

ABSORPTION OF IMMUNOGLOBULINS FROM COLOSTRUM
BY NEWBORN CALVES

By

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CHAPTER I

INTRODUCTION

The etiology of colisepticaemia in calves is very complicated. It is evident that the development of the disease depends upon the pathological type of bacteria, birth hygiene, and malnutrition. Malnutrition includes the wrong feeding of colostrum or no feeding at all (Dam, 1968). It has been well established that the single most important factor in reducing colisepticaemia in newborn calves is the gammaglobulin fraction of colostrum (Penhale et al., 1970).

The serum of the newborn calf contains very little gammaglobulin and the level does not increase appreciably until after ingestion of colostrum. In qualitative and quantitative studies of the nature of colostrum immunity, Logan et al. (1974) demonstrated that both circulating and intestinal immunoglobulins are necessary to protect the calf from colibacillosis. Systemically administered immunoglobulin protects the calf from septicaemia but affords no intestinal immunity. Therefore, the answer must lie in getting more immunoglobulin into the gut and facilitating its transfer into the bloodstream.

Adequate levels of immunoglobulin in the calf are obtained by an adequate amount of colostrum reaching the gut, but the calf must have the ability to absorb the immunoglobulin efficiently and quickly. Also, man must not act so that the transfer is blocked or reduced (Klaus et al., 1969).

This experiment was conducted to evaluate the effects on absorption of adding potassium isobutyrate to colostrum, hopefully, determining a means by which absorption of gammaglobulin from colostrum would be accelerated and enhanced. This, in turn, might decrease incidence or severity of newborn calf diseases which cost farmers, ranchers, and dairymen millions of dollars annually.

CHAPTER II

LITERATURE REVIEW

Diarrhea

Reisinger (1965) described infectious diarrhea or scours of newborn calves as characterized by a white or yellowish diarrhea which has a rapid onset and a high mortality rate. Potential causes of scours included calves receiving no placental transfer of antibody, calves exposed to coliform bacteria and/or viruses during passage through the birth canal, and poor management of calves after birth. Organisms which were found to infect the calf before or after birth began to multiply in the logarithmic phase immediately. During the first several hours, the calf's intestines were highly absorptive to bacteria as well as globulins. The γ -globulin fraction in colostrum is the most critical factor in prevention of infectious diarrhea.

For the calendar year of 1973 the United States Department of Agriculture estimated 200,220,000 calvings in the United States and estimated a calving loss of 4.4 million. The Oklahoma calving statistics were also impressive with 49,034,000 calves and a 1973 death loss of 195,000. At a calf cost estimate of \$30 per head, the 1973 United States and Oklahoma death loss would be \$130,000,000 and \$5,850,000, respectively. These statistics are conservative because many losses were not reported (Pittman, personal communication). Larouche and Black

(1973) reported neonatal calf diarrhea as the most frequently observed disease in calves less than 2 months of age in Ontario. Estimates of death losses were found to be as high as 25%. Great as these losses appear to be, it is likely that there was an even greater loss due to permanently stunted animals due to post-natal diseases.

Colostrum

Colostrum by definition is a "thin, white, opalescent fluid, the first milk secreted at the termination of pregnancy; it differs from the milk secreted later in containing more lactalbumin and lactoprotein" (Stedman's Medical Dictionary, 1972). The high mortality of dairy calves during the first 48 hours of life has intrigued researchers for years. Smith and Little (1922) in some of the earlier work used 22 calves to determine the significance of feeding colostrum. Ten calves were fed colostrum, and 12 calves were fed raw cow's milk. All 10 colostrum fed calves survived; 8 of the 12 calves fed raw milk died, 2 had chronic sepsis, and the remaining 2 were normal. In doing later work with Brucella abortus, Smith and Little (1923) found colostrum much more efficient in transporting antibodies to the newborn calf than acquisition of antibodies via ingestion of cow serum. They concluded colostrum necessary for normal health and essential for resistance to early bacterial infections.

Howe (1924) found, by analyzing blood and urine of calves following ingestion of colostrum, that there was a positive appearance of globulins and an absence of these proteins when newborn calves were only fed raw milk. He inferred the proteins found in the blood and urine of the calves was directly from colostrum and, for the most part, unaltered.

Aschaffenburg et al. (1953) tested 264 calves to determine if the survival rate was higher in calves given colostrum versus those deprived at birth. Of the 161 calves which received colostrum, 118 survived and out of 103 calves which were deprived of colostrum only 9 survived. They concluded that the aqueous phase or more specifically the immune lactoglobulin fraction contained specific antibodies which were antagonistic to prevalent potential pathogens.

Smith (1962) noticed much greater numbers of Escherichia coli were present in all regions of the alimentary tract in the majority of colostrum deprived calves in contrast to colostrum fed calves. Systemic infections with E. coli was a common sequel of colostrum deprived calves, and it generally was accompanied by diarrhea. Diarrhea did occur in young calves that had received colostrum, but in such cases there was usually no bacteriaemia present.

Based on field observations and clinical studies, Amstutz (1965) cited improper feeding of newborn calves as a major predisposing cause of infectious diarrhea. Vitamin A and antibodies supplied in colostrum were necessary for maximum protection from pathogens. While conducting studies of gammaglobulin levels in calves from herds with colisepticaemia as a problem, Dam (1968) found a drop in calf mortality when colostrum was fed regularly and as soon after birth as possible as compared with feeding mixed milk from the herd. Patt et al. (1972) compared an artificial colostrum (milk plus bovine gammaglobulin) with milk. The bovine gammaglobulin used in the artificial colostrum was of normal cow origin. Feeding the artificial colostrum resulted in a significant increase in serum gammaglobulin concentration.

Zeliger et al. (1972) pointed out that prepartum milking stimulated increased milk production over the early part of lactation, but the immunoglobulin available for the calf decreased. They concluded that this disadvantage outweighed any gain which might be made through increased milk production.

The precise role that colostrum plays in protection has not been totally resolved, but certain factors have been implicated. Agglutinins to the "K" antigen in capsulated E. coli have been suggested to be present in colostrum along with "O" antibodies which are of the class IgG and IgA (Staley, 1971). Penhale et al. (1970) demonstrated that IgG and IgM classes of serum immunoglobulines were deficient in calves which developed septicaemia. They pointed out that either or both of these classes of immunoglobulins function in mediating immunity against enterobacterial antigens.

In one study by Penhale et al. (1970), newborn calves with IgG and IgM levels of less than 0.8 and 0.2 mg per ml, respectively, died of neonatal septicaemia. However, calves with IgG and IgM levels of 5.0 and 0.6 mg per ml, respectively, developed enteric infections. Calves with postcolostral serum levels of 7.5 and 0.8 mg per ml of IgG and IgM were normal.

Staley et al. (1971) pointed out a positive linear correlation between colostral immunoglobulin concentration and serum immunoglobulin concentration attained in calves. There was also a positive relationship between maximum immunoglobulin content in colostrum fed and maximum immunoglobulin content attained in sera of calves after ingestion.

Kruse (1970a) reported highly significant differences between breeds in yield of colostrum at first milking and in concentration of immunoglobulins in the colostrum. Also individual variation within breed was found in yield of colostrum. Heifers had a lower colostrum yield and concentration of immunoglobulin than did that of cows in second or later lactations. Season had no apparent effect on concentration of immunoglobulin and total yield of colostrum. An increase in time between calving and first milking caused a significant decrease in colostrum immunoglobulin concentration. Repeatability in concentration of immunoglobulins was high between successive calvings.

Sarwar et al. (1964) noted high variation in antibody concentration in colostrum of 29 cows. He concluded that the antibody level in colostrum, as well as in the blood, were related to prior immunological history of the animal. Clinical disease, sub-clinical disease, or the carrier state determined the antibody concentration.

Wilson et al. (1972) noted preparturient intramammary vaccination with live, formalized E. coli clearly altered the immunoglobulin content of colostrum. The post-parturient mammary secretion contained greater concentrations of IgG and IgM on days 2 and 3 and greater concentration of IgA on days 2 through 28 than secretions from non-vaccinated mammary glands. The theorized mechanisms were an increase in vascular permeability, and increase selective absorption from serum to colostrum, or actual production in the mammary gland.

Colostrum, besides being very high in immunological protein concentration, also has other chemical constituents different from milk which aid in establishing good health in newborn calves (Table I). The total solids, protein, and ash components in colostrum are higher than in milk

obtained two weeks after calving. The most striking difference in composition is the high protein percentage. High levels of lactose can cause scours in calves; therefore, the low content of lactose in colostrum could be a built-in protective mechanism to reduce the incidence of early calf scours. Colostrum is higher in calcium, magnesium, phosphorus, and chlorine concentration but lower in potassium content than milk.

TABLE I
COMPOSITION OF COLOSTRUM VERSUS MILK (2 WEEKS
POSTPARTUM) OF THE HOLSTEIN COW

Measurement	Colostrum	Milk
Total solids %	23.9	12.9
Fat %	6.7	4.0
Protein %	14.0	3.1
Lactose %	2.7	5.0
Ash %	1.11	0.74
Specific gravity	1.056	1.032

Colostrum is a very rich source of vitamins. Vitamin A in colostrum is found in concentrations 4 to 25 times that of milk and Vitamin D in concentrations 3 to 10 times that of milk. Colostrum contains much higher contents of thiamine, riboflavin, vitamin B₆, choline,

folic acid, and vitamin B₁₂ than normal milk, all which could greatly influence the health of a young calf (Schmidt, 1971).

Immunoglobulins

The term immunoglobulin is general and applies to a family of high molecular weight proteins that share common physio-chemical properties and antigenic determinants. These proteins occur in the serum and other body fluids of animals and possess gamma or slow electrophoretic mobility. These proteins include all molecules with antibody activity.

