivalent to 0.033-0.067 mg/kg). Alexander et al. (1967) reported that sheep are not especially sensitive to histamine. Doses ranging from 0.2-3.2 mg/kg given intravenously produced effects from which unanesthetized sheep recovered quickly. There are no reported deaths in cattle due to histamine.

Rumen Lactic Acid

It is well known that high concentrations of lactic acid in the rumen are toxic. The concentration of lactic acid in the rumen of animals fed roughage rations is low (less than 1 mg/100 ml or not detectable) (Balch and Rowland, 1957; Jayasuriya and Hungate, 1959; Phillipson, 1952; Waldo and Schultz, 1955). Extremely high concentrations have been found when animals were fed readily available carbohydrates (Balch and Rowland, 1957; Briggs et al., 1957; Broberg, 1960; Dirksen, 1965; Dunlop and Hammond, 1965; Hyldgaard-Jensen and Simesen, 1966; Juhasz and Szegedi, 1968; Reid et al., 1957; Turner and Hodgetts, 1949-1959). Concentrations of more than 1000 mg/100 ml have been reported (Broberg, 1960; Juhasz and Szegedi, 1968). Rumen lactate does not always increase with high concentrate feeding (DeBarthe et al., 1971; Briggs et al., 1957; Reid et al., 1957). Gradual adaptation to high concentrate feeding prevents the lactate buildup (Ryan, 1964b; Tremere et al., 1968). Peak rumen lactate concentrations usually follow rumen pH inversely (Briggs et al., 1957; Dunlop and Hammond, 1965; Tremere et al., 1968). Bruno and Moore (1962) reported that at a rumen pH of 6.3 lactic acid does not accumulate and is rapidly metabolized. Rumen lactate levels reported by several workers are shown in Table 2.

Lactic acid has been shown to be absorbed from the rumen (Broberg,

TABLE 2. RUMEN LACTIC ACID VALUES REPORTED BY VARIOUS INVESTIGATORS

Animal	Treatment and/or diet	Lactic acid concentration mg/ml	Rumen pH	Signs	Investigator
Cattle	hay	< .01	6.0	none	Balch and Rowland (1957)
	grain	0.95-2.70	4.5-5.5	none	
Cattle	hay	< 0.01	...	none	Waldo and Schultz (1955)
	grain	0.58	...	none	
Cattle	hay	<0.01	7.1	none	Ghorban, Knox, and Ward (1966)
	grain	0.59	6.3	none	
Cattle	grain	2.4-3.0	4.0	lactic acidosis	Hyldgaard-Jensen and Simeson (1966)
Cattle	grain	10.0	4.0	lactic acidosis	Broberg (1960)
Cattle	grain	3.0-9.7	4.6-4.9	lactic acidosis	Dirksen (1965)
Sheep	intraruminal infusion of lactic acid	12.7-16.2	3.8-4.5	lactic acidosis	Telle and Preston (1971)
Cattle	grain	9.12	4.5	lactic acidosis	Mann (1970)
Sheep	grain	4.3	3.9	lactic acidosis	Juhasz and Szegedi (1968)
	intraruminal infusion of lactic acid	9.0	3.4	lactic acidosis	

TABLE 2. (continued)

Animal	Treatment and/or diet	Lactic acid concentration	Rumen pH	Signs	Investigator
		mg/ml			
Sheep (con't)	intraruminal infusion of glucose	9.0	3.6	lactic acidosis	
Cattle	hay and grain	$\leq$ 0.05	...	none	DeBarthe et al. (1971)

1960; Heuter et al., 1956; Williams and Mackenzie, 1965) and also from the intestines (Dunlop and Stefaniak, 1965). Waldo and Schultz (1960) reported that most of the ruminal lactate is converted to propionate, then butyrate and lastly acetate. Heuter et al. (1956) reported that lactate was metabolized to equal molar percentages of acetate and propionate. Using radioactive lactate, Jayasuriya and Hungate (1959) reported that most of the lactate is metabolized to acetate. They concluded that lactate is not an essential intermediate in the genesis of rumen propionate.

In roughage fed animals, the L(+) isomer of lactic acid predominates (Dunlop and Hammond, 1965). In lactic acidosis, the L(+) isomer diminishes and the D(-) isomer increases until the proportions are essentially equal or until the D(-) predominates (Dunlop and Hammond, 1965). Most mammalian tissues have a far greater capacity to utilize the L(+) isomer (Braide and Dunlop, 1969). The pharmacologic differences in sheep between D(-) and L(+) lactate were studied by Braide and Dunlop (1969). They reported that increasing L(+) lactate concentrations in the rumen increased the following blood parameters: pH, total lactate, pyruvate, PCO_2, bicarbonate and also lowered hemoglobin and packed cell volume. Increasing D(-) lactate in the rumen did not increase blood pH, pyruvate, PCO_2, and bicarbonate but did increase hemoglobin and packed cell volume. Increasing concentrations of D(-) lactate in the rumen produced a far greater increase in total lactate which disappeared from the blood much more slowly. After L(+) lactate was infused intravenously, the concentration of total blood lactate returned to normal within 3 hr whereas after the D(-) isomer was infused it took from 6-24 hr. After grain overload, metabolic acidosis develops due to the absorption of large

amounts of D(-) lactate which displaces bicarbonate in body fluids (Dunlop, 1967). Since the animal cannot readily metabolize the D(-) isomer, uncompensated acidosis develops (Juhasz and Szegedi, 1968).

There are numerous reports in the literature of a metabolic disorder in ruminants associated with ingestion of large quantities of readily fermentable carbohydrates. Materials such as wheat (Turner and Hodgetts, 1949-1959), starch (Krogh, 1961a), lactose (Krogh, 1960), sucrose (Krogh, 1959), glucose (Phillipson, 1952), apples (Merril, 1952), lush pasture (Annison et al., 1959a, b), mangolds (Scarisbrick, 1954), brewer's grain (Owens, 1959), fodder beets (Williams and Coup, 1959), grapes (Portway, 1957), corn (Dunlop and Hammond, 1965), and barley (Dunlop et al., 1969) have all been associated with disorders of varying severity which appear to be sufficiently similar for them to be classified under a single disease called lactic acidosis. Detailed although sometimes conflicting descriptions of the condition have been given by several authors (Broberg, 1960; Dirksen, 1970; Dunlop and Hammond, 1965; Hungate et al., 1952; Juhasz and Szegedi, 1968; Krogh, 1959, 1960, 1961a, b; Turner and Hodgetts, 1949-1959). Cattle on a roughage ration have a predominantly gram negative rumen flora (Hungate, 1966; Hungate et al., 1952; Krogh, 1959). This gram negative flora produces a volatile fatty acid (VFA) molar ratio of acetate:propionate of about 3:1 to 4:1 (Balch and Rowland, 1957; Briggs et al., 1957; Ghorban et al., 1966; Reid et al., 1957). When cattle are suddenly fed large quantities of readily fermentable carbohydrates, the rumen VFA parameters change. Propionate increases markedly (Bauman et al., 1971; Phillipson, 1952) and the total concentration of VFA also increases (Balch and Rowland, 1957; Briggs et al., 1957; Reid et al., 1957; Rumsey et al., 1969). The lowered pH alters the physiological medium for the

predominantly gram negative rumen flora. When an excessive amount of readily fermentable carbohydrates are in the rumen, a complete change in the microbial population of the rumen occurs. This change is characterized by the development of a predominantly gram positive flora consisting of amylolytic streptococci, lactobacilli and sometimes yeasts, whereas the ruminal fauna and gram negative flora originally present are destroyed or strongly reduced (Dunlop and Hammond, 1965; Hungate et al., 1952; Krogh, 1959, 1960, 1961, 1963a, b). The protozoa are killed when rumen pH reaches about 5.5. The development of a predominantly gram positive flora follows a pattern (Krogh, 1959). The first stage is a marked rise in the amounts of streptococci but only transiently, as the streptococcal flora is later depressed far below its initial level or disappears entirely. Coincident with, or following the peak in streptococcal counts, the lactobacilli start to flourish in the rumen, reaching their maximum amounts in 1 to 2 days and maintaining this level throughout acidosis. Streptococcus bovis (the primary streptococcus in the rumen) and the lactobacilli both produce lactic acid which is responsible for the pronounced and prolonged rumen acidity (Dunlop and Hammond, 1965; Hungate, 1966; Hungate et al., 1952). As lactic acid accumulates in the rumen the osmolarity of the rumen fluid increases (Broberg, 1960; Dunlop, 1967; Dunlop and Hammond, 1965; Turner and Hodgetts, 1949-1959). This hypertonic situation in the rumen draws fluid into the rumen which severely dehydrates the animal (Dunlop and Hammond, 1965). The acid-base equilibrium of the animal shifts (Bond, 1959; Broberg, 1960; Dunlop and Hammond, 1965; Huber et al., 1962; Juhasz and Szegedi, 1968). If too much lactic acid (primarily the D(-) isomer) is produced the animal develops uncompensated acidosis and dies (Juhasz and Szegedi, 1968). Other physical properties of the rumen fluid in

addition to pH and osmolarity also change. There is sedimentation of particles, yellowish-gray color and sour odor (Dirksen, 1965; Krogh, 1959). Metabolic alkalosis (hypochloremic) may appear 4 to 5 days after grain overload if there is overcompensation of the acidotic state (Dunlop, 1967). High histamine levels can lead to laminitis (Dunlop, 1967). Histamine is produced by the rumen lactobacilli (Rodwell, 1953). Rumenitis often occurs with acidosis (Dunlop, 1967; Jensen et al., 1954b). Death from lactic acidosis may occur anywhere from 1 to 5 days after grain overload (Broberg, 1960; Dunlop and Hammond, 1965).

Abnormal Rumen Metabolites

There are other metabolites produced in abnormal quantities in the rumen of high concentrate fed animals in addition to histamine and lactic acid. Ethanol has been found in very high concentrations in the rumen of animals in lactic acidosis (Allison et al., 1964a, b; Juhasz and Szegedi, 1968). Juhasz and Szegedi (1968) reported that lactic acid fermentation was accompanied by alcoholic fermentation in the rumen and the rise of ruminal alcohol levels were followed by marked elevation of blood alcohol. Ryan (1964a, b) reported marked increases of glucose, formic acid, and succinic acid in the rumen following acidosis and during gradual adaptation to a high concentrate diet. Formic acid has been reported to inhibit diamine oxidase in the rumen thus making ruminants very susceptible to toxicity by histamine (Eliassen and Sjaastad, 1968). The combination of formic acid and histamine has been shown to inhibit rumen motility and depress feed intake severely (Neumark, 1967; Neumark and Tadmor, 1968). Pyruvic acid is produced in markedly high concentrations in acidosis and high grain feeding (Broberg, 1960; Dunlop and Hammond, 1965; Juhasz and

Szegedi, 1968). Juhasz and Szegedi (1968) report that high concentrations of pyruvic acid are known to be toxic. Normally there are only trace amounts of glucose, formic, succinic, and pyruvic acid in the rumen (Hungate, 1966). The relative toxicities of these various compounds not normally present in the rumen are not known but illustrates the aberration that may occur in rumen metabolism when large amounts of readily fermentable carbohydrates are available.

