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The Blood Picture in Correlation with Bloat

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THE BLOOD PICTURE IN CORRELATION WITH BLOAT

By

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Bloat may spell disaster to any farmer on a given day. This kind of disaster is typical and not infrequent in ruminants especially cattle and sheep. Bloat affects polygastrics, why not monogastrics? Ruminants or polygastrics have a four compartment stomach utilizing protozoa and bacteria similar to a fermentation vat which catabolizes complex substrates into simple digestable nutrients while the monogastrics rely on secretions from the stomach lining. Primarily fermentation takes place in the first compartment or rumen. The end products of fermentation are water and large amounts of free gas, which can accumulate in the rumen when disposal is impaired.

Why do animals dispose of these large quantities of gas 364 days out of a year and the following day die because of the inability to discharge this gas? Some investigators suggest that the rumen is unableto function when certain soft materials such as alfalfa are the primary components of the diet. The addition of scaborous materials to the ration is believed to be beneficial for stimulating eructation and the elimination of gas. Others believe that drugs such as penicillin and aureomycin will inhibit gas production sufficiently to prevent bloat. Still another theory is based on evidence that a toxic material is present at times in sufficient quantity to cause death.

Despite voluminous amounts of research done in the field of bloat, the cause of bloat and the cause of death are still vague. The toxic theory has not been disproven completely, therefore, this seems to be as vital to the overall bloat picture as any. After choosing the toxic

theory the assumption that the toxicity would be found in the blood was made. Carbon dioxide, pseudocholinesterase, and methemoglobin were chosen as the compounds in the blood which might have the greatest potential for causing bloat symptoms or death.

REVIEW OF LITERATURE

Since the saliva of ruminants contains no ptyalin or other digestive enzymes, the only carbohydrate digestion is by microorganisms before the ingesta reaches the abomasum. However the construction of the ruminant stomach is such that large quantities of highly fermentable material may be stored for varying periods. During the storage bacteria and protozoa act upon the various constituents liberating products that are usable to the animal plus large quantities of gas which at times can be detrimental to the animal's health.

Saliva initiates digestion and the rumen and reticulum continue this process by fermentation. The major end products of fermentation are carbon dioxide, methane, and water. These are produced in voluminous amounts because the quantity of material ingested initially is excessive and highly fermentable.

A majority of the gas resulting from fermentation is eliminated via eructation. The source of the rumen gases for the main portion is from fermentation of the food ingested into the rumen. The first stomach or rumen and the reticulum carry on digestion by microorganisms wholly because no secretions are added in these two compartments. The omasum precedes the abomasum and has mucous glands occurring only at the omasum-abomasal junction; however, the main function of the omasum is trituration of ingesta. The glandular portion of the ruminant's stomach is the abomasum. Some rumen gas is eliminated rectally, but the majority which is not eructated diffuses through the rumen wall.

The possibility of the rumen wall being permeable to diffusible substances has been suggested by Rankin (44), McAnally and Phillipson (33),

Barcroft, McAnally and Phillipson (2), but the diffusion rates for ruminal gases are still unknown, Kleiber (25). Gas absorption rates are believed to be proportional to the area of mucosa exposed and the pressures exerted, Dougherty (12). Some calculations for rate of diffusion can be based on the work done by Teschendorf (47) in which he found 100 milliliters of carbon dioxide diffused through the peritoneum of a rabbit in one hour. If this rabbit peritoneum was set up proportional to a rumen, the rumen would diffuse 3.4 liters per hour.

The principal gases produced in the rumen are carbon dioxide and methane while the smaller volumes of gases are hydrogen, oxygen, nitrogen, and hydrogen sulfide. From feeding time on carbon dioxide increases for the first three or four hours then gradually drops. At this time nitrogen decreases but begins increasing about seven to eight hours after feeding, while oxygen, methane, and hydrogen remain relatively constant, (50).

Rate of gas formation has been charted by Cole, Mead, and Kleiber, (9). The rate of total gas formation reaches its lowest ebb previous to eating. Within one to one and one-half hours after eating a maximum of twenty to twenty-five liters per half hour is produced. Following this peak, the total formation rate gradually declines during a period of seven to eight hours after feeding when it returns to prefeeding levels which range from one to eight liters per one-half hour. The composition of the rumen gases is relatively constant regardless of the ration, but varies markedly with time elapsing after feeding according to Washburn and Brody (49). The total amount of gas is not affected greatly by the ration as illustrated by Cole, Mead, and Kleiber, (9), however the amount of gas formed is directly related to the amount of ration eaten.

Research workers disagree as to the sources of individual rumen gases. For instance, several workers suggest that the precursor of methane is from the sugar resulting from bacterial fermentation of cellulose (54), (22), (1), (27). On the other hand Mitchell, Hamilton, and Haines (36) have been unable to confirm these results. Carbon dioxide sources are practically unlimited but the immediate precursor as a result of fermentation is either a one-carbon or a three-carbon compound, (28). Saliva provides a rich source of carbon dioxide, (32) which ranges because of a high content of carbonates, to an equivalent of approximately 200 volumes per cent carbon dioxide.

Hydrogen gas, which is found in the rumen, stems from bacterial fermentation of carbohydrates, (55), (38), (31). Pyruvic acid, which is a product of glycolysis, can be a source of hydrogen, (53).

Hydrogen sulfide in the rumen has its most likely source in the sulfur containing organic compounds, especially amino acids. High concentrations of hydrogen sulfide of approximately seven-tenths per cent were reported by Dougherty (10) in the rumen of animals which had died of bloat three to twelve hours before the samples were taken. Concentrations of hydrogen sulfide from ten to twenty times the normal concentration were found in bloated animals, (40). However on analyses made as the gas left the rumen Kleiber, Cole, and Mead (26) found an average hydrogen sulfide content of .11% by volume and no correlation between the concentration of hydrogen sulfide and the severity of bloat. Oxygen and nitrogen sources in ruminal gas are unknown for the most part, however Quin (42) speaks of nitrogen being brought in by swallowing. Ordinarily the air has a ratio of four parts nitrogen to one part oxygen yet Kleiber,

Cole, Mead (26) point out that they have found ratios up to seven volumes nitrogen to less than one volume oxygen.