Immunoglobulins were historically, and are currently, separated into classes on the basis of their antigenic determinants or their antigenic reaction sites on the molecule. It has been shown that specific chemical features of the molecules of each class are responsible for these antigenic differences. For example, five distinct classes of human immunoglobulins have been identified and are referred to as IgG, IgA, IgM, IgD, and IgE. Three antigenically distinct classes of bovine immunoglobulins have been described. All occur in serum and in lacteal secretions and are designated IgG, IgA, and IgM (Butler, 1969).

Production of antibodies, or more specifically immunoglobulins, is initiated by the presence of antigen. Specificity of immunoglobulin molecules is maintained in that they have two and sometimes more combining sites which match accurately in an area and shape on the surface of a particular antigen. When combination occurs, various processes can take place which include (1) agglutination -- "sticking" together of particulate matter such as bacteria or blood cells, (2) precipitation -- "sticking" together of soluble antigens into visible antigen-antibody complexes, and (3) opsonization -- preparation of the antigen so that

attraction to phagocytic cells is increased (Herbert, 1970).

Schultz et al. (1971) studied the development of the bovine immune system under the effects of natural antigenic stimulation. It was found that the thymus was the first lymphoid organ to appear (42 days of gestation). The spleen was identified at 55 days of gestation, mesenteric lymph nodes were found at 100 days, and lymphoid tissue of the gastrointestinal tract (Peyer's patches) was found at 175 days of gestation. Immunofluorescence techniques aid in identifying IgM containing cells at 145 days of gestation in fetuses exposed to viral antigens. Immunoglobulins were produced in highest concentration in the spleen and lymph nodes, although some were produced in the tonsils, thymus, and bone marrow.

Humoral components which are non-specific in action are interferon, lysozyme, properdin, complement, and conglutinin. Interferon is a low molecular weight protein produced and released by many types of cells. It provides an early non-specific mechanism of acquired resistance before antibody protection (Weiser, 1971). Lysozyme (molecular weight 15,000) is an enzyme capable of breaking mucopeptide components commonly found in cell walls of bacteria. It is found in most tissues of the body including the nasal and intestinal mucous, saliva, and tears. Properdin is a high molecular weight protein (1×10^6) that, in the presence of complement, is able to kill gram positive and gram negative bacteria and inactivate some viruses (Herbert, 1970). Complement is a protein found in serum of all mammals in an inactive state. It is characterized as multiple proenzymes which require activation. There are nine distinct recognizable components designated: C1, C2, C3, C4, C5, C6, C7, C8, and C9. Complement can bind antigen-antibody complexes

causing increased phagocytic activity or in some cases can actually cause lysis of bacterial cells. Cell lysis by complement usually requires all nine components (Ingram, 1971; Herbert, 1970). Conglutinin is a naturally occurring protein found in the serum fraction of bovine blood. It is a globulin which acts with C1, C2, C3, C4 and can fix complement but is structurally and antigenically different from immunoglobulins. Conglutinin causes clumping together of bacteria or red blood cells which have already reacted with antibody and complement. Nonspecific defenses are efficient and immediate in their action. Antibodies and other specific immune agents take time to appear in sufficient strength to be protective after the first exposure to a parasite. During this delay the non-specific defenses are the primary source of protection (Weiser, 1971; Ingram, 1971; Herbert, 1970).

Colonization of E. coli requires attachment of the bacteria to intestinal epithelium. The mechanism is unknown but is associated with particular "O" and "K" antigens located on the bacterial cell surface. It has been shown that prior feeding of antiserum specific for "O" and "K" antigens of the toxic strain of bacteria prevented colonization of that strain in the small intestine and protected against diarrhea. Therefore, specific immunoglobulin class here would not seem to be as important as the speed with which they reach the proper antibacterial locality in the gut lumen.

Other bacterial strains penetrate the epithelium. In this case colostral immunoglobulins would produce antibacterial activity by the ability to activate complement. This would then be potentially bactericidal both in bacterial membrane damage and enhancement of phagocytosis (Berman, 1973).

IgG is a protein with molecular weight of 160,000 and sedimentation constant of 7S. At least 80% of the serum antibodies formed against viruses, bacteria, and toxins are of the IgG class. By using papain (a proteolytic enzyme), IgG can be split into 3 fragments (Figure 1). Two of these are non-crystalizable and retain antibody activity, and are called the Fab fragments. The other fragment is crystalizable and devoid of antibody activity and is called the Fc fragment. The IgG molecule consists of two heavy (h) chains and two light (l) chains linked together while another disulfide bond links each of the light chains to the heavy chains (Weiser, 1971).

IgG is capable of taking part in almost all antigen-antibody reactions but may not be as efficient in fixing complement and in the agglutination reaction as IgM. However, IgG is better than IgM in inactivating nonparticulate antigens such as toxins. IgG is found primarily in the blood and has characteristics of being able to pass between endothelial cells while in body fluids (Butler, 1973; Porter, 1973).

Two subclasses of IgG have been separated on their electromobility, IgG₁ and IgG₂. Both IgG₁ and IgG₂ have different immunochemical properties (Porter, 1973). IgG₁ was found by Smith (1971) to be the most concentrated immunoglobulin in colostrum. He also found estrogen and progesterone to be involved in the control of selective transport of IgG, from the blood to lacteal secretions. By examining 30 sections of mid-jejunum, Porter et al. (1972) concluded that IgG₁ is quantitatively the major immunoglobulin absorbed from colostrum, and high blood serum levels are maintained at least six weeks after birth. However, very little of IgG₁ actually passed through the mucosal gut layer to aid in

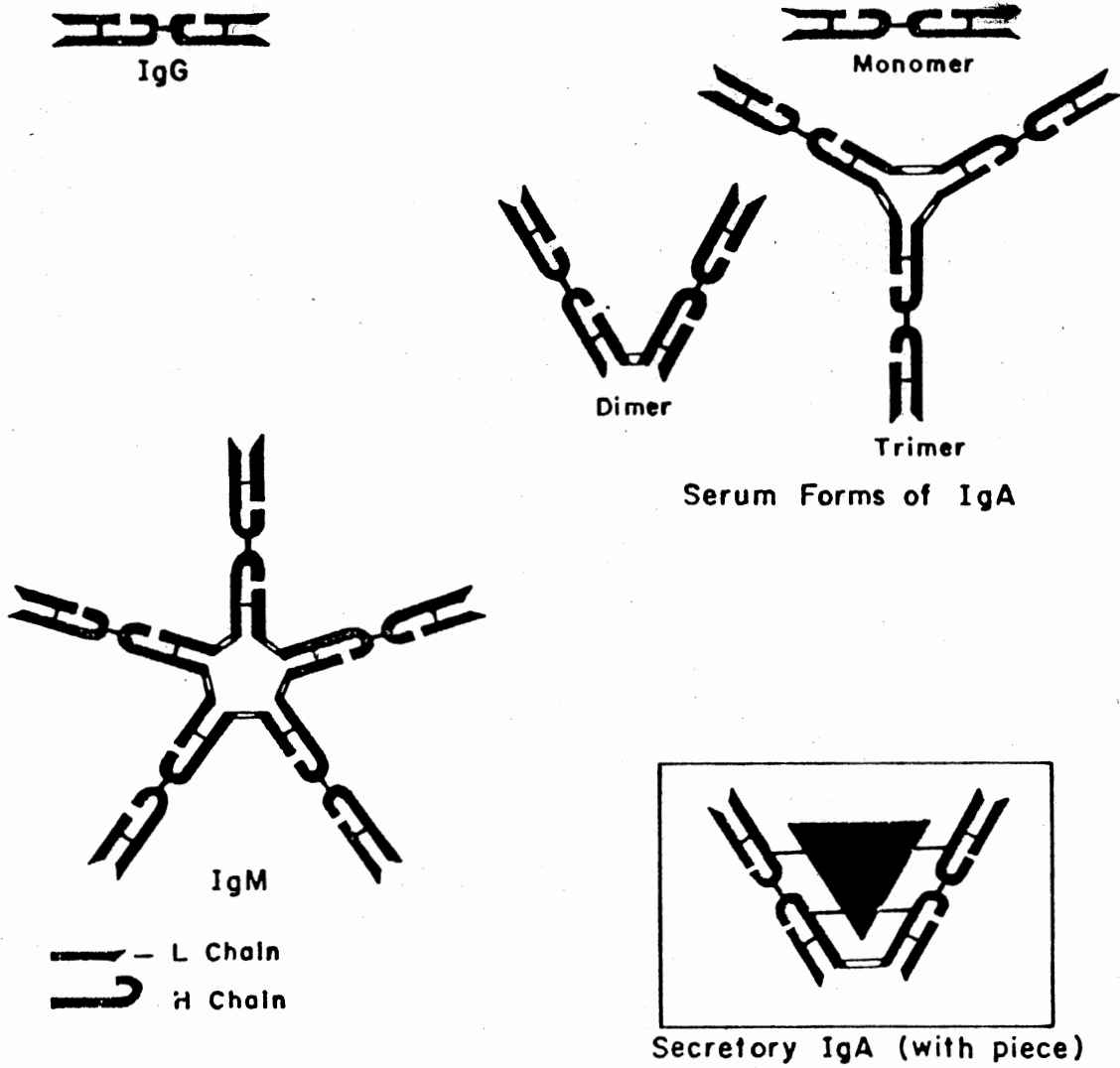


Figure 1. Structure of Immunoglobulins (Weiser, 1971)

external defense of the gut. IgG₂ had a low level in the colostrum of the dam. It was also low in the saliva and serum of the calf, but had a higher intestinal lumen concentration than IgG₁ (Porter et al., 1972).

