RESISTANCE OF THE NEWBORN CALF TO ORAL CHALLENGE WITH A SEPTICEMIA-PRODUCING ESCHERICHIA COLI

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ABSTRACT

RESISTANCE OF THE NEWBORN CALF TO ORAL CHALLENGE WITH A SEPTICEMIA-PRODUCING *ESCHERICHIA COLI*

By

Noel Edgar Johnston

Colostrum fed to the newborn calf provides immunoglobulins which are absorbed unchanged into the blood. These immunoglobulins, and other macromolecules present, are actively taken up by the intestinal epithelial cells. If bacteria are present they may also be actively transported into the blood stream by this non-specific mechanism. As colostrum promotes rapid cessation of this absorption mechanism (gut closure), it may give mechanical protection to the newborn calf as well as providing immunoglobulins. The objective of this experiment was to examine the protection provided by a colostrum diet for the newborn calf when challenged with a septicemia-producing *Escherichia coli*.

Twenty male Holstein calves were obtained soon after birth and before they had suckled. They were assigned to

four groups and fed (1) colostrum, to induce rapid gut closure and provide high serum immunoglobulin G (IgG) levels; (2) milk replacer, to give gut closure with no serum IgG; (3) polyvinylpyrrolidinone K.60 (PVP) to induce rapid gut closure with no serum IgG; and (4) normal saline, to give neither gut closure nor IgG.

The calves were given two liters of their feed on arrival at the experimental barn (within four hours of birth) and then each morning and evening. The rectal temperature was measured and a blood sample was taken before each feed. Packed cell volume, total protein, and IgG levels were quantified in the blood samples.

To give some indication of the time of gut closure, 100 ml of fresh chicken egg albumin was given orally to seven calves at 24-27 hours of age, and to 13 calves at 12 hours of age. Absorption of egg albumin into the serum was determined using an Ouchterlony agar gel diffusion test with rabbit anti-egg albumin antiserum in the center well. With this test, absorption of egg albumin ceased between 12 and 24 hours of age in most calves, including the saline-fed calves.

Total serum protein levels were significantly higher ($P<0.01$) in the colostrum-fed calves from the 12-hour sample onwards due to the absorption of colostral antibodies. As expected, calves fed the other three diets (milk replacer, PVP, or saline) did not have detectable IgG concentrations at any time.

At approximately 27 hours of age the calves were inoculated orally with 1.5×10^{10} viable organisms of *E. coli* serotype 026:K60:NM. Diarrhea was evident within 8 hours of inoculation, and *E. coli* was cultured from the small intestine of 19 calves at necropsy. Following inoculation rectal temperatures, packed cell volumes and total protein levels usually increased.

Three calves fed milk replacer died within 10 hours of inoculation. Of the 15 calves not receiving colostrum, *E. coli* was isolated from the liver and spleen of 14, and from the heart blood of 13, verifying that colostrum-deprived calves are susceptible to colisepticemia. In contrast to the colostrum-deprived calves, *E. coli* was cultured from the liver and spleen of only one of the five colostrum-fed calves, but not from any of the heart blood samples from this group.

Colostrum fed soon after birth provides the newborn calf with protection from colisepticemia, but does not prevent the diarrhea of colibacillosis. Gut closure alone does not protect the newborn calf from colisepticemia.

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Dedicated to my dear wife,

Joy,

and to our little helper,

Miriam.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Many experiments have been conducted during the past 80 years to determine how disease resistance is transferred to the newborn calf. Enteritis appears to be the major cause of neonatal deaths. White scours, or colibacillosis, is often acute and fatal, occurring in young calves up to three weeks of age. The acute form usually terminates as a septicemia (colisepticemia). Because of this, the majority of these experiments have been investigations of the role of *Escherichia coli* in diarrhea and septicemia.

Calves which receive colostrum in the first few hours of life are much more resistant to colisepticemia than those which are not fed colostrum. The maternal antibodies present in the colostrum are absorbed unchanged across the gut wall of the calf into the lymphatics and from there to the blood. The mechanism of absorption is pinocytosis ("cell drinking") in which the epithelial cells of the small intestine engulf and absorb macromolecules in a nonspecific manner. Colostral antibody proteins, foreign proteins and inert macromolecules appear

to be absorbed equally well. Cessation of absorption, or gut closure, occurs no later than 24 to 36 hours after birth, and in some calves much earlier than this.

Colisepticemia occurs mainly in very young calves and especially in those with low levels of serum antibodies. The newborn calf is particularly susceptible prior to colostrum ingestion. This is due mainly to the low levels of serum antibodies, the high levels of serum corticosteroids, and perhaps also to the nonspecific absorption mechanism of the gut. It may be that bacteria are engulfed and absorbed by the intestinal epithelial cells together with other macromolecules. From there bacteria may pass into the blood stream via the lymphatics.

Colostrum protects the newborn calf against colisepticemia by providing serum antibodies to prevent or minimize any invasion of organisms into the body. Additionally colostrum may give mechanical protection to the calf by inducing gut closure and thereby preventing bacteria direct entry into the blood.

The objective of this experiment was to examine the protective mechanisms of a colostrum diet for the newborn calf when challenged orally with a septicemia-producing *E. coli*.

CHAPTER II

REVIEW OF THE LITERATURE

Neonatal diarrhea has been the important cause of calf loss for many years. The economic loss of dead calves is obvious. Added to this are medication costs, veterinary fees, farm labor, and the unthriftiness of animals which survive.

Jensen (1893) was the first to show that the bacterium *Escherichia coli* was associated with neonatal diarrhea or "white scour." Many surveys and reviews have been published since then (Withers, 1952, 1953; Lovell, 1955; Dunne *et al.*, 1965; Gay, 1965; Sojka, 1965; Fey, 1972; Oxender *et al.*, 1973). These authors reported that white scours, or colibacillosis, was the main cause of sickness and death in young calves. In England it has been reported in two recent surveys (1968 and 1971) that colibacillosis was the cause of death of nearly half of the 100,000-200,000 calves under one month old which die each year (Logan, Stenhouse, Ormrod, Penhale, and Armishaw, 1974).

However, white scours was not necessarily due to *E. coli* (Fey, 1962). Acute septicemias caused by *Streptococcus*, *Diplococcus*, *Pasteurella*, and *Salmonella* species

may clinically resemble colisepticemia (Gay, 1965). Viruses may also cause enteric disease, and they need to be considered in a differential diagnosis (Amstutz, 1965). The viral enteric pathogens reported in the literature include enterovirus and rhinovirus (Mayr *et al.*, 1964), infectious bovine rhinotracheitis and bovine viral diarrhea viruses (Lambert and Fernelius, 1968; Steck *et al.*, 1971; Lambert *et al.*, 1974), adenovirus and parainfluenza 3 virus (Mayr *et al.*, 1964; Steck *et al.*, 1971), a reovirus-like agent (Mayr *et al.*, 1964; White *et al.*, 1970; Mebus *et al.*, 1971, 1973), and a coronavirus-like agent (Mebus *et al.*, 1973).