Numerous observations led Vanes (48) to postulate that the accumulation of ruminal gases cause inadequate respirstory movements, thus causing the animal to suffocate and at the same time be poisoned by ruminal gases absorbed into the blood as a result of the increased pressure; and therefore contribute to the animal's death. No one to date has proved the validity of this statement.

Most workers believe that a ruminal center exists, but a definite location has not been described (18). Clark finds it reasonable to assume that the ruminal center could be affected by blood composition (4). Many postulate that the rumination center correlates the activity of a number of other centers--respiratory, masticatory, and deglutitory--concerned in rumination. Wester (52), Hoflund (20), Dougherty (13), Cole, Mead, and Kleiber (9), Mead, Cole, and Regan (34), Cole, et al. (8), Clark (5), and Evans and Evans (19) have associated rumen motility with eructation.

Lindahl et al. (30) have worked on the possibility of toxic substances in bloat and reported results which give new impetus to the toxic theory. Weiss (50) has shown that large doses of potassium cyanide caused complete inhibition of all rumen motility and eructation, while minimal doses of atropine insufficient to cause inhibition of the ruminal motility, resulted in complete inhibition of the eructation reflex. Rumen motility and eructation inhibition were also reported by Clark (6) when two milligrams of histamine was injected intravenously into a sheep. Clark and Lambert (7) report that rumen paralysis was caused by feeding or

intravenous administration of sodium carbonate. They found following large dosages that eructation contractions were completely inhibited, but usually reappeared when intra-ruminal pressure was increased by insufflation. The eructation efficiency showed an inverse relationship to the carbon dioxide combining power of the blood.

A theory, expressing the possibility of a toxic substance occurring during bloat, has evolved in recent years. Greater amounts of toxins which were not listed have been found by Dougherty and Cello (14) in the rumen ingesta of animals suffering from bloat than in normal animals. These substances when filtered and centrifugated reduced blood pressure when injected intravenously into dogs. They inhibited rumen motility in sheep, stimulated respiration in intact animals and stimulated motor activity in the lower gut of dogs, goats, and sheep. The toxins were negative to hydrogen cyanide tests, but were heat stable, dialyzable through cellophane and non-volatile with steam. Dougherty and Cello (15) stated: "The relationship between the toxic factor described and the symptoms of acute indigestion and bloat in ruminants is still obscure." The source of the toxic factor in rumen ingesta and the depressor factor in crude aqueous extracts of legumes suggested that although there are apparent physiological similarities, there are many biochemical differences.

Along the same toxic theory line of thought, Shanks (45) describes a protein shock or allergy theory which can be summarized by stating that young luscious pastures and certain foods contain specific proteins which when absorbed interfere with the rumination and eructation mechanisms. This same allergy theory is substantiated by Kerr and Robertson (24), Kerr and Lamont (23), and Lamont (29). They observed a high incidence of bloat

following the administration of a second dose of trichomonal antigen seven to ten days after the initial dose. Their theory states that the animal suffering from histamine shock is unable to eructate, and bloat results.

Several authors, Dougherty (11), Nichols (39), and Weiss (51), have shown that if ruminal pressure is increased by insufflation, increased ruminal motility and eructation results. This then appears to act as a safety mechanism. Unless this neuromuscular mechanism is defective or obstructions prevent the expulsion of gas, there then could be the possibility of an inhibitory toxic element acting on the neuro reflex.

Ligation of the esophagus as done by Dracy et al. (17) provides another facet for assuming or postulating the possibility of the toxic factor in bloat. This experiment in summary allowed six ewes to graze on young alfalfa until they had a good fill. The esophagus was then ligated and in the next two hours distention was observed to a large degree but no deaths occurred. This sheds light to the effect that when the toxic element is not present death from acute bloat does not occur.

Very little research is reported in the literature about carbon dioxide in blood and its effects on bloated animals. When insufflating with carbon dioxide, Dougherty (12) found an extreme dyspnea when relatively high intraruminal pressures were present. There was not an appreciable increase in carbon dioxide content in blood from the jugular vein; however, in blood taken simultaneously from the portal vein, the carbon dioxide content increased from 0.9 to 9.4 per cent. Carbon monoxide insufflation did not inhibit respiratory movements, but did inhibit eructation.

The enzyme cholinesterase is essential for proper function of animal tissue, such as nerves, muscle and brain. Cholinesterase hydrolyzes acetylcholine into acetic acid and choline which are relatively inactive (3). Cholinesterase therefore limits the duration of acetylcholine. Acetylcholine or choline and its ester act, in their effects, similar to parasympathetic stimulation. Acetylcholine has these functions: cardiac inhibition, excitation of the smooth muscle of the digestive tract and bladder wall, secretion of saliva, tears, and sweat, dilation of the arterioles, and a fall in blood pressure. The enzyme concentration is high in many tissues, but especially so at the synaptic areas of neurons. Cholinesterase is inhibited by eserine, prostigmine, and atropine (37). An acetylcholine blocking agent (a Compound oc. 1052-1575, Sharpe and Dohme, Inc.) produced complete cessation of rumen motility, but had no effect on the esophagus or cardia as observed by Dougherty (16). Eructation occurred even when the rumen was partially filled with water.

A substance found in white clover has been isolated by British researchers (21) which inhibits the action of cholinesterase of blood. A similar substance has been isolated by Jackson and Shaw (46) in ladino clover juice concentrate. This substance reduced red cell cholinesterase activity to some degree.

A paralysis of ruminal movement was observed by Quin and Van Der Wath (43) when acetyl-choline in doses of ten milligrams or more was injected intravenously (dilution 1:1000), but the paralysis was of short duration (ten minutes).

Methemoglobin is a compound of oxidized heme and native globin. Methemoglobin is a true oxide in which one atom of oxygen combines with

one atom of iron. Best and Taylor (3) state that in poisoning by certain drugs, e.g., nitrites, chlorates, sulphates, sulphanilamide etc., the blood becomes dark in color due to the formation of methemoglobin and gives rise to a type of cyanosis to which the term toxic is applied. Methemoglobin cannot combine with oxygen in the same manner as hemoglobin and, therefore, is useless as a respiratory pigment. Methemoglobin is formed in small amounts in the circulating blood, but reducing systems in the erythrocytes prevent an accumulation in any large amounts, Dukes (18).

