SURGICAL CONSTRUCTION AND OBSERVATIONS OF MONOGASTRIC CALVES

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CHAPTER I

INTRODUCTION

Since Aristotle first noted that the ruminant stomach consisted of four separate compartments, there has been an increasing interest in the digestive physiology of ruminants. The early pursuit of knowledge in this field has been covered by Barnett and Reid (14), Hungate (59), and Blaxter (18). As early as 1685 Peyer recognized that fermentation occurred in the rumen. It was not until the era of 1875 to 1885 that Zuntz, von Tappeiner and other workers realized the role of microorganisms in ruminant digestion.

In spite of this indication of the dawn's arrival in our appreciation of the rumen, there were still many misconceptions as to the function of the ruminant stomach as noted in Navin's (86) discourse:

> When the cow is feeding or grazing, the food is swallowed without any more chewing than just to enable the animal to swallow it. It reaches the opening into the paunch or rumen in large balls or masses; this, either involuntarily or by the will of the animal, opens and lets it fall into the paunch. It is here retained, as in a storehouse, undergoing no change except softening, by whatever

> liquid or mucus it may have become mingled with in the mouth, or in the rumen, after reaching it, until the animal has leisure to begin rumination or rechewing it. Then by a sort of rolling motion commenced in the stomach, the food is carried around, and on reaching the fold or partition between it and the second stomach, a portion is separated, and by a forcible motion, thrown over the partition into the second stomach. Here the walls of the reticulum contract on it, and carrying it around, by which it is covered with more mucus, and formed into a ball which, as soon as it reaches the opening out on the floor of the oesophagean canal, is forced through it by the contraction of a spiral muscle enveloping the reticulum or second stomach. Once out of this, it is seized by the spiral muscle of the oesophagus, and carried upward into the animal's mouth. Here it is subjected to the process of final chewing and mingling with the saliva. It is then, for the second time, swallowed, and this time the opening into the rumen remaining closed, it passes on until it reaches the opening of the manyplus, or third stomach. Another portion is then separated from the mass in the rumen, which goes through the same process, and so on, until the storehouse is emptied.

By 1920 there had not been much advancement in the understanding of the passage of food through the ruminant system, as literature of that time stated that food entered the omasum only after rumination (124). In fact, there was no great stimulus to research in ruminant physiology until the classic demonstration by Barcroft, et al. (13) in 1944 showing that there was absorption of volatile fatty acids across the rumen wall.

Since 1944 there has been a tremendous amount of research which has increased man's understanding of rumen

physiology many fold. Many phases of this research have been hampered by the inability of earlier researchers to completely delete the contribution by the rumen and other forestomachs as they studied a certain parameter. Because of the foregoing a study was undertaken to develop and perfect a monogastric calf with an adequate nerve and blood supply to the abomasum, to provide a nearly physiologically complete model that could be used to add to man's understanding of bovine digestion and metabolism.

CHAPTER II

LITERATURE REVIEW

It being a basic premise of research that to understand the whole, one must study the parts, it is not surprising that there has been an attempt in ruminant physiology to study the individual stomach compartments. Evidently, one of the first attempts to produce a non-ruminant bovine was by Lubbehusen in 1939 (Appendix B) when he removed the rumen at the ruminoreticulo junction from three heifers and several bull calves. As reported by Powell (Appendix A), there was considerable proliferation of the rumen tissue in two of the heifers which were subsequently slaughtered. One of the heifers did not develop the large abdomen seen in the other two, would consume only small amounts of hay with the grain, and at the time of slaughter revealed a small rumen compartment with a capacity of 10 to 12 liters. It is interesting that this heifer was allowed to complete two lactations in which the milk had a lower fat content than her inheritance would indicate that she should produce. Wise (130) also

studied the response of calves to simple rumenectomies, as did Trautman and Schmitt (118).

Sauer and Brisson (101) in 1960 reported at least three months survival in four calves from which they had removed the rumen, reticulum and omasum. Though they did not attempt to leave the spleen or either of the vagal trunks intact, they were able to obtain animals which were alert and had normal appetites. These animals were closely studied by Lupien, et al. (70), using a variety of diets which were fortified with vitamins and minerals. Their observations included: (1) the stimulation of rumination by fiber in the diet, (2) 14 to 18 hours for food passage through the alimentary tract, (3) a decrease in blood sugar levels, (4) a slight increase in the levels of volatile fatty acids (VFA) in the peripheral blood, and (5) convulsions in one calf and sudden death in two calves which were not explained by autopsy findings.

As Williams, et al. (130) were further developing the technique for the production of monogastric calves, Stewart, et al. (107), were developing a procedure for bypassing the forestomachs of calves by the anastomsis of the esophagus into the dorsal medial portion of the abomasum. Huber (53) has used a similar procedure in lambs. Although the forestomach bypass procedure was much simpler to perform and

consequently had less inherent danger of surgical shock, ingesta was found in the forestomachs of animals prepared by this procedure. The assumption was made that this dried ingesta had been present since the time of surgery inasmuch as there was no papillary growth of the rumenal epithelium. If this assumption was substantiated by analysis of blood from the rumenal and omasal veins for VFA or by emptying of the contents of the forestomachs at the time of surgery, it was not reported. There evidently was some absorption of butyric acid from animals with forestomach bypasses since the omaso-abomasal orfice remained patent. Thomas (111) postulated that backwash does occur in these preparations. Whether the calves were prepared by the forestomach bypass or by the removal of the forestomachs, investigators have had difficulty in getting animals so prepared to survive much more than 6 months after surgery.

The digestive physiology and nutrition of bovine monogastrics is basically understood only to the extent that data from milk-fed calves can be used to estimate the probable conditions existing in the monogastric calves. Lister (68) reviewed the literature published on calves fed liquid diets and concluded that such animals existed without the influence of a functional rumen and their digestion could be assumed to be in the manner of a monogastric calf.

The neural and circulatory supply to the ruminant digestive tract has been well reviewed by Habel (42,43) and Anderson (4). Of major importance to the subject of this thesis is the innervation which is furnished by the dorsal and ventral branches of the vagus as they course posteriorly from the esophageal hiatus of the diaphragm. Approximately 4 cm. caudad to the diaphragm, the two trunks are joined via the anatomosing branch, after which the dorsal trunk sends branches to: (1) the dorsal surface of the rumen, (2) the right longitudinal rumenal groove, (3) the omasal groove and the fundic area of the abomasum, (4) the visceral surface of the reticulum which lies in contact with the rumen, and (5) the Auerbach's plexus and Meissner's plexus by the way of the celiac and cranial mesenteric plexuses. The ventral vagal trunk supplies branches to: (1) the cardia, (2) the liver and gall bladder, and (3) the pyloris and lesser curvature of the abomasum. The sympathetic fibers to the abomasum accompany the celiac artery. The celiac artery furnishes the entire arterial supply to the ruminant stomach through four major branches which are: (1) the common hepatic to the liver, duodenum, and greater curvature of the abomasum, (2) the right ruminal artery which gives off the splenic artery and then supplies the dorsal and ventral ruminal sacs, (3) the left ruminal

artery supplying the reticulum and the cranial ventral sac of the rumen, and (4) the left gastric artery which as the continuation of the celiac supplies the abomasum and omasum.

Duncan (37) studied partial and total vagotomy in the ruminant. She observed normal abomasal motility as long as the ventral vagus was left intact, while severance of both vagal trunks impaired but did not stop abomasal emptying. Of interest is that one of the lambs which had been subjected to total vagotomy developed abomasal ulceration. Hill (48) stressed the importance of the intact vagus which allowed the abomasal parietal cells to respond to gastrin. Gastrin was shown to initiate the production of acid and water which reached a volume of 30 to 35 liters in the cow per 24 hours. Hill made note of the role of the afferent fibers of the vagus in inhibiting gastric secretions upon adequate acidification of the duodenal mucosa.

Ash and Kay (10,11) have studied the stimuli to abomasal motility. Phillipson and Ash (95) state that outflow from the abomasum is controlled first by the distension of the duodenum, and secondly by the inflow from the omasum. They state that there is an increased inflow from the omasum starting 30 minutes after periodic feedings which peaks in 1 to 3 hours. At first there is a comensurate flow from the

abomasum, but soon the duodonal distention slows the outflow from the abomasum resulting in a net increase in the content of the abomasum. A more appropriate study for understanding the monogastric calf was the fluroscopic study by Benzie and Phillipson (17) which demonstrated that abomasal emptying, as judged by the disappearance of the barium milk, occurred in $3\frac{1}{2}$ hours in young calves on a liquid diet. The volume of abomasal ingesta flow of a monogastric calf has not been estimated; but with the stimulation of ingesta in the stomach, it seems logical to assume that it would exceed the 2.8 liters which Mylrea (84) demonstrated in fasted calves.

Introduction to Digestion

The consideration of the digestion of the various dietary components in a monogastric calf is again a unique problem in that the digestion is certainly not the same as that of an animal with functioning forestomachs nor is it the same as that found in a natural monogastric animal. Dukes (36) states that gastric lipase is practically absent in ruminants and lipid digestion in the mature animal is accomplished primarily through bacterial action. Wise (131), Ramsey (97,98), and Young (135) have investigated the action

of pregastric esterase which is produced by glands at the base of the tongue and in the upper esophagus. Present evidence indicates that this enzyme fulfills a definite role in lipid digestion prior to the maturation of the rumen, its importance being restricted primarily to those animals which are nursing. Ramsey was able to demonstrate a marked decrease in the release of pregastric esterase in animals not allowed to nurse. He estimated that 20% of the milk fat underwent hydrolysis in the abomasum of the nursing calf, and that this included 48% of the butyric acid. This report assumes greater importance since Ash (8,9) demonstrated that the greatest stimulation of abomasal secretions could be obtained by the presence of the short chain volatile fatty acids. Grimes and Gardner (41) have shown that calves are able to digest rations containing approximately 50% milk fat on a dry matter basis with an efficiency of 97% even though scouring is produced. The utilization of different fats by calves on liquid diets has been studied by numerous workers (19,22,49,55,57,100) with the general conclusion that calves could best use the hydrogenated vegetable oils which were homogenized, preferably with lecithin, and fortified with Vitamin E and selenium to prevent white muscle disease. Shannon and Lascelles (104) studied the lipid transport in

milk fed calves through the thoracic duct. Ingestion of 140 to 190 grams of fatty acids containing at least 12 carbon atoms, during a 24 hour period, produced yields of 100 to 160 grams of neutral lipid fatty acids in the thoracic duct. In view of the efficiency obtained in digestion trials reported above (41), it is evident that these workers were not able to measure all the absorbed lipid.

Carbohydrate digestion is another unique problem with the monogastric calf. Lister (68) gives a complete review of this problem. Huber, et al. (56) compared the apparent digestibility of various carbohydrates introduced directly into the abomasum of the calf and found that lactose and maltose were well digested. In contrast, starch and modified starches were only moderately digested and sucrose was poorly digested. This was substantiated by Dollar and Porter (32) and Huber, et al. (57) in their report that calves and lambs possess no invertase activity. Furthermore, the work of Huber, et al. (57) showed that the oral administration of sucrose gave no increase in blood glucose in the young calf. Dollar and Porter (32) and Huber, et al. (54) reported that lactase activity was found to be at a high level and maltase activity at a lower level in nursing calves. These same two groups were able to demonstrate that the two monosaccharides of lactose, glucose and galactose,

were each readily absorbed. Morrill (82) studied the production of pancreatic amylase in calves of different ages and on different diets. Measurement of activity was by incubation of the collected enzyme with starch and quantitation of the resulting reducing sugars. The author stated that this may have given values too high to reflect adequately the calf's utilization of starches; even so, the conclusion was drawn that insufficient pancreatic amylase was secreted to provide a significant contribution to the energy needs of the calf. Morrill (82) as well as Van Soest (121) discussed the possible increased importance of fermentation in the lower intestine of the monogastric calf. The efficiency of a functioning rumen is such that very little carbohydrate substrate is available for digestion in the lower intestine, however this is not necessarily the case in the monogastric calf. Van Soest (121) suggested that monogastric calves might be able to make use of bacterial fermentation in the utilization of starch, sucrose, and hemicellulose. He also posed the question as to what extent the lower intestinal flora, fauna, and digestive secretions will adapt to the newly available substrates. Larsen, et al. (65) have presented data that the VFA, produced by the fermentation of starch in the large intestine, can be absorbed and used for energy. Additional evidence indicating that the

lower alimentary tract may be important in carbohydrate digestion and absorption in the monogastric calf was demonstrated by Little, et al. (69). Their data indicated that a large proportion of the carbohydrate which was in the ileum was absorbed before reaching the rectum.