The IgM molecule has a molecular weight of 900,000 and a sedimentation coefficient of 19S. By the use of thiol (-SH) reagents, the IgM molecule can be cleaved into five similar subunits; further reduction yields two L chains and two H chains (Figure 1). By the theoretical model there appears to be five possible antigen-antibody reaction sites per molecule (Weiser, 1971). IgM is predominantly found intravascularly; very little is found in tissue fluids, and none is found in mucous secretions and sweat. In many reactions it is the most efficient type of antibody. One molecule of IgM may be more efficient than IgG. This is true primarily with particular antigens (Herbert, 1970).

The most typical form of bovine IgA is protein containing a secretory component. IgA is the principle immunoglobulin synthesized by exocrine glands, gut, and by the respiratory tract (except in the mammary gland and reproductive tract where IgG may equal or exceed production of IgA). The immunoglobulin class IgA is present in relatively large quantities in saliva, colostrum, tears, nasal and bronchial secretions, and secretions of the intestines.

It appears that serum IgA and IgA found in external secretions are not identical. Secretory IgA is a complex of IgA plus a secretory piece produced by the glandular epithelium (Figure 1). The function of this secretory piece is to protect the molecule from proteolytic enzymes. IgA has been referred to as "immunological, aseptic paint" due to its protective characteristics on epithelial surfaces (Butler, 1971).

The role of milk secretory IgA in defense of the calf is different than that of the pig. Porcine milk IgA is held at high levels during later lactation, particularly the first two weeks postpartum. Porter (1971) has suggested that low levels in bovine milk may be an immunologic adaptation to allow normal ruminal flora development and function. The goat was similar in IgA concentration as the bovine. It was pointed out that along with the pig, the horse and the rabbit, which are not ruminants and use caecal digestion, also secrete and maintain high levels of IgA in milk.

IgM and IgA play an important role in the local response of the bovine udder to intramammary challenge by bacterial antigens; therefore, it is probable that these two immunoglobulins act together in the defense mechanisms of the young calf. In the neonate the intestinal absorption of colostrum immunoglobulins is not selective, thus the antibody profile of postcolostral calf serum resembles that of colostrum. Secretory IgA and IgM make a significant contribution to passive immunity considering their respective half-lives in calf serum are only 2 and 4 days (Porter, 1972).

IgA has been identified in mucosal epithelial surfaces and IgA with its secretory piece has been detected in most external secretions. However, IgA does not appear to be exclusively the major mediator in external defense. IgG₁ is the predominant immunoglobulin in mammary secretions and IgM exceeds IgA in the gut secretions of the preruminant calf.

There is no clear evidence for a bovine analog of IgE, the immunoglobulin class associated with many common allergies in man. However, investigations are being made into immediate-type hypersensitivity in

cattle to determine whether IgE is mediating the response. There is recent evidence that human IgE is cross-reactive with a bovine counterpart which further points to a possible IgE molecule present in the bovine species (Porter, 1973).

Absorption

Staley (1971) described passive immunity as the acquisition of antibodies from an outside source. In the situation of the calf, antibodies are obtained or transferred via the colostrum from the dam. The calf is born hypogammaglobulinaemic or with very little antibody in its sera. This lack of antibody is due to the fact that immunoglobulins fail to pass the placental barrier, and there are few antibody producing cells in the newborn.

Payne and Marsh (1962) pointed out marked differences exist between domestic animal species in regard to passive immunity. There appears to be no systematic pattern. There is circulating transfer of antibodies from dam to offspring in the rabbit, guinea pig, chicken, and human. In the rat, mouse, dog, and cat there is some placental transfer and further transference after birth via colostrum. There appears to be no placental transfer in the ruminant, horse, and pig, but only colostrum antibodies acquired entirely after birth.

Studies by Porter (1973) with the use of cell-specific antiserum indicate that there is little or no transmission of immunoglobulins in utero, thus the intestinal absorption of ingested colostrum immunoglobulins supplies the passive immunity necessary for the calf in early life.

It is believed that the majority of the globulin fraction of colostrum is synthesized by the plasma cells from free amino acids in the blood (Hafez, 1968). Staley (1971) also agrees that the origin of immunoglobulins is in the plasma cell, but it is in the mammary gland where they are directly secreted to colostrum. After ingestion of colostrum the antibodies are rapidly absorbed in the intestine of the newborn, and within 1 to 3 hours after nursing, these antibodies are present in the blood. Comline et al. (1959) administered colostrum directly into the duodenum and found there was an interval of 60 to 120 minutes from the time of colostrum introduction to the appearance of colostrum proteins in the lymph. The time it took from administering colostrum to detecting colostrum antibodies was increased with the use of older animals, and the delay was not reduced by adding a more concentrated whey.

The absorption of colostrum from the intestine to the circulation can be thought of as occurring in three steps according to Staley (1971). The first step involves the engulfing of immunoglobulins by the intestinal epithelial cell. The surface of the epithelial cell is active in invaginating and extending its cytoplasm as tubules which allows an easy entrance for the protein molecules. The second step involves these tubules which "bud off" into vacuoles and are transported to the basal cell membrane. The third step occurs when the vacuole opens and discharges its contents into lymphatic channels. The colostrum protein continues through the lymphatic system to eventually be released into the circulation.

Comline (1959) showed that after absorption of colostrum in the small intestine of small calves, unchanged colostrum proteins do not

enter the portal circulation in any appreciable amounts but are carried in the lymph to the peripheral blood. With the use of electronmicroscopy Staley et al. (1972) were able to determine the ultrastructural organelle responsible for the uptake of IgG. It was found to be the tubular system of the apical cytoplasm both in the ileum and jejunum in which IgG was transported through the cell and released at the basement membrane.

Absorption is not always beneficial. Frey (1971), while doing studies with colisepticaemia, found the intestinal mucosa of newborn calves to absorb all kinds of proteins non-specifically. Along with these proteins, bacteria are absorbed and thus have a direct entrance into the circulation. Of 191 calves, 175 (91.6%) with colisepticaemia were found to be hypo- or even agammaglobulinaemic in spite of colostrum intake on the first day of parturition. Treatment by intravenous injection of 50 to 100 ml. of a colostrum serum containing 10.8% total protein into the newborn calves rendered them normal in serum gammaglobulin content.

Staley et al. (1971) learned that IgM and IgG were absorbed equally well from colostrum. The maximum concentrations of IgG and IgM found in blood were 49% and 60% of the colostrum level. They further found that by feeding daily colostrum levels of 2.5% and 3.75% of body weight maximum absorption occurred at 24 hours. There was a positive correlation between maximum immunoglobulin in colostrum and maximum immunoglobulin in blood at parturition. McCoy et al. (1970) demonstrated serum gammaglobulin increased linearly over a seven and one half hour period after ingestion of colostrum. Serum albumin also increased but α and β globulins remained constant.

In an attempt to explore the age at which absorption can occur, Staley (1971) used 7 to 8 month old fetuses and fed colostrum 38 hours after delivery. He found no absorption and concluded that there must be a gestation time comparable to normal to insure absorption.

A summary of reported data on immunoglobulin concentrations in the bovine is shown in Table II. Age of animals from which samples were taken varied from birth to adult cows. Analysis of immunoglobulin concentrations in all cases was by single radial diffusion.

Hypogammaglobulinaemia has been mentioned several times previously and is such a serious problem that the subject warrants investigation. To determine the significance of hypogammaglobulinaemia to the occurrence of E. coli infections, Dam (1968) found serum from 2 day old calves in herds with colisepticaemia to have a lower percentage of serum gammaglobulins than in calves which had no clinical signs of the disease. Antibody levels in the dam quickly decreased after the first milking. Generally, only the first 2 milkings could be expected to give colostrum of real value with respect to specific antibodies. The extremely rapid fall in the content of antibodies in colostrum could have indirectly been the cause of agammaglobulinaemia or hypogammaglobulinaemia observed in young calves due to careless administration of colostrum. Gay et al. (1965) studied the immunoglobulin levels of 178 calves under 4 days of age in an attempt to predict which would die of colisepticaemia. Fifty-three calves (29.8%) were markedly or absolutely deficient in serum gammaglobulin, 33 (18.5%) possessed low globulin levels. All but one of the deaths from colisepticaemia were in calves whose sera was markedly deficient in serum globulins.

TABLE II
SUMMARY OF DATA ON IMMUNOGLOBULIN CONCENTRATIONS
DETERMINED BY SINGLE RADIAL DIFFUSION

Serum Ig	Age	Concentration mg/100 ml	Number of Animals	Reference
IgG ₁	0 hours	6800	5	a
IgG ₁	24 hours	2400	5	a
IgG ₁	Adult	1050	100	b
IgG ₂	0 hours	350	5	a
IgG ₂	24 hours	1100	5	a
IgG ₂	Adult	790	100	b
Total IgG	0 hours	87	13	c
Total IgG	0 hours	1200	10	d
Total IgG	24 hours	22300	10	d
Total IgG	Adult	12.9	100	e
IgM	0 hours	750	5	a
IgM	0 hours	13	13	c
IgM	24 hours	1000	5	a
IgM	Adult	2.8	100	e
IgM	Adult	250	100	b
IgA	0 hours	750	5	a
IgA	24 hours	450	5	a
IgA	Adult	30	100	b

References

- | | |
|---------------------------|---------------------------------|
| (a) Porter (1971) | (d) Klaus et al. (1969) |
| (b) Mach and Páhud (1971) | (e) Penhale and Christie (1969) |
| (c) Logan et al. (1974) | |

Many researchers have found hypogammaglobulinaemic calves in spite of adequate levels of ingested colostrum (Bush et al., 1971; Mungle, 1970; Kruse, 1970c; Gay et al., 1965). Kruse (1970c) postulated repeated high frequency of hypogammaglobulinaemia and wide variation in serum immunoglobulin content in newborn calves were due to independent variables such as weight of calf, concentration of immunoglobulin in the colostrum, inherited genetic ability to absorb immunoglobulin, the amount of colostrum given, and the time at which the colostrum was fed. Kruse (1970b) found by using 141 calves and varying the first feeding from 2, 6, 10, 14, 20 hours of age, that absorption was primarily a function of time of first feeding. There was a significant drop in amount of colostrum immunoglobulin absorbed from the 2-hour feeding to the 20-hour feeding. He concluded that the normal second feeding will not reduce the frequency of hypogammaglobulinaemia. Bush et al. (1971) found immunoglobulin concentration increased markedly after ingestion of colostrum and reached a maximum peak between 24 and 48 hours after birth. Average immunoglobulin concentration decreased after 24 hours reflecting catabolism of the material or transfer to other metabolic pools. A positive linear relationship was found (.82) between grams of gammaglobulin consumed and serum level attained.