EXPERIMENTAL METHODS

Cattle and sheep were the two species of ruminants used in this experiment. The dairy cows were grade Holsteins and Guernseys, and their weights, ages, and stages of lactation were extremely variable. The sheep were also of varying weights and ages. All animals were in good flesh and healthy condition.

GENERAL MANAGEMENT

All the cattle received the same management throughout the experiment. The dairy cows were placed on pasture starting June 6, 1957. This pasture consisted of three-fourths brome and one-fourth alfalfa. Starting July 1, 1957, and daily from then on until September 1, 1957, they were on various alfalfa pastures. Previous to June 6, 1957, the ration consisted of baled alfalfa hay, corn silage, and grain. After going on pasture, the hay, silage, and grain were discontinued in order to stimulate the animals to eat as much forage as possible.

The pastures varied considerably in stage of growth and amount of weeds. The main objective was to have the animals on the pasture which appeared to have the most succulent pre-bloom growth. The animals were put on pasture at approximately 8:30 a.m. and taken off about 3:30 p.m. Water was not provided in the pastures, consequently the animals had to come to the barn to drink.

Thirteen sheep, eighteen cows, and one steer were used in this experiment. One cow (No. E161) died as the result of being cut open during acute bloat. Two other cows were sent to market because one (No. E158) was doing very poorly from what apparently was hardware, and the other (No. E168) had

been cut open because of an extreme bloated condition.

PROCEDURES FOR BLOOD ANALYSES

Cholinesterase tests were run using Michel's method (35) for determining enzyme activity. The principle of the method depends upon the ability of the enzyme to produce a change in pH of a standard buffered solution containing a standard amount of acetylcholine. This change in pH was measured in one to one and one-half hours and was rated in pH units change per hour. This then is a measure of the enzyme activity.

Cholinesterase is not a single enzyme, but rather a name for a group. This group can be subdivided into true cholinesterases and pseudocholinesterases as characterized by their tissue location, physiological function and substrate specificity.

The group analyzed was the pseudocholinesterase which is found in the plasma. A brief description of the procedure is as follows:

a. The freshly drawn heparinized blood was centrifuged at 2,000 r.p.m. for 15 minutes.

b. Two-tenths of a milliliter of plasma was placed in ten milliliters of water.

c. One milliliter of this mixture was placed with a standardized freshly mixed phosphate buffer.

d. After setting for ten minutes in a water bath at 25°C., the pH_1 was determined.

e. In one to one and one-half hours the pH_2 was determined. The calculations were computed using the formula, change in pH per hour = $\frac{(pH_1 - [pH_2 - b])f}{t}$, t for time in hours; pH_1 for the

first pH reading; pH_2 for the second pH reading; b and f are correction factors for non enzymatic hydrolysis of substrate and for the effect of pH change on enzyme activity relative to buffer capacity respectively.

CARBON DIOXIDE

The tests for carbon dioxide determinations were run on a manometric blood gas apparatus (Van Slyke-Neill). All operations and calculations were done according to Peters and Van Slyke (41). All blood samples were taken in a cold test tube. The test tubes were stoppered immediately and placed into a container which reduced the temperature to 0-10°C. All samples were kept cold until analyzed, which at times may have been nearly an hour after taking.

METHEMOGLOBIN

Methemoglobin has a characteristic light absorption at 635 millimicrons. When neutralized sodium cyanide is added, this absorption is abolished by converting methemoglobin to cyanomethemoglobin. The difference in light absorption at 635 millimicrons before and after adding cyanide is considered a measure of the methemoglobin present (46).

All analyses were determined as quickly as possible, generally within one-half hour after sampling in order to facilitate aliquots for all three tests. Efficiency was also increased when the tests were run in this order--methemoglobin, cholinesterase, and carbon dioxide. The time interval between bleeding and testing the blood was kept as constant as possible in order to minimize possible changes in the blood.

RESULTS

A mean or average was established for methemoglobin, carbon dioxide, and pseudocholinesterase between May 18, 1957, and August 5, 1957, for each animal and these values are grouped in Table I. More animals had subacute bloat than is found in the data, but samples were not run unless the animals appeared to be in distress. Most of the bloat data were difficult to obtain even though the pastures were irrigated regularly. Most of the natural bloat occurred on one day and almost all within a week.

Data for samples of blood presented in Table II were for a young chronically bloated steer. Extremely little change appeared as far as the blood composition pertaining to the methemoglobin, carbon dioxide, and pseudocholinesterase was concerned. It did seem, however, that the animal had a defective eructating mechanism because seconds after a stomach tube was inserted the animal returned to normal.

Data in Table III were obtained from sheep when they were insufflated with carbon dioxide. Carbon dioxide values in the blood increased in every case. In many instances values increased two to three times the original amounts. Very little time elapsed between the time at which insufflation commenced and increased carbon dioxide values were found in the blood.

Methemoglobin, carbon dioxide, and pseudocholinesterase checks as presented in Table IV on blood of sheep before and 30 minutes after drenching with concentrated alfalfa juice (unpublished data). While the values for methemoglobin and carbon dioxide remained constant, a significant rise in pseudocholinesterase was noted.

The data in Table V represent samples taken from a steer which bloated acutely and died within one hour.

Table I

Cow No. E86	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-18-57	4.03	.419	---	
5-24-57	---	.454	.227	
5-27-57	2.83	.428	---	
5-29-57	---	---	.105	
5-31-57	4.17	---	.109	
6- 7-57	4.23	.440	.139	
6-10-57	4.17	.468	.159	
6-15-57	3.91	.396	.211	
6-18-57	3.93	.485	.234	
6-25-57	4.84	.493	.168	
6-28-57	5.12	.488	--- (a)	(a) Bacterial reaction in reagent
7- 3-57	5.33	.534	--- (b)	(b) Bacterial reaction in reagent
7-10-57	4.95	.567	.138	
7-17-57	5.55	---	.159	
7-22-57	6.83	.493	1.32 (c)	(c) Reason unknown
8- 2-57	6.89	.420	.147	
8- 5-57	4.92	.522	.114	
9- 9-57	4.05	.434	.237	Subacute Bloat--101.5" diameter at 1:55 p.m.
9- 9-57	4.23	.396	.227	Diameter 93" at 2:45 p.m.
9-11-57	1.35	.489	.222	Subacute Bloat
	.96	.518	.240	2 3/4 hrs. later
9-17-57	1.15	.468	.172	Subacute Bloat

Table I (Cont.)

