INDUCED ENDOTOXIC SHOCK IN THE NEWBORN CALF

by 6408

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INTRODUCTION

Diarrhea in newborn and young calves has been a serious problem for centuries. The incidence of diarrhea varies greatly and depends upon many factors including the amount of colostrum ingested, the conditions of husbandry and sanitation, the climatic conditions, the immune status of the dam and thus the specific antibodies present in the colostrum.

Infectious diarrhea can be caused by bacteria, viruses, and fungi. The chief cause in young calves are certain serotypes of Escherichia coli (E. coli). The syndrome produced by these serotypes is called colibacillosis. The importance of E. coli as a primary cause of diarrhea has been debated for many years. Originally, E. coli was considered the cause of the diarrhea; later it was relegated to the role of a secondary invader. Recently, the importance of E. coli as an initiating agent in colibacillosis has apparently been reaffirmed. Current research indicates that certain viruses may initiate diarrhea and that the E. coli recovered in some cases is actually a secondary invader.

Colibacillosis, whether a primary disease or a condition developing secondarily to an initial viral infection, may run a critical and often fatal course. Colibacillosis may manifest itself as either a toxemia, a septicemia or as an enteric disease.

Peracute colibacillosis is probably a result of an E. coli toxemia characterized by collapse and sudden death. The calf is quite depressed and shows signs of shock as evidenced by pale mucosa, subnormal temperature, cold clammy skin, respiratory distress and convulsive movements.

The septicemic form is an acute condition with death occurring in untreated cases 24-72 hours after the onset of clinical signs. Anorexia is a common finding. The calves are depressed and weak. The body temperature is often elevated in early stages, falling to normal or subnormal as the disease progresses. A bacteremia can be demonstrated by recovery of E. coli from several internal organs (liver, spleen, heart blood) of calves which have just died or were euthanatized in the terminal stage. Localized arthritis is sometimes noted as a sequela in calves recovering from septicemic colibacillosis.

One explanation of the chronic form of colibacillosis is the rapid multiplication of bacterial flora in the gut shortly after the calf first nurses. Blood and mucus may be noted in the feces of normal calves within a few days of birth. This is thought to represent the shedding of neonatal epithelium which was permeable to the large globulin molecule at birth. In presence of a high bacterial population the exposed mucosa may become inflamed resulting in enteritis. The final outcome

is probably dependant upon the level of antibody the calf received during the first few hours of life. Where enteritis persists, death may result from dehydration and electrolyte imbalance.

Many signs noted in colibacillosis are a direct result of endotoxin which is one of the metabolic byproducts of the gram negative organism. Bacterial endotoxic shock, a complex yet specific type of shock, has been the subject of extensive study. It has been established that certain serotypes of E. coli produce potent endotoxins capable of producing profound signs of illness in the calf.

There have been numerous studies of the blood changes as well as the urinary and fecal loss of electrolytes in calves showing clinical signs of the enteric form of colibacillosis. It was the purpose of this study to determine the effect of a purified E. coli endotoxin from a pathogenic strain on the very young calf. The effect to be measured by changes in the temperature, pulse rate, respiratory rate, erythrocytes and leukocytes, sodium and potassium concentration of the serum, and the sodium, potassium, and chloride concentration of the urine and feces. It was anticipated that information obtained from the study might explain more fully the pathogenesis of endotoxic

shock. Treatment regimes might be modified as a result of a better understanding of the effects of the endotoxin.

The study was also utilized to accumulate information in the use of an indwelling cannula in the saphenous artery of the calf. The cannula was used in the collection of arterial blood for studies concerning the effects of endotoxin on the cardiac and respiratory system. The physiological data were recorded from the cannulated calves from birth until they had either received the endotoxin or reached ten days of age. Information concerning the cannulated calf is necessary for this animal to become an effective experimental model.

REVIEW OF THE LITERATURE

Mills (Watts, 35) noted in 1776 that diarrhea was responsible for neonatal death in many animal species. A historical review of colibacillosis has been made by Barnum et al. (1). The organism which is now known as Escherichia coli (E. coli) was first described by Escherich in 1885 following isolation from feces. The bacterium was known as Bacterium coli for many years. Jensen (1891) was first to associate the organism with a disease in young calves known as "Kalberruhr" or "Calf scours" and he later stated that the condition had existed in Denmark for at least a hundred years. Jensen was the first worker to distinguish between pathogenic and non-pathogenic strains of the bacteria. Smith et al. (1925, 1927) confirmed the presence of E. coli in white scours and demonstrated the effect of colostrum on the development of colibacillosis. They were unable to distinguish between pathogenic and non-pathogenic strains by biochemical methods.

Lovell (1937) used the precipitin tests to distinguish between strains of E. coli in calves. Kaufman (1947) and Ewing et al. (1956) employed serological tests in the differentiation of the serotypes of E. coli. Serological identification is based upon the presence of certain antigens in the bacteria: the O or somatic antigen, the H or flagellas antigen, and

the K or capsular antigen. The K antigen is divided into three types: L, B and A.

Three metabolic by-products of E. coli have been reviewed by Barnum et al. (1). They are: 1. Endotoxins which are the most important of the metabolic by-products and often are incriminated as the etiology of bacterial shock; 2. Hemolysins which have the ability to lyse mammalian red blood cells; 3. Colicines which are bactericidal substances which may be active upon other related bacteria. At least 15 colicines from E. coli have been identified. Thomlinson (34) suggests that the colicines have a role in maintaining equilibrium between the various E. coli strains.

Thomas (34) reviewed the physiological and pathological effects of endotoxins in mice, rats, guinea pigs, rabbits, cats and dogs. He suggests that the term endotoxin refers to a relatively homogenous group of toxic substances which exist as phosphorous containing, polysacchride-protein-lipid complexes in the intact cells of a wide variety of gram negative micro-organisms. The endotoxins, even those isolated from unrelated species of bacteria, are quite similar in chemical structure and properties.

Osborne (24) reports that bacterial endotoxin shock in dogs and rabbits is characterized clinically by a pronounced

chill, biphasic fever spikes, respiratory distress, marked depression, hypotension, cold clammy extremities and anorexia. Oliguria and anuria may develop and death often occurs. He suggested that the endotoxin was found in and on the cell wall and was released after the cell wall had been lysed. The endotoxin resulted in peripheral vasoconstriction followed by the pooling of blood in the splanchnic vascular bed.

Pulmonary edema has been a regular finding in gram negative toxemias. This was reported in neonatal swine by Shreeve (31) as well as in several laboratory animals (Osborne, 24, 26). Reduced blood flow and respiratory distress resulted in tissue anoxia and ischemia. Kidneys and lungs were most seriously affected. Kuida et al. (20) stated that the increase in total pulmonary vascular resistance was due to venous constriction and therefore the development of pulmonary edema was due to an increase in hydrostatic pressure.

Thomas (35) illustrated the complexity of the body's response to a single injection of an endotoxin by listing some of the mechanisms which are brought into action. They include: fever, leukopenia followed by leukocytosis, peripheral vasoconstriction and vasodilation, shock, depletion of liver glycogen, hyperglycemia, increased adhesiveness of polymorphonuclear leukocytes, heparin-preciptability of fibrinogen, impeded

phagocytosis by reticuloendothelial cells, augmentation of antibody response to protein antigens, abortion, hemorrhagic necrosis in malignant tumors, and disturbances of the reactivity of terminal blood vessels to epinephrine. Following two or more injections of endotoxin, the effects were complicated by appearance of such phenomenon as tolerance, increased resistance to bacterial infection, local and generalized Shwartzman reactions (Shwartzman (32) described a localized reaction as that produced in the rabbit by first giving a "preparing" injection of endotoxin intradermally and following with an intravenous "provoking" injection after 18 to 24 hours. Within three hours after the second injection, hemorrhagic necrosis occurred at the prepared site. A generalized Shwartzman reaction occurred when both injections were given intravenously. Extensive hemorrhagic necrosis occurred in many internal organs with bilateral cortical necrosis of the kidneys being the most characteristic lesion. He demonstrated that severe diarrhea also occurs in animals which had been sensitized to gram negative bacteria and then received a second dose whether injected or given orally.)