Closure

Staley et al. (1968) defined closure as the mechanism by which intestinal absorption of protein ceases. Closure is dependent upon species, intraluminal environment, length of postnatal life, and circulating hormones. The process of closure can be initiated by exposure of the epithelium to colostrum or milk. Bovine closure occurs

spontaneously whether colostrum is fed or not. Initiation of closure in pigs can occur with bovine colostrum whey, glucose, or even dialysis products from colostrum or milk. However, porcine closure is normally postponed until first nursing. Even after being starved for 36 to 48 hours, and then fed, absorption occurs at a reduced level, but as though first nursing had occurred. Leece et al. (1964) found that pig closure, mediated by diet, was initiated by presence of colostrum components such as carbohydrates, vitamins, and minerals, not necessarily protein per se. The pig is born fetal-like in two respects. He is born low in serum gammaglobulins, and also he is born with a primitive functioning intestinal epithelium. The colostrum from the sow quickly alters both immature states by supplying protein gammaglobulins to increase serum proteins and also supplying fractions which induce physiological changes in the intestinal epithelium (closure).

Staley et al. (1968) further described the process of closure in the calf as occurring in retrograde fashion, that is, the basal cell membrane ceases to release the envoculated product. Transport ceases and finally uptake by the tubular system ceases. Staley (1971) was able to initiate closure by the chemical, cortisone acetate. When administered to starved pigs, closure was complete at 48 hours. It was found that cortisone, ACTH, diethylstilbesterol, progesterone, and somatotrophin had no effect on stimulating closure in calves. He also concluded that proteolytic enzymes do not contribute to the mechanism of closure in calves due to the very low concentration found in the gut of calves from 3 to 21 hours of age.

McCoy et al. (1970) demonstrated that gammaglobulin levels in calves fed glucose or nothing remained at a low level for 31 hours.

Calves fed colostrum at 24 hours after feeding glucose or nothing had no effect on serum gammaglobulin levels, suggesting that the gut was impermeable to colostrum proteins after 24 hours of life. By infusing human gammaglobulin via stomach tube, Staley et al. (1972) found that day-old calves previously fed colostrum absorbed 45% as much human gammaglobulin as newborn calves. He indicated that the decline in absorption was related to the cessation of release of proteins from the intestinal cell rather than cessation of uptake.

Chemical Factors Affecting Absorption

In an attempt to increase absorption of gammaglobulin from colostrum in newborn calves, Hardy (1969c) found potassium isobutyrate to be the best compound tested. The concentration of potassium isobutyrate used was 56 millimoles per liter of colostrum whey. Other compounds tested included chloride, bicarbonate, citrate, lactate, pyruvate, formate, acetate, propionate, isovalerate, and butyrate administered as the sodium or potassium salt. The conclusion that potassium isobutyrate dramatically increased absorption was based on four calves varying in age from 7 to 14 hours. This conclusion formed the basis of our research and prompted us to use 22 calves in determining the significance of feeding potassium isobutyrate along with colostrum at first feeding.

Hardy (1969c) indicated that when immunoglobulins are administered in solutions containing sodium lactate or sodium pyruvate, increased absorption can be produced. The hypothesis proposed solvent factors act on the transfer of bovine serum globulin from the epithelial cell into the core of the villus. They may be a source of metabolic energy

for the epithelial cell. Glucose and citrate were not found to be effective in accelerating transfer. He concluded that lactate and pyruvate are not present in sufficient quantity in colostrum to maximize the absorptive process.

Balfour and Comline (1962) used radioactive isotopes to demonstrate increased absorption of colostrum proteins when inorganic phosphate was added. Glucose-6-phosphate had only a slight effect on absorption. The mechanism of action of phosphate compounds as well as lactate and pyruvate compounds was postulated to involve the intracellular metabolism of the intestinal epithelium. The compounds provide energy for absorption or more specifically for the transfer of globulins across the epithelial cells.

Deutsch and Smith (1957) could get no response in increasing or prolonging intestinal permeability beyond the first 24 hours of life with the use of diethylstilbesterol, progesterone, diethylstilbesterol plus progesterone, cortisone, and ACTH. Smith et al. (1964) treated newborn calves with $Al(OH)_3$ gel to inhibit gastric activity and prevent gastric digestion of protein. Intestinal permeability was not lengthened. Direct infusion of colostrum into the duodenum and bypassing the stomach in 48 hour calves failed to enhance absorption. This study disproved the theory that antibody transference is related to gastric development and digestion of colostrum antibodies approximately 24 hours postpartum.

Immunotherapy

A relatively new and broadening field called immunoprophylaxis or immunotherapy as discussed by Freeman (1973) has had some notable

successes in both human and veterinary medicine. Immunotherapy by definition is the administration of antibodies in various forms and by various routes into a body to intervene in infectious disease. Some of the limitations of this type of passive immunity include (a) a limited duration of protection afforded by the transferred antibody molecule due to constant body catabolism, (b) the antibody must be administered prior to exposure or invasion of the pathogen, (c) the antibody must be from the same species in which donation occurs, and (d) finally purity of the antibodies is both costly and difficult to produce.

Logan and Penhale (1972) found that immunotherapy was not completely satisfactory when they treated 13 colostrum deprived calves with IgM intravenously and then challenged them orally with a known strain of E. coli. All calves suffered severe diarrhea within 24 hours after challenge, and 11 died.

Immunotherapy looks promising in prevention and treatment of calf scours. Dosage rates as well as specific antibody ratios must be established and tested in order to be effective and practical.

CHAPTER III

MATERIALS AND METHODS

Experimental Plan

Twenty-two calves were obtained from the dairy herd at Oklahoma State University. Two breeds were used (Aryshire and Holstein) with calves blocked by breed.

Within each breed, calves were randomly assigned to two treatment groups. One group was given 2.83 milliequivalents of potassium isobutyrate per gram of gammaglobulin which was consumed immediately after birth. The control group received colostrum with distilled water immediately after birth. Colostrum was fed as quickly after birth as possible (no longer than one hour) at a rate of 2.5% of body weight. The grams of colostrum and the millileters of potassium isobutyrate required per feeding were determined by the following formula:

$$\text{kg. of colostrum fed} = \text{wt. of calf (kg.)} \times .025$$

$$\text{ml. of potassium isobutyrate added to colostrum} = \text{kg. of colostrum} \\ \times 151.4 \text{ ml potassium isobutyrate/kg. colostrum.}$$

The objective was to feed 2.83 milliequivalents of potassium isobutyrate per gram of gammaglobulin. The colostrum used in this experiment had 4.26% gammaglobulin. There were 120.8 milliequivalents of potassium isobutyrate added per kg of colostrum. The concentration of potassium isobutyrate solution was .798 meq /ml. Therefore, 120.8 meq potassium isobutyrate/kg colostrum \div .798 meq potassium

isobutyrate/ml = 151.4 ml potassium isobutyrate solution/kg colostrum.

The control group was given the exact same amount of distilled water as the treatment group received potassium isobutyrate to insure equalization of treatment volume.

Colostrum from several cows was packaged in pint and quart containers and stored at -20°C .

Blood samples were taken immediately after birth (before feeding) and at 4, 8, 12, 16, 20, 24, 48 and 72 hours thereafter. The blood samples were analyzed for packed red cell volume (hematocrit), total blood serum protein by refractometry, total gammaglobulin by microzone electrophoresis, and specific immunoglobulin fractions (IgG, IgM) by radial gel diffusion technique.

Calves were taken from the dam immediately after birth and were not allowed to nurse. The calves were weighed and placed in an individual calf stall, and dried with burlap sacks. The calves were bled then fed the calculated amount of colostrum with either potassium isobutyrate or distilled water added depending upon the randomization sequence. Colostrum was weighed to the nearest 9.1g (.02 lb) on a Toledo fan scale, and the potassium isobutyrate or distilled water was measured in a 100 ml glass graduated cylinder to the nearest 1.0 ml. Colostrum with the additive was fed via nipple bottle to a standing calf without loss or spill at birth and colostrum without additive was fed at 12 hours of age. Thereafter, each calf was fed whole milk from any Holstein cow at least 30 days into lactation twice daily at the rate of 4% of body weight per feeding. Records were kept on hematocrit and general condition of the calf at each bleeding (good, fair,

or bad) and general condition of the stool (firm or watery). Calves which needed veterinary assistance were treated, being careful to avoid injections which would alter blood chemistry.

Blood samples were obtained by jugular venipuncture with sterile hypodermic needles (size 18 x 1.5) and sterile 20 ml disposable syringes. The 15 to 20 ml of blood was allowed to clot in clean 40 ml plastic centrifuge tubes and stored at 5°C for no longer than 12 to 14 hours. After clotting had occurred (4 hours) an applicator stick was used to separate the clot from the wall of the 40 ml centrifuge tube to allow the separation of serum. Immediately after bleeding, blood was drawn into two heparinized capillary tubes and subjected to centrifugation. Hematocrit values from the two capillary tubes were averaged.

