PRENATAL AND POSTNATAL IMMUNE
RESPONSES OF THE BEEF-TYPE CALF
TO ESCHERICHIA COLI 026:K60:NM

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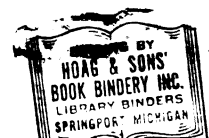TO ESCHERICHIA COLI 026:K60:NM

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ABSTRACT

PRENATAL AND POSTNATAL IMMUNE RESPONSES OF THE BEEF-TYPE CALF TO *ESCHERICHIA COLI* 026:K60:NM

By

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The humoral and local intestinal responses of fetuses and neonates to *Escherichia coli* 026:K60:NM were studied in 26 crossbred beef-type calves. *Escherichia coli* bacterin (5.0 x 10^{10} organisms) was injected *in utero* by a nonsurgical technique into the amniotic fluid of 17, 7- to 8.5-month-old fetuses (principals). Saline was injected in the same manner into 9 control fetuses. The colostrum-free newborn calves were removed from their dams after natural delivery and were maintained in separate pens and fed a milk replacer diet. Calves were divided into 10 unequally sized groups. Calves in one principal and one control group were each given an oral challenge inoculum at birth. Similarly, calves in one principal group (reimmunized at birth) and one control group each were challenged at 3 days of age. The challenge doses were 1.5 x 10^{10}, 1.0 x 10^{11} or 1.5 x 10^{11} live, unwashed homologous *E. coli* organisms. Calves were euthanatized at 1.5 to 5 days after challenge. Calves of the remaining 3 principal groups were either euthanatized at birth or were reimmunized at birth and euthanatized at 5 to 7 days or at

2 weeks of age. Control calves (3 groups) were also euthanatized at the above corresponding times.

Immunologic tests included direct bacterial and passive hemagglutination (PHA) of sera and intestinal washings; hemolytic plaque assays of intestine, mesenteric lymph nodes and spleen; and single radial immunodiffusion, radioimmunoassay and qualitative immunoelectrophoresis of sera. Antiglobulin, monospecific against bovine IgM, IgG_1 or IgG_2 and antiglobulin against whole bovine serum were produced in guinea pigs or rabbits and used in the appropriate immunologic tests. Other tests included histologic and bacteriologic examination of tissues and partial compositional analysis of amniotic fluids.

The resistance of calves to challenge was a function of previous *in utero* injection of bacterin, age when challenged and the dose of challenge organisms used. Clinical disease was observed after inoculation of newborn calves with the intermediate or the high challenge dose in one principal and one control calf, respectively. The low challenge dose failed to produce clinical disease in either the newborn principal or the control calves. Clinical disease was not observed after giving the high challenge dose to 2 other newborn principal calves or in the 3-day-old principal calves, previously reimmunized at birth, or in the 3-day-old control calves. Clinical signs after challenge were those associated with colisepticemia and toxemia. The gross and histologic lesions, however, were mild and not consistent with those usually associated with septicemia. Bacteriologic examination of tissues from the challenged calves revealed a light growth of *E. coli*.

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Principal calves, tested at birth, had serum PHA titers against the O26 antigen of *E. coli* ranging from 0 to 512, whereas all but 2 control calves were negative. It was suspected that one of the control calves had suckled and the passively acquired colostral antibody was the most probable cause for the PHA response to the test antigen. The PHA titers of intestinal washings against the same antigen were either low or negative. Similarly, the PHA titers of serum and intestinal washings against the K60 antigen of *E. coli* were either low or negative. Qualitative and quantitative analysis by radioimmunoassay of sera from selected calves revealed that the antibody activity against the O26 antigen was primarily IgM although there was also increased activity of either IgG_1 or IgG_2 or both. Immunoelectrophoretograms confirmed the presence of these classes of antibody when compared to whole bovine serum.

Reimmunization with bacterin and use of the high challenge dose given to both principal and control calves caused either a conversion of serum from negative to sero-positive or as much as a 4-fold increase in the PHA titers. The intermediate or low challenge doses caused little or no increase in the PHA serum titers. Sera from all but one of the calves was serotype specific for the O26 antigen and did not react by the PHA test with 4 other O serotypes.

Results of the hemolytic plaque assay provided evidence of the important role of IgM in the early development of the local intestinal immune system. IgM producing cells predominated and were nearly evenly distributed throughout the intestinal tissues and corresponding mesenteric lymph nodes of the majority of the calves tested.

The plaque forming response in the spleens tested was weak and inconsistent compared to the intestinal tissues. There was no direct relationship between the PHA titers of intestinal washings and the plaque forming assays from intestinal segments. Reimmunization with bacterin at birth had some stimulatory effect on the plaque forming responses whereas the major increases in plaque forming activity in the intestine occurred after challenge with the high dose. The intermediate or low challenge doses stimulated either the plaque forming or the humoral immune responses whereas the high challenge dose stimulated both responses. The principal target organs for antigenic stimulation by the test organisms were the intestine and the mesenteric lymph nodes, and these immunocompetent organs were primarily responsible for the immune responses observed.