Fey, writing in 1972, regarded viruses as being of minimal importance in diarrhea in the very young calf. However, earlier it was postulated that viruses may act synergistically with *E. coli* to cause colibacillosis (Amstutz, 1965). Recent work with a reovirus-like agent and a coronavirus-like agent by Mebus *et al.* (1971, 1973) confirms that viruses can be important etiologic agents in calf diarrhea.

Escherichia coli varies in its effect on the newborn calf. Gay (1965) divided the syndromes into three groups according to their clinical signs, bacteriology, and possible pathogenesis. These groups are: (1) septicemia or colisepticemia, (2) enteritis-toxemia, and (3) enteritis, colienteritis or white scours. Colisepticemia is an acute

bacteremia with anorexia, dehydration, prostration and death. Diarrhea may or may not be present (Fey, 1972). In the enteritis-toxemia form of colibacillosis the *E. coli* organisms multiply rapidly in the intestine. No diarrhea or bacteremia is present. The calf collapses suddenly and dies within hours of clinical onset, probably from a toxemia (Gay, 1965). A calf with the enteric form of colibacillosis suffers from mild to severe diarrhea, anorexia, and dehydration. The feces vary from firm to liquid in consistency, are grey-white in color, and are distinctively malodorous. After several days calves may become comatose, with subnormal temperatures, and die. Those that recover often remain unthrifty (Sojka, 1965).

As early as 1892 Ehrlich had shown in mice that immunity could be passed from the mother to the young via the milk. Famulener (1912) reviewed this work, and that by other researchers. He also reported on his experiments with goats that the young animal acquired its passive immunity from the antibodies contained in colostrum. He postulated that these colostral antibodies came from the maternal serum. Additionally when the newborn ingested colostrum, the antibodies were absorbed unchanged across the gut wall, but this absorption occurred only in the first few days of life.

In the 1920s a series of experiments were carried out by Smith and his co-workers (Howe, 1921, 1922, 1924; Little

and Orcutt, 1922; Orcutt and Howe, 1922; Smith and Little, 1922a,b, 1923; Smith, 1930). They showed that calves do not receive any immunity across the placenta and so are born without detectable globulins or agglutinins in their serum. They also showed that the calf receives its immunity from the colostrum which contains antibodies derived from the maternal serum. The calf, too, must ingest the colostrum soon after birth to be able to absorb these antibodies. These observations have since been verified by other authors (Smith and Holm, 1948; Jones, 1967). Later work has shown that traces of antibodies (immunoglobulins) are present in the serum of calves prior to their ingestion of colostrum (Pierce, 1955; Kniazeff *et al.*, 1969; Klaus *et al.*, 1969; Merriman, 1971). However, these trace amounts are too low to provide the calf with any degree of immunity.

Ingestion of colostrum is extremely important for the survival of the newborn calf. Smith and Little (1922a, 1923) and Smith and Orcutt (1925) showed a direct association between the early ingestion of colostrum by the newborn calf and its resistance to *E. coli* infection. Most calves deprived of colostrum died within the first week, and usually from colisepticemia. Smith and Little (1922b) found that feeding serum from normal lactating cows to newborn calves also provided protection against *E. coli* septicemia.

Aschaffenburg and co-workers in 1949 demonstrated that the active component of colostrum was found in the whey

fraction. Calves needed colostral whey, or the immunoglobulins contained in it, to survive. Newborn calves given just 80 ml of whey survived, whereas 5 out of 6 calves not receiving colostrum died. Scouring was frequent and severe when calves received only low amounts of whey. Logan, Stenhouse, Ormrod, Penhale, and Armishaw (1974) found that, even under adverse conditions, 1500 ml of pooled colostral whey fed at birth would protect 80% of calves from death from colibacillosis.

It has been found very difficult to kill colostrum-fed calves with either experimental or natural infection with virulent strains of *E. coli* (McEwan, 1950; Glantz *et al.*, 1959, 1966; Smith, 1962; Gay *et al.*, 1964). Dam (1967) was able to kill colostrum-fed calves with 3 to 4 cc of a 20-hour-old broth culture of strain K667 (O-group 78). The inoculum was injected intraperitoneally in the umbilical region at 24 to 48 hours postpartum. The calves died one to nine days later with septicemia. Colostrum-deprived calves inoculated with 0.1 cc of the broth culture died within 12 hours.

The protection colostrum and cow serum provide against the diarrhea associated with colibacillosis was not as marked as that provided against septicemia (Smith and Little, 1922a,b). This fact has also been reported by Gay *et al.* (1964), Fey (1971), Gay (1971), Logan and Penhale (1971a), and Logan, Stenhouse, Ormrod, Penhale,

and Armishaw (1974). Bovine colostrum contains high levels of immunoglobulins (IgG, IgM, and IgA) which fall rapidly during the first three days of lactation. These immunoglobulins are analogous to human IgG, IgM, and IgA in their physicochemical and antigenic characteristics (Porter, 1973). Logan and Penhale (1971b) suggested that the immunity provided by colostrum was of a complex nature involving two separate systems: (1) systemic - this was mediated largely by IgM and acted in preventing septicemia, and (2) local - this acted within the lumen of the small intestine and inhibited enteric disease. This local immunity provided by colostrum has been reported to be present in enteric diseases of other species, but does not appear to occur to any great extent in colibacillosis of calves (Logan and Penhale, 1971b). This may be related to the low levels of IgA in bovine colostrum and milk compared to the higher levels in other animals and the human (Butler, 1973). IgA in the pig and the human, for example, has a definite protective effect in the lumen of the intestine (Butler, 1973), whereas in calves IgA was much less effective than IgG or IgM (Logan, Stenhouse, Ormrod, and Penhale, 1974). In fact, in their experiment, the diarrhea seen in the calves fed IgA was similar to that of the control calves. Diarrhea was present to a lesser degree in the calves given IgG and IgM orally, but it did not occur in the calves fed colostrum. It thus appears that all three immunoglobulins (IgG, IgM,

and IgA) are necessary to provide protection against diarrhea in the calf, and they may act in synergism (Logan, Stenhouse, Ormrod, and Penhale, 1974).