Laboratory Animals and Rumen Fluid

There are only a few reports in the literature concerning inoculation of laboratory animals with rumen fluid. Hungate et al. (1952) reported the inoculation of mice with 0.5 ml of normal rumen fluid, lactic acidosis rumen fluid and rumen bacterial culture filtrates. No deaths were produced within 24 hr. Turner and Hodgetts (1949-1959) reported the effects of sheep lactic acidosis rumen fluid on mice to detect bacterial toxins. No positive results were reported. Dougherty and Cello (1952) used mice in studies on toxic factors in rumen digesta. They reported that mice were unsatisfactory (no details given). Hypotensive effects similar to histamine and a few deaths have been reported when rumen fluid was injected intravenously into dogs (Dain et al., 1955; Dougherty and Cello, 1949, 1952; Prier, 1954). Sheep have also been used for intravenous administration of rumen fluid (Dougherty and Cello, 1949, 1952; Mullenax et al., 1966; Prier, 1954; Turner and Hodgetts, 1949-1959). Hypotensive effects similar to histamine and some deaths were reported. Similar results have been reported in goats (Dougherty and Cello, 1949) and in cattle (Mullenax et al., 1966). Turner and Hodgetts (1949-1959) reported that lactic acidosis rumen fluid was without effect upon isolated perfused guinea pig ileum and isolated

perfused sheep spleen. Dougherty and Cello (1949) reported that lactic acidosis rumen fluid markedly depressed the isolated rabbit gut. Depression was abrupt and lasting; however gut activity returned after flushing. Histamine stimulated gut activity. Dunlop and Hammond (1965) administered orally to rabbits lactic acid in the same concentration as found in rumen fluid during lactic acidosis. The effect was fatal.

EXPERIMENTAL PROCEDURE

There are no reported data on metabolic changes in rumen fluid obtained from cattle dying from SDS. The present investigation was initiated to determine metabolic changes in rumen fluid obtained from roughage fed cattle, lactic acidotic cattle, and cattle fed a typical feedlot ration. When these parameters were established they were compared with those from cattle dying from SDS.

Experiment 1. Two fistulated cattle, a 638 kg Angus steer (140E) and a 400 kg Jersey cow (321D), were each fed daily 9 kg of good quality alfalfa hay for one month before sampling. Rumen fluid samples were collected before feeding hay and 1, 2, 3, 5, and 7 hr after feeding. Rumen fluid was frozen after sampling until the following laboratory analyses were made: pH, total VFA, VFA molar percentages, total lactic acid, L(+) lactic acid, and histamine. Rumen pH was determined by a pH meter*. The following procedure was used to determine VFA: 2 microliter injections of acidified (pH 1), centrifuged rumen fluid were separated on a 183 x 0.32 cm stainless steel column packed with 60-80 mesh chromosorb 101. The detector was flame ionization. Nitrogen was the carrier gas and the flow rate was 35 mg/min. Quantitation was computed on the basis of peak height using appropriate standards. A Beckman GC4 gas chromatograph was used. Total lactic acid was determined colorimetrically by the method of Barker and Summerson (1941). L(+) lactic acid was determined by the method of Hohorst (1963). A fluorometric analysis (Oates et al., 1962) was used to determine rumen histamine.

Blood samples were collected from intravenous jugular catheters before

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*Porto-matic pH Meter, Model 175, Instrumentation Laboratory Inc., Boston, Mass.

feeding and at various times after feeding. Determinations of total lactate and L(+) lactate were made on blood according to the methods of Barker and Summerson (1941) and Hohorst (1963) respectively. In addition the following were determined in blood: glucose, urea nitrogen, total serum protein, serum glutamic oxaloacetic transaminase (SGOT), packed cell volume, hemoglobin, and total and differential white blood cell count (Coles, 1967). Ethylene diamine tetracetic acid (EDTA) was used as an anticoagulant for all samples and sodium fluoride (NaF) as a preservative for blood lactate samples.

Experiment 2. Fourteen days following Experiment 1, lactic acidosis was produced in the two roughage fed animals used in Experiment 1. Each animal was given 50 g per kg of a mixture containing 97% sorghum grain and 3% molasses. The feed was introduced through a rumen fistula. Six hours after administering the feed, 5g/kg of cooked wheat starch (100% gelatinized) was also given through the fistula. Rumen fluid samples were collected before initial engorgement and at 1.5, 3, 6, 12, 24, 30, 36, 48 and 54 hr later. Rumen fluid was frozen after sampling. Blood samples were also taken at these times. The blood was collected in tubes containing EDTA and NaF. The rumen fluid and blood were analyzed as in Experiment 1.

Experiment 3. To compare metabolic changes in rumen fluid from feedlot cattle, six steers that had been in a lot on full feed for over 100 days were selected. These steers weighed 450 to 550 kg and were fed a ration consisting of 24.14% sorghum silage, 4.14% protein supplement (soybean oil meal and urea), 1% salt, 71.71% rolled sorghum grain and 0.5% trace mineral mixture*. The rumen fluid was collected with a stomach

*Z-5 Calcium Carbonate Company, Quincy, Illinois

tube and vacuum pump 6 hr after feeding. One sample was collected from each steer and analyzed as in Experiments 1 and 2. No blood samples were collected.

Experiment 4. Several fiedl trips were made to feedlots and feedlot veterinarians in western Kansas. Specimen containers were left with the veterinarians to send in fixed and frozen tissues and frozen rumen fluid from cattle succumbing to SDS. It was necessary to rely on the veterinarian's diagnosis of SDS since these were field cases. Twenty-one SDS samples were collected and sent to the Kansas State University Veterinary Diagnostic Laboratory. The previously mentioned determinations were conducted on the rumen fluid samples.

Experiment 5. Laboratory animals were inoculated with rumen fluid to determine the presence of toxins. Mice were chosen because they are easy to handle and are inexpensive. Intraperitoneal administration was chosen because absorption is necessary for a possible intraruminal toxin to elicit its effects. The dose was 0.5 ml/25g of mouse body weight. The mice were injected and then observed for 36 hr.

Experiment 5 Trial 1. The first trial was conducted using rumen fluid from the roughage fed and lactic acidotic animals in Experiments 1 and 2 respectively.

Experiment 5 Trial 2. This trial was conducted with SDS rumen fluid samples collected in Experiment 4.

Experiment 5 Trial 3. This trial was conducted with various treatments of selected toxic SDS rumen samples. The treatments were: heating to 85 to 90 C for 15 min; buffering to pH 7.0 and addition of chlortetracycline at the rate of 5 mcg/ml of rumen fluid; addition of chlortetracycline (5mcg/ml) to rumen fluid without buffering; supernatant from low speed

centrifugation (600g for 5 min); untreated rumen fluid as a control; saline plus chlortetracycline (5 mcg/ml) as a control for the antibiotic; and the supernatant from high speed centrifugation (15,000g for 15 min).

Experiment 5 Trial 4. This trial was conducted with one roughage sample from Experiment 1, a lactic acidotic sample from Experiment 2, and four SDS samples from Experiment 4. The samples were centrifuged (15,000 g for 15 min), supernatant discarded, and the precipitate removed from the centrifuge tube and lyophilized. The lyophilized material was reconstituted into a suspension 7.5 times more concentrated than normal rumen fluid. The suspension was injected intraperitoneally at 0.5 ml/25 g of mouse body weight. The suspension was also given orally with a stomach tube to some mice. Similar untreated rumen fluid samples were also used as a control.

Experiment 5 Trial 5. This trial was conducted with rumen fluid from the feedlot cattle fed a high concentrate ration (Experiment 3). These samples were centrifuged at high speed (15,000 g for 15 min) and the precipitate was reconstituted into a suspension approximately 7.5 times greater than untreated rumen fluid (these samples were not lyophilized). The concentration was based not on a dry matter basis as in the fourth trial but on an "as is" basis. The weight of the precipitate was considered in relation to the initial volume and the volume of distilled water necessary to reconstitute the precipitate.

Experiment 5 Trial 6. This trial was conducted with untreated rumen fluid samples, autoclaved samples (120 C, 1.4 kg/sq cm pressure for 20 min), and with samples treated with chlortetracycline at the rate of 1 mg/ml of rumen fluid. Before these samples were injected into mice they were cultured aerobically to determine the effectiveness of the antibiotic. The

samples used were a roughage sample from Experiment 1, a lactic acidosis sample from Experiment 2, a high concentrate sample from Experiment 3, and an SDS sample from Experiment 4. A control antibiotic sample of 1 mg/ml of saline was used also.

Experiment 5 Trial 7. This trial was conducted with the same samples used in the previous trial but treated differently. A 1.0% solution of phenol was used as a bactericidal agent. Phenol was added to untreated rumen fluid and then refrigerated overnight. Some of this fluid was used for inoculation and the rest was centrifuged (15,000 g for 15 min) and then the precipitate reconstituted as in the fifth trial. Control solutions of 0.5 and 1.0% phenol were also injected intraperitoneally into mice.

Experiment 5 Trial 8. Roughage fed rumen fluid (Experiment 1), lactic acidotic rumen fluid (Experiment 2), high concentrate rumen fluid (Experiment 3), and SDS rumen fluid (Experiment 4) were treated with antibiotic. The antibiotic was penicillin and streptomycin. Penicillin was added to the rumen fluid samples at the rate of 500 units/ml and streptomycin at the rate of 250 mcg/ml. The antibiotic treated rumen fluid was cultured aerobically and anaerobically before injecting it into mice.

Experiment 5 Trial 9. Due to inconclusive results following the addition of antibiotics (penicillin and streptomycin) to rumen fluid another trial was conducted with antibiotic treated samples. The antibiotics and quantities used were: (1) penicillin (1000 units/ml) and streptomycin (500 mcg/ml); (2) gentamicin (Gentocin^{R} - Schering; 50 mcg/ml); (3) chloromycetin (Chloramphenicol^{R} - Parke-Davis; 100 mcg/ml); (4) erythromycin (Erythrocin^{R} - Abbot; 100 mcg/ml); and (5) neomycin

(Biosol^{R} - Upjohn; 1000 mcg/ml). One SDS sample (SD-04) was chosen because of its toxicity and extensive use in previous trials. Five control solutions contained the previously mentioned antibiotics in sterile physiological saline.

Experiment 5 Trial 10. This trial was conducted to determine if the gram positive Bacillus recovered in Experiment 5, Trials 8 and 9 produced an exotoxin. Culture filtrates from these cultures were incubated, centrifuged (2,000 g for 5 min) and injected i.p. at the rate of 0.5 ml/25g mouse body weight. Thirty mice were inoculated. This was done because some gram positive bacteria produce exotoxins (Merchant and Packer, 1967).

RESULTS

Experiment 1. Rumen fluid was collected before feeding and at 1, 2, 3, 5 and 7 hr after feeding alfalfa hay. Prefeeding rumen pH for 140 E was 6.65. The pH dropped to 6.45 during the first hour after feeding, then steadily increased to the prefeeding value at the second hour, and increased above the prefeeding value at the fifth and seventh hour (6.75 and 6.85 respectively). The rumen pH for 321 D at prefeeding was 7.15 and reached the lowest value at 6.8, at the first hour. This trend was similar to that of 140 E. The rumen pH then increased to a value of 7.0 at 7 hr. These rumen pH values indicate normal rumen metabolism as would be expected following hay feeding. Histamine and L(+) lactic acid were not present in any of the rumen samples which is to be expected when cattle are fed roughage only.