Osborne (26) reported anaphylactoid shock response to single and multiple intravenous injections of known serotypes of E. coli extracts into cattle, goats, ewes, pigs and rabbits. He noted death in as short a time as 50 minutes using a single

injection intravenously. Calves given smaller daily doses developed marked resistance to the extract of the endotoxin even when given in increasing amounts. Many of the signs noted by Osborne (25) following oral ingestion of E. coli cultures were noted in calves which had been given the endotoxin intravenously.

Osborne (26) reported that when three pregnant cows were given single doses of 17-25 ml. of a cellfree supernatant fluid from standardized cultures containing approximately 10^9 cells per ml. several reactions were noted. One cow died in 27 hours, one cow aborted in six days following a marked shock reaction, and the third cow showed evidence of severe shock and was anorectic for three days. Necropsy of the third cow, following euthanasia, revealed a partially decomposed fetus.

Wray and Thomlinson (39) successfully demonstrated that E. coli endotoxin could produce a reaction similar to anaphylactoid shock in young calves. They observed marked shock when the endotoxin was given intravenously and mild shock and obvious lesions of enteritis following oral administration. They were able to show differences in susceptibility to the endotoxin challenge and to correlate the severity of the reaction to the intravenous injection with the response to intradermal injection of the same endotoxin.

Many workers have reported changes in body fluids and loss of electrolytes in young calves suffering diarrhea and dehydration. Blaxter and Wood (3) measured the water loss as well as the loss of some of the electrolytes in calves with diarrhea. The loss of sodium and potassium in the feces increased eleven times over the usual anticipated daily loss. While the loss of sodium and potassium in the urine usually declined in calves with severe diarrhea, the total loss of sodium and potassium exceeded the intake. This is thought to be due to the amount of base secreted into the intestine with little net absorption. Cantarow and Trumper (7) noted that acidosis resulted from a primary alkali deficit due to the loss of base. McSherry and Grinyer (21) have shown that great loss of sodium and potassium would lower the pH of blood as determined by the Henderson-Hasselbach equation ($pH=6.1 + \log (B\text{-}HCO_3/H_2CO_3)$ where the base is primarily sodium and potassium.

Dalton et al. (9) studied the loss of serum electrolytes in young calves with naturally occurring diarrhea and reported both isotonic and hypotonic dehydration with concurrent changes in the packed cell volume (PCV) occurring infrequently. They concluded that the hyperkalemia reported by Roy et al. (1959) was probably due in fact to the decrease in fluid intake.

Fisher (17) studied sodium, potassium and chloride concentration in serum of young calves with clinical colibacillosis. Based on these observations he divided his calves into three groups -- normal calves, calves with diarrhea which survived, and calves with diarrhea which died. There was a significant increase in the serum potassium in calves which died and a significant decrease in the serum sodium and chloride in both groups of calves developing diarrhea.

Changes in the PCV of calves exhibiting marked diarrhea and dehydration did not form a consistant pattern in dehydration. McSherry and Grinyer (21) reported that very few calves with diarrhea had changes in the PCV. This observation was also reported by Dalton et al. (9) following observation of forty calves with diarrhea. In this group only four had an increase in the PCV as the diarrhea developed. The explanation of why the PCV failed to increase as the diarrhea developed was not readily apparent. Dalton suggested that the plasma volume would be maintained in spite of general dehydration. He further suggested that the erythrocytes were either destroyed as part of the catabolic response to diarrhea or were removed from the general circulation in an attempt to prevent hemoconcentration.

In contrast, Watt (37) utilized the increase in PCV that he found in calves with diarrhea as a guide to determine the amount of fluids required in certain treatment regimes.

Braude et al. (5) and Brunning et al. (6) have utilized labeled isotopes to show that the endotoxin is taken up by polymorphonuclear leukocytes which are quickly lysed, giving rise to the leukopenia.

Blaxter and Wood (3) reported that normal calves receiving only whole milk produced little fecal material and that little or no fecal material would be eliminated on one or more days in neonatal life. They determined the normal fecal output as 0-200 grams daily containing 25-35% dry matter. They classified a fecal output of 200-500 grams per day as loose and over 500 grams a day as diarrheal. The increase in feces produced was mainly due to increased water content and a concurrent decrease in the percent dry matter.

Watt (37) noted that calves with severe diarrhea during the first month of life lost as much as 100 ml. of body fluids for each kilogram of body weight within a twelve-hour period. Such loss resulted in a ten percent decrease in body weight within the same period.

Dalton (10,11,12,13) observed that the neonatal bovine was markedly different from the neonates of many other species

including the human, kitten, puppy and piglet in that it has a remarkedly well developed kidney at birth. Studies on renal function involving diuresis demonstrated that the kidney was able to excrete large volumes of urine following loading experiments utilizing milk, water, a hypotonic electrolyte solution and an isotonin saline solution. Studies involving starvation in the neonatal calf revealed that calves had the ability to produce a distinctly hypertonic urine. This ability is of considerable significance when related to the calf's ability to withstand the effects of starvation or scours.

Dalton reported the urine pH of the neonatal calf to be approximately 5.5. The addition of ammonium chloride in different amounts for a period of one to five days resulted in an increase in excretion of urine ammonia, yet the neonatal kidney maintained the pH of the urine at approximately the same as that observed in normal calves.

MATERIALS AND METHODS

Fifteen newborn, full term, Holstein bull calves were used. The calves were entered into the experiment as soon as possible after birth. Elapsed time between birth and start of cannulation of the saphenous artery ranged from 15 minutes to three hours. The cannulation technique was a modification of the procedure described by Donawick. The calf was placed in left lateral recumbancy. The right leg was flexed and abducted laterally to allow access to the surgical site. The medial aspect of the leg over the saphenous artery was clipped, scrubbed with surgical soap, and prepared with 70% alcohol and tincture of iodine. The skin and subcutaneous tissue over the artery between the hock and the stifle was infiltrated with a local anesthetic.[a] A 3-4 cm. incision was made and the artery exteriorized for a length of 2-3 cm. Pulsation of the artery aided the location of the structure. After exposure, the distal portion of the artery was ligated with 0.3 synthetic suture material.[b] A strand of the suture was also placed under the exposed artery at the proximal end. The artery was incised and a polyethylene catheter[c] was inserted into the artery a distance of 25-30 cm. Slight pressure on the strand of suture effectively

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a 2% Lidocaine, McGaw Labs., Glendale, Calif.
b Vetafil Bengen R, Dr. S. Jackson, Washington, D. C.
c Intramedic, PE90, Clay Adams, Inc., New York, New York.

controlled the hemorrhage from the incised artery until the catheter could be inserted and advanced. When the catheter was inserted the desired distance, a ligature was placed around the artery and catheter. A three-way valve with a minimum amount of epoxy on the male aperture was inserted into the exposed catheter and the catheter was flushed with 2 ml. of a heparin solution. The incision in the skin was closed with interrupted mattress sutures and the valve and the distal end of the catheter were anchored to the skin.

Das' procedure was used in the collection of fecal samples. An area 7 cm. wide and extending laterally each way from the sacrum was clipped with an electric clipper equipped with a #40 blade. A 40 by 5 cm. strip of light weight belting was doubled and the ends stitched together. A snap was placed about 1/3 of the way toward the center from each end. An adhesive was placed on one side of the belting and the belt applied to the clipped area of the calf. A similar belt was also fastened to the buttocks of the calf approximately 4 cm. below the anus. A 3 liter plastic freezer bag was attached to the buttocks of the calf by means of the snaps on the belting.

The calves were placed in individual metabolism cages at a room temperature of approximately 70^{o}F with adequate ventilation. They were entered into the cages following catherization

and attachment of the plastic bag. The urine tray was lined with 6 mm. plastic sheeting thus permitting the collection of urine. Plastic bottles containing ice were placed in the styrofoam receptacles to keep the urine at a low temperature in an attempt to prevent bacterial growth in urine held at room temperature.

Fifteen ml. of blood was collected within 15-20 minutes following the catherization of the artery. The sample was divided into three parts -- a sample containing no anticoagulant for utilization in electrolyte studies, a sample containing disodium ethylenediaminetra-acetic acid (NaEDTA) for hematological studies including PCV, hemoglobin, and leukocyte count, and a specimen containing .1% heparin for studies on pH, PO_2, and PCO_2 of the blood. Additional samples were collected at 24-hour intervals for the duration of the experiment.

Voided urine was measured and sampled at 12-hour intervals for the first 48 hours; thereafter, samples were collected at 24-hour intervals. At time of harvesting the urine specimen, the feces were removed and a new sack attached to the belting. The fecal specimens were frozen and stored at $0^{\circ}F$. The urine specimen were stored at $34^{\circ}F$ until analysis.