Cow No. E117	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	1.94	.546	---	
5-18-57	---	.572	---	
5-22-57	4.15	.525	---	
5-29-57	3.81	---	.075	
5-31-57	4.18	---	.087	
6- 7-57	3.93	.511	.168	
6-13-57	4.11	.497	.460	
6-20-57	4.00	.517	.214	
6-24-57	4.70	.530	.162	
6-27-57	4.45	.511	2.210 (a)	(a) Bacterial action
7- 5-57	4.26	.512	.357	
7-19-57	5.49	.448	.214	
7-25-57	7.47	.548	.138	

Table I (Cont.)

Cow No. E122	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-18-57	4.05	.524	---	
5-24-57	---	.500	.215	
5-27-57	2.67	.503	---	
5-29-57	---	---	.110	
5-31-57	4.71	---	.088	
6- 7-57	4.20	.480	.145	
6-10-57	4.01	.466	.121	
6-15-57	3.93	.465	.242	
6-18-57	4.29	.440	.326	
6-25-57	4.84	.507	.162	
6-28-57	4.76	.478	---	
7- 3-57	5.21	.551	.281	
7-10-57	5.75	.514	.173	
7-17-57	5.12	.505	.156	
7-22-57	5.16	.538	.190	
8- 5-57	3.71	.609	.145	
9- 9-57	4.07	.487	.165	Subacute Bloat--2:20 p.m.
9- 9-57	---	.500	.170	After returning to normal--3:20 p.m.

Table I (Cont.)

Cow No. E125	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-18-57	3.66	.526	---	
5-24-57	---	.511	.215	
5-27-57	2.35	.399	---	
5-29-57	---	---	.107	
5-31-57	4.20	---	.110	
6- 7-57	3.74	.446	.145	
6-10-57	3.87	.462	.098	
6-15-57	3.36	.478	.179	
6-18-57	3.51	.440	.279	
6-25-57	4.55	.506	.101	
6-28-57	4.37	.488	---	
7- 3-57	4.99	.505	.421	
7-10-57	4.88	.600	.207	
7-17-57	4.88	.522	.182	
7-22-57	6.28	.522	.579 (a)	(a) Reason unknown
8- 2-57	4.59	.460	.164	
8- 5-57	3.79	.510	.190	

Table I (Cont.)

Cow No. E134	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	1.85	.546	---	
5-18-57	---	.509	---	
5-22-57	4.15	.515	---	
5-29-57	3.06	---	.038	
5-31-57	4.47	---	.092	
6- 7-57	3.84	.466	.175	
6-13-57	4.27	.475	.430	
6-20-57	4.19	.494	.222	
6-24-57	3.64	.521	.187	
6-27-57	3.79	.476	.137	
7- 5-57	4.24	.543	---	
7-19-57	4.85	.444	.249	
7-25-57	6.47	.468	.113	
8- 2-57	4.60	.487	.141	

Table I (Cont.)

Cow No. E150	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	1.94	---	---	
5-18-57	---	.476	---	
5-22-57	4.74	.506	---	
5-29-57	3.84	---	.081	
5-31-57	5.09	---	.255	
6- 7-57	4.28	.473	.182	
6-13-57	4.44	.482	.337	
6-20-57	4.97	.436	.222	
6-24-57	4.59	.488	.172	
6-27-57	4.77	.501	.496	
7- 5-57	4.69	.468	.204	
7-19-57	5.97	.422	.206	
7-25-57	7.65	.513	.040	

Table I (Cont.)

Cow No. E151	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-18-57	3.57	.545	---	
5-24-57	---	.424	.202	
5-27-57	2.34	.434	---	
5-29-57	---	---	.107	
5-31-57	4.33	---	.109	
6- 7-57	3.62	.503	.169	
6-10-57	3.85	.466	.106	
6-15-57	3.41	.467	.179	
6-18-57	3.63	.411	.279	
6-25-57	4.23	.488	.141	
6-28-57	4.45	.489	---	
7- 3-57	4.79	.625	.159	
7-10-57	4.98	.598	.207	
7-17-57	5.06	.577	.131	
7-22-57	5.64	.535	.349	
8- 2-57	4.56	.505	.152	
8- 5-57	3.40	.560	.145	

Table I (Cont.)

Cow No. E153	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-18-57	3.98	.495	---	
5-24-57	---	.398	.200	
5-27-57	2.05	.404	---	
5-29-57	---	---	.095	
5-31-57	---	---	.133	
6- 7-57	3.06	.441	.121	
6-10-57	3.38	.467	.149	
6-15-57	3.58	.425	.175	
6-18-57	3.14	.451	.225	
6-25-57	4.76	.493	.131	
6-28-57	4.00	.492	---	
7- 3-57	4.83	.523	.128	
7-10-57	4.56	.590	.147	
7-17-57	4.63	.509	.168	
7-22-57	7.34	.549	.121	
8- 2-57	6.91	.492	.137	
9-11-57	1.06	.529	.172	Subacute bloat

Table I (Cont.)

Cow No. E157	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-18-57	4.46	.440	---	
5-24-57	---	.455	.182	
5-27-57	3.02	.443	---	
5-29-57	---	---	.111	
5-31-57	3.31	---	.095	
6- 7-57	3.83	.506	.151	
6-10-57	4.03	.438	.175	
6-15-57	4.19	.469	.145	
6-18-57	3.82	.466	.199	
6-25-57	4.35	.470	.140	
6-28-57	4.21	.485	---	
7- 3-57				The animal was sold.

Table I (Cont.)