Experiment 2. Heavy starch feeding produced lactic acidosis in the two roughage fed animals used in Experiment 1 (Tables 3, 4, 5 and 6). Rumen pH dropped from pre-engorgement levels of 7.05 in both animals to 3.80 (30 hr) in 321 D (Jersey) and 3.55 (48 hr) in 140 E (Angus). Rumen L(+) lactic acid concentrations at pre-engorgement were 0 mg/ml and rose to 5.2 and 6.1 mg/ml at 24 hr for 321 D and 140 E respectively. Rumen lactate concentrations remained greatly elevated at 54 hr. Rumen histamine concentrations at pre-engorgement were 0 mcg/ml and increased to 26 mcg/ml (48 hr) and 22.8 mcg/ml (54 hr) for 321 D and 140 E respectively. Laminitis was observed in 321 D at 48 hr. Total VFA concentration showed no specific trend with time in 140 E but decreased in 321 D (154.7 to 27.5 mM/l). Acetic acid concentration and molar percentage decreased in 321 D but exhibited no marked trend in 140 E. Propionic acid concentration decreased

TABLE 3. RUMEN pH, TOTAL VFA, HISTAMINE AND LACTIC ACID CONCENTRATIONS AND VFA MOLAR PERCENTAGES FROM 321 D IN LACTIC ACIDOSIS (EXPERIMENT 2).

Time	pH	Histamine	Total lactate[a]	L(+) lactate	VFA						
					C_2		C_3		C_4		Total
					conc	%	conc	%	conc	%	conc
hr		mcg/ml	mg/ml	mg/ml	mM/l		mM/l		mM/l		mM/l
0	7.05	0	0	0	99.25	62.81	32.07	20.31	26.61	16.86	154.71
1½	6.25	0.222	> 1.28		55.46	58.71	19.57	20.72	19.42	20.56	94.61
3	5.80	0	> 1.28		29.96	66.38	8.30	18.40	6.85	15.20	45.24
6	4.95	0	> 1.28	2.369	13.38	60.44	4.93	22.14	3.88	17.40	21.12
12	4.70	0	> 1.28	4.865	14.52	48.87	8.93	29.61	6.63	21.51	27.48
24	4.10	0	> 1.28	5.191	38.16	74.59	8.54	16.69	4.46	8.70	50.20
30	3.80	5.0	> 1.28	5.842	28.25	64.74	8.41	19.25	6.99	15.99	43.18
36	3.80	13.778	> 1.28	7.036	19.18	59.89	7.90	24.66	4.94	15.43	31.95
48	3.90	26.00	> 1.28	6.276							
54	4.0	16.889	> 1.28		43.96	70.92	10.90	17.59	7.11	11.47	60.97

[a] 1.28 was the maximum detectable value by the analytical method employed.

TABLE 4. RUMEN pH, TOTAL VFA, HISTAMINE AND LACTIC ACID CONCENTRATIONS AND VFA MOLAR PERCENTAGES FROM 140 E IN LACTIC ACIDOSIS (EXPERIMENT 2).

Time	pH	Histamine	Total lactate[a]	L(+) lactate	VFA						
					C_2		C_3		C_4		Total
					conc	%	conc	%	conc	%	conc
hr		mcg/ml	mg/ml	mg/ml	mM/l		mM/l		mM/l		mM/l
0	7.05	0	0.38	0	62.05	67.23	16.23	17.56	14.04	15.19	88.88
1½	6.25	0	> 1.28		58.53	61.76	18.03	19.06	18.15	19.16	97.72
3	5.80	0	> 1.28		56.68	58.37	20.07	20.69	20.31	20.92	98.37
6	4.90	0	> 1.28	2.919							
12	4.50	1.166	> 1.28	6.055	69.23	82.12	11.27	13.36	3.80	4.50	84.30
24	4.10	0.333	> 1.28	6.055							
30	4.10		> 1.28	8.012	37.48	74.74	9.17	18.31	3.47	6.93	51.00
36	3.75	3.721	> 1.28	8.881	103.84	75.59	24.86	18.09	8.67	6.31	135.96
48	3.55		0.032	9.206	65.21	68.19	18.65	19.33	12.14	12.47	90.51
54	3.70	22.778	0.014								

[a] 1.28 mg/ml was the maximum detectable value by the analytical method employed.

TABLE 5. BLOOD L(+) LACTIC ACID, UREA NITROGEN, TOTAL SERUM PROTEINS, SERUM GLUTAMIC OXALOACETIC TRANSAMINASE, PACKED CELL VOLUME AND HEMOGLOBIN FOR 140 E AND 321 D IN LACTIC ACIDOSIS.

Sample	L(+) lactic acid	BUN	Total protein	SGOT[a]	PCV	Hb
	mg/ml	mg/100ml	g/100ml	Fu	vol%	g/100ml
140 E 0 hr	0.142	8.9	7.5	150	32	10.2
140 E 12 hr	...	5.7	7.5	90	31	10.3
140 E 24 hr	0.136	4.8	8.1	100	39	12.5
140 E 48 hr	0.190	9.6	8.7	95	46	14.8
321 D 0 hr	0.115	15.8	7.5	125	34	11.8
321 D 12 hr	0.113	9.6	6.7	125	32	10.3
321 D 24 hr	0.143	9.4	7.7	100	37	12.5
321 D 48 hr	0.137	15.4	8.7	80	47	15.6

[a] Fu - Frankel units

TABLE 6. TOTAL AND DIFFERENTIAL WHITE BLOOD CELL COUNTS FOR 140 E AND 321 D IN LACTIC ACIDOSIS.

Sample	(WBC/cumm) White blood cells[a] Total	PMN		Bands		Lymph.		Mono.		Eosin.	
		#	%	#	%	#	%	#	%	#	%
140 E 0 hr	6,500	1495	23			3900	60	195	3	910	14
140 E 12 hr	9,100	6006	66			2912	22	91	1	91	1
140 E 24 hr	10,200	6426	63	102	1	3570	35	102	1	0	0
140 E 48 hr	12,800	6912	54			4992	39	512	4	384	3
321 D 0 hr	7,900	3476	79			4029	51	158	2	237	3
321 D 12 hr	7,800	3900	50			3588	46	312	4	0	0
321 D 24 hr	5,100	2499	49			2346	46	102	2	153	3
321 D 48 hr	9,900	5643	57			3663	37	396	4	198	2

[a] Number of WBC/cumm

markedly in 321 D, and increased slightly and then decreased in 140 E. Molar percentage of propionic was maintained relatively unchanged in both animals. After engorgement, butyric acid concentration decreased markedly in 321 D but increased in 140 E before declining markedly by 12 hr. Molar percentage of butyric acid was maintained for 12 hr in 321 D but declined by 12 hr in 140 E. Total serum protein was unchanged during the course of lactic acidosis. SGOT levels decreased in both animals. Blood urea nitrogen decreased initially and then returned to pre-engorgement levels. Hemoglobin and packed cell volume increased in both animals. Total white blood cell count progressively increased in 140 E but decreased initially and later increased in 321 D. The differential white blood cell count in 140 E indicated a shift to the left but a similar shift was not noted in 321 D. Both animals had a high eosinophil count (2-4% for 321 D and 1-14% in 140 E). Blood lactate initially decreased and then increased about 100% in 140 E and about 80% in 321 D.

Both animals exhibited anorexia and depression within 12 hr. There was increased fluid in the rumen of each animal. The smell or odor of the rumen fluid from each animal became progressively more sour during the development of lactic acidosis. Severe laminitis and dehydration was seen in 321 D. After sampling at 54 hr, the rumen contents of each animal were removed and replaced with the contents from a roughage fed animal. On the fourth day after engorgement 321 D died. Fluid, cortico-steroid and antihistamine therapy was of no avail. Post mortem examination revealed only a slight abomasitis. 140 E made an uneventful recovery.

Experiment 3. The range in rumen total lactate was 0.038 to 0.078 mg/ml (Table 7). The average rumen total lactate concentration was 0.054 mg/ml. There was no histamine in any of the rumen fluid samples. The

TABLE 7. RUMEN pH, TOTAL VFA, HISTAMINE AND LACTIC ACID CONCENTRATIONS AND VFA MOLAR PERCENTAGES FROM FEEDLOT STEERS MAINTAINED ON A FINISHING RATION FOR OVER 100 DAYS (EXPERIMENT 3).

Sample	pH	Histamine	Total lactate	L(+) lactate	VFA						
					C_2		C_3		C_4		Total
					conc	%	conc	%	conc	%	conc
		mcg/ml	mg/ml	mg/ml	mM/l		mM/l		mM/l		mM/l
HC-1	5.85	0	0.230	0.005							
HC-2	5.8	0	0.313	0.009	56.83	41.86	65.26	48.12	13.55	10.01	132.64
HC-3	5.6	0	0.192	0.051	53.72	51.90	38.99	37.68	10.76	10.40	101.36
HC-4	6.2	0	0.377	0	32.14	46.53	29.30	42.36	7.77	11.09	63.91
HC-5	6.1	0	0.140	0	73.34	54.36	46.62	34.48	15.14	11.15	131.64
HC-6	5.9	0	0.394	0.020	41.48	38.74	54.53	50.92	11.06	10.33	107.57
Average	5.91	0	0.274	0.014	51.50	46.68	46.94	42.71	11.66	10.60	107.42

range in concentration of total VFA was 63.9 to 132.6 mM/l. The average total VFA concentration was 107.4 mM/l. The range in acetic acid concentrations was 32.1 to 73.3 mM/l with an average concentration of 51.5 mM/l. The average molar percentage of acetic acid was 46.7% with a range of 38.7 to 51.9%. The range in propionic acid concentrations was 29.3 to 65.3 mM/l. The average molar percentage of propionic acid was 42.7% with a range of 37.68 to 50.9%. The range in concentration for butyric acid was 7.8 to 13.6 mM/l. The average molar percentage of butyric acid was 10.6% with a range of 10.0 to 11.2%. The range in rumen pH was 5.6 to 6.2 with an average rumen pH of 5.91.

Experiment 4. The range in rumen pH was 4.5 to 7.0 with an average pH of 5.05 (Table 8). There was a wide range in rumen histamine concentration from 0 to 57.45 mcg/ml. There were eight rumen samples with 0 mcg/ml, five with less than 2.0 mcg/ml and two with high levels of histamine (27.33 and 57.45 mcg/ml).

The range in total rumen lactate was 0.023 to greater than 1.28 mg/ml. Five samples contained more than 1.28 mg/ml; eight had 0.22 to 0.59 mg/ml and only three had less than 0.20 mg/ml. The average total rumen lactate concentration was greater than 0.626 mg/ml. Three samples had no L(+) lactate while three contained 0.08 or more mg/ml (range 0.0 to 0.238 mg/ml). The range in total VFA concentration was 64.49 to 306.66 mM/l with an average concentration of 204.55 mM/l. The concentration of acetic acid ranged from 40.9 to 145.79 mM/l with an average of 93.18 mM/l. The molar percentage of acetic ranged from 36.83 to 62.07 with an average of 47.17%. The concentration of propionic acid ranged from 18.4 to 126.5 mM/l with an average concentration of 75.3 mM/l. The average molar percentage of propionic acid was 35.4 with a range of 27.9 to 41.9. The average

TABLE 8. RUMEN pH, TOTAL VFA, HISTAMINE AND LACTIC ACID CONCENTRATIONS AND VFA MOLAR PERCENTAGES FROM SDS CATTLE (EXPERIMENT 4).