Temperature, respiratory rate and pulse rate of the calf were recorded at the time of collection of the urine and fecal specimens.

The birth weight was used in the determination of the quantity of milk to be offered to the calf. The calves were fed four percent of the birthweight twice daily. Three feedings of colostrum at 12-hour intervals were given; thereafter the calves were fed from the pooled milk from the bulk tank.

Two percent tincture of iodine was applied to the umbillicus of the newborn calf. Antibiotics were not used following surgery except where the temperature became elevated. In such event, 1 million units of procaine penicillin and 1.25 grams of dihydrostreptomycin were injected intramuscularly at 12-hour intervals. During the experiment, an outbreak of colibacillosis occurred in the dairy; thereafter, 400 mg. tylosine[d] and 1 ml. of Vitamin A, D and E[e] were injected intramuscularly and two antibiotic boluses[f] were given orally to each calf shortly after birth.

All calves were handled similarly for the first 48 hours in order to establish baseline levels of certain electrolyses, urine and fecal volumes, leukocyte counts, packed cell volumes, pH and partial pressure oxygen (PO_2) and the carbon dioxide (PCO_2) of the arterial blood.

Prior to initiation of the experiment, four calves were randomly selected as the recipients of an intravenous (IV)

d Tylan 200, Corvel Laboratories, Omaha, Neb.
e ADE, Chas. Pfizer & Co., New York, N. Y.
f Sulkamycin, Norden Laboratories, Lincoln, Neb.

injection of E. coli B-055:B5 endotoxin.[g] The injection was given following the 48-hour recording. The endotoxin was administered on the basis of 1.5 mg/kg body weight in divided doses. Two calves (#6 & 14) received two -.75 mg/kg doses and calves 8 & 9 received four -.37 mg/kg doses. All physiological parameters were recorded immediately prior to the initial injection of the endotoxin and then at intervals as the toxemia developed.

Four calves were also randomly selected as recipients of endotoxin orally following the 48-hour recordings. The calves were observed and the various parameters were recorded, at 12-hour intervals.

The PCV was determined by using the microhematacrit method[h] for four minutes. The WBC were counted on an electronic particle counter,[i] and the hemoglobin was determined by the cyanmethemoglobin technique. The serum sodium and potassium were determined by flame photometry.[j]

The urine collected for a time period was measured and a sample saved for determining specific gravity, sodium, potassium and chloride. The specific gravity was established by use of a

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g Difco Laboratories, Detroit, Mich.
h Microhematacrit International Model #MB, International Equipment Co., Boston, Mass.
i Coulter Counter Model A, Coulter Electronics, Chicago, Ill.
j Coleman Flame Photometer Model, Coleman Instrument Co., Maywood, Ill.

refractometer,[k] while the sodium and potassium levels were established by flame photometry. Schales and Schales procedure was used to establish the amount of chloride.

The total feces collected for a timed period was weighed and an aliquot was removed for determination of the dry matter content, and sodium, potassium and chloride level. If the feces had a firm consistency, ten grams were used while 30 grams were weighed out in the case of a watery diarrhea. The test samples were frozen and later dried in an oven at 180°F until no further loss of weight was noted at which time the dried weight was recorded. The dried feces were pulverized and digested in 20 ml. of .75 N HNO_3 for 7-10 days at which time the suspension was filtered and the filtrate collected for future sodium, potassium and chloride determination. Chloride was determined by use of Schales and Schales procedure and the sodium and potassium were established by flame photometry.

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k TS METER, American optical, Buffalo, N. Y.

RESULTS

Fifteen Holstein male calves were used in the experiment over a three-month period. The calves ranged in weight from 30.3 kg. to 45.3 kg. The observations for all of the calves during the first 48 hours and the observations of the control calves (no endotoxin given either IV or orally) from 48 hours through 240 hours are included in Tables 1-23.

There was either a clinician or a barn attendant present at the time of delivery and the calves were not allowed to nurse. The cannulation procedure was completed as quickly after birth as feasible. Initially no antibiotics or other prophylactic procedures were used other than 2% tincture of iodine on the navel at birth and nitrofuran powder* on the cannulation incision. After calves 1, 3, 4 and 5 developed a clinical disease resembling colibacillosis as evidenced by depression and diarrhea, the prophylactic measures used in the non-experimental calves in the herd were also used in the experimental calves. One ml. of a vitamin ADE solution** and 400 mg. of tylosine*** were injected IM and two calf scour antibiotic boluses**** given orally shortly after birth reduced

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* Furacin Powder, Eaton Laboratories, Norwich, N. Y.
** ADE, Chas. Pfizer & Co., New York, N. Y.
*** Tylan 200, Corvel Lab., Omaha, Neb.
**** Sulkamycin, Norden Labs., Lincoln, Neb.

the incidence of diarrhea. The cannulation procedure produced stress on the calves; however the time required for the surgical procedure became shorter and the stress of a lesser degree as the surgery team developed proficiency in the technique. Reduced stress combined with the prophylactic procedures against colibacillosis resulted in a change in the defecation pattern. From that point on it was not uncommon for the calf to go the first 24 hours or more without defecation. One calf failed to pass any feces during the first 48 hours.

The rectal temperatures of the calves (Table 1 and 10) fluctuated considerably; however the majority of the rectal temperatures exceeding 102.5 during the first 48 hours were noted in calves which were showing signs of colibacillosis or complication from surgery. The rectal temperature mean remained steady at 101.8-101.9 for the first 36 hours, then an increase in the mean of one degree F. was noted at 48 hours. The higher temperature mean was fairly constant throughout the remainder of the experiment.

Calves 1 and 5 developed localized, cutaneous infections around the cannulation incisions. Calf 7 which was used as a control developed a septic arthritis within two weeks after the cannula was removed.

Anticipation of feeding resulted in a marked variation in the respiratory rate (Table 2 and 11) and in the pulse rate (Table 3 and 12) in the calves; however the means of the readings reveal a definite pattern. The mean pulse rate dropped from 143 per minute at birth to 130 per minute at 24 hours with a second drop to 118 per minute noted at 96 hours and a gradual decrease to a mean of 107 per minute at 240 hours.

The mean of the respiratory rate at 12 hours was 58 and did not vary until 48 hours of age when there was an increase of 12 per minute. The mean changed back to 62 at 72 and 96 hours. There was a continued decline with the 168 hour mean of 41. From this time on, the mean respiratory rate gradually increased to 55 at 240 hours.

The hemoglobin and packed cell volume (Table 4 and 13) were highest during the first 48 hours with virtually no variation in the means of the readings for the immediate postnatal, 12 hour and 24 hour tests. A decrease to a mean hemoglobin of 8.8 grams and a PCV of 26% was noted in the control calves in the 72-hour readings. The levels noted at 72 hours remained reasonably constant throughout the remainder of the experiment.

The leukocyte numbers (Table 4 and 14) decreased from a mean of 13,750 per cmm. at birth to 7,500 per cmm. at 72 hours. The 240 hour reading was 16,800 per cmm. and based on two readings.

The mean values of the serum sodium and potassium (Table 5 and 15) expressed as milliequivalents per liter (mEq) followed a consistant pattern throughout the experiment with the exception of the 240 hour reading which was markedly lower.

The volume of urine (Table 6 and 16) passed by the calves varied considerably. If the 12 and 24 hour volumes and the 36 and 48 hour volume means respectively are added together and compared to the other 24 hour readings a mean volume of 1500-1600 ml. is revealed. The specific gravity of the urine (Table 6 and 17) showed a variable pattern throughout the experimental period.

The mean urine sodium composition expressed in mEq varied from 17-70 through the ten-day experiment (Table 7 and 18). A drop in the sodium level to 17 mEq occurred on the sixth day through the eight day after which the mean increased to 26 and 35 mEq respectively the last two days. The mean urine potassium (Table 7 and 19) fluctuated from 43-103 mEq throughout the first 120 hours then remained steady at 60-65 mEq thereafter.

The mean urine chloride dropped from 57 mEq at 12 hours to 31 at 24 hours and remained constant through the 48-hour collection. From the third day on, the chloride readings were less than 2 mEq unless diarrhea existed (Table 7 and 20).

The wide variation in the defecation pattern as well as the amount and characteristics of the feces are shown in Tables 8, 9, 21, 22 and 23.