Since the abomasum and the small intestine are the primary sites of protein digestion in the bovine, with and without a functioning rumen, the monogastric calf probably is little different from the intact calf in its utilization of protein. Even so, a review of the literature still leaves many questions unanswered. Protein digestion has been adequately reviewed by Hill (48) and Thomas (110). Upon the entrance of ingesta into the abomasum, pressure receptors in both the pyloric antrum and the fundic regions react to the distention and initiate secretion. The secretory response is also activated by the presence of VFA in the abomasum (8). The mechanism by which the presence of ingesta or VFA cause the release of gastrin is not clear, although Hill (48) reported that histamine may be involved. Injection of histamine by any parental route caused the secretion of copious amounts of a highly acid gastric juice. Much of Davenport's (29,30) work furnished a better understanding of the factors involved in the release of gastrin and the secretion of acid from the parietal cells, but full

interpolation of work done on simple stomached animals to ruminant animals cannot be made. This is especially evident from the work of Davenport (31) which demonstrated damage to the gastric mucosa of dogs by VFA, whereas Ash (8) and Phillipson and Ash (95) observed that there is physiological absorption of VFA from the ruminant abomasum without damage to the gastric mucosa. Regardless of the variance between species in this regard, it is well established (110) that ingested proteins are first denatured by the acid in the abomasum and thus prepared for further action by rennin in the young calf and pepsin in the older animal. The extent of proteolysis occurring in the abomasum varies considerably for the different proteins (110). The inhibition of gastric secretion is by the sympathetic nerves that accompany the left gastric artery. Inhibition of gastric secretion also occurs by mechanisms within the abomasum which respond to decreases in the pH of the ingesta below 2 (48). Further digestion of proteinaceous material is accomplished when the ingesta enters the duodenum, primarily by the action of the enzymes of pancreatic juice. Thomas (110) has characterized the bovine pancreatic juice as a bicarbonate solution of zymogens containing 14-23% trypsinogen, 14-16% chymotrypsinogen A, 4-16% chymotrypsinogen B, 26-35% procarboxypeptidases A and B, 2-5%

nucleases, 2-5% amylase, an undetermined amount of elastase, only a trace of lipase, and up to 20% of inert protein. The control of the secretion of these enzymes is an integrated but complex function of cephalic, gastric, and intestinal stimulations. Pancreatic protein is low when dietary protein is low (±6%) and can be increased to normal levels within 24 hours when adequate protein is fed (110). Contrary to observations in simple stomached animals, there is only a slight increase in enzyme output in milk fed calves after eating (110). This is explained in that pancreatic secretion in functioning ruminants is thought to be continuous and not cyclic. The amino acids in the pancreatic enzymes and intestinal tissue represent a large pool of mobile amino acids. The extent of this reserve is shown by the fact that dietary protein accounts for 90% of the protein in gastric contents but only 14% of the protein in intestinal contents. When fasted, the intestinal tissue loses up to one-half of its protein and, upon realimentation, the intestine recovers quickly since it has first access to the absorbed amino acids (110). This large amino acid pool serves to dilute and reduce the relationship between amino acids in the diet and that in portal or peripheral blood. Many of the amino acids are absorbed as dipeptides, hydrolyzed on or within the cell, and then transferred in either

direction. Apparently, protein leaves the alimentary tract as digestive products which are larger than amino acids but enters the blood stream almost exclusively in the form of amino acids (110).

Introduction to Vitamins and Minerals

In 1926, Bechdel, et al. (15) observed that the requirements for B vitamins were not as critical for calves as for rats. Since that time, the rumen has become recognized as a potent source of B vitamin synthesis in the many and varied feeding trials and management procedures which have been studied. Woods and Tillman (133) obtained increased gains in lambs by B vitamin supplementation of synthetic diets but Clifford, et al. (25) and Oltjen, et al. (88) did not get benefits from supplementation of their synthetic diets. Meites (80) studied B vitamin requirements in domestic livestock given estrogens and concluded that estrogen treatment increased the requirements for B_{12} and thiamine. In view of the widespread use of estrogens in modern feedlot nutrition, there is need for the accurate determination of B vitamin requirements without the influence of the rumen. Annison and Lewis (6) stated that "there is little precise information available on the

organisms responsible for the (B vitamin) synthesis, the mechanism of the synthetic processes, or the factors governing them."

Johnson, et al. (62) demonstrated the need for thiamine in newborn calves by feeding a thiamine deficient synthetic diet. Supplementation with 6.5 mg thiamine per 100 kg body weight prevented the onset of deficiency symptoms which included anorexia, diarrhea, emaciation, and generalized weakness ultimately leading to death. Severe tetanic convulsions responded dramatically to 10 mg thiamine intravenously. Benevenga, et al. (16) demonstrated that thiamine deficiency apparently diminished the amount of pyruvate metabolized through acetyl-CoA, increased the carbon entering the triacarboxylic acid cycle via CO_2 fixation, and increased the blood pyruvate from 15 to 100 mg %. McElroy and Goss (79) studied thiamine deficiency in fistulated mature cows and concluded that their thiamine deficient ration produced clinical signs of deficiencies in two cows. One cow died after 12 days on the experiment and the other responded in 6 hours to intravenous injections of 50 mg of thiamine.

Brisson and Sutton (21) calculated that the minimum requirement for riboflavin for nursing calves was between 3.5 and 4.5 mg per 100 kg live weight, whereas Draper and

Johnson (34) concluded that the minimum riboflavin requirement was only 1.3 to 1.4 mg per 100 kg. Weise, et al. (128) listed the symptoms of riboflavin deficiency as anorexia, hyperemic areas on the gums which became hemorrhagic upon digital pressure, similar hyperemic lesions at the oral canthi and along the edges of the lips, tenacious, chalky salivary secretions, excessive lacrimations, alopecia of the abdomen, and diarrhea. Injections of 5 mg of riboflavin to a 32 kg calf alleviated the symptoms for 24 hours only. Oral supplementation with riboflavin at the rate of 5 mg per day produced dramatic improvement in 3 days. McElroy and Goss (78) were unable to produce riboflavin deficiency symptoms in a mature Jersey cow on their riboflavin deficient diet and found 21-25 ug of riboflavin per gm of dried rumen contents.

Johnson, et al. (61) were unable to demonstrate a need for niacin by newborn calves in their first trial with a synthetic diet. In later trials, Hopper and Johnson (51) lowered the dietary tryptophan content to 0.17% and produced a niacin deficiency as evidenced by a profuse diarrhea and weakness. Intravenous injection of 6 mg niacin for four continuous days alleviated all further deficiency signs for the balance of the 30 day trial. Urine excretion studies indicated niacin synthesis within the calf. Within 2 days

a diet containing 0.10% tryptophane produced symptoms similar to those seen in the niacin deficiency in two out of three calves, with death occurring in one calf on the third day.

A pyridoxine deficiency was reported by Johnson, et al. (60) in calves on a synthetic milk diet. The symptoms of anorexia, poor growth, sluggishness and epileptic seizures leading to death were prevented by supplementation with 6.5 mg pyridoxine per 100 kg live weight. Thomas and Okamoto (114) also used a synthetic milk diet to produce pyridoxine deficiencies in newborn calves. Their calves exhibited uremia and an absence of body fat at death. One calf exhibited convulsions prior to death.

The vitamin B_{12} minimum daily requirement for calves is 44 to 88 ug per 100 kg body weight according to the work of Lassiter, et al. (67). Calves on B_{12} deficient rations exhibited anorexia, poor growth, muscular weakness, enlarged kidneys with numerous white foci in the cortex, and eventually death. Draper, et al. (35) produced B_{12} deficiencies in a group of calves on synthetic diets and calculated a minimum requirement of 44-62 ug vitamin B_{12} per kg body weight per day. Their calves showed symptoms and lesions similar to those observed by Lassiter's group. In addition, demyelination of the brachial and sciatic

nerves, as well as obstructive jaundice due to a lack of patency of the bilary duct, was found at autopsy. Smith and Loosli (104) reported that the minimum B_{12} needed in calves when given parenterally is similar to the requirements of sheep, rats, pigs and chickens on a ug per kg basis, and that parental administration is thirty-five times more effective than oral administration.

Pantothenic acid deficiency symptoms in new born calves have been reported by Sheppard and Johnson (102) when synthetic rations were fed. Supplementation with 19.5 mg per 100 kg body weight appeared to prevent the lesions of lobar pneumonia, muscle edema, softening of the cerebrum, and demyelination of the sciatic nerve and spinal cord.

Biotin deficiency in calves fed synthetic milk diets was described by Weise, et al. (127) as being primarily a paralysis of the hindquarters. The parenteral administration of 0.1 to 1.0 ug biotin per day apparently cured the condition. Oral administration of 10 ug biotin per day prevented the deficiency.

An apparent choline deficiency was produced by Johnson, et al. (63) in calves 7 days of age but could not be produced in calves over 16 days of age, probably due to internal synthesis. The choline deficiency symptoms in the

7 day old calves included anorexia and dypsnea; fatty degeneration of the liver was seen upon necropsy.

Folic acid requirements of lambs were studied by Draper and Johnson (33). The only indication of a folic acid deficiency that they reported was an undifferentiated leucopenia which tended to predispose the lambs to pneumonia. Total white cell counts of 2,600 per mm^3 would rise to 7,900 per mm^3 within 2 weeks with folic acid supplementation.

A requirement for ascorbic acid could not be demonstrated for calves on a synthetic ration by Weise, et al. (126). Sorensen (107) has reported vitamin C deficiency in cattle which apparently had a heritable defect which prevented either synthesis or adequate assimilation of vitamin C.

Vitamin A is a known requirement of cattle. Eaton, et al. (38) studied the carotene requirement necessary to prevent an elevation in cerebrospinal fluid pressure and concluded that the minimum level was 10.6 mg carotene per 100 kg body weight. This is equivalent to approximately 4,200 I.U. of vitamin A and is three times the maintenance requirement published by the National Research Council (85).

The vitamin D requirements for the young calf are more difficult to assess due to the interaction of calcium and phosphorous. Thomas and Moore (112) found that a level of

400 I.U. vitamin D per 100 kg live weight was sufficient to prevent rickets in their calves which were maintained on a well balanced diet with respect to the calcium-phosphorous ratio. This same level is listed as the minimum vitamin D requirement by the English standard (2). Huber and Thomas (58) stated that higher levels are probably necessary for optimum growth and health and for this reason most commercial calf starters in the United States are fortified with 2200 to 8800 of vitamin D per kg ration.

The requirements of the young calf for vitamin E are perhaps more complicated than are his requirements for vitamin D. The apparent level required to prevent muscular dystrophy is dependent on the type of ration, general health (113), and the selenium intake (20,2). Thomas and Okamoto (113) found that 49 mg alpha-tocopherol per 100 kg body weight would not maintain normal plasma vitamin E concentrations when calves were placed on a synthetic diet containing lard and iron salts, but that lower tocopherol intakes in calves on a whole milk diet resulted in normal plasma levels. Blaxter, et al. (20) demonstrated that an intake of 6.6 mg tocopherol per 100 kg live weight was sufficient in calves on diets of reconstituted dried skim milk which contained no lard, cod-liver oil, or other glyceride of unsaturated fatty

acids. The addition of 15-18 ml. cod-liver oil per calf per day to these diets produced muscular distrophy in spite of the supplementation of up to 50 mg of the tocopherol per calf per day.