Cow No. E158	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-18-57	4.05	.458	---	
5-24-57	---	.486	.184	
5-27-57	2.15	.470	---	
5-29-57	---	---	.103	
5-31-57	3.40	---	.126	
6- 7-57	3.31	.463	.175	
6-10-57	3.61	.468	.156	
6-15-57	3.80	.473	.151	
6-18-57	3.38	.467	.208	
6-25-57	4.99	.501	.093	
6-28-57	4.36	.479	---	
7- 3-57	5.14	.585	.129	
7-10-57	4.68	.580	.147	
7-17-57	5.03	.509	.140	
7-22-57	5.07	.526	.108	
8- 2-57	6.70	.471	.147	
8- 5-57	4.42	.500	.114	
9- 5-57	1.38	.498	.192	Subacute bloat 12 p.m. 96.5 in. dia.
9- 5-57	1.63	.515	.340	1 p.m. 80.0 in. dia.

Table I (Cont.)

Cow No. E161	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	
5-18-57	3.56	.526	---	
5-24-57	---	.518	.217	
5-27-57	2.69	.514	---	
5-29-57	---	---	.095	
5-31-57	3.25	---	.116	
6- 7-57	4.11	.506	.139	
6-10-57	4.12	.489	.137	
6-15-57	3.71	.491	.153	
6-18-57	3.82	.432	.270	
6-25-57	4.17	.507	.182	
6-28-57	4.72	.527	---	
7- 3-57	5.00	.540	.418	
7-10-57	5.01	.585	.173	
7-17-57	5.62	.532	.177	
7-22-57	4.69	.545	.805	Reason unknown
8- 2-57	5.20	.492	.147	
8- 5-57	4.06	.538	.159	
9-11-57	.92	.515	.280	Acute bloat
9-11-57	1.02	.467	.250	Cut open
9-20-57	---	---	---	Animal died

Table I (Cont.)

Cow No. E162	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo. cholinesterase Change pH/hr.	Remarks
5-18-57	3.32	---	---	
5-24-57	---	.466	.177	
5-27-57	2.02	.437	---	
5-29-57	---	---	.100	
5-31-57	---	---	.151	
6- 7-57	3.02	.470	.144	
6-10-57	3.53	.436	.178	
6-15-57	3.35	.456	.151	
6-18-57	3.12	.413	.234	
6-25-57	4.54	.450	.037	
6-28-57	4.11	.453	---	
7- 3-57	4.89	.536	.339	
7-10-57	4.52	.565	.138	
7-17-57	5.23	.505	.164	
7-22-57	6.42	.402	.145	

Table I (Cont.)

Cow No. E163	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	1.85	.446	---	
5-18-57	---	.433	---	
5-22-57	4.61	.463	---	
5-29-57	3.73	---	.140	
5-31-57	4.20	---	.073	
6- 7-57	4.02	.352	.168	
6-13-57	4.42	.456	.390	
6-20-57	4.72	.456	.333	
6-24-57	3.89	.448	.187	
6-27-57	4.20	.434	.936 (a)	(a) Bacterial action
7- 5-57	4.55	.420	.566 (b)	(b) Bacterial action
7-19-57	5.59	.452	.208	
7-25-57	6.64	.466	.147	
8- 2-57	7.04	.437	.162	
8- 5-57	4.59	.516	.114	

Table I (Cont.)

Cow No. E164	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	1.95	.499	---	
5-18-57	---	.497	---	
5-22-57	4.19	.502	---	
5-29-57	3.88	---	.159	
5-31-57	4.44	---	.099	
6- 7-57	3.92	.438	.148	
6-13-57	4.42	.488	.400	
6-20-57	4.64	.513	.313	
6-24-57	3.46	.513	.298	
6-27-57	4.45	.488	2.230 (a)	(a) Bacterial action
7- 5-57	4.56	.491	2.020 (b)	(b) Bacterial action
7-19-57	4.89	.466	.231	
7-25-57	7.66	.541	.154	
8- 5-57	4.70	.567	.100	

Table I (Cont.)

Cow No. #165	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	1.69	.533	---	
5-18-57	---	.472	---	
5-22-57	4.02	.495	---	
5-29-57	3.11	---	.065	
5-31-57	4.12	---	.092	
6- 7-57	---	.432	.162	
6-13-57	4.00	.409	.390	
6-20-57	3.81	.443	.381	
6-24-57	3.24	---	.159	
6-27-57	3.98	.429	.278	
7- 5-57	4.04	.424	---	
7-19-57	5.23	.381	.162	
7-25-57	6.85	.456	.105	
8- 2-57	7.17	.421	.156	

Table I (Cont.)

Cow No. E166	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	2.07	.518	---	
5-18-57	---	.510	---	
5-22-57	4.24	.435	---	
5-29-57	---	---	.064	
5-31-57	4.38	---	.153	
6- 7-57	---	.465	.141	
6-13-57	4.39	.494	.400	
6-20-57	4.32	.493	.253	
6-24-57	3.45	.478	.215	
6-27-57	3.93	.476	.160	
7- 5-57	4.77	.472	2.730 (a)	(a) Bacterial action
7-19-57	5.29	.443	.202	
7-25-57	6.83	.497	.156	
8- 5-57	4.27	.502	.121	

Table I (Cont.)