A number of workers have looked at the levels of serum immunoglobulins and their effect in preventing colibacillosis (Gay *et al.*, 1965; Penhale, 1965; McEwan *et al.*, 1970; Penhale *et al.*, 1970; Selman, de la Fuente, Fisher, and McEwan, 1971; Irwin, 1974). Calves which survived were found to have high serum levels of immunoglobulins while those which died had low levels (Gay *et al.*, 1965; McEwan *et al.*, 1970; Penhale *et al.*, 1970; Irwin, 1974). Calves with intermediate levels were susceptible to diarrhea. Some of these recovered, especially when treated, and those that died usually did not have septicemia (Penhale *et al.*, 1970). So for calves to survive, both serum and intestinal immunoglobulins must be present in adequate quantities (Logan, 1974).

The picture becomes more complex when we consider the fact that some calves which have been fed adequate amounts of colostrum in their first 24 hours still have low levels of immunoglobulins. Fey and Margadant (1961) reported that 5 of 46 "normal" calves they examined were hypogammaglobulinemic (10.9%), while 21 of 22 colisepticemic calves (95.5%) were severely hypogammaglobulinemic or even agammaglobulinemic. Smith (1962) found that 6 of 52 calves left with their mothers for the first two days of life were deficient in immunoglobulins.

Other workers have also found that some calves which were supposedly fed colostrum were deficient in serum immunoglobulins (Gay, 1965; Smith *et al.*, 1967; Klaus *et al.*, 1969; Fey, 1971; Logan, McBeath, and Lowman, 1974). Selman, McEwan, and Fisher (1971a,b) found that mothered calves attained significantly higher levels of serum immunoglobulin at 48 hours of age than non-mothered controls. Calves born out-of-doors, and thus allowed to suckle, had significantly higher serum immunoglobulin levels than those born in-doors (Selman, de la Fuente, Fisher, and McEwan, 1971). In-door calving usually meant removal of the calf from the mother and bucket feeding. Suckling usually resulted in higher serum immunoglobulin levels than did bucket feeding (Smith *et al.*, 1967; Selman, de la Fuente, Fisher, and McEwan, 1971). Fey (1971) reported that 175 of 191 calves with colisepticemia (91.6%) were hypo- or agammaglobulinemic, even though they all received colostrum on their first day of life. However, no details of amounts or times of feeding were given. Selman *et al.* (1970) found that calves which did not suckle within 8 hours of birth were not able to absorb adequate amounts of immunoglobulins. Later, Selman, de la Fuente, Fisher, and McEwan (1971) decreased this critical time for first ingestion of colostrum to 6 hours. Even so, Gay *et al.* (1965) found that some calves had lost the capacity to absorb colostral globulins by 4 to 6 hours

after birth. Selman, McEwan, and Fisher (1971b) examined 50 calves in an experiment and found that all these calves absorbed adequate amounts of colostral immunoglobulins. These calves were personally fed, indicating that some cases of agammaglobulinemia may be from calves not nursing or failing to drink colostrum.

Absorption of molecules by the small intestine of the newborn calf occurs by pinocytosis. This primitive mechanism ("cell drinking") is accomplished by the folding and interiorizing of the surface membrane of the cell (Lecce, 1966b; El-Nageh, 1967a). In the calf this appears to be a rather non-specific mechanism. A number of macromolecular substances such as bovine serum proteins, egg white proteins, insulin, dextran, and polyvinyl pyrrolidone have been shown to be absorbed unchanged into the serum of the newborn calf (Deutsch and Smith, 1957; Balfour and Comline, 1959b; Pierce, 1961; Pierce *et al.*, 1964; Lecce, 1966a; Hardy, 1969). Smaller molecules are readily absorbed also, but are either broken down or excreted by the kidneys (Deutsch and Smith, 1957; Pierce *et al.*, 1964). The absorption of colostral immunoglobulins has been regarded as non-selective, with the immunoglobulin profile of post-colostral calf serum resembling that of the colostrum (Pierce and Feinstein, 1965; Klaus *et al.*, 1969; Porter, 1971). However, work done by Hammer *et al.* (1968) has shown that IgG is absorbed more efficiently than IgM.

These findings were supported by those of Penhale *et al.* (1973).

The actual absorption of the macromolecules by the intestinal epithelial cells starts as an invagination of the plasma membrane which then forms vesicles and vacuoles in the brush border region. These vacuoles move to a large supranuclear vacuole. From there the macromolecules pass into the Golgi complex and thence through the cytoplasm to the intercellular space. They then penetrate the basal membrane and enter the lymph vessels. This mechanism was described by Kraehenbuhl *et al.* (1967) using ferritin absorption in the newborn rat as a model. These authors used electron micrographs to show in detail this process. Kraehenbuhl and Campiche (1969) carried out further work on the pig with similar results. The vacuoles shown so clearly in the electron micrographs represent the protein-containing vacuoles first postulated by Smith (1925) in calves from birth to 3 days of age. Comline *et al.* (1951a,b) explained the mechanism of absorption in the calf, and the significance of these vacuoles. They showed that the absorbed material was passed almost completely into the lymph with very little entering the portal venous system. Immunoglobulins could be detected in the thoracic duct lymph within 80-120 minutes of being introduced into the duodenum, and between 12 and 25% could be recovered from the lymph within 300 minutes (Balfour and Comline, 1962).

Colostrum has been found to contain substances which accelerate the absorption of globulins and other macromolecules from the small intestine of the calf (Balfour and Comline, 1959a, 1962; Hardy, 1969). Balfour and Comline (1962) found that fresh colostral whey was best in promoting rapid absorption of globulin. Hardy (1969) reported that sodium lactate and sodium pyruvate were similar to colostral whey in their facilitation of absorption of IgG and polyvinyl pyrollidine (PVP), an inert macromolecule with a molecular weight of 160,000. Potassium isobutyrate was the most effective of the compounds tested, but these substances are not found in colostral whey at the levels used in this experiment. McEwan *et al.* (1970) found there was a tendency for the amount of globulin absorbed to increase with the amount of colostrum fed. This also suggests that factors are present in colostrum which promote the absorption of immunoglobulins.

Famulener (1912) and Smith and his co-workers in the 1920s reported that the absorption of colostral antibodies into the blood stream was only possible in the first few days of life. Since then the recommended time for first ingestion of colostrum by the calf has been shortened to within 15 minutes of birth (Reisinger, 1965).

A number of factors appear to be involved in this shut-down of the absorption mechanism (gut closure), but the actual process of gut closure is not fully understood

(Fey, 1972). Hill (1956) proposed the theory that the development of gastric activity over the first few days of life resulted in destruction of the immunoglobulins by proteolysis. However, work by Smith and Erwin (1959) showed that gastrointestinal enzyme development was not the primary reason for gut closure. Fey (1971) further examined the effect of pepsin on the immunoglobulin molecule. He found that absorption of the pepsinized immunoglobulins still occurred in the newborn calf. So maturation of the gastric enzymes does not appear to affect time of gut closure.