Four calves were given endotoxin orally after the 48 hour recordings were made. The endotoxin was added to milk or water and the calf allowed to drink the solution.

Calf 11 received 2.2 mg/kg at 48 hours and 7 mg/kg at 96 hours of age. The calf did not reveal visable effects to either the 48 or 96 hour challenge. The percent dry matter of the fecal material (Table 26) collected after the initial challenge was only 5 percent; however the calf had had diarrhea previous to the challenge.

Calf 12 received 3.4 mg/kg. There was no indication the calf was affected by the endotoxin.

Calf 13 received 7 mg/kg of endotoxin. The calf had passed watery feces for 12 hours prior to the challenge and the diarrhea continued for 24 hours post challenge (PC). Attempts to collect the feces and urine separately failed. The calf exhibited some depression and shallow respirations PC; however significant changes were not noted in the blood (Table 27).

Calf 15 was given 7 mg/kg endotoxin. The endotoxin did not have visable effects on the calf. The calf did not defecate during the 12 hours following challenge; however the

calf had watery diarrhea during the 12-24 hour post challenge period. The amount and dry matter were not determined.

The volume and composition of the urine (Table 28) which was voided and examined following the oral challenge did not vary from urine collected from control calves showing clinical signs of colibacillosis.

Four calves were challenged at the 48th hour with endotoxin administered intravenously. Calves 6 and 14 received an initial injection of .75 mg/kg and calves 8 and 9 were given .375 mg/kg. All four calves exhibited marked depression and ataxia within five minutes post injection (PI) of the endotoxin. Within ten minutes, the calves were prone. The respiratory rate was reduced from a 48 hour mean of 70 to 8-10 per minute within 15 minutes PI. The pulse rate increased from a 48 hour mean of 132 to 150 at the same PI time interval. The rectal temperatures ranged from 100.5 to 102 until the terminal stages when a temperature of 2-3^{o}F was noted.

Calves 6 and 14, which received the .75 mg/kg dosage showed depression and cold, clammy extremities in less time than calves 8 and 9. Calf 14 could barely raise his head off the floor within 20 minutes PI.

The four calves were down and comatose within 40 minutes PI and had an irregular respiratory pattern. Depression of the

rate to four per minute was noted at 60 to 90 minutes; however at different times the calf appeared to be ventilating the lungs and the rate would increase to 40 to 50 per minute for a short time.

Calf 6 died 150 minutes PI while calf 14 died in 135 minutes. The terminal stages were marked by an erratic pulse and respiratory pattern. Calf 6 had a variation in the pulse from 80 to 120 per minute ten to fifteen minutes prior to death. Ten minutes before death, the calf had six to eight shallow, irregular respiratory movements per minute. Respirations ceased three minutes before the heart stopped. The pulse rate was 90 at this time.

Calves 8 and 9, which received .375 mg/kg of endotoxin, developed a similar, but slower pattern. Calf 8 died 210 minutes PI; while calf 9 was euthanatized 300 minutes PI. The calf was in agony; however the fourth injection of endotoxin did not cause death within an hour.

None of the calves urinated following the injection of the endotoxin.

Calves 6, 8 and 9 exhibited a watery diarrhea (Table 25). The plastics which were attached to the calves did not fit tightly enough to catch all of the fluid feces when the calf was prone. The three calves passed small amounts at intervals

and samples were collected as noted in the table. Calf 14 did not pass feces following the challenge.

Significant changes were not noted in the PCV or hemoglobin levels (Table 25); however a marked drop in the WBC was noted. A precipitous drop from 7,000 to 1,400 in 65 minutes PI was noted in calf 8; while calf 14 had a decline from 11,100 to 3,700 in 20 minutes PI.

TABLE 1

Rectal temperatures (^{o}F) taken at 12-hour intervals for first 48 hours

Calf	Hours of Age			
	12	24	36	48
1	----	103.2	----	103.6
2	----	101.2	----	101.4
3	----	101.1	----	105
4	----	103	104	105
5	102.5	102.5	102.2	103.4
6	103	102	101	104
7	100	101.2	101	103.8
8	100.8	101.8	102.7	101.2
9	----	----	----	----
10	----	102.8	103	102
11	101.8	100.6	101.5	103
12	103	102.5	101.6	101.6
13	101	100.4	101.4	103
14	102	101.8	100.7	----
15	101.8	101	102	101.6
Mean	101.8	101.8	101.9	103

TABLE 2

Pulse rate per minute taken at 12-hour intervals for first 48 hours

Calf	Hours of Age			
	12	24	36	48
1	----	132	----	----
2	----	120	----	----
3	----	104	----	160
4	----	140	----	----
5	150	135	120	120
6	150	120	144	144
7	120	108	120	132
8	132	144	144	132
9	----	----	----	----
10	----	100	108	120
11	144	132	114	100
12	150	144	144	144
13	150	144	144	144
14	144	130	130	----
15	150	132	144	132
Mean	143	130	131	132

TABLE 3

Respiratory rate per minute taken at 12-hour intervals for first 48 hours

Calf	Hours of Age			
	12	24	36	48
1	----	----	----	60
2	----	72	----	60
3	----	----	----	110
4	----	100	120	148
5	60	72	52	60
6	72	60	60	52
7	60	48	52	64
8	36	60	48	48
9	----	----	----	----
10	----	40	36	48
11	60	46	48	60
12	60	60	80	60
13	60	60	54	84
14	60	54	60	----
15	54	48	60	54
Mean	58	60	61	70

TABLE 4

Packed cell volume (PCV-percent), hemoglobin (Hb-grams/100 ml), and leukocytes (WBC-per cmm) at 0, 24 and 48 hours of age

Calf	Hours of Age 0			24			48		
	PCV	Hb	WBC	PCV	Hb	WBC	PCV	Hb	WBC
1	29	9	10,700	26	8.5	12,000	25	9	---
2	28	8.6	9,200	23	8	6,800	28	8.6	9,200
3	35	11.5	10,000	26	10.9	8,200	28	10.5	7,600
4	23	7.7	12,000	24	6.8	12,000	18	7.2	6,400
5	32	10.5	10,800	28	9.5	9,000	28	9.5	9,000
6	30	10	12,500	30	10		26	9	6,700
7	35	12	20,900	33	11.5	22,500	34	12	17,300
8	45	13	16,200	40	12	7,900	38	12	7,000
9	38	10.7	11,900	33	9.2	16,000	31	8.2	13,700
10	34	9.7	20,800	31	9.1	16,200	----------		
11	----------			43	13	13,200	47	13.7	8,000
12	----------			32	9.7	11,300	30	9.2	8,700
13	33	9.2	19,300	----------			37	12	17,200
14	----------			34	10.7	16,300	31	9.2	11,100
15	21	6.5	----------	----------			18	5.5	4,400
Mean	32	9.8	13,750	31	9.9	12,600	30	9.7	9,700

TABLE 5

Sodium (Na) and potassium (K) concentration in milliequivalents (mEq) at birth, 24, and 48 hours

Calf	Hours of Age 0		24		48	
	Na	K	Na	K	Na	K
1	143	8.5	153	6.2	122	5.4
2	137	8.5	---	---	133	6.1
3	142	8.5	150	4.8	130	3.8
4	139	6.9	149	6	147	6
5	141	5.5	146	11.6	131	9.6
6	138	11.8	152	12.4	181	15.1
7	133	7.8	122	6.6	129	7.4
8	137	11.5	116	5.8	140	11.1
9	143	5.7	137	5.1	121	4.4
10	154	6.6	130	---	141	7.7
11	150	5.1	137	8.4	170	9.7
12	---	---	150	7	123	7.2
13	141	5.4	123	6.3	124	6
14	146	8.6	143	6.4	139	4.8
15	176	6.3	---	---	---	---
Mean	144	7.6	139	7.2	138	7.5

TABLE 6

Volume (ml) and specific gravity of urine collected at 12-hour intervals for the first 48 hours

Calf	Hours of Age 12		24		36		48	
	Vol.	Sp.Gr.	Vol.	Sp.Gr.	Vol.	Sp.Gr.	Vol.	Sp.Gr.
1	125	1.020	1,275	1.017	NS	NS	550	1.034
2	725	1.011	650	1.013	NS	NS	2,300	1.010
3	65	ND	540	1.005	NS	NS	75	1.018
4	25	1.027	325	1.028	700	1.035	275	1.038
5	35	1.026	530	1.023	725	1.018	**	**
6	350	1.023	2,250	1.011	250	1.036	575	1.017
7	700	**	2,200	**	1,100	1.009	**	**
8	900	1.021	1,150	1.015	1,400	1.008	500	1.019
9	NS	NS	NS	NS	NS	NS	NS	NS
10	**	**	800	1.015	550	**	1,500	1.018
11	NS	NS	1,050	1.011	**	**	800	**
12	800	1.007	900	1.010	750	1.024	950	1.015
13	NS	NS	575	1.014	700	1.017	**	**
14	550	1.036	850	1.017	**	**	**	**
15	NS	NS	1,600	1.013	850	1.009	450	1.005
Mean	430	1.021	1,050	1.015	780	1.019	800	1.019

NS - No sample taken to lab.