An attempt to review the available literature concerning mineral requirements and the metabolism of these minerals has left the writer in a state of frustration, especially when attempting to take this current knowledge and interpret it on the basis of probable needs of the monogastric calf. Horvath (52) and Cragle (27) have reviewed the problem and each concluded that there were many more questions to be answered than facts to be stated. The National Research Council (85) has recommended that a 50 kg calf receive a ration containing 0.77% calcium and 0.66% phosphorous. Tillman (115) has recommended the following concentrations of minerals in cattle rations: calcium 0.4%, phosphorous 0.3%, magnesium 0.1%, potassium 0.65%, sodium 0.2%, sulfur 0.15%, copper 10 parts per million (ppm), iron 100 ppm, manganese 30 ppm, cobalt 0.1 ppm, iodine 0.3 ppm.

The exceptions to the recommendations of the National Research Council and Tillman have been found to be so numerous that they can serve only as a general guide for the monogastric bovine. Some of the complicating factors which various research projects have revealed are:

1. Horvath (52) mentioned the variation in mineral requirements and utilization which are associated with Vitamins D and E.

2. Hibbs and Conrad (46) demonstrated the anti-vitamin D activity associated with diets high in carotene.

3. Payne and Chamings (91) stated that ruminants have relatively a greater "pool" of minerals in the lumen of the gut and eliminate a greater proportion of calcium and phosphorous by way of the feces.

4. Horvath (52) summarized work showing that calcium and magnesium utilization is altered by certain lipids resulting in calcium-oleate-phosphate complexes in the rat. The fact that such lipids, as contrasted to butterfat, are used at levels up to 25% would indicate that their influence in the ration of a monogastric calf would have to be determined by experimentation.

5. Horvath (52) stated that lactose is known to alter utilization and retention of calcium.

6. Storry (108) reported that the pH of the proximal portions of the intestine may influence calcium absorption based on the diffusion ultrafiltrable calcium.

7. Wise, et al. (132) determined the acceptable calcium/phosphorous ratios in calves to be between 1 and 7, while Chandler, et al. (24) concluded that the ratios should be between .66 and 3.94.

8. Chandler and Cragle (23), Cragle, et al. (28), Perry, et al. (93), and Yang and Thomas (134), all were able to show that in some calves there is a net absorption of calcium from the abomasum, whereas in others a net absorption is not evident.

9. Cragle and his co-workers (92,117) have studied the effect of parathyroid extracts in cattle.

10. There are complex interactions among the components of the diet and water supply as reviewed by Underwood (119), Mills (81), and the Symposium on Interaction of Minerals (109).

11. Andrews and Isaacs (5) demonstrated that cobalt administered to lambs reduces the accumulation of ingested copper.

12. Hogue and Walker (50) reported on the action of a dietary factor which was heat-labile and alcohol-extractable and capable of increasing the selenium and vitamin E requirement.

13. Mayer, et al. (76) have summarized numerous factors involved in calcium homeostasis, including the effect of the parathyroid hormone and thyrocalcitonin.

14. Powell, et al. (96) reported an increase in the cadmium content of the diet caused a decrease in serum zinc levels in calves.

15. Van Campen (120) stated that both cadmium and zinc depressed copper uptake in rats.

16. Hendriks (45) has shown that increased aldosterone secretion by the adrenal cortex can contribute to hypomagnesemia. An increase in adrenal cortical activity could be expected following drastic surgery such as removal of the forestomachs of calves in preparing monogastric calves.

Review of Blood Constituents

There has not been an adequate study of the various blood constituents in the monogastric calf. Lupien, et al. (70) studied the blood levels of glucose, total volatile fatty acids (TVFA), and actone bodies in the blood of four calves from which the first three stomach compartments had been removed. A gradual decline in blood glucose concentrations in these monogastric calves was observed which was similar to what would be expected from an intact calf.

Blood glucose concentrations reached the 40 to 50 mg per 100 ml range by the 12th to 15th week. One of their calves had a marked increase in blood glucose after the 23rd week, with the final level of 85 mg prior to convulsions and death at the 30th week. The levels of blood acetone bodies and TVFA compared closely with intact controls maintained on a rigid milk diet. Thomas (111) published data obtained on fore-stomach-bypass calves. He stated that plasma acetate levels varied from 14 to 45 ug/ml; at 4-7 months, plasma choles-terol was approximately 2.00 ug/ml, plasma cholesterol esters averaged about 0.35 ug/ml, and plasma triglycerides were approximately .40 ug/ml. Abbassy (1) studied the blood acetate and free fatty acids of forestomach bypass calves. He was able to demonstrate an increase in blood acetate from 0.76 ug/ml. at 30 days of age to 1.15 at 60 days of age, but this increase was not as rapid or as high as that observed in intact calves. The free fatty acids decreased in the period from 30 to 60 days of age from 260 to 180 micro-equivalents per liter in the controls and from 150 to 135 microequivalents per liter in the bypass calves.

Much of the work done in the digestive physiology of the young ruminant is of value in anticipating probable values for the monogastric calf. The work of Kennedy, et al. (65), McCandless and Dye (76), Hibbs, et al. (47), and

McCarthy and Kesler (77) has served to emphasize the decrease in blood glucose concentration in the young bovine as the rumen develops. Ratcliff, et al. (99) demonstrated that this decrease was not dependent on the development of the rumen but proceeded when a strict milk diet prevented normal rumen development. Lupien, et al. (70) confirmed this with their monogastric calves. Several workers (70, 47) have reported an increase in blood glucose levels in calves which had nonfunctioning rumens; this increase occurred after 7 weeks of age which was after the initial decline in blood glucose seen in calves with functioning rumens.

Manns, et al. (71,72,73) have done considerable work, demonstrating that propionate and butyrate play a role in insulin secretion. Jones (64) points out that Mann's work would have been more meaningful had he been able to analyze the canulated pancreatic veins for glucagon as well as insulin. Jones supported the postulation of Phillips, et al. (94) that butyrate induces the release of glucagon. No reference has been found citing work which would demonstrate that pancreatic function changes in its ability to respond to the VFA as the age of the calf increases.

Coles (26) gives normal values for bovine serum cations as: calcium 10.2 mgm/100 ml., phosphorous 5.2 mgm/100

ml., potassium 4.8 mEq/L, and sodium 142 mEq/L. The potassium levels in calves demonstrated a weekly decrease from 2.7 at the 2nd week of life to 2.3 at the 10th week. Horvath (52) has suggested that potassium may be a factor in the survival of calves with a forestomach bypass. Ginsburg (40) listed the following normal ranges for adult cattle: calcium 11.0 to 12.6 mg/100 ml., phosphorous 7.0 to 7.5 mg/100 ml., sodium 128 to 137 mEq/L, potassium 5.6 to 7.0 mEq/L. Marsh and Swingle (74) found lower values for range cattle in Montana (Ca 9.8 mg/100 ml. and P 3.7 mg/100 ml.) and noted that the phosphorous level of younger animals was higher. Payne (90) determined blood calcium and phosphorous levels in 279 cows and reported the mean calcium and phosphorous levels to be 10.65 and 5.23 mg/100 ml., respectively, with the explanation that younger animals tended to have higher levels of each.

Weber (125) determined the average serum protein in 15 animals between the age of 2 weeks and 12 months; his observations indicated variations from the mean of 7.9 were not influenced by either age or sex. Ginsburg (40) reported normal serum protein levels of 6.8 to 7.18 mg/100 ml.

Many of the areas discussed in the preceeding paragraphs need much further investigation. Such investigations

would be greatly aided by the existence of a monogastric bovine that was as nearly physiologically normal as possible. This investigation was designed to perfect the construction of such a bovine monogastric animal with an intact vagus and spleen. The animals successfully constructed by this means were studied for possible variation in serum potassium, serum sodium, serum calcium, serum phosphorous and blood glucose.

CHAPTER II

METHODS AND MATERIALS

General Surgical Procedure

The surgical procedure was changed as the project progressed inasmuch as one of the objectives was to develop a more physiological model of the monogastric calf. For this reason the surgical technique will be discussed in reference to each of the experimental animals. Holstein bull calves were obtained from a local dairy when three to thirty days of age. These calves were placed in a constant temperature room and fed a commercial milk replacer[1] twice daily. A 12% protein preparation of mixed rolled grains and alfalfa hay was made available ad libitum. The calves were acclimated to their new surroundings prior to surgery. An attempt was made to operate on each calf at three weeks of age; however, this was dependent on the availability of calves in line with the schedule of the experiment. The

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[1]Kaffa, Kraft Foods Inc., Chicago, Ill.

age of three weeks was selected so that the calves could be given some time to learn to eat, become accustomed to nursing the milk replacer from the bucket, and develop a more extensive blood supply in the area of the surgery. At the same time, it was realized as a calf passed 3 weeks of age, rumen development would proceed to such a degree that the large amount of tissue to be removed would increase the danger of surgical shock.

Twenty-four hours prior to surgery, each calf was given parenteral injections of 1 million units of procaine penicillin, 1.25 gm of dihydrostreptomycin, and 360 mg of neomycin sulfate. Twelve hours prior to surgery each calf was administered 1 gm nitrofurazone and 500 mg of neomycin sulfate orally. This regime was instituted to prevent any subclinical infections at the time of surgery. All grain and hay was withheld for 24 hours and all liquids for 8 hours prior to surgery. On the morning of surgery, the hair was clipped closely with a No. 40 Oster clipper. The area clipped extended on the left side from a point anterior to the scapula caudally to the posterior surface of the hind limb, and from the spinous processes ventrally to a point across the mid-line.

Prior to the time the surgery was scheduled, an adequate amount of supplemental fuilds was prepared and assembled.

At least three liters of citrated blood were collected for each surgical procedure. The blood was collected in 1 liter rubber stopper bottles which had been prepared by inserting 200 cc of 3% sodium citrate into each, subjecting the bottle to steam sterilization, and a vaccuum drawn on the bottle. Into each bottle so prepared, 800 cc of whole blood was drawn directly from the donor and adequately mixed with the citrate solution. This preparation will be designated as blood in this report. Also available at the time of surgery were the following: 1,000 ml bottles of sterile 5% glucose in distilled water (D-5-W), 1,000 ml bottles of sterile 5% glucose in 0.85% sodium chloride solution (D-5-S), 1,000 ml bottles of a sterile balanced electrolyte solution (Elc), and 500 ml of a commercial protein hydrolysate.[1]

Light surgical anesthesia was attained by the intravenous administration of thialbarbitone[2] to effect. The trachea was then entubated which in turn was connected to a mechanical ventilator and nonrebreathing system equipped to meter methoxyflurane.[3] The calf was then restrained in right

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[1]Ambex, Elanco Products Co., Indianapolis, Ind. (A mb.)

[2]Kemithal, Fort Dodge Laboratories, Fort Dodge, Iowa.

[3]Ibid.

lateral recumbency in preparation for surgery. A 2.5 inch, 14 gauge needle was inserted into the left jugular vein for the administration of 250 mg of oxytetracyline and supplemental fluids during surgery. The surgical area was prepared by three scrubbings with a 3% hexachlorophene soap,[1] dried, and disinfected with a 1:1,000 tincture of roccal.[2]

Exposure of the abdominal viscera was accomplished by the use of a V-shaped incision exposing both the abdomen and thorax. The skin incision was started in the left paralumbar fossa about one inch below the transverse processes of the lumbar vertebrae and extended ventrocranially, approximately two inches posterior to and parallel to the costal arch. The incision was continued to a point just ventral to the sixth intercostal space. The second incision was begun just ventral to the thoracic vertebrae in the 6th intercostal space and extended ventrocaudally to the termination of the 7th rib and then across the costal cartilages to the termination of the first incision (Figure 1). The incision was deepened to include the underlying structures and the serosal surfaces of the thoracic and abdominal cavities. Forceps were placed

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[1]Phisohex, Winthrop Laboratories, New York, New York.