Laboratory Procedures

Colostrum

An aliquot of colostrum was weighed into a 25 ml centrifuge tube. The casein was precipitated by AOAC procedure (Association of Official Agricultural Chemists, 1965), and the supernatant decanted. Total protein was determined by Kjeldahl analysis and calculated on basis of whole colostrum. Total gammaglobulin in the colostrum sera was determined by microzone electrophoresis and calculated on whole colostrum basis.

Serum Total Protein

After clotting, the blood serum was separated by centrifugation at 2,500 rpm for 15 min. The serum was drawn off by disposable micropipets

and aliquots transferred to two 5 ml stoppered plastic test tubes. Labels were immediately applied and the serum was analyzed for total protein by use of a Bausch and Lomb refractometer as described by Lecoq (1962). Samples were stored at -20°C until all calf samples were collected.

Total Gammaglobulin

Serum samples were thawed at room temperature and exposed to electrophoresis with the microzone system as described by Elliott (1966). This system separates serum protein into albumin, alpha-, beta-, and gammaglobulin fractions according to their intrinsic differences in migration when exposed to an electric field (Carpenter, 1965; Elliott, 1966). The gammaglobulin fraction was figured on a percentage of the total of albumin, alpha-, beta-, and gammaglobulin and expressed as grams per 100 ml of serum.

Serum and Colostral IgG

Quantitative determination of bovine IgG was obtained using a Pentex kit from Miles Laboratories, Inc., Kankakee, Illinois. This kit allowed the determination of serum IgG on the principle of single radial diffusion (Butler, 1969; Mancini et al., 1965; and Butler, 1971). Each plate was prepared incorporating monospecific anti-bovine IgG serum in buffered agarose. Each plate allowed 17 assays with an accuracy of $\pm 10\%$ within a range of 500 to 4000 milligrams IgG per 100 ml serum. Four bovine IgG reference standards were provided. All samples were randomized within each plate. Four reference standards with concentrations of 500, 1000, 2000, 4000 mg per 100 ml were used

on each plate. Each 12 hour blood sample was duplicated and averaged.

Serum or colostrum was pipetted into predetermined wells using disposable micropipets. After 13 samples and four standards were applied, a few drops of water were pipetted into the moisture trough bordering the immunodiffusion plate to insure constant humidity. The plate was covered by a lid and left undisturbed at room temperature (27°C) for 18 hours. After incubation, these plates were photographed, and the diameter of the precipitin rings was measured to the nearest 0.1 mm with the aid of a Bausch and Lomb magnified scale.

Serum IgM

Essentially, the same procedure was used to determine serum IgM and IgG levels. Four standards again were used on each plate but the standard concentrations were 60, 130, 320, 520 mg per 100 ml of serum. Measurements of assays had an accuracy of $\pm 10\%$ within a range of 60 to 520 milligrams per 100 ml serum. Incubation and final determination of serum concentration was the same as with serum and colostrum IgG.

Analysis of Data

A split plot design was utilized in which the main plot consisted of calves randomly assigned to a treatment within breeds. The subplots consisted of time periods within the main plot. Analysis of variance was performed to test for differences among treatments, breeds, and periods.

CHAPTER IV

ABSORPTION OF IMMUNOGLOBULINS FROM COLOSTRUM BY NEWBORN CALVES^{1, 2, 3}

Summary

The effect of adding potassium isobutyrate to colostrum on absorption of total gammaglobulin, IgG, and IgM in newborn calves was determined. Calves received colostrum with 2.83 meq potassium isobutyrate per gram of gammaglobulin or colostrum with distilled water within an hour after birth. Blood samples were taken at nine intervals for determination of gammaglobulin, IgG and IgM concentrations.

Calves fed colostrum with distilled water had consistently higher serum levels of total gammaglobulin, IgG and IgM. Holstein calves apparently were more efficient than Ayrshire calves in absorbing total gammaglobulin, but differences between breeds were not significant for specific IgG and IgM. An estimate of overall efficiency of gammaglobulin absorption within 24 hours was calculated to be 35.7 ± 3.14 (mean \pm standard error) for the control group and 24.7 ± 3.14 for the group given potassium isobutyrate added to the colostrum. Potassium

¹Journal article of the Agricultural Experiment Station, Oklahoma State University, Stillwater.

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isobutyrate had a negative effect on colostrum protein absorption.

Introduction

The significance of colostrum in reducing the incidence and severity of colisepticemia in the newborn calf has been noted by various workers (Smith, 1962; Amstutz, 1965; Dam, 1968). Penhale et al. (1970) found the gammaglobulin fraction of colostrum to be the most important single factor in this increased resistance to infection. Bush et al. (1971) found a positive linear correlation between colostrum immunoglobulin consumed during the first day after birth and serum immunoglobulin level at 24 hours. Approximately 68% of the variation in serum globulin levels was accounted for by immunoglobulin intake. In later work (Mungle, 1972), in which intake of gammaglobulin was either .3 or .6 g per unit of metabolic size, 50% of the variation in serum immunoglobulin was attributed to level of γ -globulin intake. Kruse (1970c) suggested that hypogammaglobulinaemia may be associated with variables such as weight of calf, concentration of immunoglobulin in colostrum, time after birth at which colostrum is fed, and lack of inherited genetic ability to absorb immunoglobulins. In a series of experiments, Hardy (1969) infused bovine serum γ -globulin with lactate, pyruvate, and salts of certain fatty acids into the duodenum of anesthetized calves. Potassium isobutyrate proved to be the most beneficial in increasing absorption of γ -globulin.

This study was conducted to investigate the effect of adding potassium isobutyrate to colostrum on absorption of immunoglobulins by newborn dairy calves.

Experimental Procedures

Twenty-two calves (12 Holstein, 10 Ayrshires) obtained at birth from the University herd were randomly assigned within breeds to two treatment groups. The treatments involved the following additions to colostrum given at the first feeding after birth: (a) 2.83 meq potassium isobutyrate per gram of γ -globulin, or (b) control, distilled water. The volume of distilled water added was equivalent to the volume of solution containing potassium isobutyrate. The colostrum was a pooled batch obtained from Holstein cows within 12 hours after calving. It contained 4.26 g total γ -globulin per 100 ml colostrum.

Each calf was removed from its dam immediately after birth (before nursing). Colostrum was fed at the rate of 2.5% of body weight within an hour after birth and again 12 hours later. After 24 hours the calves were fed whole milk from Holstein cows 30 days into lactation. Blood samples were taken immediately after birth (before feeding), and 4, 8, 12, 16, 20, 24, 48, and 72 hours afterwards to determine serum globulin concentrations. These samples were analyzed for packed cell volume (hematocrit), total blood serum protein by refractometry (Lecoq, 1962), total γ -globulin by microzone electrophoresis (Elliot, 1966), and specific immunoglobulin fractions (IgG, IgM) by radial gel diffusion technique described by Fahey and McKelvey (1965). A commercial source⁴ of purified antigen and antisera was used for the IgG and IgM assays. Concentration of these fractions in the serum samples was determined by comparison with specific antigen standards, utilizing the

⁴Pentex immunological assay kits from Miles Laboratories, Inc., Kankakee, Illinois.

linear relationship between precipitate ring diameters on antibody-agar plates and the logarithm of immunoglobulin concentration. Total protein content of colostrum was determined by Kjeldahl procedure and the proportion of γ -globulin by microzone electrophoresis (Elliot, 1966).

Results and Discussion

Serum Total Gammaglobulin

Serum gammaglobulin concentration before nursing was typical of newborn calves (Logan et al., 1974); however, the values increased rapidly following intake of colostrum (Figure 2). The average time at which maximum concentration of gammaglobulin occurred in the blood was 26.9 and 21.1 hours after birth for the control and treatment groups. The difference between groups was not statistically significant ($P > .05$) as there was considerable variation among calves within groups. Within the control group, the time at which peak concentration occurred ranged from 8 to 48 hours, whereas the range was 4 to 72 hours in the group receiving potassium isobutyrate in the colostrum. Since no samples were taken in the intervals between 24 to 48 hours and 48 to 72 hours, there is a possibility that the peak concentration in a few calves occurred at some intermediate point during these intervals. There was no relationship between the time that peak concentration was observed and the maximum serum concentration of γ -globulin attained. One of 22 calves had serum gammaglobulin below 0.6 g/100 ml of blood serum throughout the period of study, although general health and hematocrit were normal.

Calves were fed 106 g gammaglobulin/50 kg body weight within 24 hours after birth. Total gammaglobulin serum levels at 24 hours were 1.02 and .73 g per 100 ml for the control and treatment groups. These were somewhat lower than values obtained by Bush et al. (1971) in response to feeding a comparable amount of gammaglobulin. Mungle (1972) observed serum gammaglobulin concentrations of .32 and .73 g/100 ml in response to feeding colostrum containing 22.6 and 45.2 g γ -globulin/50 kg body weight prior to 24 hours.

Overall, there was evidence that calves fed colostrum with potassium isobutyrate added had less total gammaglobulin absorbed than did calves fed control colostrum ($P < .08$). Considering individual periods after birth, differences between treatments were statistically significant ($P < .05$) only at 24 and 48 hours (Figure 2). There was evidence of treatment period interaction ($P < .11$) which explains the larger differences at 24 and 48 hours. It was apparent that adding potassium isobutyrate to colostrum at the first feeding after birth was not helpful in obtaining either more rapid absorption or higher serum concentrations of total gammaglobulin.

Although the amount of potassium isobutyrate in relation to amount of γ -globulin consumed was essentially the same in this work as in that reported by Hardy (1969c), the effect on absorption was different. Hardy (1969c) used anesthetized calves and infused colostrum with I_{131} labeled γ -globulin directly into the duodenum. It is interesting to note that Patt et al. (1972) found that adding histamine to colostrum for calves resulted in serum gammaglobulin concentration consistently lower than that obtained by feeding colostrum with no additives.