ND - Sample analyzed. No results recorded.

** - Fecal contamination of sample precluded measurement and/or analysis.

TABLE 7

Sodium (Na), potassium (K), and chloride (Cl) composition (mEq) of urine collected at 12-hour intervals for the first 48 hours

Calf	Hours of Age 12			24			36			48		
	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl
1	28	6	ND	80	31	ND	NS	NS	NS	56	70	ND
2	26	30	ND	53	37	ND	NS	NS	NS	90	30	8
3	72	60	6	50	52	3	NS	NS	NS	72	55	6
4	77	30	69	90	48	6	67	88	5	20	104	11
5	47	47	73	90	90	20	32	32	4	**	**	**
6	45	55	81	35	40	13	140	105	14	47	100	15
7	**	**	**	27	30	28	100	61	12	**	**	**
8	62	57	103	90	ND	99	61	ND	74	95	ND	135
9	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
10	**	**	**	77	75	87	**	**	**	62	56	11
11	NS	NS	NS	40	50	13	**	**	**	**	**	**
12	54	60	52	62	100	80	55	150	132	44	120	29
13	NS	NS	NS	70	105	11	90	300	3	**	**	**
14	60	45	16	72	50	12	**	**	**	**	**	**
15	NS	NS	NS	ND	40	4	15	30	3	8	50	26
Mean	52	43	57	64	57	31	70	109	31	55	73	30

NS - No sample taken to the lab.

ND - Sample analyzed. No results recorded.

** - Fecal contamination of sample precluded analysis.

TABLE 8

Grams, percent dry matter (%DM), and total dry matter (TDM) in feces collected at 12-hour intervals for first 48 hours

Calf	Hours of Age 12			24			36			48		
	Grams	%DM	TDM	Grams	%DM	TDM	Grams	%DM	TDM	Grams	%DM	TDM
1	190	47	89	130	28	37	93	37	34	NF	NF	NF
2	NF	NF	NF	NF	NF	NF	**	**	**	95	28	27
3	295	47	138	NF	NF	NF	140	50	70	205	36	73
4	NF	NF	NF	NS	NS	NS	90	32	29	NF	NF	NF
5	270	38	103	NF	NF	NF	NF	NF	NF	180	16	29
6	115	43	50	NF	NF	NF	NF	NF	NF	400	35	140
7	**	**	**	255	22	56	NF	NF	NF	305	6	18
8	NF	NF	NF	25	40	10	145	42	61	100	31	31
9	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
10	**	10	**	**	13	**	200	10	20	200	28	56
11	30	38	12	220	31	68	**	**	**	**	**	**
12	NF	NF	NF	NF	NF	NF	550	21	115	350	22	77
13	NS	NS	NS	NF	NF	NF	NF	NF	NF	**	**	**
14	NF	NF	NF	NF	NF	NF	**	**	**	225	24	54
15	NF	NF	NF	NF	NF	NF	230	33	76	300	20	60

NF - No feces passed during the time period.
NS - Sample not taken to lab.
** - Diarrhea - Amount of feces could not be determined or urine and feces mixed which precluded analysis.

TABLE 9

Milliequivalents (mEq) of fecal sodium (Na), potassium (K), and chloride (Cl) in samples collected at 12-hour intervals for 48 hours

Calf	Hours of Age 12			24			36			48		
	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl
1	101	14	62	62	18	34	55	23	6	NF	NF	NF
2	NF	NF	NF	NF	NF	NF	**	**	**	45	18	6
3	85	20	74	NF	NF	NF	27	22	25	25	22	35
4	NF	NF	NF	NS	NS	NS	44	16	12	NF	NF	NF
5	55	20	6	NF	NF	NF	NF	NF	NF	75	24	95
6	100	12	24	NF	NF	NF	NF	NF	NF	35	13	24
7	**	**	**	69	22	16	NF	NF	NF	40	20	80
8	NF	NF	NF	55	17	12	85	22	12	60	18	11
9	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
10	85	18	35	75	19	32	80	29	57	42	20	17
11	35	23	31	15	16	6	NF	NF	NF	13	19	ND
12	NF	NF	NF	NF	NF	NF	100	18	11	70	21	91
13	NS	NS	NS	NF	NF	NF	NF	NF	NF	**	**	**
14	NF	NF	NF	NF	NF	NF	**	**	**	50	21	ND
15	NF	NF	NF	NF	NF	NF	58	21	57	65	24	11

NF - No feces passed during the time period.
NS - Sample not taken to lab.
** - Diarrhea - mixing of feces and urine precluded analysis.
ND - No data recorded.

TABLE 10

Rectal temperatures (^{o}F) of control calves from 72 to 240 hours of age

Calf	Hours of Age			
	72	96	120	144
1	102	102.4	104.5	102.4
2	102.6	102.8	104.6	103.8
3	104.5	104.5	103	102.8
4	105	104.5	102.5	103.6
5	102.5	102.5	103	104
7	102.6	101	100.5	102.5
10	102.6	102	103	103
Mean	103.1	102.8	103	103.1

Calf	168	192	216	240
1	102.8	103	103.5	103
2	102.7	102.5	102.6	104
3	103.6	103	102.2	102
4	102	102.2	101.5	103
5	103	102.5	103.8	102
7	103	102	103	103
10	103	104	105	----
Mean	102.9	102.7	103.1	102.8

TABLE 11

Pulse rate per minute of control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	----	----	----	100
2	----	----	----	144
3	120	96	100	96
4	140	132	120	120
5	180	144	120	120
7	108	108	120	90
10	96	110	112	96
Mean	129	118	114	109

Calf	168	192	216	240
1	84	96	90	80
2	112	120	110	100
3	120	120	102	100
4	120	144	96	120
5	120	120	120	144
7	100	90	96	96
10	96	110	100	----
Mean	107	115	102	107

TABLE 12

Respiratory rate per minute of control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	44	----	----	24
2	44	----	----	75
3	72	72	48	50
4	114	96	52	60
5	84	52	60	60
7	54	54	60	30
10	24	36	48	36
Mean	62	62	54	48

Calf	168	192	216	240
1	24	40	44	30
2	60	48	42	72
3	48	54	54	42
4	30	60	48	92
5	52	48	54	48
7	42	36	48	48
10	36	48	88	----
Mean	41	48	54	55

TABLE 13

Packed cell volume (PCV-percent) and hemoglobin (Hb-grams/100 ml) of control calves from 72 to 240 hours

Calf	Hours of Age 72		96		120		144	
	PCV	Hb	PCV	Hb	PCV	Hb	PCV	Hb
1	25	9.5	---	---	27	8.2	29	8.2
2	25	9.5	---	---	27	8.2	23	7.1
3	29	10.1	29	9.1	28	9.2	31	9.2
4	16	5	19	6.2	19	5.9	19	6
5	29	8.7	27	8	27	8.2	25	8
7	30	10	35	10.4	31	9.4	33	9.5
10	29	8.5	---	---	31	10.1	29	9.2
Mean	26	8.8	28	8.4	27	8.5	27	8.2

Calf	168		192		216		240	
1	30	9	30	9	29	9	---	---
2	22	6.9	22	7.2	26	8.1	---	---
3	---	---	29	9.2	31	9.3	31	9
4	19	5.9	19	5.9	19	5.9	---	---
5	25	8	26	8	27	7.7	26	7.7
7	32	9.5	31	9.2	30	9.1	29	8.8
10	26	8.3	30	9.5	29	9.1	---	---
Mean	26	8	27	8.3	27	8.3	29	8.3