[2]Zephrian, Winthrop Laboratories, New York, New York

on the cartilage of the 7th rib and traction applied to reflect the V-shaped section of the thorax and abdomen while incising the diaphragm to the eosphageal hiatus. At this point the surgical technique was varied from calf to calf to obtain the most nearly physiologically normal monogastric preparation.

After the surgical procedure was completed in the abdominal cavity in each trial, the closure was accomplished by a standardized procedure, which was initiated by the aspiration of the fluids from the thoracic and abdominal cavities. The exhaust hose in the mechanical ventilator was closed in order to inflate the lungs and thereby reduce any atelectasis. The incision in the diaphragm was repaired with continuous interlocking sutures using No. 2 chromic catgut. The 6th and 7th ribs were realigned and secured with 4 stainless steel wire sutures placed in a figure-8 manner at regular intervals along the two ribs between the vertebral articulation and the costal cartilages. The serosal surfaces were brought into apposition by interlocking continuous sutures using No. 2 catgut. The muscle layers were sutured by simple interrupted sutures of the same material. When the last stitch in the thoracic musculature was placed, but not tied, a set of Mayo-Hegar forceps was inserted over the suture, and

between the continuous suture of the pleura. The exhaust of the hose was again closed allowing the lungs to completely fill the thoracic cavity. At this time, the forceps were removed, the suture tied, and the exhaust hose released. Skin closure was completed with cruciate stitches using a synthetic suturing material.[1]

After surgery was completed, the calves were removed to a recovery area where the temperature was maintained at 85 degrees F. One million units of vitamin A were administered intramuscularly with 3 ml vitamin B complex. D-5-S (1,000 ml) was administered by slow intravenous drip over a four hour period during recovery. Daily injections of 3 ml vitamin B complex were administered whenever the calf was being maintained on intravenous fluids. As soon as the calf had begun oral feedings and digestion appeared to be normal, blood samples were obtained from the left jugular vein and thereafter at approximately weekly intervals. At each sampling, 10 ml of the heparinized blood was taken along with 100 ml of blood to which no anticoagulant was added. All samples were obtained at 8 a.m. prior to the

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[1]Vetafil, Bengen, West Germany and imported by Dr. S. Jackson, Washington, D. C.

first feeding and at least 7 hours after the last feeding of the previous day.

Individual Surgical Procedure

Trial No. 1

The first calf to have surgery was thirty days old. The surgical procedures were modifications of the technique of Sauer and Brisson (101). In an attempt to reduce the amount of rumen tissue left attached to the cardia, the diaphragm was dissected away from the esophagus permitting complete exposure of the cardia. Three Doyen intestinal clamps were placed on the rumen approximately 3 cm. posterior to the cardia. The rumen was transected in such a manner as to leave one clamp on the posterior segment and two on the anterior segment. The spleen was freed from its anterior and dorsal attachments and left attached to the rumen. The celiac artery was identified so that the splenic and rumino-reticulo branches could be double ligated and severed. The greater omentum was ligated near the bifurcation of its arteries and transected so that the larger portion was left attached to the rumen. Three Doyen intestinal clamps were then placed on the abomasum near the omaso-abomasal orfice and the abomasum transected between the two forceps nearest

the omasum. The rumen, reticulum, omasum, spleen, and the greater omentum were then removed from the abdominal cavity. The proximal end of the abomasum was closed by a double layer of Cushing-Lambert sutures. Approximately two inches lateral to this closure, an incision was made in the abomasum equal in length to the width of the rumen segment which had been left attached to the cardia. These two openings were then approximated and joined serosa to serosa by a continuous stitch of No. 2 chromic catgut. This closure was reinforced by a row of continuous Lambert sutures. The incisions in the diaphragm, thorax, and abdomen were closed as previously described.

During surgery, 2,000 ml of blood and 2,000 ml of D-5-S were administered. Sixteen hours after surgery, the calf was in obvious shock and an additional 1,000 ml of D-5-S was administered with 500 ml of citrated blood intravenously.

Trial No. 2

The surgery on the second calf was accomplished after observing the necropsy of the first calf. After exposure of the abdominal viscera, transection of the rumen was accomplished at a point 7 cm posterior to the cardia after the three Doyen clamps were applied. The incision was made

between the posterior two clamps. A temporary continuous basting stitch of Vetafil was then placed over the more posterior clamp, the clamp removed, and the stitch tightened to invert the stump of the rumen segment. The spleen, posterior rumen, reticulum, omasum, and a large part of the greater omentum were removed by the same technique used on calf No. 1. The stump of the abomasum was not closed but rather inverted with a temporary basting stitch similar to that used on the anterior segment of the rumen. The suture lines in the rumen and abomasum were brought into close approximation and the two organs joined by two rows of continuous Lambert sutures using No. 2 chromic catgut. Both basting sutures were removed by traction and the anastomosis examined closely for patency and possible leakage. Abdominal and thoracic closure was routine.

During surgery, 3,000 ml of blood and 500 ml of Ambex were administered. More blood and fluids were administered during the first two postoperative days. The calf eagerly nursed 16 ounces of milk replacer 24 hours after surgery. Forty-eight hours after the operation, the calf was fed one quart of milk, muzzled, and placed on hay bedding. When it was determined that the calf had been able to remove the muzzle during that first night, the decision was made to use only rubber mats, with no hay or muzzles. Twice a day

feedings were continued throughout trial No. 2. Body weight and blood samples were obtained on days 5 and 12 after surgery.

Trial No. 3

This calf was 42 days of age at surgery and though considerably older than desired, was the only one available. The exposure of the stomachs was accomplished in the usual manner. In an attempt to maintain some neural control of the abomasum, the dorsal and ventral branches of the vagus nerve were located as they passed through the esophageal hiatus of the diaphragm and were followed posteriorly to the site of the anatomasing branch. The ventral branch of the vagus and the lesser omentum were dissected free from the underlying rumen, omasum, and anterior abomasum, posterior to the point at which the abomasum was to be transected. Electrocautery was used to control the hemorrhage from the severed omasal arteries and veins contained in the lesser omentum. The portion of the ventral vagus which had been thus freed from the stomachs was then left enveloped in the lesser omentum until the latter was reattached after the completion of the anastomosis. The rumen, abomasum, and dorsal vagus were transected and the

anastomosis completed as described in trial No. 2, even though the procedure was considerably more difficult in this older calf.

During surgery the calf received 2500 ml blood and 1500 ml D-5-S. Twelve hours post operative, 500 ml Ambex and 500 ml D-5-S were administered. Sixteen hours after surgery the calf was offered 4 ounces of water which was taken readily. Thirty minutes later, the calf was given 8 ounces of milk replacer which caused immediate vomition. After this experience the calf was maintained on fluids given intravenously until 64 hours after surgery, at which time it was able to stand unassisted and consume 1 quart of milk replacer twice daily. Blood samples were taken on the seventh and fourteenth days.

Trial No. 4

Calf No. 4 was 46 days old on the day of surgery and therefore about twice the age desired. Surgery was completed with the same techniques used in trial No. 3. This calf, however, did not gain enough strength to stand or take any milk replacer.

Trial No. 5

Surgery was performed on this calf at only 7 days of age since older calves were not available. Due to the problems encountered in previous operations, and the favorable results reported by Sauer and Brisson (101), the decision was made to again transect the ventral branch of the vagus. Except for the transection of the ventral branch of the vagus, the technique was the same as that used on the two previous calves.

Fluids, blood, and antibiotics were used to maintain the calf for the first 24 hours after surgery. At that time the calf drank four ounces of water and nursed four ounces of milk replacer while standing, but was not as alert as previous calves. Twice daily feedings of milk replacer were continued with supplementation by intravenous fluid and blood when dehydration and anemia became evident on the 6th and 7th days after surgery. On the 8th day, the calf was euthanized.

Trial No. 6

This calf was only 5 days old at the time of surgery, and was again the only one available at time of surgery. In an effort to increase the survival rate, increase the

animal's resistance to stress, and to prepare a more physiological monogastric, the decision was made to leave the spleen in this animal. After the Doyen clamps were placed on the anterior rumen and the rumen transected, the spleen was separated from the rumen by blunt dissection and allowed to remain secured by its diaphragmatic and dorsal attachments. The celiac artery was identified and the ruminal and reticular branches isolated, ligated, and severed. The omaso-abomasal artery was identified and retained, as was the corresponding vein, providing a nearly normal blood supply to the abomasum. The ventral branch of the vagus was retained by the technique previously described. The abomasum was transected as in prior operations and anastomosis completed.

During surgery, 3,000 ml of blood and 2,000 ml of D-5-S were administered. The calf was maintained on fluids for 68 hours prior to an oral feeding. Milk replacer was fed three times a day from the first feeding until death on the 5th postsurgical day.

Trial No. 7

This calf was 15 days old at the time of surgery. In an effort to again ascertain the wisdom of transection of the ventral branch of the vagus and splenectomy, these two

procedures were reincorporated into the procedure for this animal. Blood (2,000 ml) and D-5-S (2,000 ml) were administered during surgery. Fluid therapy but not oral feeding was given until death of the calf approximately 60 hours after surgery.

Trial No. 8

This calf was 22 days of age at time of surgery. After a review of the problems experienced with the previous seven calves, it was concluded that the primary problems had been (1) adhesions developing in the suture line of the anastomosis due to the coalescence of the denuded tissue which had been inverted in suturing, and (2) interference with the circulation to the abomasum due to torsions of the abomasum and displacement of the arterial trunk supplying the abomasum. To correct these deficiencies, further alterations in the surgical procedure were made. Preparation of the calf for surgery, anesthesia, and exposure of the abdominal viscera were not changed. The greater omentum was dissected away from the rumen as close to the ruminal attachment as possible. The omentum was then secured to the abdominal wall with Allis forceps in such a manner as to maintain the intestine in the posterior abdomen away from the operative site and lessen the chance of possible bacterial

contamination. The ventral branch of the vagus nerve as well as the anastomosing nerve to the dorsal branch were retained as previously described. In addition, the omaso-abomasal veins were carefully dissected away from the wall of the omasum and retained. The spleen was retained with its normal circulation. The ruminal and reticular branches of the celiac were ligated and transected. The omaso-abomasal artery was identified as it continued from the celiac artery and carefully retained. The numerous small omasal arteries were controlled by electrocoagulation. The Doyen clamps were placed on the rumen and the rumen transected 7 cm. posterior to the cardia. A temporary basting stitch was placed on the anterior segment of the rumen. The abomasum was similarly transected just posterior to the omaso-abomasal junction, and the anastomosis completed by the use of a double row of continuous Lambert sutures using No. 2 chromic catgut. Both basting stitches were removed and a good separation of the suture lines obtained by the passage of a stomach tube in order to inflate the abomasum to moderate distension. Bacterial cultures were taken from the serosal surface of the anastomosis to check for possible contamination during the surgical procedure. The omentum was then released from the abdominal wall and sutured to the serosa of the abomasum in such a way as to

stabilize the position of the organ and thus prevent displacement or torsion. The greater omentum was also sutured around the exposed arteries and veins supplying the abomasum to stabilize the vessels and prevent herniation of the abdominal viscera through the space between the vessels and the abomasum.

During surgery the calf was given 2,000 ml of blood and 2,000 ml of Elc. The calf was maintained on fluids the first postsurgical day. On the second day the calf was given four oral feedings of six ounces of milk replacer. On succeeding days the calf was fed three times daily with increasing amounts of milk replacer to meet the calf's dietary needs. Regular blood samples and weights were taken through the 60 days this calf was kept on experiment. On the 45th day, the calf managed to reach a cotton rag on the outside of his pen; in attempting to swallow the rag, he became choked and salivated profusely. The calf was placed under general anesthesia with thiamylal sodium,[1] and the rag pushed into the stomach with a stomach tube. Recovery and return to feed at the next feeding were uneventful. On the 70th day the monogastric animal was placed in an outside

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[1]Surital, Parke-Davis and Company, Detroit, Michigan.

pen and permitted increasing amounts of the rolled grain mixture containing 12% protein, but no hay. This calf died 90 days after surgery.