Sample	pH	Histamine	Total lactate	L(+) lactate	VFA						
					C_2		C_3		C_4		Total
					conc	%	conc	%	conc	%	conc
		mcg/ml	mg/ml	mg/ml	mM/l		mM/l		mM/l		mM/l
SD-01	4.5	0	> 1.28								
SD-02	5.5	0.222	0.12								
SD-03	7.0	0	0.02		40.90	62.07	18.42	27.93	6.57	9.98	64.49
SD-04	4.95	1.222	> 1.28	0	116.41	38.46	126.46	41.76	59.88	19.77	306.66
SD-06	5.1		> 1.28								
SD-07	4.95	27.333	> 1.28								
SD-08	5.0	0	0.30	0							
SD-09	5.4	0	0.27								
SD-10	4.9		0.22		60.84	36.83	69.41	41.99	34.99	21.16	168.59
SD-11	4.6	0.167	0.54								
SD-12	5.4		> 1.28								
SD-13	4.8	0	0.37		126.75	46.35	108.10	39.54	38.54	14.10	266.93
SD-14	5.1	0	0.16		73.00	51.80	38.23	27.13	29.69	21.06	142.53
SD-16	4.9	0	0.45		145.79	52.38	85.81	30.83	46.69	16.67	277.41
SD-17	4.9	0	0.59	0.086	129.67	45.14	114.94	40.01	42.62	14.83	288.15
SD-18	4.7	57.445	0.51	0	52.06	44.33	40.86	34.76	24.52	20.90	121.67
SD-19	4.6	0.222		0.238							
SD-20		0.389		0.136							
Average	5.05	5.8	[a]0.63	0.069	93.18	47.17	75.28	35.42	35.44	17.3	204.55

[a] Average of all 0.63; average of those below 1.28 = 0.33

concentration of butyric acid was 35.44 mM/l with a range of 6.57 to 59.88 mM/l. The average molar percentage of butyric was 17.3 with a range of 9.98 to 21.2%.

The SDS specimens submitted for study came from several feedlots in Kansas and one from Nebraska. More samples were sent in from Garden City, Kansas than any other cattle feeding area. This probably does not reflect incidence of SDS but rather cooperation of feedlot veterinarians. Of the eighteen SDS cases in which sex was known, four were heifers and fourteen were steers. The age of the 20 SDS cattle was 18 to 24 months except for two cases (16 and 30 months). The estimated weight of the SDS cattle ranged from 341 to 545 kg with an average weight of 426 kg. The ration was specified in only three cases as a finishing ration but since the average weight was greater than 425 kg, it can be assumed that the SDS cattle were on a finishing ration. The estimated number of days on feed was specified in only seven cases. The range was from 70 to 140 days with an average of 109 days on feed. Since the cattle averaged over 425 kg it may be assumed that the majority were on feed for over 100 days. Four SDS cattle were noted to be healthy. No history was given for eleven SDS cases, so it was assumed that these were healthy cattle. One SDS steer was seen alive at 2:15 P.M. one afternoon and found dead at 3:00 P.M. that same afternoon. Death was observed in two SDS cattle. Dyspnea was observed immediately before death in one animal (SD-09). Post mortem examination showed the trachea nearly occluded at the thoracic inlet by edema and swelling. SD-13 was also observed just prior to death. The animal stood and trembled for a few moments, then collapsed and died without struggling. The animal was posted within 35 to 40 min after death. There were few lesions (no gas in the subcutaneous tissues of the neck

and shoulders; only a few small hemorrhages in the trachea; and a slight enteritis).

There were no necropsy lesions in seven SDS cases. The occurrence of lesions was as follows: rumenitis (SD-02 and SD-10); abomasitis (SD-09 and SD-11); enteritis (SD-06 and SD-07); friable liver (SD-02); pale liver (SD-06 and SD-10); pale kidney (SD-06); rumen tympany (SD-06 and SD-17); gas in tissues (SD-07); cervical and/or tracheal hemorrhages (SD-07, SD-10, SD-11, SD-14, SD-16, SD-17 and SD-19) and a granulomatous lesion (SD-15) adjacent to the larynx and probably not related to SDS. The necropsy lesions and other information (Table 9) for these SDS cases agree in general with those reported by Anthony (1971a, b) and Turner (1971).

Experiment 5 Trial 1. There were no deaths in mice due to intraperitoneal injection of rumen fluid from cattle fed roughage (Table 10). Injection of rumen fluid taken from cattle 24 hr after initiation of grain overload and exhibiting lactic acidosis proved to be fatal whereas rumen fluid obtained from cattle during the first 24 hr was not toxic. The average mortality rate for lactic acidosis rumen fluid obtained between 0 to 54 hr was 30.9% (23.8% for 140 E and 38.1% for 321 D). The average mortality rate for lactic acidosis rumen fluid obtained between 12 to 54 hr was 43.3% (33.3% for 140 E and 53.3% for 321 D). Apparently changing cattle abruptly from high roughage to high grain feeding either produces or releases a substance toxic to mice. All deaths in mice were between 12 and 24 hr post injection.

TABLE 9. HISTORY AND NECROPSY LESIONS OF "SUDDEN DEATH" CATTLE.

Animal no.	Location	Sex	Age	Wt.	Ration	No. days on feed	History	Necropsy lesions
	Kansas		months	kg				
SD-01	Potwin	M(c)	30	545.4	finishing	130	healthy day before death	none
SD-02	Herington	F		454.5		140	healthy	friable liver, rumenitis
SD-03	Humboldt, Neb.	M(c)		heavy	finishing			
SD-06	Colby	M(c)	16	431.8			not vaccinated with Clostridium perfringens type C or D	tympany; enteritis; pale liver and kidney
SD-07	Colby	F		431.8				gas in tissues; cervical hemorrhage; enteritis
SD-08	Garden City			433.1	finishing	100		
SD-09	Dodge City	F	24	409.0			death was observed; difficult respiration then died	abomasitis
SD-10	Garden City	M(c)	24	545.4			seen alive at 2:15 and dead at 3:00	rumenitis; cervical and tracheal hemorrhage; pale liver
SD-11	Dodge City	F	24	340.9				abomasitis and tracheal hemorrhage
SD-12	Potwin	M(c)	24	545.4		70	healthy	
SD-13	Garden City	M(c)	18	454.5			observed death--trembled for a minute and then collapsed and died without struggling	unclotted blood; no hemorrhage in trachea; no gas in subcutaneous tissues

TABLE 9. (continued)

Animal no.	Location	Sex	Age	Wt.	Ration	No. days on feed	History	Necropsy lesions
	Kansas		months	kg				
SD-14	Garden City	M(c)	18	386.3		100		
SD-15	Garden City	M(c)	18	363.6		90	no rumen fluid collected	granulomatous tissue around larynx
SD-16	Scott City	M(c)	18	454.5				tracheal and cervical hemorrhages
SD-17	Leoti	M(c)		431.8				tympany; tracheal and cervical hemorrhages
SD-18	Beloit							
SD-19	Garden City	M(c)	24	545.4		135		cervical and tracheal hemorrhages
SD-20	Oakley							

Table 10. Mortality of mice injected (i.p.) with rumen fluid from healthy roughage fed cattle or from cattle exhibiting lactic acidosis (Experiment 5, Trial 1).

Animal no.	Treatment[a]	Time after feeding	No. mice injected	No. died	Mortality	Time of death
		hr			%	hr
140 E	R	0	3	0	0	.....
140 E	R	3	3	0	0	.....
140 E	L	3	3	0	0	.....
140 E	L	12	3	0	0	.....
140 E	L	24	3	0	0	.....
140 E	L	30	3	1	33	24
140 E	L	36	3	1	33	24
140 E	L	48	3	1	33	24
140 E	L	54	3	2	66	24,24
321 D	R	0	3	0	0	.....
321 D	R	3	3	0	0	.....
321 D	L	3	3	0	0	.....
321 D	L	12	3	0	0	.....
321 D	L	24	3	2	66	24,24
321 D	L	30	3	0	0	.....
321 D	L	36	3	2	66	24,24
321 D	L	48	3	2	66	24,24
321 D	L	54	3	2	66	24,24

[a] R = Roughage fed cattle
L = Lactic acidotic cattle

Experiment 5 Trial 2. The average mortality rate for SDS samples injected intraperitoneally into mice was 63.3% (Table 11). Only four SDS samples produced no deaths; four produced two deaths; and ten produced three deaths (three mice were injected per sample). The average mortality rate of 63.3% for SDS samples was greater than for the lactic acidosis rumen fluid (30.9% and 43.3% after 12 hr). Deaths occurred sooner after injection of SDS rumen fluid (as early as 6 hr post injection with several being recorded at 8, 10, 12 and 14 hr) than after injection of lactic acidosis rumen fluid.

Table 11. Mortality of mice injected (i.p.) with SDS rumen fluid. (Experiment 5, Trial 2).

Animal no.	No. mice injected	No. died	Mortality	Time of death
			%	hr
SD-01	3	3	100	8,12,24
SD-02	3	3	100	10,24,24
SD-03	3	2	66	12,14
SD-04	3	3	100	12,14,24
SD-06	3	3	0	8,12,12
SD-07	3	0	0	...
SD-08	3	2	66	8,12
SD-09	3	2	66	24,24
SD-10	3	2	66	12,24
SD-11	3	3	100	6,12,12
SD-12	3	0	0	...
SD-13	3	3	100	12,14,24
SD-14	3	0	0	...
SD-16	3	3	100	24,24,24
SD-17	3	3	100	8,8,10
SD-18	3	0	0	...
SD-19	3	3	100	10,14,14
SD-20	3	3	100	24,24,24
SD-22	6	6	100	12,12,12, 12,12,12
SD-26	6	6	100	12,12,12, 12,14,24
SD-27	6	4	66	14,24,24,33

Experiment 5 Trial 3. Heating SDS samples at 85 to 90 C for 15 min,

buffering to pH 7.0, adding chlortetracycline at the rate of 5 mcg/ml and subjecting them to low speed centrifugation did not alter their toxicity (Table 12). The supernatant from high speed centrifugation (15,000 g for 15 min) was not toxic to mice. Apparently the toxic principle was spun down during centrifugation. The toxic principle appears to be heat stable, pH stable, and not sensitive to chlortetracycline administered at a dosage lethal to rumen microbes (Radisson, 1955). There was more variation between the four SDS samples than between the various treatments except for the supernatant from high speed centrifugation of rumen fluid. Deaths were first produced at 9 hr. There was no apparent relationship between the time of death and treatment of SDS rumen fluid.

Experiment 5 Trial 4. The pattern of deaths produced by untreated roughage, lactic acidotic, and SDS rumen fluid samples was similar to those of Experiment 5 Trials 1 and 2, respectively (Table 13). But mortality was 100% from the intraperitoneal injection of the precipitate from high speed centrifugation of roughage fed, lactic acidotic, and SDS rumen fluid. Deaths were first produced at 6 hr; several deaths were observed before 12 hr. Oral administration of the precipitate was not toxic. No deaths were produced from oral administration of 0.5 ml of the precipitate from roughage fed, lactic acidotic, or SDS rumen fluid. Apparently the toxic principle is inactivated in or not absorbed from the mouse gastrointestinal tract.

Experiment 5 Trial 5. Untreated rumen fluid from cattle in a feedlot was very toxic as evidenced by 100% mortality (Table 14). The precipitate from high speed centrifugation of these samples produced 100% mortality as in Experiment 5 Trial 4 with precipitates from roughage fed, lactic acidotic, and SDS rumen fluid. Deaths were first observed at 5 hr and 75% were dead within 13 hr.

Table 12. Mortality of mice injected (i.p.) with fractions of SDS rumen fluid obtained by differential centrifugation and treated with antibiotic and/or heat (Experiment 5, Trial 3).