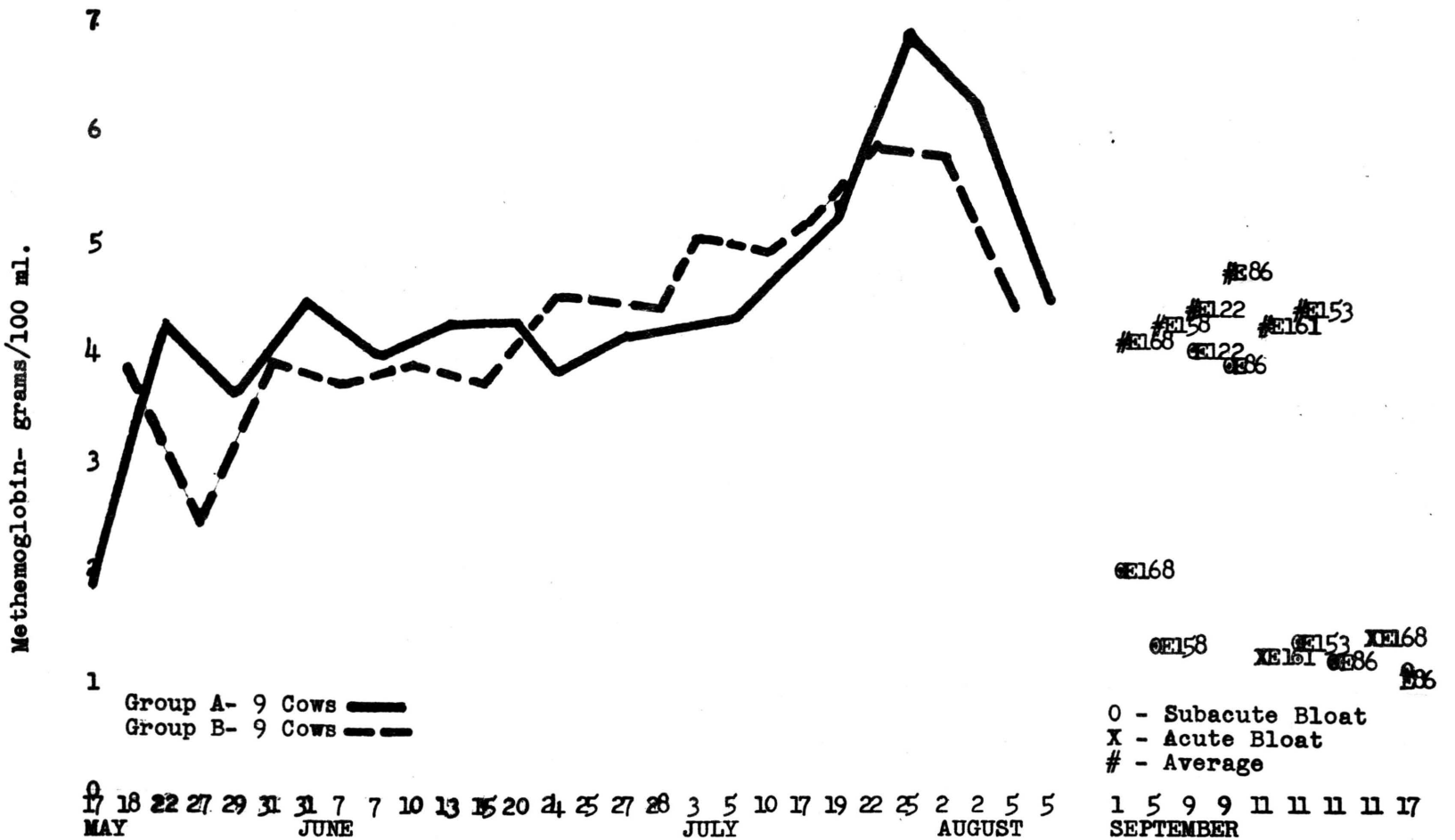
Cow No. E167	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	1.81	.445	---	
5-18-57	---	.446	---	
5-22-57	4.06	.495	---	
5-29-57	---	---	.083	
5-31-57	4.67	---	.097	
6- 7-57	---	.461	.159	
6-13-57	3.77	.451	.370	
6-20-57	3.61	.465	.298	
6-24-57	3.79	.483	.187	
6-27-57	3.59	.476	.235	
7- 5-57	3.34	.445	.457 (a)	(a) Bacterial action
7-19-57	5.44	.438	.206	
7-25-57	6.39	.475	.173	

Table I (Cont.)

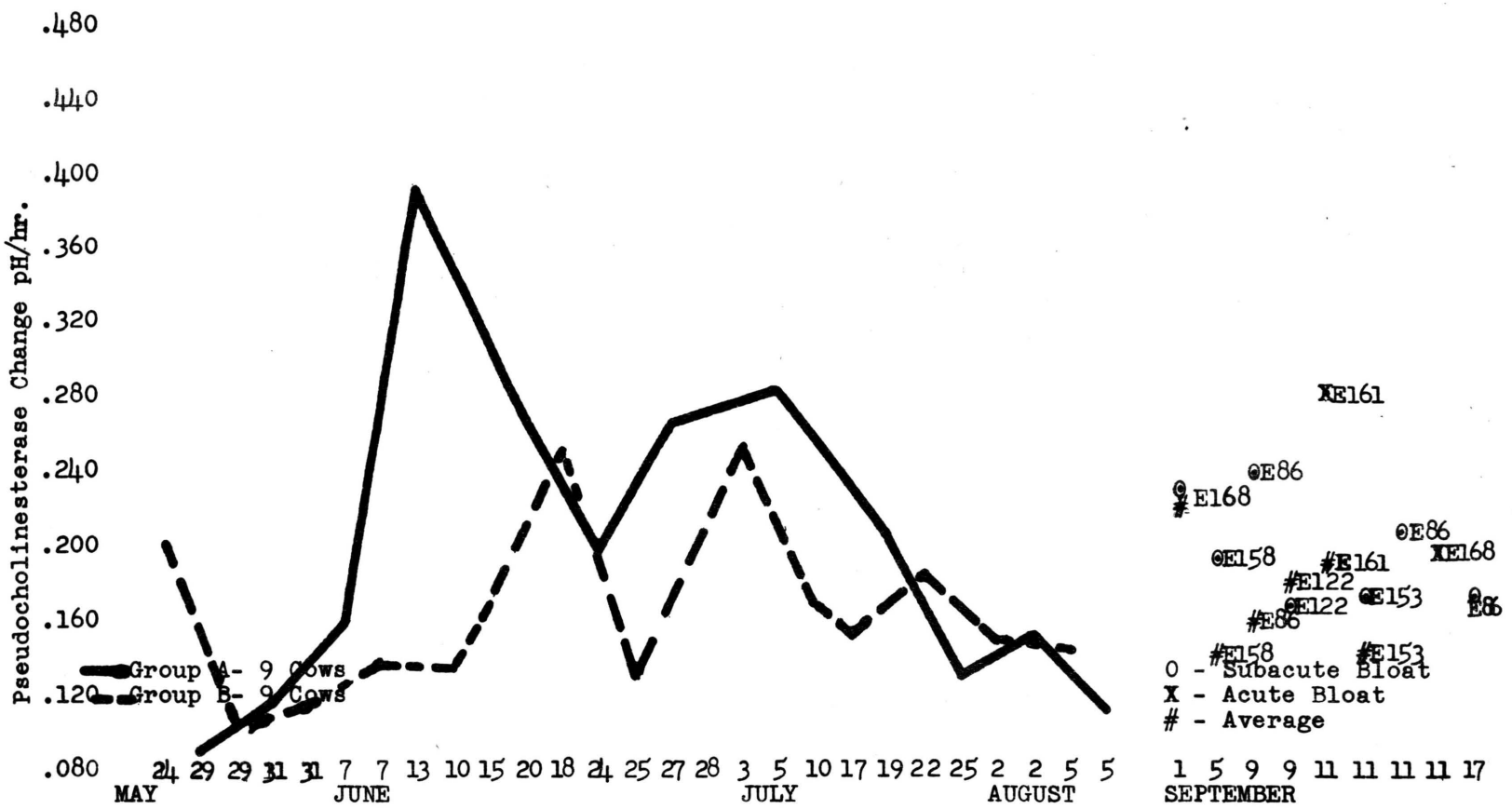
Cow No. E168	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
5-17-57	1.55	.534	---	
5-18-57	---	---	---	
5-22-57	3.67	.497	---	
5-29-57	---	---	.077	
5-31-57	4.67	---	.116	
6- 7-57	---	.461	.147	
6-13-57	3.94	.475	.355	
6-20-57	3.76	.526	.269	
6-24-57	3.75	.513	.205	
6-27-57	4.24	.510	.288	
7- 5-57	4.28	.571	.425	
7-19-57	4.73	.463	.178	
7-25-57	6.57	.556	.141	
9- 1-57	2.06	.559	.226	Subacute Bloat
9-11-57	1.42	.596	.195	Acute Bloat
9-11-57	1.45	.417	.202	After animal was cut open