Trial No. 9

Calf No. 9 was 23 days old at time of surgery. The surgical procedure used was the same as that used on calf No. 8. During surgery, 2,000 ml of blood and 1,000 ml of Elc. were administered. Fluids were again given for the first 30 postsurgical hours, at which time 6 ounces of water were administered orally. On the second day after surgery, milk replacer was given four times a day in ever increasing amounts starting with 8 oz. per feeding. Further feedings were increased according to the animal's appetite until the total daily consumption was two gallons. Regular blood samples and weights were taken through the 60 days this monogastric animal was on experiment and at various intervals through the nine months of survival.

Eighty days after surgery, this calf was placed in a concrete outside pen and fed a complete hog ration instead of the milk replacer. At 120 days after surgery, he was moved to a small lot containing green bermuda grass and continued on the hog ration. The monogastric calf was not

observed eating any of the grass, but did develop marked inanition and died 9 months after surgery.

Trial No. 10

This calf was 21 days old on the day of surgery. The surgical technique used on calves 8 and 9 was also employed on this calf. During surgery, the calf received 2,000 ml of blood, 2,000 ml of Elc. and 1,000 ml of D-5-S. The monogastric calf was maintained for 32 hours on intravenous fluids and at the end of this time was allowed to nurse 4 oz. of milk replacer. For the next 48 hours it was allowed to nurse at 8 hour intervals. At 8 a.m. on the morning of the 4th day, the calf refused milk and showed symptoms of occlusion of the upper alimentary tract. At 2 p.m., an exploratory lapratomy was performed in the right flank, under thiamylal sodium anesthesia. The calf was assisted to standing position at 12 p.m. and given water; at 1 a.m. it nursed 4 ounces of milk replacer. The calf accepted normal feedings for 3 days, refused the first feeding on the 9th post surgical day, and died later that day.

Trial No. 11

This was a normal animal on which surgery was not performed. Control blood samples and weights were taken

from this calf at the same time samples were taken from calves No. 8 and No. 9.

Laboratory Procedure

At each sampling period, 110 ml of whole blood were withdrawn from the left jugular vein. Ten ml of the whole blood were added to heparinized test tube; this sample was then used to determine the hemoglobin (Hb), the packed cell volume (PCV), the total leucocyte count (WBC), and the differential count of the leucocytes. The other 100 ml of the sample were not heparinized; 2 ml of the whole blood were immediately diluted with 10 ml of glass distilled water to hemolyse the red blood cells and later used to determine blood glucose. The balance of the sample was allowed to clot and the serum was harvested by centrifugation. The serum was then frozen for later determinations.

The Hb was determined, after first lysing the red blood cells, by direct photometry. The PCV was determined by centrifugation of a 1 ml sample in a Wintrobe tube. Differential leucocyte counts were obtained from blood smears stained with Wright's stain. Leucocyte numbers were determined by the standard microscopic procedure after first diluting with 3% acetic acid and placed in the counting chamber.

To the 12 ml hemolysed sample collected for glucose analysis, 4 cc of 2% $Ba(OH)_2$ and 4 cc of 2% $ZnSO_4$ were added to precipitate the proteins of the blood. The barium and zinc solutions were previously adjusted in such a manner to assure that a solution of pH 7.2 resulted from the mixing of equal quantities of the two preparations. One ml of the protein free filtrate was then used to determine blood glucose concentrations according to the methods of Nelson (87) and Somogyi (105).

A 2 ml aliquot of previously prepared serum was used for the determination of the serum phosphorous. The determination was made by the photometric method described by Fiske and SubbaRow (39). A 1 ml serum aliquot was titrated to determine the serum calcium concentration by the method of Ward et al. (122), Pappenhagan and Jackson (89) and Bachre, et al. (12).

A portion of the serum sample was used for the determination of serum sodium and serum potassium levels. The determinations were made with a Baird Atomic KY2 Flame Photometer* following the procedure of Mosher, et al. (83). Serum protein determinations were made by direct photometry

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*Baird Atomic Inc., 33 University Road, Cambridge, Massachusetts.

using a 0.5 cc of serum in the Bausch and Lomb serum protein Refractometer.*

In order to compare the absorption of dietary sugars and the clearance of postprandial blood glucose levels, 10 samples were taken on an hourly basis from calves 8 and 9 as well as from calf 11, the intact control. Samples were taken at 7 and 8 a.m. after a fast from the previous feeding at 12:00 p.m. Immediately after the 8 a.m. feeding, a normal feeding of milk replacer supplemented with two ounces of glucose was given to each calf. Hourly samples were then collected until 12:00 a.m. A normal feeding of milk replacer was fed without the glucose immediately after the 12:00 a.m. sampling. Further samples were taken until 4 p.m. As previously described, all samples were hemolyzed and blood glucose determined after the method of Nelson (87) and Somgyi (105).

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*Bausch and Lomb, Inc., Rochester, New York, 14602.

CHAPTER IV

RESULTS

Results of Surgery

Because this report describes the techniques for the development of an experimental model, both positive and negative results along with the errors which were manifested in each surgical trial are reported.

Trial No. 1

Though adequate fluids and blood volume were maintained during surgery, the length of surgery and the necessity of developing certain techniques contributed to produce a grave surgical shock in this animal. This calf died within 16 hours after surgery. Autopsy revealed a torsion of the abomasum resulting in the interference of circulation to the affected tissue and an ischemia. Death was undoubtedly hastened by the postsurgical shock, which was, in turn, partly due to the torsion.

Trial No. 2

Calf No. 2 made an excellent recovery from the surgery. In 40 hours, this calf was able to rise to its feet unassisted and nurse 1 quart of milk replacer. Following the procedure of Sauer and Brisson (101), on day 2 the calf was muzzled and bedded on hay. During the first night, the calf dislodged his muzzle and attempted to eat his hay bedding. The next morning he was found choked. The calf was removed from the presence of the hay and observed closely for a period of four hours. At the end of this period, the calf had corrected the choke as the result of excessive salivation and attempts to swallow. This experience motivated the removal of all hay bedding and the installation of rubber mats in the stalls. Five days after surgery, this animal weighed 99 pounds, had a WBC of 9,850 and a Hb of 12.5. The differential count was normal. The daily intake of milk increased to one gallon daily and on day 12, additional weight measurements and blood samples were taken. Though the calf had gained 0.5 lbs. per day up to 102.25 lbs., the WBC had increased to 16,200 and the Hb had decreased to 9 gm per cent. The differential count was normal. Undue restraint may have been used in obtaining the blood samples on day 12 since the calf's condition deteriorated rapidly

after the blood was collected. Symptoms of shock appeared in 11 hours resulting in death in 12 hours. On autopsy, a rupture of the line of sutures at the anastomosis was found which must have been preceeded by an abcess in this area as evidenced by the increased WBC twelve hours before death.

Trial No. 3

Calf No. 3 made a normal recovery from anesthesia, being alert and able to stand 18 hours after surgery. At this time the calf took four ounces of water without evidence of discomfort. When fed 8 ounces of milk 30 minutes later, vomition was immediate. Consequently, no further oral feeding was attempted until day 3 and maintenance was accomplished by intravenous fluids. On day 3, one quart of milk replacer was given morning and afternoon with supplemental intravenous fluids; and though the calf did not stand to nurse, there was no further vomition. By day 5, the calf was standing for his feedings and obviously growing. Blood samples taken on day 7 revealed a leucocytosis as evidenced by a 36,850 WBC with an accompanying marked neutrophilia (segmented neutrophils 43%, stab cells 32%, lymphocytes 25%). The Hb was 11.5 gms %. On day 8, a dose of 250 mg of oxytetracyline was added to the daily

antibiotic therapy in addition to the standard penicillin, streptomycin, and neomycin regime. The calf continued to improve through day 11. On day 12, it was noticeably weaker. Blood samples obtained on day 14, revealed a 71,000 WBC (seg 66%, stab 10%, lymphocytes 19%, and monocytes 4%). The Hb was down to 6 gms %. Prostration and death occurred 12 hours after the blood samples were taken. Autopsy revealed an ulceration of the duodenum, ulceration around the suture line, and generalized fibrinous peritonitis. There was also an abcess in the umbilical vein and an Escheria coli septic arthritis which had existed in spite of all the antibiotics which the calf had received.

Trial No. 4

This calf did not recover fully from anesthesia and began regurgitation of stomach contents 24 hours after surgery. Death occurred 30 hours after surgery. Necropsy revealed a torsion of the abomasum and proximal duodenum with interference at the arterial blood supply resulting in necrosis.

Trial No. 5

This 7 day old calf vomited during recovery and it was feared that there was danger of inhalation pneumonia. This

fear increased when the calf exhibited moderate dyspnea on days 2 and 3. At autopsy no evidence of pneumonia was found. Marked salivation with some vomition was noted with each feeding from day 2 until the calf finally refused part of the milk feeding on the sixth day after surgery. The calf became much weaker and would not accept any milk by noon of the 7th day. On day 8, the calf was euthanized when it became evident that his condition was terminal. Necropsy revealed a complete stasis of the abomasum with grain and undigested fiber present in the abomasum which no doubt was present at the time of surgery. This was accompanied by marked inflamation of the abomasal mucosa.

Trial No. 6

This 5 day old calf recovered from anesthesia in the usual time but was noticeably slower in regaining its strength than some of the older calves. The calf did not stand until day 3 postsurgery and was maintained until this time with intravenous fluids. Oral feeding was begun on the third day after surgery with the feeding of 24 ounces of milk replacer; the feeding resulted in a mild colic. The intensity of the colic diminished with subsequent feedings and disappeared after the third feeding. On the fifth postsurgical day, the calf was dehydrated, unable to rise,

and very depressed. Blood (500 ml) and D-5-S (3,000 ml) were administered intravenously but the calf died within six hours. Autopsy revealed the cause of death to be rupture of the suture line as a result of adhesions which permitted the accumulation of feed in the area of the anastomosis.

Trial No. 7

This 14 day old calf did not recover sufficiently from surgery to start oral feeding. It was unable to stand after surgery and was in moderate to severe shock from surgery to death. Necropsy findings indicated the cause of death to be peritonitis resulting from a perforating ulcer of the abomasum. The ulcer was caused by the displacement of the arterial trunk by the abdominal viscera causing a deficiency of blood to the abomasal wall which resulted in tissue death. At the line of anastomosis there was a marked adhesion resulting in the accumulation of a considerable amount of ingesta in the cul de sac thus formed.