Animal no	Treatment[a]	No. mice injected	No. died	Mortality	Time of death
				%	hr
SD-04	A	6	6	100	11,11,11,11,11,13
SD-04	B	6	6	100	13,13,24,24,24,24
SD-04	C	6	6	100	13,13,13,13,24,24
SD-04	D	6	5	83	11,11,11,13,13
SD-04	E	6	5	83	13,13,24,24,24
SD-04	F	6	1	17	31
SD-06	A	6	1	17	24
SD-06	B	6	0	0	...
SD-06	C	6	1	17	24
SD-06	D	6	4	66	24,24,24,32
SD-06	E	6	6	100	11,11,13,13,24,24
SD-06	F	6	0	0	...
SD-16	A	6	2	33	12,12
SD-16	B	6	4	66	14,22,22,27
SD-16	C	6	4	66	22,22,22,22
SD-16	D	6	6	100	12,12,22,22,22,22
SD-16	E	6	3	50	11,12,24
SD-16	F	6	0	0	...
SD-17	A	6	4	66	22,22,25,31
SD-17	B	6	3	50	22,22,31
SD-17	C	6	4	66	22,22,22,22
SD-17	D	6	6	100	10,12,14,14,22,22
SD-17	E	6	6	100	9,9,10,11,12,12
SD-17	F	6	1	17	31
Control	G	3	0	0	...

[a] A - Supernatant from low speed centrifugation heated at 85-90 C for 15 minutes.
B - Supernatant from low speed centrifugation treated with antibiotic (chlortetracycline 5 mcg/ml rumen fluid) and buffered to pH 7.0.
C - Supernatant from low speed centrifugation treated with antibiotic (chlortetracycline 5 mcg/ml rumen fluid).
D - Supernatant from low speed centrifugation.
E - Untreated rumen fluid.
F - Supernatant from high speed centrifugation (greater 15,000 g) of rumen fluid.
G - Saline plus antibiotic (chlortetracycline 5 mcg/ml saline).

Table 13. Mortality of mice given intraperitoneally or orally rumen fluid that had been centrifuged and the precipitate lyophilized (Experiment 5, Trial 4).

Animal no.	Animal treatment	Time after feeding	Rumen fluid treatment[a]	Route of administration	No. mice injected	No. died	Mortality	Time of death
		hr					%	hr
321 D	Roughage	3	None	i.p.	3	0	0	...
321 D	Roughage	3	None	oral	3	0	0	...
321 D	Roughage	3	PR	i.p.	3	3	100	6,6,6
321 D	Roughage	3	PR	oral	3	0	0	...
321 D	Lactic acidosis	48	None	i.p.	3	2	66	13,13
321 D	Lactic acidosis	48	None	oral	3	0	0	...
321 D	Lactic acidosis	48	PR	i.p.	3	3	100	8,8,9
321 D	Lactic acidosis	48	PR	oral	3	0	0	...
SD-04	SDS	...	None	i.p.	3	3	100	13,24,24
SD-04	SDS	...	None	oral	3	0	0	...
SD-04	SDS	...	PR	i.p.	3	3	100	6,13,13
SD-04	SDS	...	PR	oral	3	0	0	...
SD-06	SDS	...	None	i.p.	3	2	66	11,13
SD-06	SDS	...	None	oral	3	0	0	...
SD-06	SDS	...	PR	i.p.	3	3	100	10,10,10
SD-06	SDS	...	PR	oral	3	0	0	...
SD-16	SDS	...	None	i.p.	3	3	100	13,24,24
SD-16	SDS	...	None	oral	3	0	0	...
SD-16	SDS	...	PR	i.p.	3	3	100	11,24,24
SD-16	SDS	...	PR	oral	3	0	0	...
SD-17	SDS	...	None	i.p.	3	3	100	11,13,24
SD-17	SDS	...	None	oral	3	0	0	...
SD-17	SDS	...	PR	i.p.	3	3	100	6,10,24
SD-17	SDS	...	PR	oral	3	0	0	...
Control[b]				oral	3	0	0	...

[a] None - Untreated rumen fluid.
PR - Precipitate from high speed centrifugation (greater than 15,000 g for 15 min), lyophilized and then reconstituted into a suspension 7.5 times as concentrated as untreated fluid.

[b] Control - Oral administration of 0.5 ml saline.

Table 14. Mortality of mice injected (i.p.) with rumen fluid from feedlot cattle fed a high concentrate, low roughage ration (Experiment 5, Trial 5).

Animal no.	Treatment[a]	No. mice injected	No. died	Mortality	Time of death
				%	hr
HC-1	None	6	6	100	13,13,13,13,13,25
HC-1	PR	4	4	100	5,9,9,9
HC-2	None	6	6	100	13,13,13,13,13,13
HC-2	PR	7	7	100	4,5,5,6,9,9,13
HC-3	None	6	6	100	13,13,25,25,25,25
HC-3	PR	3	3	100	9,13,24
HC-4	None	6	6	100	13,13,25,25,25,25
HC-4	PR	3	3	100	6,9,24
HC-5	None	6	6	100	9,13,13,13,25,25
HC-5	PR	3	3	100	9,9,24
HC-6	None	6	6	100	13,13,13,13,13,25
HC-6	PR	6	6	100	6,6,9,9,9,13

[a] None = Untreated rumen fluid
PR = Precipitate from high speed centrifugation (greater than 15,000 g) reconstituted into a suspension 7.5 times as concentrated as untreated rumen fluid.

Experiment 5 Trial 6. This trial (Table 15) was conducted to determine if bacteria are the cause of mice deaths. Untreated rumen fluid samples from roughage fed, lactic acidosis, SDS, and high concentrate samples (feedlot samples) were used as controls. The mortality rate for the untreated control samples duplicated the results of Experiment 5, Trials 1, 2 and 5. Chlortetracycline was added to the samples at the rate of 1 mg/ml. The samples were cultured aerobically before inoculation. Only the high concentrate sample showed any growth on the plate. As expected the antibiotic treated roughage sample produced no deaths but the lactic acidosis treated sample produced one death and the SDS sample produced three deaths (three mice were used for each sample). The high concentrate sample which produced growth on the culture plate had a mortality rate of

100%. An antibiotic control solution of 1 mg/ml saline produced no deaths. Autoclaved samples from these same rumen fluid samples were also used. No deaths were produced from any autoclaved samples.

Table 15. Mortality of mice injected (i.p.) with untreated, autoclaved, and antibiotic treated roughage fed, lactic acidotic, SDS and high concentrate rumen fluid samples (Experiment 5, Trial 6).

Animal[a] no.	Treatment[b]	No. mice injected	No. died	Mortality	Time of death
				%	hr
321 D 3 hr	A	3	0	0	...
321 D 3 hr	B	3	0	0	...
321 D 3 hr	C	3	0	0	...
321 D 54hr	A	3	3	100	14,14,14
321 D 54hr	B	3	1	33	32
321 D 54hr	C	3	0	0	...
SD-04	A	3	3	100	14,14,14
SD-04	B	3	3	100	24,24,24
SD-04	C	3	0	0	...
HC-4	A	3	3	100	14,14,24
HC-4	B	3	3	100	24,24,32
HC-4	C	3	0	0	...
Control	D	3	0	0	...

[a] 321 D 3 hr - Roughage fed rumen fluid
321 D 54hr - Lactic acidotic rumen fluid
SD-04 - SDS rumen fluid
CH-4 - High concentrate rumen fluid

[b] A - Untreated rumen fluid
B - Antibiotic (chlortetracycline at the rate of 1 mg/ml) treated rumen fluid.
C - Autoclaved rumen fluid
D - Control antibiotic solution (chlortetracycline added to saline at the rate of 1 mg/ml).

Experiment 5 Trial 7. The phenol treated samples (roughage, lactic acidosis, SDS and high concentrate) produced growth in aerobic (blood agar) and anaerobic cultures (thioglycolate). Therefore a 1% phenol solution is not an effective bacteriacidal agent for the organisms encountered in this study. All the phenol treated rumen fluid samples and a 1% and 0.5%

control phenol solutions caused convulsions in the mice. The convulsions started within 30 sec after injection and lasted for 30-40 min. After the convulsions stopped the mice appeared to be normal for 36 hr. Addition of phenol to the rumen fluid samples did not alter the mortality rate of any of the samples or their precipitates (Table 16).

Table 16. Mortality of mice injected (i.p.) with phenol treated rumen fluid and precipitate from high speed centrifugation (15,000 g) of phenol treated rumen fluid (Experiment 5, Trial 7).

Animal[a] no.	Treatment[b]	No. mice injected	No. died	Mortality	Time of death
				%	hr
321 D 3 hr	A	6	0	0	...
321 D 3 hr	B	6	6	100	14,14,14,14,14,14
321 D 54hr	A	6	4	66	24,24,24,24
321 D 54hr	B	6	6	100	6,10,10,14,14,14
SD-04	A	6	6	100	14,14,14,14,14,14
SD-04	B	6	6	100	10,14,14,14,14,14
HC-4	A	6	6	100	24,24,24,24,24,24
HC-4	B	6	5	83	14,14,14,14,14
Control	C	3	0	0	...
Control	D	3	0	0	...

[a] 321 D 3 hr - Roughage fed rumen fluid
321 D 54hr - Lactic acidotic rumen fluid
SD-04 - SDS rumen fluid
HC-4 - High concentrate rumen fluid

[b] A - Phenol added to rumen fluid to make a 1% solution.
B - Precipitate from high speed centrifugation of 1% phenol treated rumen fluid.
C - 0.5% phenol control solution
D - 1.0% phenol control solution

Experiment 5 Trial 8. Penicillin (500 units/ml) and streptomycin (250 mg/ml) were used to treat roughage, lactic acidosis, SDS and high concentrate rumen fluid samples. This fluid was cultured aerobically and anaerobically before inoculation as in Experiment 5, Trial 7. The cultures produced growth indicating either insufficient antibiotic or the presence

of resistant organisms. The organisms cultured were Streptococcus and Bacillus. The antibiotic treated fluid was injected as treated and some was centrifuged (15,000 g) to determine if antibiotic treatment altered the toxicity of the precipitate. Untreated samples were used as controls. The untreated samples duplicated the mortality rates produced in previous trials. The addition of penicillin and streptomycin did not alter the mortality rate for the SDS sample but did lower the mortality rate for the lactic acidosis sample (from 66% to 0%) and the high concentrate sample (100% to 16.7%) (Table 17). The antibiotic did not alter the toxicity of the precipitates from centrifugation of lactic acidosis, SDS and high concentrate samples but did lower the mortality rate of the roughage sample precipitate from 100 to 50%.

Table 17. Mortality of mice injected (i.p.) with untreated and antibiotic treated rumen fluid and rumen fluid precipitate (Experiment 5, Trial 8).