Table IA
A summary of data from Table I

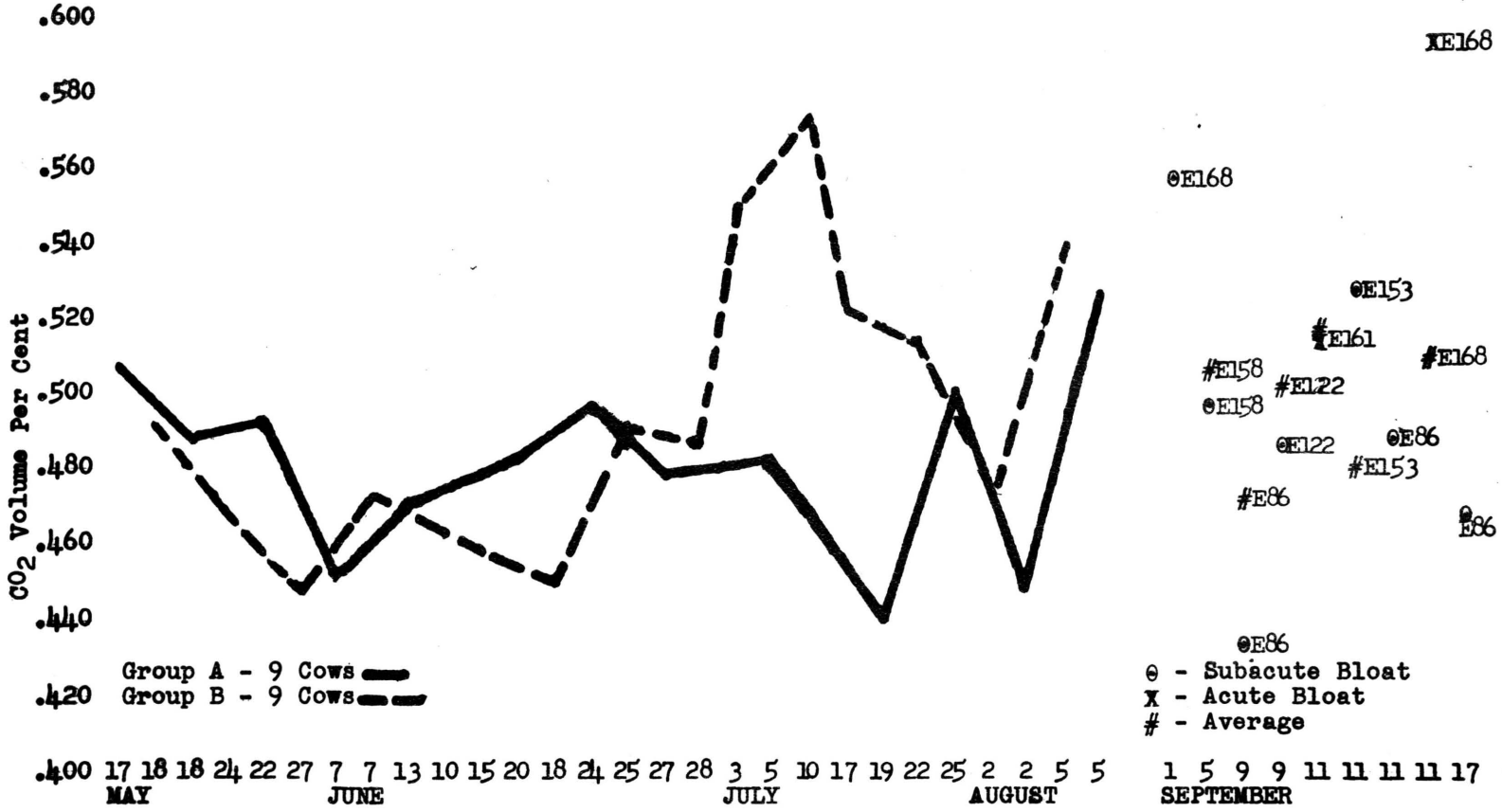
Cow No.	Hb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.
Average values for normal or control animals			
E 86	4.78	.472	.159
E 117	4.37	.520	.208
E 122	4.43	.504	.179
E 125	4.20	.492	.184
E 134	4.11	.495	.178
E 150	4.75	.477	.220
E 151	4.12	.508	.174
E 153	4.32	.481	.148
E 157	3.91	.464	.150
E 158	4.27	.496	.142
E 161	4.25	.516	.183
E 162	4.01	.466	.155
E 163	4.58	.445	.192
E 164	4.40	.500	.211
E 165	4.27	.445	.195
E 166	4.36	.482	.187
E 167	4.05	.462	.201
E 168	4.12	.511	.220
Over-all average	4.29	.485	.181
Average values for bloated animals			
E 86	2.18	.462	.212
E 122	4.07	.487	.165
E 153	1.06	.529	.172
E 158	1.51	.507	.266
E 161	0.97	.491	.265
E 168	1.75	.533	.213
Over-all average	1.92	.502	.216