TABLE 14

Leukocyte count (per cmm) of control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	6,200	----	11,400	8,400
2	6,300	----	10,400	7,900
3	6,700	6,400	10,000	12,200
4	6,300	9,200	13,700	12,600
5	4,100	6,300	8,800	8,800
7	11,500	10,500	12,600	16,500
10	11,100	----	15,300	8,100
Mean	7,500	8,100	11,700	10,600

Calf	168	192	216	240
1	9,700	10,650	10,700	----
2	7,900	8,900	9,600	----
3	----	10,900	----	----
4	8,700	9,100	9,100	----
5	9,200	9,900	13,500	11,500
7	----	----	21,700	22,100
10	13,400	11,300	10,300	----
Mean	9,800	10,100	12,500	16,800

TABLE 15

Serum sodium and potassium concentrations (expressed in milliequivalents) of control calves from 72 to 240 hours

Calf	Hours of Age 72		96		120		144	
	Na	K	Na	K	Na	K	Na	K
1	138	4.8	---	---	130	5.4	117	5.7
2	130	5	---	---	136	5.4	141	6.2
3	135	4.8	136	8	124	10.6	126	3.7
4	139	5	153	9.7	142	10.9	175	4.5
5	133	4.7	139	6.3	142	10.3	118	8.8
7	151	8	126	9.1	133	4.9	135	6.2
10	137	7.4	---	---	137	6.6	132	5.6
Mean	138	5.7	139	8.3	135	7.7	135	5.8

Calf	168		192		216		240	
1	134	5.7	128	8	132	6.8	---	---
2	138	5.8	130	7.4	134	6.1	---	---
3	---	---	133	6.6	158	5.7	117	10.9
4	141	4.8	146	6	146	6	---	---
5	139	9.4	138	7.7	127	7.7	131	5.9
7	133	7.9	138	11.5	124	8.5	122	3.6
10	167	10.1	143	5.1	142	5.6	---	---
Mean	142	7.2	136	7.5	138	6.6	123	6.8

TABLE 16

Urine volume (ml) voided by control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	2,250	1,700	1,650	1,550
2	2,300	2,200	1,500	1,400
3	1,400	2,000	1,475	2,250
4	550	1,000	715	1,750
5	240	1,775	1,700	1,750
7	1,500	800	1,900	2,500
10	1,600	550	550	NS
Mean	1,550	1,430	1,380	1,870

Calf	168	192	216	240
1	600	3,300	2,400	1,800
2	560	2,850	2,450	1,900
3	1,375	2,550	1,400	1,500
4	2,200	2,450	1,250	2,500
5	1,400	850	2,050	2,000
7	2,150	2,100	2,500	2,000
10	1,050	1,850	3,100	NS
Mean	1,340	2,280	2,160	1,950

NS - No sample submitted

TABLE 17

Specific gravity of urine voided by control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	1.007	1.011	1.011	1.012
2	1.010	1.010	1.013	1.019
3	1.029	1.011	1.011	1.011
4	1.035	1.031	1.024	1.007
5	1.012	1.018	1.009	1.011
7	NS	1.028	1.012	1.013
10	1.008	1.005	1.028	NS
Mean	1.017	1.016	1.016	1.012

Calf	168	192	216	240
1	1.011	1.007	1.006	1.009
2	1.019	1.011	1.008	1.009
3	1.006	1.008	1.017	1.010
4	1.007	1.012	1.020	1.006
5	1.026	1.020	1.024	1.023
7	1.014	1.012	1.013	1.013
10	1.006	1.006	1.010	NS
Mean	1.013	1.011	1.017	1.012

NS - No sample submitted

TABLE 18

Composition of urine -- millequivalents of sodium -- voided by control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	52	38	25	16
2	60	60	65	28
3	98	32	18	10
4	22	25	54	32
5	70	60	60	7
7	----	12	7	10
10	21	20	65	----
Mean	54	41	49	17

Calf	168	192	216	240
1	10	11	30	29
2	22	21	38	40
3	10	19	40	35
4	32	39	65	17
5	25	10	21	28
7	8	2	2	6
10	14	30	47	----
Mean	17	19	35	26

TABLE 19

Composition of urine -- Milliequivalents of potassium -- voided by control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	37	68	68	55
2	28	85	90	106
3	72	70	68	58
4	108	80	95	46
5	25	90	55	76
7	----	124	95	65
10	30	18	225	----
Mean	50	76	100	68

Calf	168	192	216	240
1	45	45	40	50
2	70	54	55	52
3	40	52	95	75
4	46	64	58	65
5	110	120	87	76
7	93	58	65	74
10	40	38	50	----
Mean	63	62	65	65

TABLE 20

Composition of urine -- Milliequivalents of chloride -- voided by control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	****	****	****	****
2	****	****	****	****
3	6.3	4.1	****	****
4	6.1	****	3.2	****
5	****	****	****	****
7	----	54.8	42.8	11.6
10	51	39	****	----

Calf	168	192	216	240
1	****	****	****	****
2	****	****	****	****
3	12.7	****	2.6	****
4	****	****	****	****
5	****	3.6	****	****
7	****	14.5	****	****
10	30	41	****	----

**** less than 2 mEq ---- no sample

TABLE 21

Fecal quantity (grams) and percent dry matter (% DM) of control calves from 72 to 240 hours

Calf	Hours of Age 72		96		120		144	
	Grams	%DM	Grams	%DM	Grams	%DM	Grams	%DM
1	---	---	46	33	---	---	---	---
2	---	---	---	---	---	---	---	---
3	---	---	---	---	---	---	---	---
4	275	32	110	27	---	---	---	---
5	---	---	120	32	---	---	---	---
7	---	---	55	7	---	---	220	26
10	---	---	135	30	6	28	190	16

Calf	168		192		216		240	
1	---	---	---	---	---	---	120	26
2	---	---	---	---	---	---	95	33
3	27	39	---	---	---	---	220	15
4	---	---	80	35	25	30	90	29
5	135	29	75	35	180	16	---	---
7	270	22	---	---	72	14	98	35
10	120	22	150	19	---	---	---	---

TABLE 22

Grams of dry matter in feces collected from control calves from 72 to 240 hours

Calf	Hours of Age			
	72	96	120	144
1	----	15	----	----
2	----	----	----	----
3	----	----	----	----
4	88	30	----	----
5	----	38	----	----
7	----	4	----	57
10	----	41	2	30

Calf	168	192	216	240
1	----	----	----	31
2	----	----	----	31
3	11	----	----	33
4	----	28	8	26
5	39	26	29	----
7	59	----	10	35
10	26	30	----	----

TABLE 23

Milliequivalents of sodium (Na), potassium (K), and chloride (Cl) in feces from control calves from 72 to 240 hours

Calf	Hours of Age 72			96			120			144		
	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl
1	--	--	--	--	--	--	--	--	--	--	--	--
2	--	--	--	--	--	--	--	--	--	--	--	--
3	--	--	--	--	--	--	--	--	--	--	--	--
4	--	--	--	41	44	17	--	--	--	--	--	--
5	--	--	--	35	20	23	--	--	--	--	--	--
7	--	--	--	40	32	61	--	--	--	27	40	28
10	--	--	--	66	22	28	40	21	34	30	44	11
Mean				46	30	32	40	21	34	29	42	20

Calf	168			192			216			240		
	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl
1	--	--	--	--	--	--	--	--	--	31	18	16
2	--	--	--	--	--	--	--	--	--	--	--	16
3	40	36	12	--	--	--	--	--	--	32	20	80
4	--	--	--	28	28	12	35	54	12	30	40	12
5	41	21	11	32	24	11	25	24	31	--	--	--
7	24	36	10	--	--	--	45	48	5	35	50	5
10	27	46	11	37	26	5	--	--	--	--	--	--
Mean	33	35	11	32	26	9	35	42	16	32	32	28

TABLE 24

Composition of fecal samples collected from calves challenged intravenously

Calf No.	Minutes Post Injection	Amount (grams)	Dry Matter (percent)	Na	K	Cl
				Milliequivalent		
6	150##	150**	3	50	26	66
8	35	60	35	60	19	18
	50	12	45	55	25	85
	90	7	43	68	18	95
	120	125**	3.3	92	26	98
	165	150**	2	70	29	107
	210##	55**	3.6	72	26	130
9	65	100**	2.3	108	56	130
	110	90**	3.3	114	29	99
	170	150**	1.6	116	36	68
	240	150**	2	52	26	73
	300##	15**	3	35	24	62
14	135##	None				

- Death ** - Incomplete recovery of sample

TABLE 25

The packed cell volume (PCV), hemoglobin (Hb), leukocytes (WBC), and serum sodium (Na) and potassium (K) levels in 48-hour calves at the time of injection and at varying post injection intervals (PI).