Holstein calves attained a higher concentration of serum gamma-globulin than Ayrshire calves by 24 hours after birth ($P < .05$). Kruse (1970c) found that Red Danish calves had significantly lower efficiency for absorption of gammaglobulin than Black and White Danish or Jersey calves. In contrast, Bush et al. (1971) and Mungle (1972) found no significant differences among breeds in ability to absorb gammaglobulin from colostrum.

Assuming the plasma volume of a day-old calf to be 93 ml per kilogram of body weight (McEwan, 1968), overall efficiency of gamma-globulin absorption within 24 hours was calculated to be 35.7 ± 3.14 (standard error) for the control group and 24.7 ± 3.14 (standard error) for the group with potassium isobutyrate added to the colostrum. The difference between groups was statistically significant ($P < .05$). The value for the control group was similar to those reported by others, and may be near the maximum that can be achieved. Bush et al. (1971) observed a 45% efficiency at 24 hours in 27 calves and Mungle (1972) found the efficiency to be 33.2% in 30 calves. Differences in efficiencies could be due to the differences in the amount of gammaglobulin consumed from the colostrum. It would be reasonable to assume that intestinal degradation of some gammaglobulin would decrease availability to the circulation. It is then logical to assume that a higher percentage of gammaglobulin would escape degradation if a larger amount were consumed.

Serum IgG and IgM

Serum concentrations of IgG and IgM were very low in calves at birth (less than 0.125 and .020 g/100 ml), but, as with total

gammaglobulin, quickly increased after consumption of colostrum. This is in agreement with other investigators (Mungle, 1972; Klaus et al., 1969). Average serum concentrations of IgG reflected the pattern of feeding (Figure 3) in that there was an increase 8 hours after feeding, after which there was a drop in concentration. Concentration increased after feeding at 12 hours and continued to a maximum at 20 hours, declining again by 24 hours. Presumably, the decline between 8 to 12 hours post feeding was due to metabolism of IgG (Pierce, 1951; McDougall, 1965). Maximum serum levels attained during the first three days of life were at 20 hours (Figure 3) which was similar to results obtained by Klaus et al. (1969) and Mungle (1972). Over the 72 hour period, the total absorption of IgG differed significantly ($P < .08$) between treatment groups. Again, colostrum with water resulted in a uniformly higher serum concentration than did colostrum treated with potassium isobutyrate.

Differences between groups in serum IgM values were similar to those observed in regard to serum gammaglobulin and IgG. Serum IgM concentration was significantly higher ($P < .03$) in the control group.

It is apparent from the present study that the calf is not totally devoid of IgG or IgM at birth (Figures 3 and 4). Small amounts of IgG and IgM are synthesized by the bovine fetus (Osburn, 1973). In most of the calves the consumption of colostrum led to a rapid increase in total serum gammaglobulin, IgG, and IgM concentrations within 24 hours. The time at which maximum IgM concentration occurred in the treated and control group was identical to that of gammaglobulin. However, the maximum serum IgG concentration in both groups occurred at 20 hours.

The exact mechanism by which potassium isobutyrate affects absorption is not known. It is possible that the potassium isobutyrate could

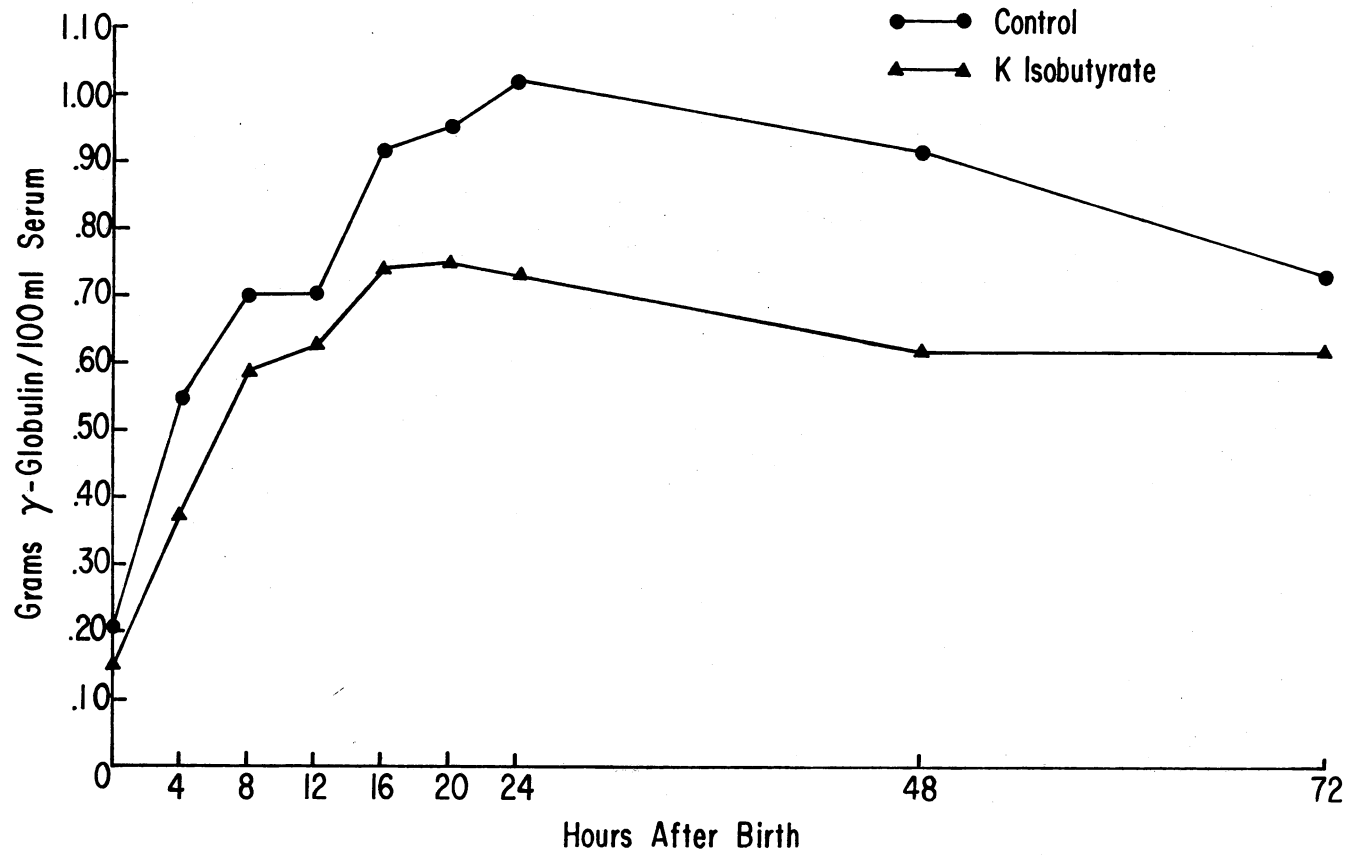


Figure 2. Concentration of Total Gammaglobulin in Blood Serum of Calves at Intervals after Birth

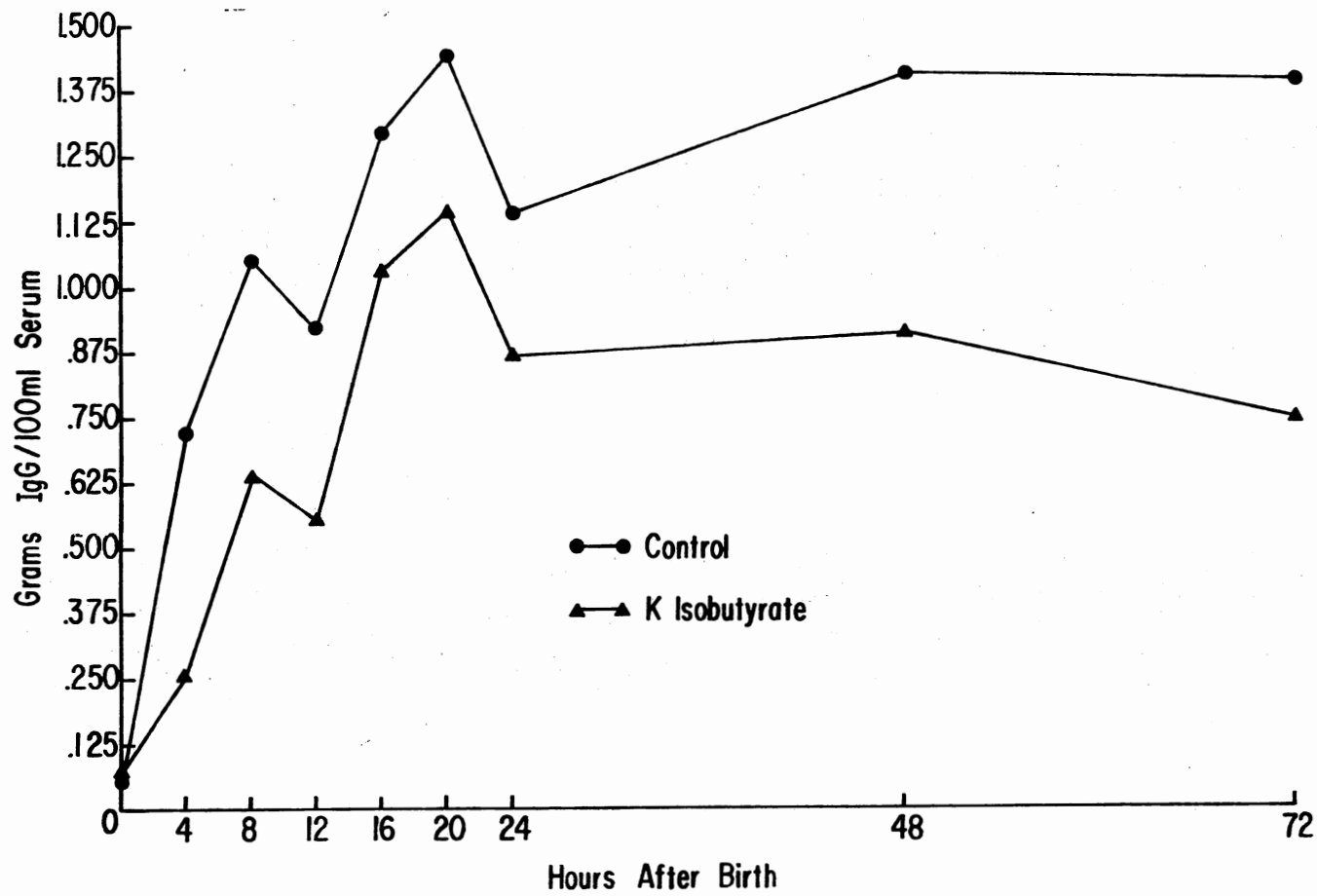


Figure 3. Average Concentration of IgG Serum of Calves at Different times after Birth