Animal[a] no.	Treatment[b]	No. mice injected	No. died	Mortality	Time of death
				%	hr
321 D 3 hr	A	3	0	0	...
321 D 3 hr	B	6	0	0	...
321 D 3 hr	C	6	3	50	24,24,24
321 D 54hr	A	3	2	66	14,24
321 D 54hr	B	6	0	0	...
321 D 54hr	C	6	6	100	14,14,24,24,24,24
SD-04	A	3	3	100	14,14,24
SD-04	B	6	6	100	14,24,24,24,34,34
SD-04	C	6	6	100	14,14,14,14,24,24
HC-4	A	3	3	100	14,14,24
HC-4	B	6	1	17	34
HC-4	C	6	6	100	14,14,24,24,24,24

[a] 321 D 3 hr - Roughage fed rumen fluid
321 D 54 hr - Lactic acidotic rumen fluid
SD-04 - SDS rumen fluid
HC-4 - High concentrate rumen fluid

[b] A - Untreated rumen fluid
B - Rumen fluid treated with penicillin (500 units/ml) and streptomycin (250 mcg/ml).
C - Precipitate from high speed centrifugation (15,000 g) of rumen fluid

Experiment 5 Trial 9. There was a variation in mortality rate due to the various antibiotic treatments (Table 18). The mortality rates were as follows: (1) penicillin-streptomycin, 33%; (2) gentamicin, 0%; (3) chloromycetin, 100%; (4) erythromycin, 100%; (5) neomycin, 50%. No deaths were produced from the control antibiotic solutions. Bacillus was cultured from the abdominal cavity of the dead mice. This was the same Bacillus spp. cultured from the rumen fluid used in Experiment 5, Trial 8.

Table 18. Mortality of mice injected (i.p.) with SDS rumen fluid treated with various antibiotics (Experiment 5, Trial 9).

Animal[a] no.	Treatment[b]	No. mice injected	No. died	Mortality	Time of death
				%	hr
SD-04	A	6	0	0	...
SD-04	B	6	6	100	24,24,24,24,33,33
SD-04	C	6	6	100	14,24,24,24,24,33
SD-04	D	6	3	50	24,24,33
SD-04	E	6	2	33	24,33
Control a	A	3	0	0	...
Control	B	3	0	0	...
Control	C	3	0	0	...
Control	D	3	0	0	...

[a] SD-04 - SDS rumen fluid
Control - Antibiotic treated physiological saline

[b] A - Gentamicin (Gentocin[R] 50 mg/ml - Schering) added at the rate of 50 mcg/ml.
B - Erythromycin (Erythrocin[R] 5.0 mg/ml - Abbot) added at the rate of 100 mcg/ml.
C - Chloromycetin (Chloramphenicol[R] 100 mg/ml - Parke-Davis) added at the rate of 100 mcg/ml.
D - Neomycin (Biosol[R] - Upjohn) added at the rate of 1000 mcg/ml.
D - Penicillin and streptomycin added at the rate of 1000 units/ml and 500 mcg/ml respectively.

Experiment 5 Trial 10. Injection of the culture filtrates from Experiment 5, Trial 9, produced no deaths.

DISCUSSION

Experiment 1. When hay was fed the rumen pH dropped from a pre-feeding value of 7.15 to a 7 hr postfeeding value of 6.8 in 140 E and from 6.65 to 6.45 in 321 D. This suggests moderate VFA production in the rumen (Hungate, 1966). Lactic acid would not be expected to be present when the rumen pH is this high (Bruno and Moore, 1962). There was no ruminal histamine. This agrees with the low concentrations of ruminal histamine in roughage fed animals reported by Ahrens (1967), Long (1970), Sanford (1963), Sjaastad and Stormoken (1963) and Van der Horst (1961). The data suggest that rumen function was normal in the roughage fed animals. Therefore these animals served well as roughage control animals.

Experiment 2. When lactic acidosis was produced in 321 D and 140 E the rumen pH decreased as expected. This has been reported by others (Broberg, 1960; Dirksen, 1970, Dunlop and Hammond, 1965; Hungate et al., 1952; and Turner and Hodgetts, 1949-1959). The lowest rumen pH recorded for each animal was 3.80 and 3.55 for 321 D and 140 E respectively. The following rumen pH values for lactic acidosis have been reported: 4.5 (Mann, 1970); 4.0 (Broberg, 1960 and Dunlop and Hammond, 1965); 3.8 (Bond, 1959); and 3.6 (Juhasz and Szegedi, 1968). The value of 3.55 for 140 E is as low a rumen pH value as has been reported. This animal survived. It is doubtful if an animal would survive if the rumen pH dropped much below 3.55. Dirksen (1970) reported that the initial decrease in pH was due to an increase in total VFA concentration but that later the marked and prolonged depression of pH was due to an increase in lactic acid concentration. This was not the case in 321 D or 140 E. The total

VFA concentration did not increase in either animal. The total rumen lactic acid concentration increased almost fourfold in 140 E (0.038 to greater than 1.28 mg/ml) and in 321 D (0 to greater than 1.28 mg/ml) in the first 90 min after engorgement. On the basis of a rapid increase in rumen lactic acid concentration and the lack of an increase in total VFA concentration, it is concluded that rumen lactic acid accounted for the initial and prolonged depression in rumen pH.

The total VFA concentration decreased markedly in 321 D but showed no trend with time in 140 E. Total VFA concentration has been reported to decrease in lactic acidosis (Allison et al., 1964; Hungate et al., 1952; Phillipson, 1952; and Telle and Preston, 1971). Reid et al. (1957) reported that total VFA concentration decreases when rumen pH drops below 5. This is due to a shift in rumen microflora caused by a lowered rumen pH (Dunlop and Hammond, 1965; Krogh, 1959, 1960, 1961a,b, 1963b; and Turner and Hodgetts, 1949-1959). The precipitous drop in total VFA concentration in 321 D indicates a severe change in the normal rumen fermentation.

The total VFA concentration in 140 E remained essentially unchanged. This has also been reported for lactic acidosis by Uhart and Carroll, 1967. Apparently the aberration in rumen metabolism was not as severe in 140 E as in 321 D. This may account for the demise of 321 D even though the rumen pH was lower in 140 E.

The molar percentages of acetic, propionic and butryic remained essentially unchanged throughout the course of lactic acidosis. This agrees with the results of Allison et al. (1964), Tremere et al. (1968) and Uhart and Carroll (1967). Dirksen (1970 and Phillipson (1952) reported increases in propionic acid with a narrowing of the acetic:propionic ratio

during lactic acidosis.

The pre-feeding trace concentrations of lactic acid were in the range of values reported for roughage fed animals (Balch and Rowland, 1957; Waldo and Schultz, 1955). The trend of a rapid increase in rumen lactic acid which occurred in 140 E and 321 D has often been reported (Broberg, 1960; Dirksen, 1970; Dunlop and Hammond, 1965; Hyldgaard-Jensen and Simesen, 1966; Juhasz and Szegedi, 1968; MacKenzie, 1967; and Turner and Hodgetts, 1949-1959). Tremere et al. (1968) reported that the highest concentration of lactic acid coincided with the lowest rumen pH. This also occurred in 140 E and 321 D.

Blood L(+) lactate values in 140 E and 321 D both declined initially from pre-engorgement values but by 24 to 30 hr the levels were increased by 50% or more. There are no reports of initial decreases in blood lactate concentration in lactic acidosis but there are several reports of increases in blood lactate concentration (Broberg, 1960; Dirksen, 1965, 1967; Dunlop, 1967; Dunlop and Hammond, 1965; Hyldgaard-Jensen and Simesen, 1966; and Turner and Hodgetts, 1949-1959). The highest blood L(+) lactate values for 321 D (0.19 mg/ml) and for 140 E (0.21 mg/ml) correspond closely with 0.20 mg/ml reported by Broberg (1960).

The rumen histamine values at pre-engorgement were 0 mcg/ml for both 321 D and 140 E. This agrees with the very low concentrations reported in roughage fed animals (Ahrens, 1967; Dunlop, 1967; and Sjaastad, 1967a). Histamine accumulation in the rumen was noted at 12 hr in 140 E and 30 hr in 321 D. The highest concentrations of rumen histamine were 22.78 (54 hr) and 26.00 mcg/ml (48 hr) for 140 E and 321 D respectively. High rumen histamine values in lactic acidosis have also been reported (Ahrens, 1967; Dain et al., 1955; Dunlop, 1967; and Turner and Hodgetts, 1949-1959).

Histamine is not absorbed from the rumen if there is an acid pH (Dunlop et al., 1965), so most of the histamine probably remained in the rumen and was not absorbed (rate of removal of rumen contents including histamine was probably slowed due to rumen atony). In the rumen of a roughage fed animal, histamine is rapidly inactivated (Sjaastad and Kay, 1966, 1970). There are no reports of histamine inactivation in lactic acidosis cattle or sheep. Laminitis occurred in 321 D. The role of histamine in bovine laminitis has been discussed by MacLean (1966) and Nilsson (1963). Nilsson (1963) relates low blood histamine and high local tissue histamine to laminitis in cattle. MacLean (1966) relates laminitis in cattle to high blood histamine. Both workers reported variable blood levels of histamine throughout the course of laminitis. Nilsson (1963) reported that blood histamine is considered physiologically inactive since it is in a bound form. Histamine concentration in the blood of 321 D and 140 E was not determined.

Total serum protein and blood urea nitrogen (BUN) showed no trend with time. SGOT levels decreased. These results differ with those reported by Dirksen (1965, 1967, 1970) and Hyldgaard-Jensen and Simesen (1966). The reported increases in PCV and hemoglobin occurred in both animals. This has also been documented by others (Dirksen, 1965, 1967, 1970; Dunlop and Hammond, 1965; Hyldgaard-Jensen and Simesen, 1966; Juhasz and Szegedi, 1968; and Turner and Hodgetts, 1949-1959). Increases in PCV, hemoglobin, total protein and BUN are merely signs of dehydration (Dirksen, 1965, 1967; and Dunlop and Hammond, 1965). It is not clear why the BUN and total protein did not increase in 321 D and 140 E. 321 D exhibited clinical signs of severe dehydration (eyes sunken and decreased skin tone and turgor) while 140 E did not appear

dehydrated. Dehydration is due to large amounts of fluid being drawn into the rumen from the blood and tissues due to increased osmolarity of the rumen fluid. This fluid sequesters in the rumen leaving the animal dehydrated. The volume of fluid in the rumen of both 140 E and 321 D increased greatly.

In summary lactic acidosis in 140 E and 321 D was characterized by increased rumen and blood lactic acid, increased rumen histamine, decreased or unchanged VFA concentrations and molar percentages, and dehydration.

Experiment 3. The average rumen pH of the feedlot steers used as controls was 5.9. This is lower than what would be expected for a roughage fed animal and yet well above that seen in lactic acidosis (Hungate, 1966). The average total concentrations of VFA was 107.4 mM/l. This is somewhat less than might be expected on a high concentrate ration but part of this may be explained by the fact that the samples were collected 6 hr after feeding. The molar ratio of acetic to propionic was almost 1:1 (46.7% acetic and 42.7% propionic). On high grain rations the narrow acetate: propionate ratio is due to an increase in the amount of propionic acid and is not due to a decrease in the amount of acetic acid (Bauman et al., 1971; and Davis, 1967).

The average rumen lactate concentration (0.274 mg/ml) was much lower than that observed during lactic acidosis in Experiment 2. Low levels of rumen lactic acid in cattle adapted to high grain rations have been reported by DeBarthe et al. (1971), Ryan (1963b), and Tremere et al. (1968).

There was no rumen histamine in any of the six feedlot steers. There are no reports of lowered rumen histamine concentration due to adaptation to high grain as there is in rumen lactic acid. It is probable that adaptation results in lower rumen lactic acid levels from lower production

and/or increased lactate fermenters. The same balance may also exist with rumen histamine. More work needs to be done to establish rumen histamine concentration in cattle fed and adapted to high concentrate rations.