Time	PCV (%)	Hb (gm/100 ml)	WBC (cmm)	Na	K (milliequivalents)
Calf 6					
Challenge	26	9	6,700	151	5.1
20 min. PI	--	--	--	103	4.1
35 min. PI	--	--	--	151	7.4
75 min. PI	--	--	--	119	6.5
110 min. PI	26	9	--	129	6.8
160 min. PI	Death				
Calf 8					
Challenge	37	11.5	7,000	--	--
30 min. PI	--	--	--	124	6.1
65 min. PI	41.5	13	1,400	146	5.4
90 min. PI	41.5	12.5	1,800	--	--
115 min. PI	39	12.1	2,100	134	11
155 min. PI	40	12.1	1,800	146	5.6
210 min. PI	Death				

TABLE 25 (Continued)

The packed cell volume (PCV), hemoglobin (Hb), leukocytes (WBC), and serum sodium (Na) and potassium (K) levels in 48-hour calves at the time of injection and at varying post injection intervals (PI)

Time	PCV (%)	Hb (gm/100 ml)	WBC (cmm)	Na	K (milliequivalents)
Calf 9					
Challenge	29	8.1	8,500	128	4.7
45 min. PI	31	8.7	3,300	133	5
65 min. PI	30	8.2	2,300	129	4.3
90 min. PI	29	8.1	2,600	--	--
150 min. PI	--	--	--	140	4.1
175 min. PI	--	--	--	136	4.4
300 min. PI	-- Death	--	--	137	5.4
Calf 14					
Challenge	31	9.2	11,200	139	4.8
5 min. PI	34	10.7	12,300	131	5.1
20 min. PI	38	11	3,700	125	4.6
35 min. PI	31	10.1	3,500	125	4.9
55 min. PI	--	--	--	133	6.6
85 min. PI	33	10.4	5,100	132	5.6
105 min. PI	31	9.5	2,700	141	6.6
135 min. PI (Death)	31	10.1	2,150	--	--

TABLE 26

Composition of feces collected from calves given endotoxin orally at 48 hours of age

Time	Amount (grams)	Dry Matter (%)	Na (mEq)	K (mEq)	Cl (mEq)
Calf 11 Challenge					
plus 12 hours	68	5	69	36	50
plus 24 hours	20	16	13	22	30
plus 72 hours	115	20	40	34	10
Calf 12 Challenge					
plus 24 hours	38	26	43	24	16
plus 48 hours	70	20	68	40	21
Calf 13	The calf had severe diarrhea starting twelve hours before the challenge and the diarrhea continued for 24 hours. This precluded samples being collected for analysis.				
Calf 15 Challenge					
plus 12 hours	None				
plus 24 hours	Severe diarrhea - No sample				
plus 48 hours	40	35	62	38	100

TABLE 27

Changes in packed cell volume (PCV), hemaglobin (Hb), leukocytes (WBC), and serum sodium (Na) and potassium (K) of calves challenged orally with endotoxin at 48 hours of age

Time	PCV	Hb	WBC	Na(mEq)	K(mEq)
Calf 11					
Challenge	47	13.6	8,000	169	9.7
plus 12 hours	46	14.1	8,300	134	7.8
plus 24 hours	42	13.6	11,300	141	8.2
plus 48 hours	42	12.7	10,400	141	6.6
plus 72 hours	45	14	18,000	140	4.8
Calf 12					
Challenge	30	9.2	8,700	125	6.3
plus 24 hours	30	9	7,000	156	7.4
plus 48 hours	31	9.8	6,500	143	5.1
Calf 13					
Challenge	37.5	12	17,200	124	6
plus 6 hours	41	12.5	18,400	139	8
plus 12 hours	47	14.7	18,700	154	8.6
Calf 15					
Challenge	18	5.5	4,400	205	10
plus 2 hours	16	5.3	3,700	144	7
plus 4 hours	18	5.5	4,500	132	4.8
plus 7 hours	15	4.9	5,000	144	7.1

TABLE 28

Volume and composition of urine from calves which were given endotoxin orally at 48 hours of age

Time	Volume (ml)	Sp. Gr.	Na (mEq)	K (mEq)	Cl (mEq)
Calf 11 Challenge					
plus 12 hours	600	1.020	1.8	144	24
plus 24 hours	1,050	1.012	3	72	12
plus 72 hours	2,200	1.011	2.2	56	2
Calf 12 Challenge					
plus 24 hours	500	1.006	16	100	27
plus 48 hours	2,750	1.009	--	--	--
Calf 13	The calf had severe diarrhea starting twelve hours before the challenge and the diarrhea continued for 24 hours. This precluded samples being collected for analysis.				
Calf 15 Challenge					
plus 12 hours	1,350	1.010	5	80	9
plus 24 hours	1,200	1.010	9	74	Trace
plus 48 hours	2,000	1.004	12	25	Trace

DISCUSSION

The significance of colibacillosis as a major problem to the cattleman as well as the dairyman is established; however the chronic or enteric form which is characterized by diarrhea of many hours or even several days duration with the resulting dehydration and loss of condition is easier recognized and has been studied in more detail. In contrast, the septicemic and enteric-toxemic forms are of a much more acute nature and the calf is sometimes found dead or in severe shock as evidenced by marked depression, cold extremities and mouth, and depressed respiration. Diarrhea is not a consistant clinical finding in the acute colibacillosis and may not be observed before the calf expires. The septicemic and the enteric-toxemic forms are considered to be clinical manifestations of the effects of the endotoxin which is a metabolic by-product of E. coli and they have not been studied as thoroughly as has the enteric form.

The procedure described by Donawick for permanent cannulation and subsequent collection of blood samples from the arterial system was utilized and certain physiological parameters were recorded. These parameters, which have not been previously reported for the cannulated calf, were deemed necessary to aid in the development of the cannulated calf as an experimental model for studying the effects of the endotoxin on the pH of

the blood and the changes in the partial oxygen and carbon dioxide pressures.

The calves were placed in lateral recumbancy which was a change from the procedure used by Donawick. It was determined that this positioning, rather than standing, would provide the best surgical approach in the newborn calf.

The calves were all cannulated and observed for 48 hours and the calves which did not receive endotoxin were studied a total of 240 hours. Although there were no uncannulated calves to serve as controls, the findings which were recorded agree with the previously published observations on the neonatal calf and there does not appear to be any basis for assuming that the cannulated calf is not an acceptable, otherwise normal, neonate. The calves showed no clinical signs or physiological changes which would indicate that the cannula was not well tolerated. There was no indication of impairment or loss of function to the leg which received the cannula with the resulting occlusion of the saphenous artery. It appears that compensatory circulation is available to the anatomical area normally served by the artery.

There was some concern that the increase in the mean rectal temperature at 48 hours and persisting throughout the experiment indicated that there was a possible reaction to the

cannula. The rectal temperatures of 24 calves which were in the same barn at approximately the same season of the year were recorded. It was found that the lowest temperature mean (101.5) was on day one. There was an increase to 102.4 at 48 hours and the highest mean temperature for the ten-day period was at 72 hours (103.1).

The calves were kept in a dairy barn near other non-experimental calves and the bacterial flora of the area produced certain problems which precluded the collection of all of the desired information. Natural outbreaks of apparent colibacillosis in some of the control calves precluded determining all of the desired parameters, especially data on the urine and feces. If the diarrhea was severe, some of the watery feces escaped from the plastic bag and ultimately drained into the urine container. The mixing of the feces and urine prevented analysis of the urine and also posed problems in determining the amount of feces that was passed by the calf. Similar illnesses in the calves which were to receive the endotoxin masked some of the response to the challenge. This was a problem in attempting to determine if the reaction shown by the calf was to the experimental endotoxin or if there was also additional effect because the calf was the victim of a natural infection.

In spite of these limitations, the overall observations of the calves as reported should serve as guidelines for further use of the cannulated calf as a useful experimental animal. There was no evidence to indicate that the physiological parameters of the cannulated differ from the non-cannulated newborn.