Trial No. 8

This was the first calf which had been available at three weeks of age when surgery was scheduled. The modifications of the surgical techniques at this stage of the

experiment, as explained in the method and materials were definitely an improvement in preventing (1) adhesions in the area of the anastomosis, (2) torsion of the abomasum and duodenum, and (3) necrosis of the wall of the abomasum due to deficient circulation. Cultures taken from the serosal surface of the suture line at the time of surgery were negative. Forty hours after surgery, the calf was fed orally for the first time, and this was followed by smaller feedings four times daily. This regime supplied the nutritional needs of the calf without overloading the capacity of the abomasum. Diarrhea developed on the 4th postsurgical day and persisted intermittently throughout the experiment. This diarrhea could not be altered by any of the antidiarrheal preparations used and was attributed to changes in neural control of the abomasum associated with fibers transferred from the dorsal to the ventral vagus by the anastomosing branch. There was an indication that the diarrhea was nutritional in etiology because the diarrhea abruptly stopped and was slow in returning when the calf escaped from his stall on the 39th postsurgical day. During this period the calf ate enough alfalfa hay that he was choked for approximately 6 hours. Table 1 presents the hematological findings at the different sampling periods. Table 2 presents the blood chemistry determinations in

TABLE I

HEMATOLOGICAL VALUES FOR CALF NO. 8

Day of Age	Day After Surgery	Hemoglobin	PCV	WBC	Differential Count %						Degree of RBC Anisocytosis
					Seg Cells	Stab Cells	Lymphocyte	Monocyte	Eosinophil	Basophil	
30	7	15.5	49	9,300	37	2	52	7	1	1	+++
38	15	11.5	38	6,750	26	1	70	3			+++
52	28	11.2	36								
59	35	12	37	7,900	24	1	68	3	3	4	++
66	42	10	29.5	6,100	27	1	60	6	6	6	+++
73	49										
80	56	10	34	9,250	25	1	69	1	2	2	++
87	63	9	30.5	9,250	13	1	72	1	13		+++

TABLE II

BODY WEIGHT AND BLOOD CHEMISTRY ON CALF NO. 8

Day of Age	Days After Surgery	Serum Sodium mEq/L	Serum Potassium mEq/L	Serum Phosphorus Mgm/100ml	Serum Calcium Mgm/100ml	Blood Glucose Mgm/100ml	Serum Protein	Body Weight Pounds
30	7	<110	1.3	1.11	15.6	24		
38	15			2.07	15.6	35	5.2	
44	21	<110	2.3	1.25	13.5		5.4	85
52	28	<110	2.8	1.38	13.2	52	5.5	92
59	35	<110	1.0	.64	13.4		5.4	93
66	42	<110	3.5	2.11	13.0	27		100
73	49	<110	1.5	1.33	12.9	36		112
80	56	<110	2.01	.68	12.7	34		125
87	63							126

Trial No. 8. The hair was clipped from the body of this calf on day 21 since it was feared the calf might swallow its own hair and produce hair balls in its alimentary tract.

Trial No. 9

This calf was also of the desired age at the time of surgery, being 23 days old. This calf exhibited excess salivation after each feeding for the first five days after surgery. A four times per day feeding schedule was followed with this calf; however, this animal developed such an appetite that in 12 days it was consuming 1.5 gallons of milk replacer per 24 hours. This calf did not develop the chronic diarrhea which was observed in calf No. 8. On the 30th postsurgical day, this calf refused the first feeding of the day and exhibited symptoms of mild colic. By noon, its appetite had returned and there was no indication of the cause of the problem. After the 60 days in which samples were collected, the calf was acclimated to an outside pen and a 12% protein rolled grain ration. Tables 3 and 4 present the results of various parameters measured in Trial 9. After approximately 100 days the calf was transferred to a grass lot and continued on the grain ration. Occasional samples were taken at selected intervals. The animal was

TABLE III

HEMATOLOGICAL VALUES FOR CALF NO. 9

Day of Age	Days After Surgery	Hb	PCV	WBC	Differential Count %						Degree of Anisocytosis
					Seg Cells	Stab Cells	Lymphocytes	Monocytes	Eosinophils	Basophils	
30	7	10.5	35	14,000	33	1	65	1	1		++
36	13	12.5	38	12,550	13	2	62	4	1		+++
49	26	11.7	36								
56	33	12.5	38	10,700	26	1	65	7	1		++
63	40	12.5	34	8,050	13	2	75	8	2		+++
70	47	11	34	9,150	14		80	6			+++
77	54	11	36	14,250	38	3	49	5	5		++
84	61	9.5	31	10,850	16		67	11	6		++
245	222										
230	197	13.2		7,550							

TABLE IV

BODY WEIGHT AND BLOOD CHEMISTRY ON CALF NO. 9

Day of Age	Days After Surgery	Serum Sodium mEq/L	Serum Potassium mEq/L	Serum Phosphorus Mgm/100ml	Serum Calcium Mgm/100ml	Blood Glucose Mgm/100ml	Serum Protein	Body Weight Pounds
36	17	<110	3.9	2.46	15.2	38	5.6	
42	19	<110	2.9	1.25	14.2		5.6	114
49	26	<110	3.3	4.95	13.9	66	5.8	122.5
56	33	<110	3.9	4.64	14.2		6.2	127.3
63	40	<110	4.5	5.38	15.6	64		137.3
70	47	<110	1.3		13.8	46		
77	54	<110	1.4	1.02	13.3	25		148.5
214	191					83		
218	195					103		
220	197					104		
230	207					84		
238	215					73		
245	222					50		
247	224					57		
252	229					50	6.6	

observed to gradually decline in condition and finally reach a plateau in body weight. At necropsy, nine months after surgery, there was very little body fat and ample evidence that the diet had failed to provide adequate caloric intake of the type of carbohydrates that the calf was able to digest and absorb. There was no evidence of a bacteremia or septemia that one would expect to see in an animal with a protein deficiency. The abomasum was approximately 45 cm long and 30 cm at its greatest diameter. Macroscopic and microscopic examination failed to indicate any hyperplasia of the small amount of rumen tissue which had been left attached to the cardia in order to complete the abomasal anastomosis (Figs. 1, 2, 3, and 4).

Trial No. 10

This calf was also at the optimum age of 21 days at time of surgery and was essentially normal in its recovery from surgery. No indication of any abnormality was noted until refusal of the first feeding of the fourth postsurgical day. Passage of a stomach tube resulted in the immediate return of gastric ingesta. With this indication of increased intragastric pressure and probable occlusion of the upper alimentary tract, a right flank exploratory laparotomy was

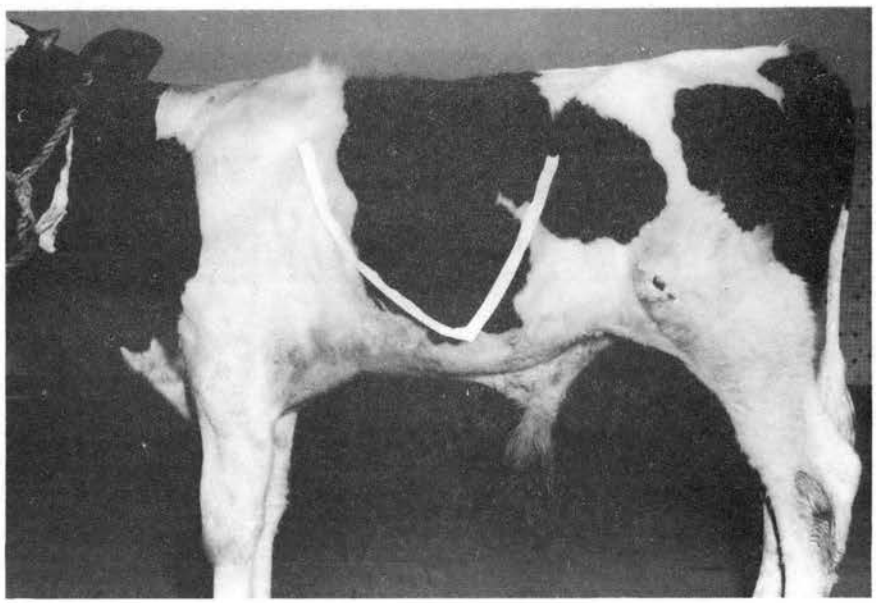

Figure 1. Calf No. 9, 150 Days After Surgery With Site of Incision Indicated

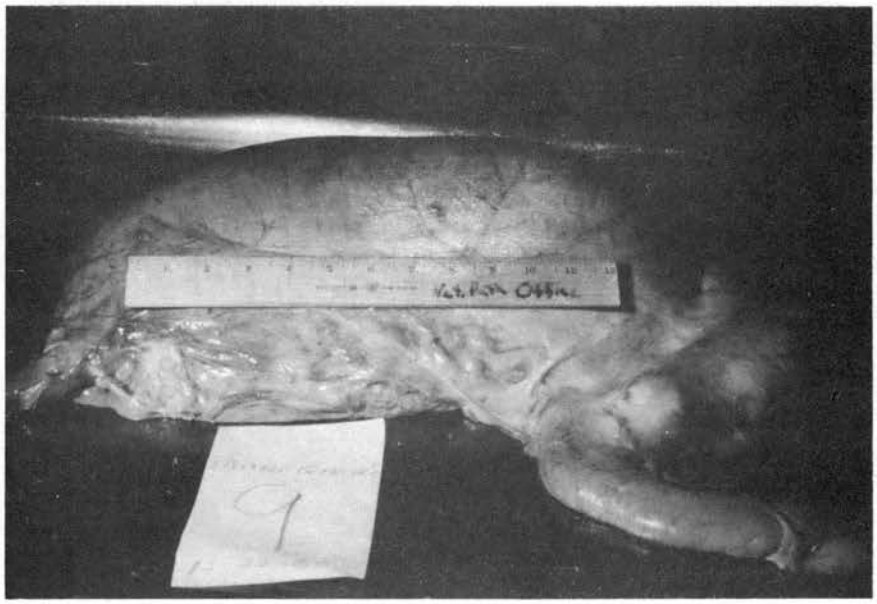

Figure 2. Exterior of Abomasum, Esophagus and Duodenum of Calf No. 9 at the Time of Necropsy Following Death Due to Inanition

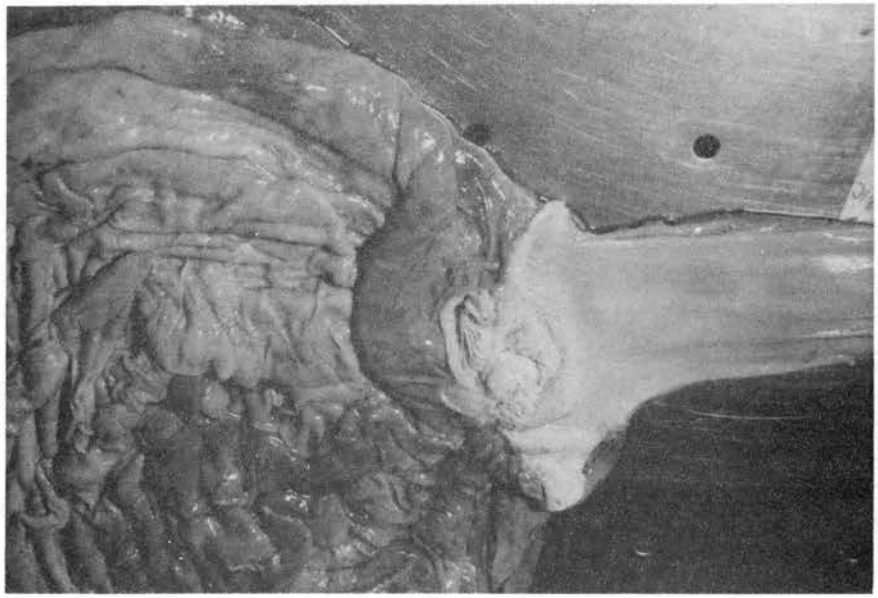

Figure 3. Internal Surface of Esophagus and Abomasum of Calf No. 9 at Necropsy

Figure 4. Site of Anastomosis of Abomasum to Cardial Region of Rumen.

performed. Exposure of the abdominal viscera revealed a greatly distended abomasum which showed no evidence of peritonitis nor ischemia. The pylorus was noted to be at an acute angle to the rest of the abomasum and duodenum was empty. Moderate traction was applied to pull the duodenum away from the abomasum. This resulted in the correction of the pyloric occlusion and allowed the immediate passage of ingesta into the duodenum. No other abnormalities were observed at this time and the abdominal incision was closed. Within nine hours, the calf was on its feet drinking water. It then took the normal four feedings per day for the next three days. On the tenth day after surgery, the calf refused all feedings until it died in mid-afternoon. Necropsy revealed that the cecum had passed through an opening in the omentum in such a way as to encroach on that portion of the omentum occupied by the omaso-abomasal artery and thus impair the circulation to the abomasum. Death was attributed to the so-called "strangulation fluid" as reported by Amundsen (3). According to Amundsen, this fluid contains a non-bacterial endotoxin which is evidently produced by the tissue of the alimentary tract when it is in an ischemic state.

Glucose Tolerance Study

Glucose tolerance studies were conducted on calves 8, 9, and 11. Three separate trials were conducted at weekly intervals. The average value for each hour from the three studies is shown on the graph in Figure 5.