Immunoelectrophoretograms of serum from all control calves tested at birth showed the presence of low levels of IgG_1 and/or IgG_2. This gamma globulin probably represented natural or nonspecific antibody. Immunoelectrophoretograms also showed the presence of an unidentified gamma globulin in whole bovine serum which may represent a subclass of either IgG_1 or IgG_2.

PRENATAL AND POSTNATAL IMMUNE RESPONSES OF THE BEEF-TYPE

CALF TO *ESCHERICHIA COLI* 026:K60:NM

By

David P. Olson

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INTRODUCTION

Neonatal diseases of young calves cause great economic losses

to the livestock industry each year. Associated with many epizootics

in young calves are various pathogenic strains of *Escherichia coli*

(*E. coli*). The disease producing strains of *E. coli* are either

primary or secondary pathogens and may cause enteritis or septicemia.

Enteric colibacillosis, or calf scours, is probably the most common

disease of young calves resulting in eventual death or reduced growth

rate and stunting. Colisepticemia is also common in young calves

and often results in acute deaths.

Little progress has been made in developing effective chemo-

therapeutic drugs to directly inhibit or kill pathogenic strains of

E. coli. The apparent failure in developing reliable and effective

chemotherapeutic drugs for treatment or prevention of colibacillosis

may be due to several factors, such as 1) the complex pathogenesis

of the disease, 2) development of resistance to drugs by the

organisms, and 3) nonspecific mode of action of many of the commonly

used drugs.

Considerable interest and important advances have developed in

the field of immunobiology during the past 15 years. As a result,

much has been learned about the ontogeny of the humoral and cellular

immune systems of man and animals. Bovine fetuses are capable of

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developing detectable immune responses to a variety of antigens. The purpose of the present research was to study the immunocompetence of bovine fetuses and neonates to *E. coli* and to evaluate prenatal and postnatal immunization as a means of preventing infection.

OBJECTIVES

The objectives of this research were:

1. To induce a primary intestinal and humoral immune response in bovine fetuses to a killed inoculum of *Escherichia coli* (*E. coli*) 026:K60:NM bacterin by *in utero* injection into the amniotic fluid.

2. To induce a secondary intestinal and humoral immune response in bovine neonates by oral reinjection with the homologous organism.

3. To compare the resistance to oral challenge of neonatal calves previously injected *in utero* with either *E. coli* bacterin or saline or reinjected with bacterin at birth.

4. To quantitate and qualitate the immune responses of calves by selected immunoassays.

REVIEW OF LITERATURE

Escherichia coli Infection in Calves

Colibacillosis is a term used to identify a group of diseases
of domestic livestock, poultry and man. These diseases are either
caused by or are associated with several pathogenic strains of
Escherichia coli (*E. coli*) which is a gram-negative, rod-shaped
bacterium, classified under the Family *Enterobacteriaceae*. Jensen,
in 1893, was the first to observe the association of *E. coli* with
white scours in calves (Sojka, 1965b). Colibacillosis usually
refers to the disease as it occurs in the young animal and is often
regarded as one of the most important clinical infections of neo-
natal calves, lambs and piglets. Newborn animals can be exposed
to pathogenic strains of *E. coli* from the environment at a very
young age, even when the fetus is passing through the birth canal
at parturition (Smith and Orcutt, 1925; Dunne *et al.*, 1956; Gay,
1965). Gay (1964a) stated that under most ordinary farm condi-
tions, it was impossible to successfully isolate the newborn calf
from contamination from the herd environment. The use of the term
colibacillosis has more recently been broadened to include other
diseases of young animals such as gut edema in weaned pigs and
infection of young birds (Barnum *et al.*, 1967). *Escherichia coli*
can also cause infections of the mammary gland and urinary tract

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of adult animals. The incidence of these other types of infections in older animals is generally infrequent and the term colibacillosis is usually not applied to them.

Colibacillosis has been recognized since the eighteenth century as an economically important disease of calves. A number of investigators have recorded the incidence of colibacillosis and other causes of mortality in calves from various countries throughout the early 1900's (cited by Sojka, 1965b). A review by Lovell (1955) also included a survey of other published data on the early incidence of colibacillosis in calves. The conclusion was that colibacillosis caused the most serious economic losses in newborn animals. Gay (1965) stated that diseases in young calves are responsible for economic losses and that colibacillosis caused by *E. coli* is the most common neonatal infection. Similarly, Sojka (1965b), referring to colienteritis as calf scours or white scours, stated that this disease was probably the most common disorder of young calves. This opinion was also supported by Amstutz (1965) and Fey (1972).