Experiment 4. The SDS rumen samples showed large variations in histamine, lactic acid, VFA and pH values. The range in rumen histamine concentration was from 0 to 57.4 mcg/ml. The range in rumen total lactic acid concentration was from 0.0226 to greater than 1.284 mg/ml. The range in total VFA concentration was from 64.49 to 306.66 mM/l. The range in rumen pH was from 4.5 to 7.0. The wide range in these parameters of rumen function are probably indicative of different rations, varying stages of adaptation in different feedlots and possibly the presence of borderline and subclinical lactic acidotic cattle in some cases. Some SDS samples had extremely high total VFA concentrations. It is possible that these animals were consuming a high energy ration. If the animals succumbed to SDS soon after eating, rumen fermentation would have continued after death and since the rumen microbes were well adapted to fermenting the high concentrate ration VFA production would have continued resulting in a high VFA concentration.

The high rumen total lactate concentration in some SDS cattle suggest that possibly some of these animals were not well adapted to high concentrate rations. In an extensive study of 1072 feedlot cattle, 98% had less than or equal to 0.05 mg/ml and 89% were less than or equal to 0.01 mg/ml (DeBarthe et al., 1971). It is difficult to conclude whether or not the high rumen lactate concentrations of some of the SDS cattle were abnormal without knowning the values of other cattle in the feedlot. Lactic acid does not elicit its pharmacological or physiological effects like a toxin

and very high levels must be reached for it to be toxic. A toxin elicits its effects in very low concentrations. Lactic acid cannot be considered toxic in the classical sense (ammonia concentration as low as 0.6 to 1.0 mg/100 ml in the blood is lethal whereas lactic acid is not toxic even at a blood level as high as 200 mg/100 ml, Juhasz and Szegedi, 1968). Lactic acidosis has been recognized as a problem for 20 years whereas SDS is a relatively new problem. Lactic acid is unlikely to be the cause of SDS because some SDS cases had no rumen lactic acid and high concentrations of lactic acid produce signs whereas no signs have been reported in SDS. Most SDS cattle have been in the feedlot for over 100 days while rumen lactic acid is a problem when cattle are started on feed.

There is no pattern to rumen histamine concentrations of SDS cattle. Most of the values were low (less than 1.0 mcg/ml). It is unlikely that histamine is involved in SDS because of the low values for almost all of the SDS cattle. More research is needed to establish rumen histamine values for cattle on high concentrate rations. From the results presented in Experiments 3 and 4 it is unlikely that ruminal histamine is a problem when cattle are fed high concentrate rations. Oral and/or intraruminal infusion of histamine has not been found to be toxic (Dickinson and Huber, 1972; Sjaastad and Kay, 1966). Oral histamine has been found toxic only when sheep were treated with a histaminase or diamine oxidase inhibitor, aminoguanidine (Sjaastad, 1967g) or with formaldehyde (Eliassen and Sjaastad, 1968). It is unlikely that either situation would be of practical significance in cattle feeding operations. On high roughage rations there is rapid inactivation of ^{14}C histamine in the rumen (Sjaastad, 1967g; and Sjaastad and Kay, 1966). It is not known if this same inactivation occurs in the rumen of cattle fed high concentrate rations. Very

little histamine is absorbed from the rumen (Crevasse, 1963; Dickinson, 1969; Dickinson and Huber, 1972; Dunlop et al., 1965; McDonald, 1963; Neumark, 1967; Neumark and Tadmore, 1968; Shinozaki, 1957; Sjaastad and Kay, 1966; and Taylor, 1968) especially at low rumen pH (Dunlop et al., 1965). Since little histamine would reach the systemic circulation via ruminal absorption it is unlikely that systemic concentrations would ever be high enough to kill an animal as suddenly as occurs in SDS.

The low rumen pH of most of the SDS cattle may be due to several factors: cattle on high concentrate rations have a low rumen pH (Experiment 3); many of the cattle may have succumbed to SDS after eating therefore the rumen pH would have decreased due to active fermentation; and fermentation may have continued after death especially in the summer heat. Thomson (1969) reported that 24 hr after death rumen pH decreases 0.4 pH unit as measured by pH meter and 1.0 as measured by pH indicator paper. Most of the SDS samples were probably collected within 12 hr after death and then frozen. The pH was measured with a pH meter after the samples were thawed. The lowest rumen pH (4.5) was not probably low enough to have killed any of the SDS cattle.

Experiment 5 Trial 1. The data presented suggests that there are no toxic or lethal principles in rumen fluid from roughage fed animals. This agrees with the observations of Dougherty and Cello (1949, 1952) and Turner and Hodgetts (1949-1959). Lactic acidosis rumen fluid had a mortality rate of 30.9%. Lactic acidosis rumen fluid has been shown to be somewhat toxic (Dain et al., 1955; Dougherty and Cello, 1949, 1952; Dunlop and Hammond, 1965; Hungate et al., 1952; Mullenax, 1966; and Turner and Hodgetts, 1949-1959). These results are similar to those reported by Hungate et al. (1952) where lactic acidosis rumen fluid produced less than

50% mortality in mice. The deaths produced in the mice from lactic acidosis rumen fluid could possibly be due to bacteria, gram negative bacterial endotoxin, gram positive bacterial exotoxin, low pH (due to lactic acid and/or VFA), toxic amines (histamine) or another unknown toxic principle. Abrupt change from high roughage to high grain rations creates an environment in the rumen suitable to produce and/or release this toxic substance.

Experiment 5 Trial 2. Rumen fluid from SDS cattle was much more toxic than lactic acidosis rumen fluid on the basis of a higher mortality rate and earlier deaths. There are no reports in the literature on toxicity of rumen fluid from finished or fattened cattle from a feedlot. Most of the work has been conducted with either roughage fed or lactic acidotic rumen fluid. Prier (1954) reported on the toxicity of rumen fluid collected in a slaughter house but the ration or condition of the cattle was not mentioned. A possible explanation for the increased toxicity of rumen fluid from cattle maintained on high grain rations could be the concentration of a toxic principle produced by high grain feeding. Mullenax et al. (1966) reported that gram negative bacterial endotoxin could be released when the gram negative bacteria are killed off due to a lowered rumen pH causing a shift from a predominantly gram negative to a predominantly gram positive rumen flora. This would be an unlikely situation in SDS cattle maintained on high concentrate rations because they are already adapted to high concentrate rations and little or no change in rumen flora occurs after 4 wk (most SDS cattle have been on full feed for over 100 days). Rumen lactic acid levels are much lower in cattle adapted to high concentrate rations (DeBarthe et al., 1971; Ryan, 1964b; and Tremere et al., 1968) so lactic acid would not account

for the increased toxicity of SDS rumen fluid. Very high levels of VFA have been found in SDS rumen fluid (Experiment 4) but this is unlikely to result in toxicity as shown by Dougherty and Cello (1949). The concentration of rumen histamine varies greatly. Samples with no rumen histamine were found to be very toxic.

Krogh (1963b) reported on gram positive rumen flora in cattle with lactic acidosis. These organisms (Streptococcus bovis and Lactobacillus) are probably also present in large numbers in cattle adapted to high grain rations. These organisms are not known for their pathogenicity except for Streptococcus bovis in feedlot bloat (Hungate, 1966) and this is not a toxic or septicemic condition. These organisms are not known to produce a potent exotoxin. Little work has been done on the rumen flora of cattle maintained on high grain rations. Other gram positive or negative organisms may exist which could possibly be infectious or toxin producing in the rumen of cattle maintained on high grain rations. The SDS rumen fluid had different physical properties than the roughage or lactic acidotic fluid: more viscous and transluscent. These factors could influence the toxicity of the fluid when injected.

Experiment 5 Trial 3. This trial was conducted to study the effects of the following factors: exotoxin (by heating to 85 to 90 C for 15 min); pH (by buffering to pH 7.0); infectious agent (by adding chlortetracycline at the rate of 5 mcg/ml); and a toxin of molecular weight greater than 10,000 (by high speed centrifugation, 15,000 g for 15 min). The treatments of heat, buffering and antibiotic did not alter the toxicity of the SDS rumen fluid. The toxic principle is heat stable, pH stable and not sensitive to chlortetracycline at a dosage lethal to rumen microbes (Radisson, 1955). The supernatant from high speed centrifugation of SDS

samples was not toxic (no deaths). Apparently the toxin is of high molecular weight or is adsorbed to a large molecule. The supernatant would contain water and rumen fluid metabolites (VFA, lactic acid, histamine, etc.). Since the supernatant is nontoxic, VFA, lactic acid and histamine present in the concentrations of these samples can be assumed not to be the toxic principle. Dougherty and Cello (1949) reported that VFA are not toxic. Dougherty and Cello (1949) reported a toxic factor in rumen fluid that was nonvolatile, heat stable and dialyzable. Later work by Dougherty and Cello (1952) reported that the toxic factor was not inactivated by trypsin or pepsin digestion; not ether extractable; not inactivated by treatment with nitrous acid or histaminase (diamine oxidase); and was pH stable within a pH range of 1.2 to 12.2. Turner and Hodgetts (1949-1959) reported that the toxic effects of lactic acidotic rumen fluid were not inhibited by antihistamine therapy. The results presented here agree in general with that reported in the literature on toxic factors in rumen fluid.

Experiment 5 Trial 4. A mortality rate of 100% was produced by intraperitoneal injection of lyophilized precipitate from high speed centrifugation (15,000 g) of untreated rumen fluid from roughage fed, lactic acidotic and SDS cattle. The 100% mortality from the precipitate from centrifugation of roughage fed rumen contents was surprising. The roughage fed samples produced earlier deaths than precipitate from centrifugation of lactic acidotic or SDS rumen fluid samples. The precipitate from high speed centrifugation would contain bacteria, protozoa, high molecular weight material and particulate matter. Therefore deaths could be due to any of these factors. The supernatant would contain water and metabolites (VFA, lactic acid, histamine, etc.) present

in the rumen fluid. This same lyophilized precipitate was administered orally to mice and no deaths were produced. Many toxins (Staphylococcus aureus exotoxin and Clostridium botulinum exotoxin) are known to be toxic when given orally. These toxins either elicit their effect in the gastrointestinal tract or are absorbed in the acidic medium in the stomach or slightly alkaline medium of the intestinal tract. The results of Experiment 5, Trial 3 indicate that the toxic factor is pH stable so it is unlikely that it would be inactivated due to pH changes in the stomach or intestines of the mouse. The results of Dougherty and Cello (1952) show that the toxic principle they found was not inactivated by either pepsin or trypsin digestion. Therefore it is unlikely that digestive enzymes inactivated the toxic principle. There was no suggestion of depression or gastrointestinal distress. These results suggest that a toxin is probably not the lethal factor. Microbes and particulate matter could possibly be destroyed or inactivated by the mouse gastrointestinal tract. If bacteria are the cause of death, then different organisms are probably involved when comparing roughage fed rumen fluid (predominantly gram negative flora) with lactic acidosis and SDS rumen fluid (predominantly gram positive flora). Mullenax et al. (1966) isolated an endotoxin from gram negative rumen bacteria therefore the mice deaths reported here may have resulted from an endotoxin rather than a true infection. The results of untreated roughage fed, lactic acidosis and SDS rumen fluid samples duplicated those of Experiment 5, Trials 1 and 2.