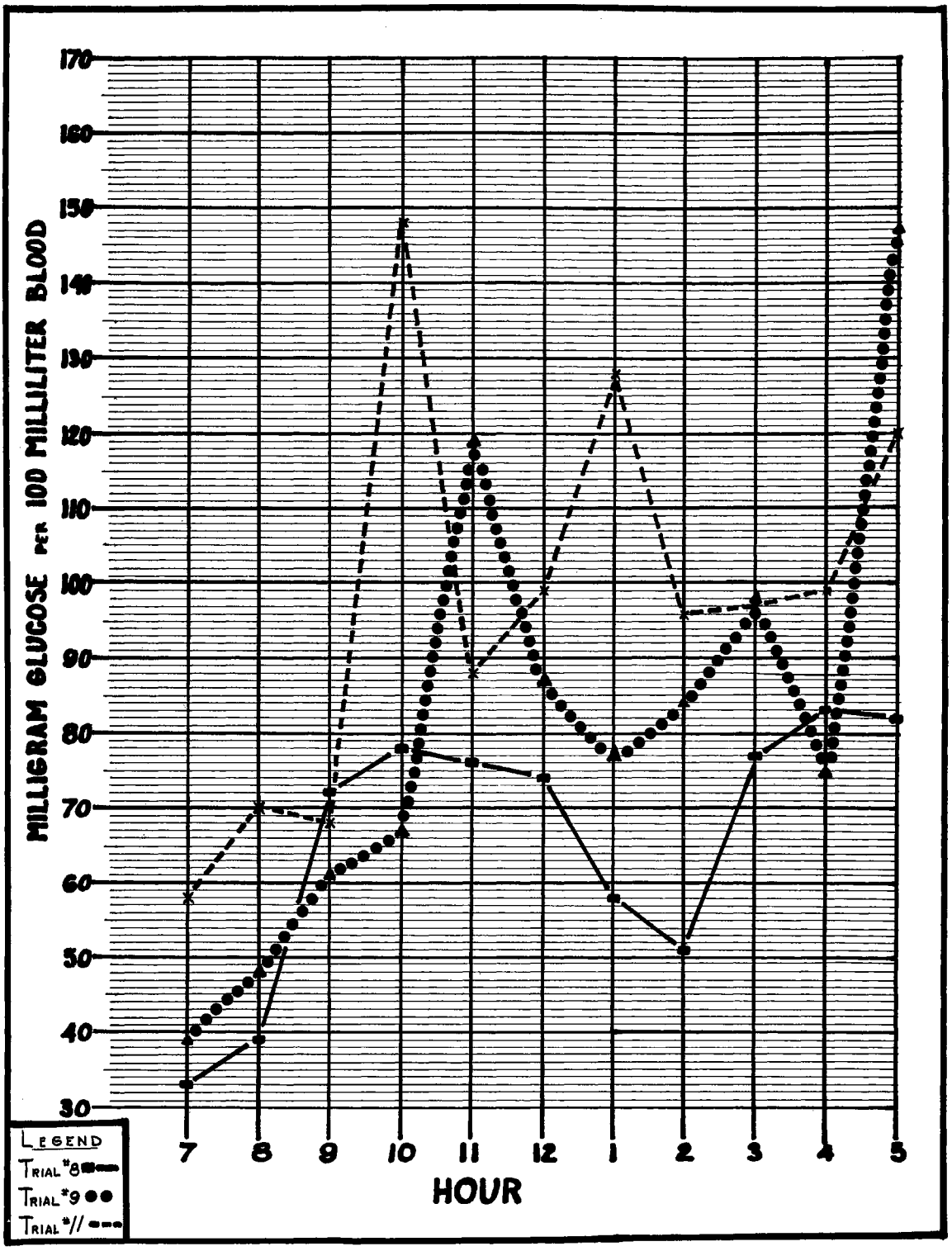

Figure 5. Gluscose Tolerance Study

CHAPTER V

DISCUSSION

Trials 8, 9 and 10 demonstrated the feasibility of constructing bovine monogastric calves with intact spleens while retaining the ventral trunk of the vagus. The death of Calf No. 10 can only be attributed to the failure to place sufficient sutures in the omentum as it was positioned around the celiac artery. The resulting space became a trap for the cecum and resulted in the impairment of the circulation to the abomasum and the production of the strangulation fluid (4) leading to shock and death. This condition undoubtedly existed to some extent at the time of the repeat surgery on Calf No. 10, but due to the prominence of the dilated abomasum and the pathology existing in this area, the condition of the cecum was not noted. Particular care was taken in turning the calves after surgery in an attempt to avoid torsions, particularly after the poor results experienced with Calf No. 1. The fact that this reoccurred in Calf No. 4 provided sufficient evidence that the problem was not due to postsurgical handling of these animals but due to failure,

in the first seven calves to build a physiological and anatomically sound attachment for the stomach of this new monogastric animal.

The presence of adhesions at the line of anastomosis in three of the first seven calves demonstrated the need for the inflation of the stomach after the completion of the anastomosis as was performed on the last three calves. The fact that this procedure has not proven necessary in the anastomosis of other portions of the alimentary tract can undoubtedly be attributed to a difference in motility of the involved areas. Observation of the abomasum and intestine during surgery revealed the greater motility of the intestine.

Evidence of the etiology of the impaired circulation and ulceration which were present in calves 3 and 7 is indicated by the work of Duncan (37) in which one out of three lambs with transected ventral branches of the vagus developed similar ulceration. Sauer and Brisson (101) encountered a similar problem in three of eight calves when they were doing total vagotomies. This seems to indicate that the innervation to the arterial supply was not entirely consistent and that vasoconstriction might have occurred in some of the calves not having an intact ventral vagus.

The variation in innernervation to the abomasum may have also been involved in the gastric stasis observed in Calf No. 5 following surgery. The fact that abomasal emptying occurred in the limited number of sheep and calves of Duncan (37), and in the eight calves of Sauer and Brisson (101) as well as in three out of the four calves subjected to total vagotomy in this experiment, may indicate that this dependence on vagal innervation occurs only in a small percent of the calves. On the other hand, there may be some other explanation for the gastric stasis observed. The necropsy on Calf No. 5 did not reveal any damage to the sympathetic nerve supply nor any distension of the duodenum which would inhibit gastric motility (95). The scope of observations made in this investigation was not sufficient to explain the gastric stasis.

During periods of rest, these calves were observed to exhibit the rumination cycle. The reverse wave of esophageal distension could be seen traveling up the esophagus to the oral cavity, followed by attempted mastication and subsequent swallowing. It was concluded that very little gastric material accompanied the regurgitation phase of the cycle inasmuch as the mastication phase was primarily a grinding of the teeth with very little material in the mouth to be masticated. The process of swallowing after mastication

undoubtedly involved a greater volume due to the inclusion of saliva as studied by Ash and Kay (11). The rumination observed in these monogastric calves was apparently initiated by a cerebral stimulus received by the rumination center of the medulla (43), since previous investigators have failed to show that the abomasum has any afferent fibers to this center (10, 37, 42, 116). The work of these investigators also indicates that any material which was regurgitated to the mouth for remastication was simply that material which happened to be in the anterior abomasum when the negative pressure was established in the terminal portion of the esophagus. It seems most improbable that there were any coordinated movements of the abomasum which would move ingesta to the region of the cardia prior to rumination. The hypothesis that rumination was initiated by a central stimulus was further substantiated by the observation that all rumination was observed during periods of tranquility without any correlation to the interval of time after feeding. It was not possible to confirm the observations of Lupien, et al. (70) that more fibrous diets increase the periods of rumination since only Calf No. 9 was placed on a diet with a high degree of fiber. Based on observations of this one animal no difference in the number of rumination

cycles or the time spent in mastication was noted. This was consistent with the known gastric sites which initiate stimulation of rumination observed by Ash and Kay (11) since all of the known sites were removed with the forestomachs, except for the cardia.

Although it was intended to submit all calves to surgery at three to four weeks of age according to the procedure of Sauer and Brisson (101), it was not possible to do so as the animals were not available. As shown in Table 5, this resulted in the performance of surgery on three calves that were younger than the preferred age. The observations on these calves reaffirmed the previous conclusion that three week old calves are preferred for this surgery. The younger calves appeared to be slower in recovering from surgery than the older calves while surgery on the older calves was more difficult and required more time due to the massive development of the forestomachs.

The diarrhea which was present in Calf No. 8 could not be explained by any observation made during the course of this experiment. Due to the fact that the diarrhea did not respond to antibiotics, sulfonamides, or intestinal protectives, it was postulated that the diarrhea was due to a physiological disturbance. Why only this calf showed diarrhea could not be explained. It is not likely that the

TABLE V

VARIATIONS IN SURGICAL PROCEDURE AND THEIR EFFECT ON SURVIVAL

	Calf Number									
	1	2	3	4	5	6	7	8	9	10
VARIATION IN SURGICAL PROCEDURE										
1. Vagus intact			X	X		X		X	X	X
2. Spleen intact								X	X	X
3. Inflation of abomasum								X	X	X
4. Omentum attached to abomasum & celiac artery								X	X	X
AGE AT SURGERY (Days)	30	38	42	46	9	5	14	22	23	21
RESULTS OF SURGERY										
1. Adhesions at anastomosis line		X	X			X				
2. Torsion of abomasum &/or duodenum	X			X						
3. Impaired circulation	X		X	X			X			X
4. Ulceration			X	X			X			
5. Post-operative shock	X									
6. Peritonitis		X	X			X	X			
7. Gastric stasis					X					
8. Death attributed to surgical procedure	X	X	X	X	X	X	X			X
9. Surgery satisfactory								X	X	

diarrhea was due to a deficiency of a dietary component because the ration was well fortified with all known vitamins and minerals. However, the diarrhea ceased for approximately three days after the animal ate alfalfa hay which implied that either added fiber content, a change in the flora of the intestines, or a combination of both was corrective.

Both of the calves from which extensive data were obtained, i.e. Calf No. 8 and Calf No. 9, showed relatively normal hemograms. The first samples from each calf taken 7 days after surgery indicated some hemoconcentration, however this was probably due to the amount of blood given at the time of surgery while attempting to prevent surgical shock. There was evidently a mild leucocytosis in Calf No. 9 fifty-four days after surgery; this was also accompanied by a shift to the left of Shillings index. However, there was no evidence of any clinical infection at this time. Except for the 54 day sample from Calf No. 9, the differential count, the white blood cell count, packed cell volume, and hemoglobin values of these calves was essentially normal.

Blood chemistry studies on these calves indicated that the serum protein was relatively stable although the values obtained were lower than those usually reported (125,40).

Serum phosphorus and serum calcium values showed relative decreases during the experiment. This is in line with the observations of Marsh and Swingle (74) and Payne (90) who observed higher serum calcium and phosphorus levels in younger animals. The low serum sodium levels found in this experiment cannot be explained on the basis of the observations made. In the procedure used, all values were reported as less than 110 mEq/L, whereas normal values of 128 to 137 are reported in the literature. The serum potassium levels obtained in the range of values reported by Horvath (52) who showed a decrease in serum potassium from the second to the tenth week of life. The trial reported herein was not of sufficient length to measure the levels of serum potassium for more than 9 weeks after surgery. At this time however, the serum potassium concentrations of both calves were found to be less than 50 per cent of expected normal values. In view of the continued performance of these calves past this period it is difficult to either support or contradict the hypothesis of Horvath (52) that potassium is a prime factor in the survival of monogastric calves.

The great variation that was observed in the different parameters studied in these two calves is an indication of

an interference to normal hemostasis. This interference was the result of the change in the digestive physiology of these experimental animals and the elimination of the normal digestive pool of the rumen.

The low blood glucose which was evident (68) in these animals confirms the work of Lupien, et al. (70) indicating that monogastric calves follow the same pattern as do intact calves with respect to a decrease in blood glucose at about 8 to 10 weeks of age. Of interest (Table 4) is the fact that Calf No. 9 exhibited an increase in blood glucose after the original decrease. Similar findings were reported by Lupien, et al. (70) and Hibbs, et al. (47). Further investigation is needed in order to determine the mechanism whereby blood glucose levels are changed with age, and the importance of the pancreas in this process.