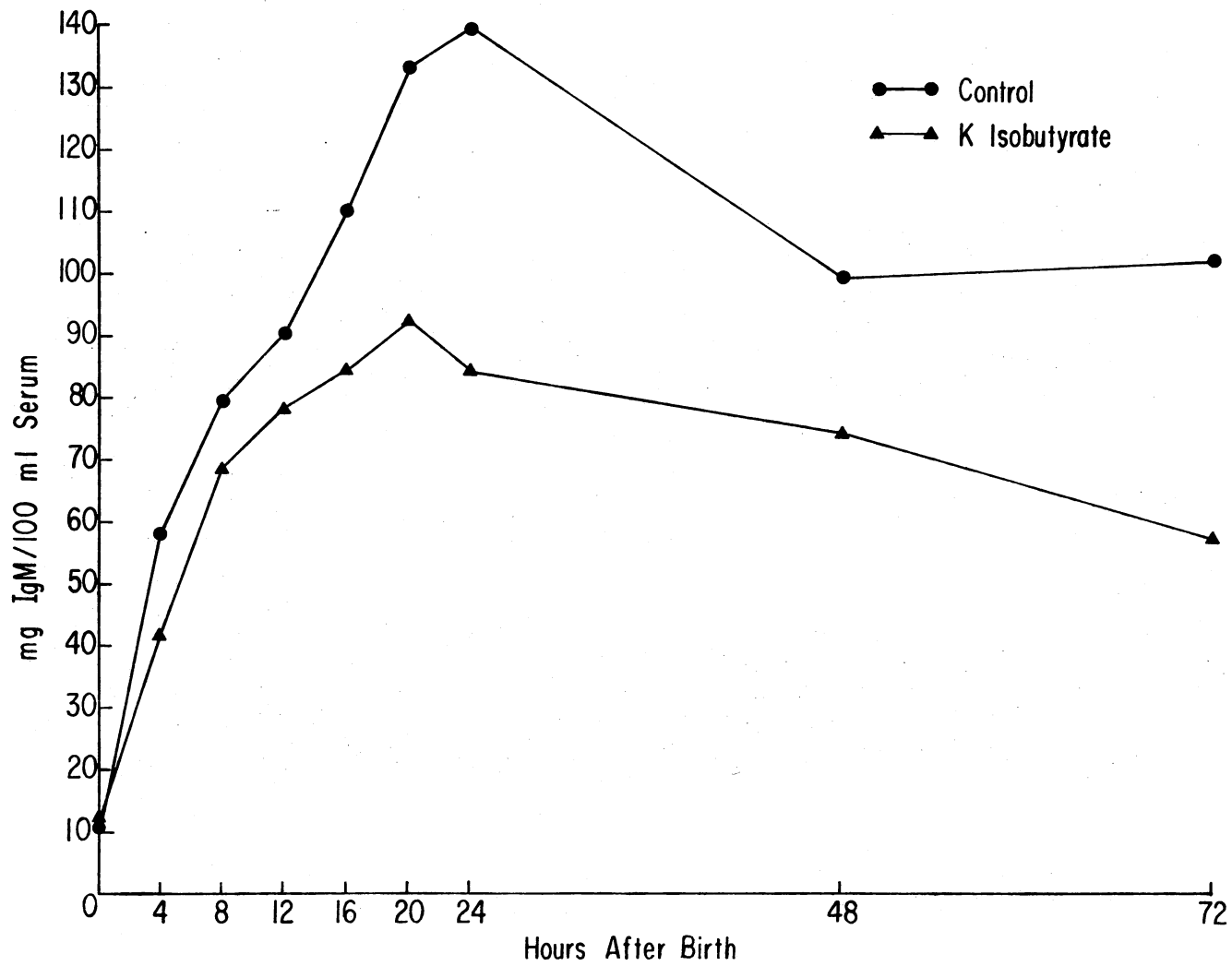


Figure 4. Average Concentration of Serum IgM in Calves at Intervals after Birth

affect the gammaglobulin so that the metabolic degradation of it by the host would be enhanced.

In this experiment, potassium isobutyrate had a negative effect on colostrum protein absorption. There might be physical as well as chemical limitations to the maximum amount of immunoglobulins which can be transported and absorbed from colostrum in the neonate. For example, if maximum efficiencies were in the 10% range, there would be a much greater possibility to increase efficiency. It is possible that there is a maximum efficiency of absorption that can be attained. With efficiencies in the range of 33-45%, we may have reached that limit.

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APPENDIXES

TABLE III
 ABSORPTION EFFICIENCIES OF TOTAL GAMMAGLOBULIN

Breed and Calf Number	Efficiency, %
<u>Treatment Group</u>	
Holstein 360	31.88
Holstein 567	31.88
Holstein 39	37.99
Ayrshire 48	25.33
Holstein 569	9.17
Holstein 52	30.57
Ayrshire 580	10.04
Ayrshire 56	19.65
Holstein 582	27.07
Ayrshire 588	21.40
Ayrshire 76	26.20
<u>Control Group</u>	
Ayrshire 29	27.51
Ayrshire 566	25.76
Holstein 561	41.05
Ayrshire 37	47.60
Holstein 51	19.65
Ayrshire 55	20.96
Holstein 61	35.81
Ayrshire 57	13.10
Holstein 67	32.31
Holstein 593	63.32
Holstein 594	65.94

TABLE IV

SERUM IMMUNOGLOBULIN CONCENTRATIONS IN CALVES THAT RECEIVED COLOSTRUM AND WATER (GRAMS/100 ML)

Calf No.	Ig	Age in Hours								
		0	4	8	12	16	18	24	48	72
57	Total Ig	.07	.15	.26	.21	.41	.20	.37	.36	.29
	IgG	.02	1.11	.27	.13	.39	.30	.87	.09	.22
	IgM	.001	.023	.030	.032	.035	.035	.037	.032	.035
55	Total Ig	.19	.49	.71	.46	.88	.45	.67	.53	.46
	IgG	.01	.37	1.37	.40	.53	1.37	.43	.46	.70
	IgM	.001	.053	.128	.068	.118	.128	.101	.058	.058
29	Total Ig	.18	.38	.60	.63	.68	.71	.81	.97	.50
	IgG	.02	.40	1.10	.84	1.41	1.41	1.41	.93	1.15
	IgM	.001	.038	.061	.077	.114	.090	.090	.071	.048
37	Total Ig	.32	1.12	.87	1.02	1.29	1.62	1.41	1.29	1.00
	IgG	.42	1.45	2.16	2.26	2.58	2.47	2.00	3.35	3.20
	IgM	.053	.149	.102	.197	.183	.170	.170	.147	.197
566	Total Ig	.15	.50	.72	.62	.68	.89	.74	.91	.54
	IgG	.07	.51	.89	.58	.89	1.04	.86	1.63	.74
	IgM	.001	.069	.101	.081	.106	.157	.178	.088	.082
51	Total Ig	.25	.17	.25	.31	.39	.58	.70	.65	.59
	IgG	.01	.58	.19	.09	.34	.63	.25	.36	.48
	IgM	.027	.031	.043	.39	.57	.11	.11	.15	.21

TABLE IV (Continued)

Calf No.	Ig	Age in Hours								
		0	4	8	12	16	18	24	48	72
593	Total Ig	.09	.29	.79	.89	1.23	1.41	1.55	1.59	1.24
	IgG	.01	.13	.29	.28	1.81	1.29	2.19	1.56	1.62
	IgM	.001	.022	.059	.075	.123	.194	.255	.123	.148
594	Total Ig	.22	.55	.72	.83	.93	1.24	1.73	.92	.80
	IgG	.03	.91	1.27	1.33	.98	1.87	1.27	1.58	1.71
	IgM	.003	.072	.098	.085	.114	.123	.142	.085	.085
561	Total Ig	.20	.96	1.26	1.07	1.52	1.10	1.14	.93	1.03
	IgG	.01	.80	1.28	1.98	1.59	1.98	.879	2.65	1.88
	IgM	.001	.082	.095	.127	.170	.158	.213	.162	.072
61	Total Ig	.14	.40	.69	.78	.83	.90	.96	.70	.66
	IgG	.03	.71	1.35	1.26	1.93	1.86	.95	1.80	2.39
	IgM	.003	.064	.092	.132	.098	.149	.117	.117	.124
67	Total Ig	.42	1.03	.93	.97	1.27	1.36	1.16	1.21	.92
	IgG	.17	.92	1.44	1.13	1.77	1.57	1.33	1.13	1.28
	IgM	.040	.044	.059	.081	.095	.150	.129	.064	.069

TABLE V

SERUM IMMUNOGLOBULIN CONCENTRATIONS IN CALVES THAT RECEIVED COLOSTRUM AND K-ISOBUTYRATE (GRAM/100 ML)