Experiment 5 Trial 5. Rumen fluid samples were collected from six steers that were on a high grain ration for over 100 days. These cattle were considered to be controls to cattle succumbing to SDS since they were

fed a typical finishing ration. The 100% mortality from untreated samples was even higher than the 63.3% mortality rate obtained from SDS samples (Experiment 5, Trial 2). The precipitate from high speed centrifugation of these samples produced 100% mortality as did the precipitate from centrifuged roughage fed, lactic acidotic and SDS rumen fluid samples. On the basis of the results of Experiment 5, Trials 3 and 4, pH, heat labile exotoxin, absorbable high molecular weight toxin and rumen metabolites (VFA, lactic acid, and histamine) can be ruled out as the toxic agents. Rumen bacteria seem to be the most probable cause. It is generally assumed that rumen microbes are not pathogens (Hungate, 1966) but it is possible that pathogens (probably gram positive facultative anaerobes) could proliferate in the rumen of cattle fed high grain rations.

Experiment 5 Trial 6. The results from the antibiotic treated rumen fluid samples were inconclusive. The mortality rates for the roughage fed, lactic acidotic, SDS and high concentrate samples were 0, 50, 100 and 100% respectively. The mortality rates for the same untreated samples were 0, 100, 100 and 100%, respectively. The mortality rate was lowered only for the lactic acidotic sample. The mortality rate was 0% for autoclaved samples. In addition to killing bacteria autoclaving will also inactivate any heat labile toxin, primarily exotoxin, (Merchant and Packer, 1967). In Experiment 5, Trial 3 the samples were heated to temperatures that would denature or inactivate exotoxins and no difference in mortality rate was produced. Gram negative bacterial endotoxins are heat stable (Landy and Braun, 1964) but only the roughage fed fluid would have had a predominantly gram negative flora. Turner and Hodgetts (1949-1959) reported that autoclaving did not alter the toxicity of lactic acidotic rumen fluid but the data presented here definitely shows a

difference. Only the high concentrate sample produced growth on the bacterial culture plate. Even though the lactic acidotic and SDS samples produced no growth they had mortality rates of 50 and 100% after treatment with chlortetracycline but 0% mortality for both after autoclaving. On the basis of these results bacteria cannot be ruled out as a cause of mice deaths.

Experiment 5 Trial 7. The samples used in Experiment 5, Trial 6 were used in this trial. Phenol (1.0% solution) was chosen as the bacteriacidal agent but proved ineffective as growth was produced in both aerobic and anaerobic cultures. Phenol also caused convulsions in the mice. The phenol treated samples produced the same mortality rates as produced by untreated rumen fluid in previous trials. The phenol per se did not cause any deaths because the phenol control solutions (0.5 and 1.0%) had a 0% mortality rate. Because of bacterial culture growth, phenol was considered to be an unsuitable agent and no definite conclusions could be drawn.

Experiment 5 Trial 8. The addition of penicillin (500 units/ml) and streptomycin (250 mcg/ml) lowered the mortality for the lactic acidotic and high concentrate samples but not for the SDS sample. Since the cultures produced growth and since the mortality rate for the SDS sample was 100%, it may be concluded that either resistant organisms were present or an insufficient quantity of antibiotics was used. The antibiotics did not alter the toxicity of the precipitates from lactic acidotic, SDS and high concentrate samples but did alter the toxicity of the roughage fed sample. Even though the results are somewhat inconclusive, bacteria appear to be involved in the mice deaths.

Experiment 5 Trial 9. The following antibiotics were used on one

SDS (SD-04) sample: penicillin-streptomycin; gentamicin; chloromycetin; erythromycin; and neomycin. The respective mortality rates were: 33%; 0%; 100%; 100% and 50%. Penicillin-streptomycin, gentamicin and neomycin were effective in preventing and reducing the mice deaths. Since 1 mg of gentamicin contains more activity than 1 mg of the other antibiotics, gentamicin was the most potent antibiotic used. Penicillin-stretptomycin gave inconclusive results in Experiment 5, Trial 8, but when the dosage was double for this trial, the mortality rate was lowered to 33%. Also a larger dosage of neomycin was used and a lower mortality rate (50%) resulted. The dosages of chloromycetin and erythromycin were those commonly used in tissue culture work. This quantity apparently was not enough to kill all the bacteria in high grain rumen fluid. It would appear that the bacteria developed resistance to chloromycetin and erythromycin, however this is unlikely because these two antibiotics, for economic reasons, are rarely used in mature cattle.

The Bacillus cultured from the dead mice is apparently the cause of the mice deaths. The Bacillus is unlikely to be a true pathogen if found in the rumen (Hungate, 1966). However, when injected intraperitoneally it may overwhelm the defense mechanisms of the mouse. Bacillus spp. are not known to produce an exotoxin but there is the possibility that they might since they are gram positive organisms.

Experiment 5 Trial 10. Bacillus was cultured from the dead mice in Experiment 5, Trial 9. To determine if this Bacillus produced an exotoxin, culture filtrates from the Bacillus cultures were injected intraperitoneally into mice. No deaths were produced. From these results and those of previous trials, it can be concluded that the lethal agent from rumen fluid is probably bacterial and not a toxin. Further work is needed to

determine the pathogenicity of some of the microbes in the rumen and their relation to malfunctions in rumen physiology and animal health.

CONCLUSION

A summarization of the results with fistulated and feedlot cattle led to the following conclusions:

(1) Since histamine and L(+) lactic acid were absent in the rumen fluid from the roughage fed cattle and because the pH was not below 6.45, it is apparent that the rumen fermentation was normal. Therefore these animals served well as roughage control animals.

(2) Engorgement of two fistulated cattle with grain produced lactic acidosis due to an accumulation of lactic acid as the pH progressively fell to below 4.0. The clinical signs of anorexia, depression, dehydration were produced by excessive levels of lactic acid. It is concluded that both animals were in metabolic acidosis. Dehydration was evidenced by increased PCV and hemoglobin concentration. Rumen histamine reached extremely high concentrations in both animals (above 22 mcg/ml). The animal with the highest ruminal lactic acid and histamine concentration developed laminitis and eventually died. It is concluded that the laminitis was due to a systemic and/or local histamine reaction. The VFA concentration was markedly lowered in one animal but showed no trend with time in the other even though a large quantity of substrate was in the rumen.

(3) Rumen fluid collected from six feedlot steers revealed no histamine, a low concentration of lactic acid, a moderately high total VFA concentration, a narrow acetic:propionic acid ratio and a pH below 6. These parameters of rumen metabolism suggested that the cattle had become adapted to a high concentrate ration.

(4) Rumen fluid collected from cattle succumbing to SDS revealed

wide variations in lactic acid concentration, histamine concentration, total VFA concentration and pH. Since the rumen fluid or some SDS cattle had only a small quantity of lactic acid and no histamine it is probable that neither is involved in SDS. The wide variation in these parameters of rumen metabolism indicate varying adaptation to high concentrate feeding, different rations, varying time of death after eating and possibly subclinical or borderline lactic acidosis in some cases.

A summarization of the results with inoculation of rumen fluid intraperitoneally into mice led to the following conclusions:

(1) Roughage fed rumen fluid was not toxic when injected into mice. The mortality rate was 30.9% when lactic acidosis rumen fluid was injected and 63.3% when SDS rumen fluid was injected. The mortality rate was 100% when high concentrate rumen fluid from six feedlot steers was used. Apparently high grain feeding creates an environment in the rumen favorable to the development of a toxic principle in mice.

(2) The rumen metabolites of VFA, lactic acid and histamine are not the toxic principle because there was no trend between mouse deaths and samples with either high or low concentrations of these metabolites. Furthermore the supernatant from high speed centrifugation which would contain low molecular weight rumen fluid metabolites was not toxic when injected intraperitoneally into mice.

(3) The toxic principle is heat stable (to 90 C), pH stable, not sensitive to 1% phenol, not sensitive to low concentrations of antibiotics, not spun down by low speed centrifugation and not absorbed when given orally. The toxic principle is spun down by high speed centrifugation. The toxic principle is rendered nontoxic by autoclaving and by high levels of antibiotics. On the basis of these results it is concluded that bacteria

are the cause of mice deaths.

SUMMARY

Investigations were conducted on the Sudden Death Syndrome, (SDS), a relatively new problem in the feedlot industry. In this study SDS cattle are apparently healthy cattle in feedlots dying suddenly and without sign of sickness or exhibiting lesions indicative of other disease conditions. Rumen fluid from SDS cattle was compared with rumen fluid from cattle maintained on an all roughage ration, cattle in which lactic acidosis was produced through grain engorgement and healthy cattle in a feedlot which had been maintained on a high energy finishing ration for over 100 days.

The rumen fluid from the lactic acidotic cattle was characterized by high concentrations of lactic acid and histamine, low pH and variable total VFA concentration due to an upset in the normal rumen metabolism. The rumen fluid from the feedlot steers used as controls had moderately high total VFA concentration, narrow acetate:propionate ratio, low lactic acid concentration, no histamine and pH below 6. The rumen fluid from SDS cattle had variable concentrations of lactic acid, histanine and total VFA. There was no relationship between SDS and rumen pH, histamine, lactic acid and total VFA concentrations.

To determine if a rumen toxin was present in the rumen fluid of cattle succumbing to SDS, mice were injected intraperitoneally with rumen fluid from SDS, roughage fed, lactic acidotic and cattle maintained on a high concentrate finishing ration. The mortality rates were 0% for roughage fed rumen fluid, 30.9% for lactic acidotic, 63.3% for SDS and 100% for the high concentrate rumen fluid. Neither pH, heat labile exotoxin nor rumen metabolites (VFA, lactic acid and histamine) were

involved in the toxicity of SDS or high concentrate rumen fluid. Autoclaving and high dosages of antibiotics reduced the toxicity thereby indicating that the toxic agent in rumen fluid for mice was bacteria.

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RUMEN FLUID FROM "SUDDEN DEATH," LACTIC ACIDOTIC AND HEALTHY CATTLE AND ITS TOXIC EFFECT IN MICE

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Investigations were conducted on the Sudden Death Syndrome, (SDS), a relatively new problem in the feedlot industry. In this study SDS cattle are apparently healthy cattle in feedlots dying suddenly and without sign of sickness or exhibiting lesions indicative of other disease conditions. Rumen fluid from SDS cattle was compared with rumen fluid from cattle maintained on an all roughage ration, cattle in which lactic acidosis was produced through grain engorgement and healthy cattle in a feedlot which had been maintained on a high energy finishing ration for over 100 days.

The rumen fluid from the lactic acidotic cattle was characterized by high concentrations of lactic acid and histamine, low pH and variable total VFA concentration due to an upset in the normal rumen metabolism. The rumen fluid from the feedlot steers used as controls had moderately high total VFA concentration, narrow acetate:propionate ratio, low lactic acid concentration, no histamine and pH below 6. The rumen fluid from SDS cattle had variable concentrations of lactic acid, histamine and total VFA. There was no relationship between SDS and rumen pH, histamine, lactic acid and total VFA concentrations.

To determine if a rumen toxin was present in the rumen fluid of cattle succumbing to SDS, mice were injected intraperitoneally with rumen fluid from SDS, roughage fed, lactic acidotic and cattle maintained on a high concentrate finishing ration. The mortality rates were 0% for roughage fed rumen fluid, 30.9% for lactic acidotic, 63.3% for SDS and 100% for the high concentrate rumen fluid. Neither pH, heat labile

exotoxin nor rumen metabolites (VFA, lactic acid and histamine) were involved in the toxicity of SDS or high concentrate rumen fluid. Autoclaving and high dosages of antibiotics reduced the toxicity thereby indicating that the toxic agent in rumen fluid for mice was bacteria.