The data reported in Figure 5 in reference to glucose tolerance studies indicate that there is more rapid absorption of glucose in intact animals than in monogastric calves. This more rapid absorption would give higher and earlier peaks in blood glucose levels in intact calves. This probably does not reflect a difference in the blood glucose control mechanisms in monogastric and intact calves but rather to a quicker passage of the milk into the intestine.

It is unfortunate that further observations were not made on these calves until their death. It would seem from the data which are available, that the primary problem in maintaining life in the monogastric calves is that of furnishing sufficient utilizable energy to sustain life. In consideration of the work of Grimes and Gardner (41) indicating that calves are able to digest rations with approximately 50 per cent milk fat on a dry matter basis, it would seem that the logical ration for a monogastric calf would be one that approached this fat percentage. The material would need to be fed in such a manner that the calf was required to nurse, since the nursing reflex is evidently a requirement for the secretion of pregastric esterase as reported by Ramsey and coworkers (97,98). The inclusion of milk fats would also make available sufficient butyric acid to stimulate gastric secretions (8). On the basis of information available in the literature and data presented in these trials, it is difficult to anticipate the magnitude of digestion and to calculate the energy furnished by any fermentation in the lower intestines of the monogastric calf. Morrill (82) and Van Soest (121) have suggested the possible importance of lower bowel fermentation.

The failure of any of the monogastric calves in this experiment to develop convulsions similar to those reported

by Lupien, et al. (70) may have been due to a higher fortification of the rations with the B vitamins. A description of the convulsive syndrome observed in their calves is suggestive of either a thiamine deficiency as described by Johnson, et al. (62) or a pyridoxine deficiency as described by Johnson, et al. (60).

CHAPTER VI

SUMMARY

Ten Holstein calves were used to perfect a technique for the surgical construction of a bovine monogastric animal. The preparations were characterized by an intact abomasum without a rumen, omasum, or reticulum. A successful surgical procedure was developed which retained both the spleen and the ventral branch of the vagus. Two surviving monogastric calves were used to study blood glucose, serum protein, serum potassium, serum sodium, serum calcium, and serum phosphorus levels at weekly intervals. Hemograms were also completed weekly in which packed cell volumes, hemoglobin, total leucocyte counts, and differential leucocyte counts were determined.

Hemograms of the monogastric calves were not significantly different from those of intact calves. Serum phosphorus and serum calcium levels of the monogastric calves were lower than those found in intact calves. The serum potassium and serum sodium levels were comparable to the

levels of intact calves though final levels of serum potassium in the monogastric calves were lower than those normally reported for intact calves. Serum protein levels were stable at a level below those normally reported for intact calves.

Blood glucose levels followed the normal regression found in intact calves at 8 to 10 weeks of age. A subsequent rise in blood glucose at 30 weeks of age is comparable to similar values evident in other trials (71,48).

One of the two surviving calves, Calf No. 9, lived for 9 months after surgery. Death was primarily due to inadequate caloric intake.

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APPENDIX A

January 31, 1952

Dr. George E. Stoddard
Assistant Professor
Dairy Husbandry
Iowa State College of
Agriculture and Mechanic Arts
Ames, Iowa

Dear George:

We were glad to have your letter of January 28 informing us that you are continuing to pursue vigorously the many problems concerning the relation of rumen activities and functions with milk and fat formation. Our Dr. R. E. Lubbehusen is the gentleman who actually performed the operations to remove the rumen for our earlier research work. Therefore, I have asked Dr. Lubbehusen to give us a written report of the method of procedure for doing this work. We are attaching this report which was originally written right after the work was done back in 1939. We hope this will serve your purpose.

George, you know we are always glad to help in any research projects that will be to the benefit of agriculture or the advance of research in the field of agriculture. We will gladly supply you with all information that we have on this subject. I am sorry that we do not have the facilities and animals that we feel that we can afford to devote to further study in this field at this time. Then too, we feel that you and others like you are in a better position to carry this work to its ultimate conclusion than we are with our facilities at our Research Farm and laboratories.

You are considering this problem from a very interesting approach when you are considering the possibilities of by-passing the rumen without removing it. I am somewhat puzzled as to how you will be able to perform an operation that will permit you to by-pass the rumen at will and still

use the rumen at will. In other words, if I understand you correctly, you will permit the animal to use the rumen, then you will avoid the use of the rumen vice-versa. I hope you succeed in this and if you do, I would like to know just how the job is accomplished.

I only wish I had the time and the opportunity to work more closely with you on this project.

Now as to the number of animals that we have used for this type of work. When we originally approached this problem from the angle of producing rumenless animals, Dr. Lubbehusen practiced on bull calves until he developed a satisfactory technique and until he proved that he could do the job and the calf would live. Following that we had him remove the rumen from 3 Holstein heifer calves as described in his report. I am sure you can appreciate that these calves did not grow at a normal rate. They were somewhat retarded in development. As they grew, 2 of the 3 developed relatively large middles and were observed chewing their cuds much more frequently than the third animal. Either fortunately or unfortunately, when these animals were approximately one year old, one of the individuals that was developing a large middle suddenly died. On post-mortem examination we found that this individual had developed a rather large rumen and this rumen was a double rumen, that is, it had 2 compartments but seemed to be functioning rather efficiently.

Following this discovery we slaughtered the other heifer that had a large middle and found practically the same condition in this animal. The third heifer, the one that had not developed a large middle, the one that would consume only a small amount of hay even when fed hay ad libitum, was grown out and when she reached breeding size she was bred. She conceived and dropped a normal calf and was continued in our herd through 2 complete lactations.

She never developed a large middle, would never consume much hay, and chewed her cud very little. We expected to use this animal to feed fermented material, etc., that we had demonstrated influenced fat percentage of milk when fed to animals on limited roughage, etc., to determine whether or not we could influence the fat content of this animal's milk. You may recall that fermented feed and rumen material from normal animals when fed to such animals did influence the fat content of the milk. This specific cow did produce milk

with lower fat content than her inheritance would indicate she should, but we were never able to get this individual cow to take feed that had been fermented or to which rumen material had been added. She would immediately go off feed and quit eating when such products were added to her ration. Therefore, we were never able to use her for the purpose for which we had produced her.

At the end of 2 lactation periods of failure in this regard we slaughtered the animal so we could examine her rumen, etc. We found that this animal had a very small rumen compartment. In fact, at most, would hold only a few gallons of material perhaps $2\frac{1}{2}$ or 3 gallons when filled to capacity.

The above is roughly our experience on the removal of rumen from animals and the resulting attempt to feed them. I am afraid Nat Allen has not kept me too well informed on the progress that they are still making in this field. Perhaps it is more my fault than Nat's because I have not followed him on the subject. Neither have I had any report from Bill Tyznik on his further work at the Ohio Station. I would appreciate very much having a copy of any such report. Do you think it advisable for me to write both Nat Allen and Bill Tyznik and ask them for the latest they have on the subject?

George, I do not know how I am going to find the time needed to go back in all of our records and bring up to date and report to the Society all of the work that we have done on this subject since our last publication. I appreciate that this should be done but we have so many other things to occupy our time that I am afraid that we may not get to this. We have aimed not to hold anything back from you or anyone else who has inquired on this subject. I hope you know that our neglect in this field is not for any purpose but just because we have felt that we could not spare the time. I hope you will continue to keep me informed on your work and if at any time you feel that we can be of any assistance do not hesitate to let us know.

With personal regards and best wishes for your continued success in this field, and hoping that you will eventually secure all the answers, I am

Sincerely,

E.B. Powell, Director
Research Department

APPENDIX B

The following is a copy of our Dr. R. E. Lubbehusen's report on the operations, etc., as written in 1939 following his successful operations:

"The operating technique was evolved through the trial and error method, and although it is still imperfect, we have, nevertheless experienced a fair amount of success, as judged on the basis of survival and apparent continued health of rumenectomized calves.

We have found the most desirable age period for the operation to be between the ages of fourteen and twenty days. Prior to the age of fourteen days, the calf, as a rule, does not possess the reserve vigor necessary to withstand the post operative shock. If the animal is older than twenty-one days, there is usually a marked distention of the rumen, rendering the organ much more difficult to manipulate.

We withhold all food, including liquids, for a period of at least twelve to eighteen hours prior to the operation, which is usually performed in the morning. We anesthatize with one-half of the recommended dosage of Nembutal (one-half grain per 5 pounds of body weight) followed by ether in the amount necessary to produce complete relaxation. Through experience, we have found that calves of this age will succumb to a full dose of Nembutal. There is no question but what post operative shock contributes to this undesirable result.

The operative area is prepared in the usual manner by first clipping the hair, then shaving the area and rendering the skin partially sterile through the use of a good antiseptic preparation. The hair coat adjacent to the area, which is, incidentally on the left side, is wet down and covered with sterile towels. The line of incision begins posteriorly in line with the twelfth intercostal space and is extended forward in a curved line just ventral to the costal cartilages. Depending upon the size of the calf, the length of the incision measures from eight to ten inches to allow ample freedom of movement during the operation and sufficient drainage

thereafter. Insofar as is possible, we try to avoid transverse section of the various muscles comprising the abdominal wall in this region. The rumen is immediately adjacent to the incision, but it is necessary to break down its dorsal attachment to the spleen before it can be withdrawn any appreciable distance. This splenic attachment can be broken down by blunt digital dissection. Care should be taken not to carry this dissection too far or there may be danger of rupturing the splenic artery and vein. After this dissection is completed the rumen is then retracted and a metal hernia clamp is placed just below the line of amputation. This line is marked by the rumeno-reticular groove. After the hernia clamp is applied, make a three to four inch incision in the wall of the rumen and through the opening; remove the contents by flushing with a warm saline solution. This flushing is important, in order to decrease the probability of extensive peritoneal infection. Before amputating the rumen, we usually ligate the larger branches of the right and left ruminal artery. This precautionary measure virtually eliminates the probability of post operative hemorrhage. In order to facilitate suturing, we allow about a half to three-quarters of inch of rumen tissue to protrude above the clamp. The cut tissues are brought into apposition by first using a continuous suture of the mucous membrane, followed with a mattress suture of the muscular and serous coats. For the former we use a No. 0 catgut and for the latter a No. 1 catgut. After releasing the hernia clamp, the abdominal incision is closed by three series of sutures, namely for the peritoneum, muscular wall, and skin respectfully. In suturing, allowance is made for ample drainage at the ventral end of the incision. As a rule, the patient does not lose over a half pint of blood. However, regardless of this fact, we usually like to give about 300 C C's of physiological saline solution intravenously. During the post operative period, one should watch the temperature rather closely. In one instance where the temperature indicated the probability of an intra-peritoneal infection, we apparently succeeded in warding off unfavorable sequelae, through the use of sulfanilamide."

VITA

Donald Eugene Williams

Candidate for the Degree of

Master of Science

Report: SURGICAL CONSTRUCTION AND OBSERVATIONS OF MONOGASTRIC CALVES

Major Field: Physiology

Biographical:

Personal Data: Born April 4, 1927 in Tahoka, Texas, the son of Nat H. and Georgia M. Williams.

Education: Attended Louisiana State University in 1944, transferred to Texas Agricultural and Mechanical College in October of 1944, after interruptions in U. S. Army and intervening work at McMurry College and North Texas State College, received Doctor of Veterinary Medicine at Texas A & M College in 1951; attended Oklahoma State University in school year 1964-65, completed requirements for Master of Science in August 1969.

Professional Experience: Practiced veterinary medicine in Abilene, Texas, 1951-53, practiced veterinary medicine in Ada, Oklahoma, from 1953 until present time. Current practice limited to the bovine animal; member of Oklahoma and American Veterinary Medical Associations, American Association of Bovine Practioners, and American Society for Study of Breeding Soundness.