The marked increase in the sodium and chloride levels in the feces of calves which were showing diarrhea reinforces earlier reported observations of the losses of the two electrolytes. Diarrhea results when there is an accumulation of fluid in the intestine and it is excreted in the feces. In the normal animal there is a continual movement of water from the blood to the intestinal lumen and a counter movement of fluid from the lumen into the blood. If the absorption of water from the lumen exceeds the flux from the blood into the intestinal lumen, the net flow is out of the lumen and diarrhea will not be present. If the opposite situation exists, there is a net flow of water into the lumen and diarrhea results. A bi-directional flux also effects electrolytes, especially sodium and chloride, and their net flow is altered in diarrhea.

The absorption of water is quite possibly the result of both an active and a passive process in many instances; however the net flow is markedly affected by osmotic changes. Water

absorption may depend upon the active absorption of an ion such as sodium which is absorbed by an active process or the water may follow it passively down an osmotic gradient.

Changes in osmotic activity within the lumen of the intestine are the bases for many of the theorys regarding the increased flow of fluids into the intestine or the possible concurrent decrease of absorption from the lumen. There is some evidence that the E. coli organism may split the large macromolecule present in the intestine into many smaller molecules with the resulting increase in the osmolarity. Interference with the absorption of sodium or other osmotically active ions would result in an increase of fluid in the intestine and alterations in capillary permeability would allow the escape of colloids and other particles which have an action to change the osmolarity of the content of the intestine. It may be that the action of the endotoxin can cause the increase in the capillary permeability as well as affecting the intestional wall so that it is a membrane which is unable to absorb digested materials but functions as a semipermeable membrane and allows osmosis. Chloride is apparently lost as a result of the increased peristalysis or other causes of failure of the ion to be absorbed. Potassium is not lost into the intestine to as

great a degree unless there is a catabolic state present in the calf in addition to the diarrhea.

The amount of endotoxin injected on a mg/kg basis appeared to influence the onset and depth of the endotoxic shock as well as the length of time that the calf survived. There appeared to be a supression of defecation in the two calves which received .75 mg/kg since one calf did not defecate and the second calf passed a small amount compared to the other two calves.

The onset of diarrhea appeared to be influenced by the pattern of defecation prior to challenge. Calf 10 had passed 535 grams of feces just prior to the challenge and the watery diarrhea was first noted in an hour; while calf 9 passed several small amounts of normal feces for 90 minutes and the diarrhea did not start until 120 minutes PI. It was not possible to fasten the plastic bags to collect all of the feces when it was watery. The problem was more serious when the calf was prone.

The kidney of the newborn calf is much more functional at birth than that of many neonates. If there has been a loss of electrolytes into the lumen of the intestine, a marked reduction in the excretion of sodium and potassium in the urine results.

The newborn calf also has a greater ability to concentrate urine and conserve fluids in cases of dehydration.

The effects of the E. coli endotoxin given IV were most pronounced on the leukocytes of the blood. The leukopenia developed within an hour or less and it persisted until the calves expired several hours later. The explanation for the leukopenia that develops in a condition such as coliform mastitis is that there is a pooling of the leukocytes in the area of the infection. The work with Isotope labeled endotoxin has shown that the endotoxin is quickly absorbed by the leukocyte (neutrophil) and lysed in minutes. The absorption and subsequent destruction of the leukocyte by the endotoxin is a more likely explanation for the rapidly developing leukopenia as there was no area of infection for the pooling to take place in the IV injected calf. It would appear that the use of a leukocyte count taken from a depressed calf with other clinical signs of acute colibacillosis would be a definite diagnostic aid in determining if the depression was due to the diarrhea and electrolyte loss or if it was primarily due to the action of the endotoxin.

Even though there was a leukopenia present, the injection of the endotoxin did not alter either the sodium and potassium level of the blood or the hemoglobin and PCV reading. This

experiment tends to substantiate previous observations that the PCV does not usually decline in cases of diarrhea in calves and questions Watt's (1967) formula for using the PCV as an indication for the amount of replacement fluids needed. The PCV probably does not accurately measure fluid loss because of either a concurrent destruction of the erythrocytes by the infectious agent or more likely that there is a withdrawal of some of the cellular constituents from the circulation to preclude hemoconcentration from developing.

If a severe leukopenia is present, the initial treatment should be directed at counteracting the probable endotoxic shock. In clinical cases, the endotoxic shock is often preceded by a bacteremia and a bacterial infection indicates the concurrent treatment with antibiotics, sulfas, or nitrofurans in an attempt to control the pathogenic bacterial population.

Pulmonary edema, which was noted at necropsy of the calves which received the endotoxin intravenously and is also reported in the literature, may preclude the use of massive volumes of replacement fluids such as balanced electrolytes except in cases of severe dehydration. In situations where dehydration dictates fluid therapy, thought should probably be given to administering part of the volume by a route other than directly into the blood stream.

The oral challenge of the endotoxin did not appear to affect the calves even though they were given much larger doses on a mg/kg basis. It is probable that endotoxin is destroyed or inactivated in the stomach or that there is a dilution and slower absorption.

SUMMARY

Calves were studied in which an indwelling cannula was inserted into the saphenous artery and maneuvered into the posterior aorta. Several physiological parameters were recorded in the calves from birth through ten days of age. The data accumulated included the rectal temperature, pulse rate, respiratory rate, and the volume of feces and urine in 12-hour periods was recorded for all of the calves. Blood samples were collected for the determination of the hemoglobin, packed cell volume, and leukocyte count as well as the serum sodium and potassium concentrations. Aliquots of feces and urine were collected for analysis of the sodium, potassium and chloride levels. The specific gravity of the urine and the percentage of dry matter in the feces was established. The procedure did not create any marked deviations in cannulated calves when compared to similar observations in uncannulated calves.

The effect of purified E. coli endotoxin administered either intravenously or orally to 48 hour old calves is reported. Intravenously administered endotoxin resulted in the rapid onset of endotoxic shock with death occurring within three to five hours. Anuria was noted in all calves. A watery diarrhea was also observed with the sodium, potassium and chloride levels of the feces elevated. A significant

finding was the rapidly developing leukopenia which developed as early as 20 minutes post injection and persisted until death.

Significant changes were not noted in any of the parameters recorded in the calves which received the endotoxin orally. This negative finding indicates that orally administered endotoxin did not affect the calves during the observed post challenge period.

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INDUCED ENDOTOXIC SHOCK IN THE NEWBORN CALF

by

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Fifteen newborn Holstein bull calves were used in a study of the effects of induced endotoxic shock on certain physiological parameters in the newborn calf. A polyethylene cannula was inserted into the saphenous artery a distance of 25-30 cm. so that the tip of the cannula was in the posterior aorta. The procedure allowed collection of repeated samples of arterial blood.

The rectal temperature, pulse rate, respiratory rate, and the volume of feces and urine eliminated in 12-hour periods were recorded for all calves. Blood samples were collected for determination of the hemoglobin, packed cell volume, and leukocyte count as well as the serum sodium and potassium concentration. Aliquots of feces and urine were collected for analysis of the sodium, potassium, and chloride levels. The specific gravity of the urine and the percentage of dry matter in the feces was established.

All calves were studied during the first 48 hours of life and seven were observed for an additional eight days. Recordings of data were made at 24-hour intervals. The cannulated calves were studied to determine if there were any deviations in any of the physiological parameters from those previously reported for the normal bovine neonate. The calves did not show any discomfort or complications from the cannula. No meaningful

deviations in the studied parameters from those reported for normal calves were detected.

Four of the calves were challenged intravenously with a purified E. coli endotoxin (B-055:B5) at 48 hours of age. A rapidly developing shock syndrome as evidenced by profound depression and cold extremities was noted. Death resulted within 135-300 minutes after the initial injection of the endotoxin.

Diarrhea was noted in three of the four calves; however, none of the calves urinated during the post injection period. No significant changes were noted in the hemoglobin or packed cell volumes of the calves. A leukopenia developed as quickly as 20 minutes after the initial challenge. Pulmonary edema was a consistant necropsy finding.

Four calves were challenged orally with the same endotoxin. No clinical signs or physiological changes were noted which could be attributed to the ingested endotoxin.

It is suggested that the leukopenia which develops may serve as a diagnostic aid in differentiating bacterial endotoxic shock from the more common enteric form of colibacillosis which is marked primarily by dehydration and electrolyte depletion. The pulmonary edema should indicate the judicious use of replacement fluids when given intravenously.