Graph 1. Comparison of Average Methemoglobin Values



Graph 2. Comparison of Average Pseudocholinesterase Values



Graph 3. Comparison of Average CO_2 Values

Table II

Steer No. E173	Whole Blood		Plasma	
Date	Mlb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
8-30-57	2.00	.505	.270	
8-30-57	1.29	.506	.172	Subacute bloat
9- 3-57	1.94	.516	.241	Subacute bloat
9- 4-57	1.91	.470	.271	
9- 5-57	2.17	.575	.270	Subacute bloat
9- 5-57	2.39	.576	.245	10 min. after gas released
9- 5-57	2.08	.564	---	4 hours later
9- 6-57	2.03	.508	.165	Normal
9-16-57	1.75	.577	.335	Subacute bloat
9-19-57	1.24	.595	---	Subacute bloat
9-23-57	1.59	.696	.250	Subacute bloat

Table III

Sheep No.	Date	Time of Insufflation	CO_2 Volume Percent
2	8-26-57	Before	.268
		15 min. after	.333
		30 min. after	.375
		45 min. after	.462
		60 min. after	.447
		100 min. after	.535
3	8-27-57	Before	.240
		15 min. after	.452
		30 min. after	.474
		45 min. after	.464
		60 min. after	.489
		90 min. after	.482
4	8-28-57	Before	.168
		15 min. after	.197
		30 min. after	.255
		45 min. after	.365
		60 min. after	.369
		100 min. after	.425
5	8-28-57	Before	.384
		15 min. after	.426
		30 min. after	.481
		45 min. after	.494
		60 min. after	.498
		100 min. after	.501

Table IV

Sheep No.	Date	Drenching	CO_2 Vol. percent	MHb. gr./ 100 ml.	Pseudo-cholinesterase Change pH/hr.
55	8- 6-57	Before	.534	7.74	.232
		After	.508	6.54	.307
55	8- 9-57	Before	.543	8.08	.222
		After	.541	7.19	.374
58	8- 6-57	Before	.502	5.18	.263
		After	.525	4.40	.275
58	8-14-57	Before	.500	2.76	.212
		After	.531	4.04	---
60	8- 6-57	Before	.539	5.38	.253
		After	.548	4.28	.175
60	8-14-57	Before	.494	3.02	.212
		After	.486	4.76	.398
62	8- 6-57	Before	.532	4.58	---
		After	.579	4.66	---
62	8- 9-57	Before	.562	5.47	.253
		After	.532	5.10	.374
64	8- 9-57	Before	.490	5.58	.242
		After	.468	6.12	.384
64	8-14-57	Before	.537	2.73	.212
		After	.527	4.24	.256
66	8- 9-57	Before	.497	4.17	.212
		After	.506	5.33	.364
66	8-14-57	Before	.494	2.89	.283
		After	.491	4.42	.211

Table V

Steer No. E156	Whole Blood		Plasma	
Date	MHb. gr./ 100 ml.	CO_2 Vol. percent	Pseudo-cholinesterase Change pH/hr.	Remarks
6-19-57	4.40	.435	.293	
7-15-57	4.70	.447	.121	Sample taken ½ hour before death
7-19-57	4.01	.457	.408	Sample taken as animal died
				The color of the blood ½ hour before death was cherry red while at death it was dark brown.

DISCUSSION OF RESULTS

Data in Table I in which animals bloated naturally showed a significant change in the methemoglobin values. The tests made on methemoglobin from May 17 through May 27 should be considered experimental errors because during this time an error in procedure was noted. Methemoglobin values did decline in almost every situation during bloat. The analysis of variance reveals highly significant differences between normal and bloated animals. The actual physiological significance of depressed methemoglobin values is not well understood, but it may lead to new knowledge regarding the causative agent in bloat. All tests on bloated animals were run in duplicate and many in triplicate.

Chronic bloating in animals does not appear to have a similar picture when compared with natural bloat. This is recorded in Table II from the data taken on a chronic bloating steer. The possibility remains that this steer's blood composition never returned to normal, therefore, giving no noticeable change when blood was taken while bloated. The normal samples were difficult to obtain because of the animal's susceptibility to bloat.

The data in Table III show strong indications as to the rapidity at which carbon dioxide diffuses through the rumen wall into the blood stream. Values reached from two to three times the amount in the blood before starting and still no distressing symptoms were noted.

An interesting observation can be made in Table IV. It will be noted that the pseudocholinesterase activity increased 80% of the time. Analysis of variance reveals highly significant differences between the

pseudocholinesterase activity before drenching and after drenching. The physiological reasons behind the increased pseudocholinesterase are unknown, but it is known that the pseudocholinesterase is capable of hydrolizing non-choline esters, e.g. tributyrin, as well as acetylcholine. Pseudocholinesterase reaches its highest activity when the acetylcholine concentration is low, whereas it has very slight hydrolytic action at the higher concentrations of acetylcholine.

The results obtained in Table V appear rather dramatic; however, it must be taken into consideration that the normal values are somewhat limited. Notice should be taken of the fact that the pseudocholinesterase activity increased almost three-fold while the methemoglobin values dropped rather significantly. Again the physiological significance is unexplainable.

SUMMARY AND CONCLUSIONS

1. The methemoglobin values of bloated animals were significantly (analysis of variance) lower than for normal animals. Methemoglobin values decreased while pseudocholinesterase activity increased from two to three-fold, when blood was tested on an acutely bloated steer one-half hour before death and just as the animal died.
2. An analysis of variance reveals highly significant differences between the pseudocholinesterase activity before and after drenching sheep with alfalfa juice.
3. Carbon dioxide diffuses very rapidly through the rumen wall. Increased carbon dioxide values up to two or three times the normal amount did not reveal any ill effects on the animals.
4. The blood composition of one animal, which was subject to chronic bloating, showed no appreciable difference between a bloated and normal condition.

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