Payne and Marsh (1962a,b) regarded gut closure as an "all-or-none" phenomenon. They reported that their work in piglets had shown that, once an intestinal epithelial cell had been exposed to protein and absorbed it, further absorption by that cell ceased.

Another theory states that absorption ceases when all the pinocytotic activity of the intestinal cell membrane is "used up" (Lecce, 1966b, 1973; Broughton and Lecce, 1970; Rundell and Lecce, 1972). This gut closure would be expected to start in the duodenum and end with the ileum. Accordingly, this has been reported in pigs (Lecce, 1966a, 1973). Micromolecules as well as macromolecules can cause gut closure. Lecce (1966a) was able to cause gut closure in piglets with 300 mEq of glucose, showing that it could occur independently of colostrum or protein. Time does

not seem to be an important factor, because starved piglets were able to absorb PVP even at 86 hours of age (Lecce and Morgan, 1962) or gammaglobulin at 106 hours of age (Payne and Marsh, 1962a).

Another contribution to our understanding of the process of gut closure has been made by El-Nageh (1967b). Using fluorescent-labelled colostral globulins he observed that the protein was absorbed by all the epithelial cells in the villi of calves 6 hours old. In calves about 53 hours old the fluorescence (demonstrating the absorption of the globulins) was limited to the apical end of the villus. El-Nageh related this to the normal cellular renewal of the epithelium. Cells are continually lost from the apex of the villus and replaced by cells which migrate from the base to the apex. Thus, in the older calf, the only "original" epithelial cells left were on the tips of the villi, the rest having been extruded into the lumen and digested. El-Nageh quoted authors who have found that the intestinal epithelium of the newborn calf was totally renewed by 1.6 to 2 days of age. However, Sunshine *et al.* (1971) stated that in the suckling rat, and probably in other newborn animals, this turnover rate of intestinal epithelium was much slower than the 1.6 to 2 days that El-Nageh quoted. Rundell and Lecce (1972) found that cessation of absorption was not a direct consequence of the turnover of the intestinal epithelial

cell population, at least in the mouse, rabbit, guinea pig, and hamster.

Moog (1955, 1962) and Halliday (1959) have shown in the mouse and rat that the levels of intestinal alkaline phosphatase increase to a peak at the time of gut closure. Daniels *et al.* (1972) found a two-fold increase in endogenous corticosteroid levels at this time. Injections of large doses of cortisone acetate or deoxycorticosterone acetate into a mouse or rat caused gut closure up to 9 days earlier than usual (Clark, 1959; Halliday, 1959; Moog, 1962). Gut closure normally occurs between 14 and 17 days of age in the mouse, and at 18 to 20 days in the rat. Payne and Marsh (1962b) found that the response of the piglet was similar to that of the mouse and rat. Gut closure occurred earlier in starved piglets injected with cortisone acetate. Bilateral adrenalectomy of the 18-day-old rat caused a delay in gut closure by up to four days (Daniels and Hardy, 1972). This closure was not permanently delayed by adrenalectomy, indicating that some other factor, or factors, are involved in causing gut closure in addition to corticosteroids.

Previously, in 1957, Deutsch and Smith had reported on their investigations of the permeability of the calf and goat intestine. Calves were injected with diethylstilbestrol, progesterone, cortisone, or adrenocorticotrophic hormone, with no noticeable change in the time of

gut closure. They concluded that, in the herbivore, hormonal factors did not appear to be responsible for the cessation of absorption by the small intestine.

Clarke and Hardy (1971a,b) suggested that gut closure occurred in two stages. Cells take up macromolecules from the intestinal lumen and then expel them through their lateral or basal surfaces into the lacteal circulation. At some time a change occurs in the characteristics of these cells. They lose their ability to release macromolecules into the circulation, but can still absorb them from the intestinal lumen. The second stage of closure occurs with progressive loss of the ability to take up macromolecules. This is seen in the terminal ileum and can take up to 16 days, as shown in the newborn piglet and goat.

Lecce (1973) agreed with the findings of Clarke and Hardy (1971a) in the piglet, and found that the time of uptake by the ileal area was about three weeks. Both phases of absorption (uptake by the cells and transport into the circulation) were found to be affected by the volume and the components of the diet.

The increased susceptibility of the newborn animal to septicemia may be due to the macromolecular transport system, because bacteria may be taken up into the intestinal cells and transported into the circulation by this non-specific absorption process (Lecce and Morgan, 1962;

Lecce, 1973; Coalson and Lecce, 1973). So perhaps the ingestion of colostrum not only protects the newborn animal by providing local and systemic immunoglobulins, but also by inducing rapid gut closure to prevent the absorption of intestinal bacteria.

CHAPTER III

MATERIALS AND METHODS

Male Holstein calves were obtained soon after birth from a large commercial dairy farm 20 miles from Michigan State University. These calves were removed from their dams at birth before suckling, and received their first experimental feed within three to four hours. They were housed in individual pens inside a heated barn.

Measurements

Blood samples were taken from the jugular vein of the calves when they arrived, and then before each feeding, which was approximately 10 a.m. and 10 p.m. each day. A sample of fresh blood was drawn into a capillary tube[a] to determine packed cell volume and plasma protein concentration. The packed cell volume was measured using a micro-capillary centrifuge and reader.[b] A Goldberg refractometer[c]

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[a]Red-Tip Heparinized Capillary Tubes, Sherwood Medical Industries, St. Louis, Mo. 68108.

[b]International Micro-Capillary Centrifuge, Model MB, and International Micro-Capillary Reader. International Equipment Company, Boston, Mass.

[c]T. S. Meter (total solids), American Optical Co., Buffalo, New York 14215.

was then used to measure the total protein of the plasma in the capillary tube. The remaining blood sample was allowed to clot and the serum removed and frozen at -20 C. The serum IgG levels in the first four blood samples (0-36 hours) were measured by radial immunodiffusion.[a] Rectal temperatures were measured prior to obtaining each blood sample.

Experimental Diets

The calves were allocated to the four experimental groups in the order of their arrival, with five calves in each group. Group I calves were fed a diet of pooled colostrum. Colostrum from the first two milkings of several Holstein cows was pooled and frozen as first and second milkings. Colostrum-fed calves received first-milking colostrum for their first three feeds, and second-milking colostrum thereafter. A commercial milk replacer[b] was used for the second group of calves. The third group of calves were fed a 4% solution of polyvinylpyrrolidinone

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[a]Quantitative Kit for IgG 64-472-1. Miles Laboratories, Inc., Elkhart, Ind. 46514.

[b]MMPA Premium Calf Milk Replacer, Michigan Milk Producers Association, Detroit, Mich.