Calf No.	Ig	Age in Hours								
		0	4	8	12	16	18	24	48	72
48	Total Ig	.15	.35	.63	.46	.94	.99	.73	.73	.97
	IgG	.02	.17	.54	.28	.61	1.25	.39	1.55	.67
	IgM	.001	.001	.097	.104	.111	.111	.128	.073	.068
76	Total Ig	.20	.30	1.20	.81	.91	.92	.80	.40	.60
	IgG	.06	.87	1.00	.57	1.35	1.45	.93	1.75	.02
	IgM	.001	.020	.052	.094	.082	.094	.114	.094	.067
580	Total Ig	.13	.15	.18	.26	.72	.41	.36	.44	.19
	IgG	.00	.04	.02	.11	.17	.33	.54	.24	.07
	IgM	.031	.040	.038	.056	.064	.048	.041	.078	.041
588	Total Ig	.07	.16	.37	.46	.50	.32	.56	.54	.61
	IgG	.01	.12	.59	.81	1.17	1.38	1.22	1.26	1.13
	IgM	.002	.040	.059	.059	.063	.077	.082	.064	.057
56	Total Ig	.12	.53	.53	.41	.51	.46	.57	.50	.45
	IgG	.00	.34	1.00	.95	.85	1.70	.31	.20	.14
	IgM	.002	.069	.110	.096	.090	.102	.053	.060	.064
567	Total Ig	.18	.38	.64	.67	.97	.80	.91	.93	.87
	IgG	.01	.16	1.19	.97	1.05	1.36	1.14	1.25	.78
	IgM	.001	.033	.069	.069	.060	.094	.087	.069	.044

TABLE V (Continued)

Calf No.	Ig	Age in Hours								
		0	4	8	12	16	18	24	48	72
39	Total Ig	.21	.43	.58	.66	.73	1.00	1.08	.80	.66
	IgG	.06	.86	1.00	.57	1.35	1.45	.93	1.75	1.57
	IgM	.028	.049	.064	.094	.113	.106	.113	.113	.052
582	Total Ig	.09	.22	.46	.98	.69	.72	.71	.60	.60
	IgG	.01	.27	.21	.13	.67	1.04	.94	.87	.17
	IgM	.001	.041	.060	.050	.035	.076	.030	.041	.038
52	Total Ig	.24	.42	.69	.77	.95	.97	.94	.88	.90
	IgG	.01	.27	.77	.48	1.62	.30	.77	.66	.86
	IgM	.001	.057	.062	.092	.138	.100	.108	.092	.072
360	Total Ig	.18	.48	.70	.82	1.07	1.13	.91	.70	.60
	IgG	.66	.21	.55	.69	1.20	.46	1.32	1.15	.63
	IgM	.038	.073	.094	.101	.122	.107	.094	.088	.088
569	Total Ig	.22	.67	.56	.65	.61	.54	.43	.29	.31
	IgG	.06	.16	.18	.16	.94	1.64	.87	.36	.55
	IgM	.028	.041	.047	.047	.051	.106	.078	.049	.043

TABLE VI
GENERAL INFORMATION ABOUT CALVES

Calf No.	Birth	Breed	Sex	Weight (kg)	Treatment group ^a
360	10-11-72	Hol.	F	34.5	TRT.
29	10- 5-72	Ayr.	M	44.1	CONTR.
566	10-24-72	Ayr.	F	33.6	CONTR.
561	10-12-72	Hol.	F	42.3	CONTR.
567	10-25-72	Hol.	F	50.0	TRT.
37	10-12-72	Ayr.	M	33.6	CONTR.
39	10-18-72	Hol.	M	49.1	TRT.
48	11-22-72	Ayr.	M	37.3	TRT.
569	10-26-72	Hol.	F	38.2	TRT.
51	11-23-72	Hol.	M	40.9	CONTR.
52	11-26-72	Hol.	M	38.6	TRT.
55	11-30-72	Ayr.	M	40.0	CONTR.
580	12-11-72	Ayr.	F	39.1	TRT.
61	1-10-73	Hol.	F	49.1	CONTR.
56	12-12-72	Ayr.	M	35.0	TRT.
57	12-17-72	Ayr.	M	29.5	CONTR.
582	12-17-72	Hol.	F	34.1	TRT.
67	1-29-73	Hol.	M	45.0	CONTR.
588	1- 6-73	Ayr.	F	32.2	TRT.
593	1-24-73	Hol.	F	45.0	CONTR.
76	2- 2-73	Ayr.	M	40.5	TRT.
594	2- 7-73	Hol.	F	49.1	CONTR.

^aTRT = K-isobutyrate + colostrum. CONTR. = water + colostrum.

TABLE VII
PEAK ABSORPTION PERIODS

Treatment ^a	Calf No.	Time of Maximum Ig Level (hr.)	Maximum Ig Level Attained In Blood mg/100 ml	Time of Maximum IgG Level (hr.)	Maximum IgG Level Attained In Blood mg/100 ml	Time of Maximum IgM Level (hr.)	Maximum IgM Level Attained In Blood mg/100 ml
CONTR.	57	16	410	4	1115	20	38
CONTR.	55	8	710	8 & 20	1375	8 & 20	128
CONTR.	29	48	970	16-24	1414	16	114
CONTR.	37	20	1620	48	3345	12 & 72	197
CONTR.	566	48	910	48	1628	24	178
CONTR.	51	24	700	4	575	72	206
CONTR.	593	48	1590	24	2191	24	255
CONTR.	67	20	1360	16	1774	20	150
CONTR.	594	24	1730	20	1868	24	142
CONTR.	561	16	1520	48	2651	24	213
CONTR.	61	24	960	72	2389	20	149
TRT.	48	20	990	48	1548	24	128
TRT.	76	8	1200	20	1835	24	114
TRT.	580	12	810	24	543	48	78
TRT.	588	72	610	20	1277	24	82
TRT.	56	24	570	20	1704	8	110
TRT.	567	16	970	48	1245	24	86
TRT.	569	4	670	20	1642	20	105
TRT.	39	24	1080	48	1754	16 & 24-48	113
TRT.	582	12	980	20	1039	20	75
TRT.	52	20	970	16	1623	16	137
TRT.	360	20	1130	16	1203	16	122

^aTRT = potassium isobutyrate. CONTR. = water.

TABLE VIII
HEMATOCRIT (%) VALUES OF CALVES FROM BIRTH TO 72 HOURS

Calf	0	4	8	12	16	18	24	48	72
	<u>Age in Hours</u>								
Holstein 360	27.5	23.0	21.5	25.8	22.0	21.5	27.0	22.0	22.5
Holstein 567	45.5	46.1	44.0	47.0	42.5	44.0	44.0	44.5	39.5
Holstein 39	45.0	37.5	35.2	35.5	33.6	35.0	36.0	38.0	36.5
Ayrshire 48	43.0	37.5	39.5	41.0	39.0	37.0	37.0	36.0	39.0
Holstein 569	42.1	33.0	31.1	31.0	31.0	30.5	31.5	31.0	31.1
Holstein 52	38.0	33.0	32.0	33.0	31.0	32.1	30.5	32.0	31.5
Ayrshire 580	45.1	46.0	42.1	48.0	51.5	53.0	50.1	55.0	46.5
Ayrshire 56	54.5	48.1	53.5	56.0	57.0	62.1	55.0	58.0	63.0
Holstein 582	34.5	31.0	34.5	36.0	28.6	27.5	29.5	28.0	29.0
Ayrshire 588	40.0	41.1	41.0	44.5	45.5	42.0	46.0	43.5	43.0
Ayrshire 76	37.0	37.0	37.0	35.0	34.0	33.0	33.0	33.5	32.5
Ayrshire 29	31.5	34.6	28.5	31.3	31.2	30.1	30.9	32.3	31.2
Ayrshire 566	46.0	47.1	46.5	47.5	41.0	46.0	46.9	46.2	44.0
Holstein 561	49.5	46.0	46.0	47.1	43.9	45.0	47.5	46.1	46.2
Ayrshire 37	48.5	44.0	43.5	44.0	44.0	44.0	44.5	46.3	46.0
Holstein 51	44.0	36.0	36.0	35.0	36.0	39.0	37.5	39.0	34.0
Ayrshire 55	40.1	41.2	38.5	43.0	40.5	37.0	37.5	39.5	41.5
Holstein 61	42.0	48.1	46.2	45.0	47.0	50.5	50.0	45.5	46.5
Ayrshire 57	33.5	31.0	27.5	29.5	28.0	29.0	26.0	27.0	25.0
Holstein 67	44.0	38.5	37.0	37.5	34.1	44.0	41.0	34.0	34.0
Holstein 593	37.0	37.5	34.5	35.0	33.5	32.5	32.0	36.0	32.5
Holstein 594	53.0	44.0	40.0	44.0	43.0	44.0	47.0	41.5	44.0

TABLE IX
ANALYSIS OF VARIANCE FOR TOTAL GAMMAGLOBULIN,
IgG AND IgM IN CALVES

Source	df	Total Gammaglobulin MS	IgG MS	IgM MS	Efficiency MS
Main Plot Analysis	21				
Treatment	1	1.460*	629878*	32327**	675*
Breed	1	1.822**	395313	3406	759
Treatment x Breed	1	.019	6207	1297	98
Animal (Breed x Treatment)	18	.417	1744173	5823	165
Sub-Plot Analysis ^a	176				
Period	8	1.045	3308721	22045	
Period x Treatment	8	.054	194895	1799	
Period x Breed	8	.061	72782	738	
Period x Breed x Treatment	8	.013	97693	845	
Animal x Period (Breed x Treat- ment) ^b	144	.032	158072	693	

^aError mean square for testing treatment, breed, and treatment x breed.

^bError mean square for testing period and all interactions with period.

*** P < .01

** P < .05

* P < .10

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