K.60 (PVP)[a] in a 56.7 mM solution of sodium lactate[b] to aid its uptake by the intestinal epithelial cells (Hardy, 1969). Normal saline[c] was the fourth experimental feed. Two liters of the experimental feed was given on arrival and then each morning and evening. If the calf was unwilling to drink, or too weak to do so, the feed was given via stomach tube. Group three and four calves received 80 ml of a 50% dextrose solution[d] given intravenously, or in a few calves intraperitoneally, at each feeding, to provide energy.

Inoculation

At about 27 hours[e] of age the calves were inoculated orally with *E. coli* serotype 026:K60:NM.[f] The *E. coli*

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[a]Polyvinylpyrrolidinone K.60 (45% Solution). Average Molecular Weight 160,000. Matheson Coleman and Bell, Manufacturing Chemists, Norwood (Cincinnati), Ohio 45212.

[b]Sodium Lactate Syrup, 60% FW112.0. Matheson Coleman and Bell, Manufacturing Chemists, Norwood, Ohio 45212.

[c]Sodium Chloride Crystals, Analytical Reagent. Mallinckrodt Chemical Works, St. Louis, Mo. 63160.

[d]Dextrose Solution, 50%. Diamond Laboratories, Inc., Des Moines, Iowa 50304.

[e]Calves 1, 3 and 4 were inoculated at 48, 33 and 30 hours of age, respectively.

[f]Supplied by Dr. Gordon R. Carter, Michigan State University, East Lansing, Michigan 48824. The initial culture was obtained from Dr. Paul J. Glantz, Pennsylvania State University, University Park, Pa.

was grown in trypticase soy broth at 37 C for 24 hours, then centrifuged and washed once with normal saline. The *E. coli* organisms were resuspended in saline and then viable count was determined. The inoculum was made up to contain 1.5×10^{10} viable organisms.

Gut Closure Determination

The first seven calves were given 100 ml of fresh chicken egg albumin orally when 24 to 27 hours old to test for gut closure. The remaining 13 calves were given the egg albumin at about 12 hours of age. Their sera were tested for the presence of chicken egg albumin using an Ouchterlony agar gel diffusion system,[a] with rabbit anti-chicken egg albumin anti-serum[b] in the center well.

Pathology and Microbiology

The calves were necropsied as soon as possible after death. Some calves died during the night, so several hours had elapsed between death and necropsy. Nine calves were killed either because they were comatose, or because fresh tissues were required for microbiological and histological examination. Calves were usually killed with an electric

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[a]Agarose: agar for Immunoelectrophoresis. Fisher Scientific Co., 15800 W. McNichols Rd., Detroit, Mich. 48235.

[b]Rabbit Antiserum to Chicken Egg Albumin, 64-115-2. Miles Research Division, Miles Laboratories, Inc., Elkhart, Ind. 46514.

shock (110 volts); however, a few were killed with an intravenous injection of succinylcholine.[a]

Each calf was examined grossly at necropsy. Specimens of liver, spleen, heart blood, duodenum, and ileum were taken aseptically for routine bacteriological examination. In seven calves specimens of coiled colon were frozen and tested for the presence of reovirus and coronavirus using the fluorescent antibody technique.[b] Sections of liver, spleen, lung, kidney, adrenal, duodenum, jejunum, ileum, coiled colon, and mesenteric lymph nodes were taken for histological examination. These were fixed routinely in 10% buffered neutral formalin, paraffin embedded, and cut at 6 μ. The sections were stained with hematoxylin and eosin.

Seven isolates of a non-hemolytic *E. coli* from the liver, spleen, or heart blood, and 15 from the small intestine of these calves were tested for agglutination with specific antiserum[c] to serotype 026:K60:NM, using a simple slide agglutination test.

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[a]"Sucostrin" Succinylcholine Chloride Injection U.S.P. E. R. Squibb and Sons, Inc., New York, N.Y. 10022.

[b]Modified Technique of that described in "Laboratory Methods for Detecting Calf Diarrhea Viruses," 1973. Norden Laboratories, Lincoln, Neb. 68501.

[c]Antiserum to *E. coli* serotype 0:26:K60:NM obtained from Dr. Paul J. Glantz, Pennsylvania State University, University Park, Pa.

CHAPTER IV

RESULTS

A total of 25 calves started in the experiment, but five were excluded because of sickness or death prior to inoculation. These included one calf that died with pneumonia (#2), a second that died with bloat (#6), and a third calf that died with diarrhea and septicemia (#17). The two other calves (#15 and #19) were showing signs of diarrhea prior to inoculation and so were not included in the experiment (see Table 10). The four calves which were cultured were septicemic, even though two were being fed colostrum (#15 and #17).

The calves were inoculated orally with the *E. coli* organisms at about 27 hours of age, except for #1 (colostrum) at 48 hours, #3 (milk replacer) at 33 hours, and #4 (PVP) at 30 hours. Following inoculation rectal temperatures usually had increased at the next measurement 12 hours later, and from this point declined before death (Table 1). Three of the calves died during the night less than 10 hours after inoculation, and before their next examination. The increases in rectal temperature at the next measurement following inoculation are shown in

Table 1. Rectal temperature (in degrees Fahrenheit)

	Sampling times from arrival (0 hours)					
	0	12	24	36	48	60
COLOSTRUM						
#1	100.0	100.5	98.0[1]	98.8[1]	98.2*	99.0[1,2]
#7	100.8	101.4	102.8*	103.0	102.8	killed
#11	102.0	101.2	102.6*	102.6	100.8	died
#21	102.4	101.0	101.8*	103.4	killed	
#25	101.0	101.2	100.8*	102.0	killed	
MILK REPLACER						
#3	100.2	99.0[1]	99.8[1]	*99.8[1]	96.0[1]	died
#8	101.6	102.0	100.8*	102.8	died	
#12	101.0	100.8	102.2*	died		
#16	99.0	102.0	101.6*	died		
#22	102.2	99.4	101.2*	died		
PVP						
#4	100.8	101.4	101.6	*103.0	101.0	died
#9	101.2	100.8	101.6*	100.2	101.2	killed
#13	100.4	102.2	101.4*	102.3	103.4	killed
#18	102.4	101.2	101.0*	102.2	killed	
#23	101.4	100.4	102.8*	103.8	killed	
SALINE						
#5	102.0	101.8	102.0*	103.6	died	
#10	100.4	101.0	101.2*	104.2	died	
#14	100.8	101.4	102.0*	103.0	died	
#20	100.8	101.2	100.0*	101.6	94.0	died
#24	100.8	100.8	102.0*	103.0	killed	

*This indicates the time of oral inoculation with 1.5×10^{10} viable organisms of *E. coli* serotype 026:K60:NM.

[1]These low temperatures are very likely due to a faulty thermometer.

[2]Killed 48 hours after inoculation.

Table 2. The normal diurnal variation in rectal temperature of the calf was confounded with the changes in rectal temperature due to the infection.

Table 2. Rectal temperature change (in degrees Fahrenheit) 12 hours after inoculation

COLOSTRUM	MILK REPLACER	PVP	SALINE
+0.8	0	+1.4	+1.6
+0.2	+2.0	-1.4**	+3.0
0	*	+0.9	+1.0
+1.6	*	+1.2	+1.6
+1.2	*	+1.0	+1.0
Mean +0.7	+1.0	+1.1*** +0.6**	+1.6

*Died before next temperature measurement.

**This drop in temperature affects the mean of the PVP group.

***Mean of PVP group excluding the -1.4 value.

After inoculation the packed cell volume increased in 15 of the calves, decreased in two (both fed colostrum), and was not able to be measured in three (Table 3). Post-inoculation changes in packed cell volume are given in Table 4.

Table 3. Packed cell volume

Calf	Sampling times from arrival (0 hours)					
	0	12	24	36	48	60
COLOSTRUM						
#1	44	38.5	41	42	45*	45[1]
#7	58	52	59*	54	51	killed
#11	50	50	52*	66	74	died
#21	39	30	32*	45	killed	
#25	30	28	27*	30	killed	
MILK REPLACER						
#3	39	39.5	43	*51	50	died
#8	41	40	47*	50	died	
#12	36	37	37*	died		
#16	32	32	36*	died		
#22	34	34	44*	died		
PVP						
#4	20	18	17	*20	21	died
#9	40	45	47*	51	-	killed
#13	33	32	30*	32	45	killed
#18	28	28	28*	34	killed	
#23	37	32	33*	35	killed	
SALINE						
#5	40	41	42*	50	died	
#10	57	54	57*	60	died	
#14	35	32	33*	37	died	
#20	40	37	36*	47	51	died
#24	31	31	30*	33	killed	

*This indicates the time of oral inoculation with 1.5×10^{10} viable organisms of *E.coli* serotype 026:K60:NM.

[1]Killed 48 hours after inoculation.

Table 4. Packed cell volume change 12 hours after inoculation

COLOSTRUM	MILK REPLACER	PVP	SALINE
0	+8	+3	+8
-5	+3	+4	+3
+14	*	+2	+7
+13	*	+6	+11
+3	*	+2	+3
+5.0	+5.5	+3.4	+6.4

*Died before next packed cell volume measurement.

The mean 12-hour and 24-hour levels of total plasma protein (pre-inoculation) were significantly higher ($P<0.01$) in the calves on the colostrum diet, probably due to absorption of immunoglobulins (Table 5). In addition, the total

Table 5. Mean total plasma protein from arrival (0 hours) in g/100 ml

Mean value (5 calves)	0	12	24 hours
Colostrum	4.70	5.54	6.24
Milk replacer	4.74	4.38	4.98
PVP	4.54	4.46	4.58
Saline	4.52	4.28	4.36

Table 6. Total plasma protein in g/100 ml

Calf	Sampling time from arrival (0 hours)					
	0	12	24	36	48	60
COLOSTRUM						
#1	4.7	5.5	6.0	7.0	7.3*	7.4[1]
#7	5.0	5.5	6.5*	6.6	6.5	killed
#11	4.8	6.0	6.4*	8.2	9.7	died
#21	4.5	6.0	6.8*	9.0	killed	
#25	4.5	4.7	5.5*	6.5	killed	
MILK REPLACER						
#3	5.2	4.6	5.2	*7.0	7.0	died
#8	4.8	4.6	5.7*	6.0	died	
#12	4.4	4.5	4.5*	died		
#16	4.3	4.0	5.0*	died		
#22	5.0	4.2	4.5*	died		
PVP						
#4	3.8	4.5	4.4	*4.3	4.5	died
#9	4.6	4.9	4.7*	5.1	-	killed
#13	4.8	4.2	4.5*	4.8	6.5	killed
#18	4.5	4.0	4.5*	4.8	killed	
#23	5.0	4.7	4.8*	5.0	killed	
SALINE						
#5	4.0	3.9	4.0*	4.5	died	
#10	4.8	4.2	4.5*	4.7	died	
#14	4.6	4.2	4.5*	5.1	died	
#20	4.8	4.6	4.3*	5.5	5.5	died
#24	4.4	4.5	4.5*	4.9	killed	

*This indicates the time of oral inoculation with 1.5×10^{10} viable organisms of *E. coli* serotype 026:K60:NM.

[1]Killed 48 hours after inoculation.

plasma protein levels increased in every calf after inoculation possibly because of dehydration associated with the diarrhea (Table 6).

One of the seven calves given chicken egg albumin at about 24 hours of age was still able to absorb some of these protein molecules, as determined by the agar gel diffusion test. This calf (#3) was fed milk replacer (Table 7). Only one of the 13 calves fed egg albumin at about 12 hours of age did not absorb any detectable amount. This calf (#13) was in the PVP group.

Table 7. Absorption of chicken egg albumin into the serum as an indicator of the presence of intestinal absorption of macromolecules

Egg albumin given	Absorbed	Colostrum	Milk replacer	PVP	Saline	Total
24-27 hours old	+	0	1*	0	0	1
	-	2	1	2	1	6
		2	2	2	1	7
12 hours old	+	3	3	2	4	12
	-	0	0	1**	0	1
		3	3	3	4	13

*Calf #3

**Calf #13

Serum IgG levels increased in the five colostrum-fed calves for at least 36 hours after their first feed

(Table 8). These levels were not measured beyond 36 hours. This significant increase in serum IgG levels was not seen in the other groups (Table 9). Calf #21 (in the colostrum group) and #9 (in the PVP group) had significant levels of IgG in their first blood sample, indicating that these calves probably nursed prior to being removed from the dam.

Table 8. Mean serum IgG levels in colostrum-fed calves (mg/100 ml)

Calf	Sampling times from arrival (0 hours)			
	0	12	24	36 hours
#1	0	1490	1290	2250
#7	0	980	2090	3000
#11	170	2300	3000	3550
#21	1150*	2700	3000	5200
#25	0	1200	3250	3400
Mean	264*	1734	2652	3480

*The high value of #21, probably due to having obtained some colostrum before removal from the dam, unduly increases the initial mean serum IgG level.

The microbiological results are shown in Table 10, together with the approximate time of death after inoculation. A non-hemolytic *E. coli* was cultured from the small intestine of 19 of the 20 calves in the experiment. Of the 15 calves not receiving colostrum, *E. coli* was

Table 9. Levels of immunoglobulin G (mg/100 ml)

Calf	Sampling times from arrival (0 hours)			
	0	12	24	36 hours
COLOSTRUM				
#1	0	1490	1920	2250
#7	0	980	2090	3000
#11	170	2300	3000	3550
#21	1150*	2700	3000	5200
#25	0	1200	3250	3400
MILK REPLACER				
#3	270	270	440	510
#8	0	0	0	0
#12	0	0	0	-
#16	0	0	0	0
#22	140	115	100	-
PVP				
#4	240	120	175	220
#9	1540*	1920	480	980
#13	0	0	0	0
#18	0	0	0	0
#23	0	0	0	0
SALINE				
#5	0	0	0	0
#10	0	0	0	0
#14	0	0	0	0
#20	0	0	0	0
#24	0	0	0	0

*There is a good possibility that these calves suckled before being removed from their mothers.

Table 10. Microbiological cultures taken at necropsy

Calf	*E. coli* cultured from: Liver	Spleen	Heart blood	Small intestine	Time of death after inoculation
COLOSTRUM					
#1	-	-	-	H	killed 48 hours
#7	-	-	-	L	killed 24 hours
#11	-	-	-	M	died 30 hours
#21	L	L	-	H	killed 20 hours
#25	-	-	-	M	killed 11.5 hours
MILK REPLACER					
#3	H	H	H	H	died 24 hours
#8	L-M	L-M	L-M	M	died 15 hours
#12	M	M	L	H	died 10 hours
#16	H	M	H	H	died 8 hours
#22	L	L	L	H	died 8 hours
PVP					
#4	L	L	M-H	M	died 24 hours
#9	H	H	H	H	killed 8 hours
#13	L	L	-	M	killed 21 hours
#18	H	H	H	H	killed 9 hours
#23	L	L	L	M-H	killed 17 hours
SALINE					
#5	M-H	M-H	M-H	H	died 16 hours
#10	*	*	*	M-H	died 15 hours
#14	H	H	H	**	died 8 hours
#20	H	H	H	H	died 23 hours
#24	L	L	L	M-H	killed 10.5 hours

Table 10 (continued)

Calf	*E. coli* cultured from: Liver	Spleen	Heart blood	Small intestine	Time of death after inoculation
EXCLUDED FROM EXPERIMENT					
#2	not cultured				died with pneumonia
#6	L	L	L	L	died with bloat
#15***	-	-	H	H	diarrhea before 24 hours
#17***	L	L	L	H	died before 24 hours old
#19	H	H	H	H	diarrhea before 24 hours

L, M, H represent light, medium and heavy growth of non-hemolytic *E. coli*, respectively.

*Moderate growth of *Klebsiella* and *Proteus* species.

**Heavy growth of *Proteus*, *Streptococcus*, and *Citrobacter* species.

***These calves were in the colostrum group before being excluded from the experiment.

isolated from the liver and spleen of 14, and from the heart blood of 13. *Escherichia coli* was cultured from the liver and spleen of one of the five colostrum-fed calves, but not from the heart blood. Only one of the five colostrum-fed calves died and that was 30 hours after inoculation, while the other four were killed to obtain fresh specimens. The five calves fed milk replacer died most rapidly after inoculation, three of these calves surviving less than 10 hours after inoculation. Two of the PVP-fed calves were killed because they were convulsing with opisthotonos, bellowing, and front-leg crossing. The third calf was killed in a comatose state, and the fourth in a weak condition. The fifth PVP calf died within 24 hours of inoculation. Four of the saline-fed calves died within 24 hours of inoculation and the fifth calf was killed in a comatose condition.

A frozen section of the coiled colon of seven calves was tested with fluorescent antibody for reovirus and coronavirus (calves 18, 19, 20, 21, 22, 24 and 25). Calf #22 was positive for coronavirus.

The experimental calves which did not receive colostrum showed the typical signs of colisepticemia (Fey, 1972). The five calves in the colostrum group evidenced the diarrhea and some dehydration of the enteric form of colibacillosis (Fey, 1972). They remained bright and alert except for #11, which died 30 hours after inoculation. Nineteen

of the 20 calves had diarrhea within eight to ten hours of inoculation. Calf #20, which became bloated, did not have diarrhea, but at necropsy was found to have atresia of the rectum, and to have died from colisepticemia.

At necropsy, the calves not receiving colostrum showed signs of dehydration with congestion of the small intestine and other tissues. Petechial hemorrhages in various parts of the body suggested septicemia. The colostrum-fed calves were similar except that generalized hemorrhages were not present. No significant histological change was detected between the groups of calves. The only difference seen was the presence of large vacuoles in the intestinal epithelial cells of those calves which had received PVP as a feed. These vacuoles did not stain with hematoxylin and eosin, oil red O, Best's carmine, or periodic acid-Schiff stains.

Six of the seven isolates of non-hemolytic *E. coli* from the liver, spleen, or heart blood were positive to the antiserum of serotype 026:K60:NM. Eight of the 15 isolates of *E. coli* from the small intestine were also positive against the antiserum test.

CHAPTER V

DISCUSSION

Colostrum fed to the group one calves was expected to cause rapid gut closure and provide these calves with high levels of serum immunoglobulins. In contrast, the ability of the milk replacer diet to stimulate the gut closure mechanism was unknown, but certainly no local or systemic immunoglobulins were expected in calves on this diet. PVP K.60 is an inert macromolecule with an average molecular weight of 160,000, which is comparable to the molecular weight of IgG (150,000). Therefore, the PVP diet was used as a feed to simulate IgG in causing rapid gut closure, but providing the calf with no serum immunoglobulins. The PVP diet included a 56.7 mM solution of sodium lactate to promote uptake of the PVP as previously reported by Hardy (1969). The saline-fed calves were expected to have a delay in gut closure since there was supposedly little stimulation of the absorption mechanism by a salt solution (Lecce, 1966a). In addition, these calves would be agammaglobulinemic and therefore highly susceptible to colibacillosis.

As predicted, colostrum provided the five calves in group one with the highest serum IgG levels, averaging 34.8 mg/ml at 36 hours. These values are in agreement with those reported for suckled calves at 24 hours (Logan, McBeath, and Lowman, 1974). In contrast to calves fed colostrum, the calves fed milk replacer had very low or zero levels of IgG. They had no resistance to the *E. coli* and died quickly with colisepticemia. These represented the typical colostrum-deprived calf.

One calf in the PVP group (#9) very likely obtained some colostrum before being separated from his mother because the serum IgG was 15.4 mg/ml on arrival. However, the serum IgG level decreased by 24 hours and this calf was found to have colisepticemia following inoculation with *E. coli*. The other 4 calves on the PVP diet as well as the saline-fed calves did not have any measurable serum IgG with the test employed. Again as with the milk replacer diet, these calves lacked significant serum IgG concentration and were found to have septicemia at necropsy.

The significant increase in total plasma protein of the colostrum-fed calves compared to the colostrum-deprived calves was mainly due to the increase in serum immunoglobulin levels. Although the total protein levels were altered by changes in the packed cell volume, this was not the major cause of the difference in plasma proteins.

Of the calves fed colostrum, two calves (#7 and #11) had high packed cell volumes of 50% and greater indicating

dehydration. The packed cell volume of calf #11 increased to 74% before it died with severe diarrhea and dehydration, 30 hours after inoculation. Although this calf clinically appeared to have severe colibacillosis, cultures of liver, spleen, and heart blood were negative. Colostrum does not protect all calves from colibacillosis if challenge is high (Gay *et al.*, 1965; Dam, 1967). Logan, Stenhouse, Ormrod, Penhale, and Armishaw (1974) reported the loss of two of ten colostrum-fed calves from colibacillosis.

Another calf on the colostrum diet (#21) was killed 20 hours after inoculation, and light growths of *E. coli* were cultured from the liver and spleen, but not from the heart blood. It is quite possible that the level of contamination had built up in the calf pens by the time calf #21 arrived, and it could have been infected with *E. coli* prior to inoculation. Added evidences of calf pen contamination were that calf #17 died from colisepticemia before 24 hours old, and calves #15 and #19 had started scouring before they were 24 hours old. The pens were being cleansed before each new calf was introduced, but evidently not well enough. So there is a strong possibility that calf #21 became infected at birth, during transport, or on arrival, before it could be protected by the colostral antibodies. Logan and Penhale (1971b) found that colostrum is essentially prophylactic in action and has little effect

once diarrhea has started. While IgG levels in calf #21 were 11.5 mg/ml when it arrived and increased to 52 mg/ml after 36 hours, these levels still did not prevent the septicemia. It may be that the blood-borne bacteria in calf #21 were being cleared from the blood by the reticulo-endothelial system and so bacteria were cultured from the liver and spleen but not from the heart blood. Smith and Halls (1968) concluded from their work that the reticulo-endothelial system was the principal defense system against *E. coli* bacteremia, and that this protection was comprehensive when calves had obtained good immunoglobulin levels from the colostrum. Perhaps this calf would have overcome the septicemia and survived had it not been euthanatized. It would be interesting to know the serum IgM level of this calf because Logan and Penhale (1971b) showed that IgM was the principal immunoglobulin in the prevention of septicemia.

There have been suggestions that bacteria may enter the blood system in newborn calves via the macromolecular absorption system that transfers colostral antibodies from the gut to the blood. Therefore, bacterial invasion would be slower if the gut closure were complete. The calves on the saline diet were not expected to have gut closure prior to inoculation. However, most of the calves on all four diets had complete gut closure by 24 hours of age.

Egg albumin given at various times after birth was used to test the calves for gut closure.

Gut closure had not occurred in one calf (#3) on the milk replacer diet by about 24 hours of age. However, the egg albumin given at about 24 hours of age was not detected in the serum until over 24 hours later, in a serum sample taken from the heart blood after the calf died. This may have been a false positive. However, in one of the saline-fed calves (#14) a similar situation occurred. This calf was given the egg albumin at about 12 hours old but evidence of egg albumin in the serum was only seen in the last serum sample which, again, was obtained from the heart blood after death. This too was over 24 hours after being given the egg albumin. So these two cases were accepted as absorption of the egg albumin indicating gut closure was incomplete at the time of feeding egg albumin.

Although gut closure was very likely complete at the time of *E. coli* inoculation in all five calves on the PVP diet, all five had evidence of colisepticemia. Two of these showed central nervous disturbances about eight hours after inoculation indicating possible encephalitis. Calf #13 was negative for *E. coli* in the culture of heart blood and only very light growths of *E. coli* were obtained from the liver and spleen. This calf was euthanatized at 21 hours after inoculation in a depressed and comatose

state. None of the egg albumin given to calf #13 at 12 hours of age was absorbed into its serum, indicating gut closure had already occurred. Thus gut closure did not protect this calf from septicemia or death, although the infectious process may have been delayed. The other four calves probably experienced gut closure somewhere between 12 and 27 hours of age.

Similarly calves on the saline diet died rapidly following *E. coli* inoculation in spite of the fact that gut closure appeared to be complete prior to inoculation. Although one calf had a septicemia due to *Klebsiella* and *Proteus* species, this may have occurred in the terminal stages. In one saline calf no *E. coli* was cultured from the small intestine although it had diarrhea. However, a heavy growth of *Proteus*, *Streptococcus*, and *Citrobacter* species was cultured. Unfortunately, no fluorescent antibody testing for coronavirus or reovirus was done on this calf. But in the one calf positive for coronavirus (#22) a non-hemolytic *E. coli* was cultured from the small intestine as well as from the liver, spleen, and heart blood, indicating the difficulty in controlling the agents causing bacterial and viral enteric infections when studying neonatal diseases.

It is interesting to note that the four calves excluded from the experiment and cultured for bacteria at necropsy all had a colisepticemia although two of

these calves (#15 and #17) were being fed colostrum. If a calf is infected early in life before it is protected systemically, and locally in the intestine, by the colostral immunoglobulins, the infectious process is rarely reversed (Logan and Penhale, 1971a,b).

The vacuoles seen in the intestinal epithelium of the calves fed PVP were due to the absorption of these inert macromolecules. Uptake of PVP by the mucosal epithelial cells has been reported in calves (Hardy, 1969), and positive correlation was found between the ability of the intestinal segment to take up PVP and the presence of vacuolated cells in that segment (Clarke and Hardy, 1971a).

There is a suggestion that the PVP calves would have survived longer than the milk replacer and saline calves had they not been euthanatized. However, each of the PVP calves had colisepticemia. The proof of protection is in the survival of the calf rather than in delaying the time of death.

Gut closure may assist in protecting the newborn calf, but once septicemia occurs the calf has little resistance without serum immunoglobulins. The *E. coli* given to the calf orally may have entered the blood stream through the pharynx, or further along the gastrointestinal tract. In this case any protection provided by gut closure would be of little use.

CHAPTER VI

CONCLUSIONS

1. Colostrum fed early after birth provides the newborn calf with protection against colisepticemia.

2. Colostrum appears to be mainly prophylactic and is not as effective if the calf is infected prior to receiving adequate amounts of colostrum.

3. If the infective dose is high, colostrum does not protect the newborn calf from the diarrhea of colibacillosis.

4. Gut closure does not protect newborn colostrum-deprived calves from colisepticemia.

5. Calves fed with polyvinylpyrrolidinone K.60, milk replacer, and normal saline achieved gut closure by about 24 hours of age indicating age may be more important than diet in determining gut closure.

6. It is very difficult to completely disinfect calf pens contaminated with a pathogenic *E. coli*.

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