Colibacillosis in young calves has been divided into three forms according to clinical signs, bacteriology and possible pathogenesis (Gay, 1965). The 3 forms include: 1) septicemia or colisepticemia, 2) enteritis, white scours, infectious calf scours or colienteritis, and 3) enteritis-toxemia. The clinical signs of each of the 3 forms may at times be distinctive enough to be helpful in making a differential diagnosis (Gay, 1965). The clinical signs of colisepticemia in young calves are those of an acute disease and

include sudden anorexia, rapid dehydration, increased cardiac and respiratory rates, prostration and death. Diarrhea may or may not accompany the acute clinical episode. The predominant clinical signs of the enteric form are mild to severe diarrhea, anorexia and dehydration. The feces have a pasty to watery consistency and are also malodorous and colored yellow to grey-white. Calves may die after several days of scouring or they may slowly recover but remain unthrifty. The enteritis-toxemia form is characterized by sudden collapse, extreme prostration and death within 6 to 16 hours after clinical onset. There is no diarrhea or bacteremia but, rather, a massive proliferation of mucoid strains of *E. coli* of the A type of K antigen in the small intestine (Fey, 1972).

The gross lesions of the three forms of naturally occurring colibacillosis are not distinctive but variable and dependent on the severity of the clinical disease. Often there are signs of generalized dehydration with sunken eyes, smearing of diarrhea fluid around the perianal region and distention of the intestine with pasty to watery, straw-colored feces. The microscopic lesions of colisepticemia may include multiple petechial and ecchymotic hemorrhages of the spleen, epicardium and endocardium, catarrhal enteritis and enlarged, hemorrhagic lymph nodes. A fibrinous peritonitis and polyarthritis may also be seen. In cases of colisepticemia resulting in acute deaths, there may be few of the above lesions. In addition to the above lesions, purulent meningoencephalitis has been reported in animals that have lived for several days with colisepticemia. In mild cases of colienteritis, the only

lesions are congestion of the lungs, intestine and mesentery. More severe colienteritis results in either a catarrhal, mucopurulent or hemorrhagic enteritis (Barnum *et al.*, 1967). Osborne (1967) used multiple injections of a composite inoculum of *E. coli* to produce experimental colibacillosis. The lesions included severe gastroenteritis and multiple lesions of the microvasculature such as vascular stasis, thromboemboli, edema and hemorrhage.

There are reports of natural and experimental cases of colibacillosis in calves in which most or all of the common dairy and beef breeds of cattle are affected (Amstutz, 1965). This would suggest "...that colibacillosis is a disease that affects calves of every breed" (Fey, 1972). There seems, however, to be a common impression among many researchers of calf diseases and veterinary laboratory diagnosticians that colibacillosis occurs more frequently in calves of the dairy breeds than in the beef or dual-purpose breeds of cattle. This would further suggest that dairy calves are more susceptible (less resistant) to colibacillosis than beef or dual-purpose calves and that the latter may have some sort of natural resistance to infection. Sojka (1965b) has stated that "There appears to be a lower death rate in beef and dual purpose herds..." when referring to calf mortality in general rather than specifically to colibacillosis. There is little information to support an hypothesis of a possible interbreed difference in susceptibility or resistance to colibacillosis (Gyles, C. G., personal communication, 1974). Fey (1972) cited an example in Denmark of a higher incidence of colisepticemia in one dairy breed

as compared to another. A recent survey (Olson, D. P., unpublished data, 1975) of data from The Missouri Veterinary Diagnostic Laboratory showed a significantly higher (P = <0.05) incidence of colibacillosis in dairy calves compared to beef or dual-purpose calves examined from 1970 through 1973. Additional information such as sex, management factors and colostrum intake was not available.

The clinical signs of infectious diseases caused by other bacteria, viruses, protozoa or parasites may be confused with those of colibacillosis and make a differential diagnosis difficult. For example, "...white scours, diarrhea in the newborn calf, infectious calf diarrhea, etc., are not necessarily coli infections" (Fey, 1972). Likewise, acute septicemias caused by *Streptococcus, Diplococcus, Pasteurella* and *Salmonella* spp. may also appear clinically similar to colisepticemia (Gay, 1965). A definitive diagnosis in these cases must be made by isolation and identification of the primary causative organism(s) whenever possible.

Theobald Smith was one of the early workers to study the bacterial flora of calves affected with diarrhea and confirmed the importance of *E. coli* as a causative organism. However, further research since that time has shown that there are a number of other noninfectious factors which can contribute to the cause of colibacillosis and illustrate the complexity of this disease syndrome. Accordingly, colibacillosis has been referred to as a complex of etiologies rather than an entity by itself (Lovell, 1955; Dunne *et al.*, 1956). Other factors which may influence the incidence of colibacillosis include those directly affecting the host, such as

age, species, genetics, nutritional levels of the dam and calf,

pre-existing disease and season of the year. Included are husbandry

factors such as sanitation, crowding, cold and dampness and feeding

equipment (Reisinger, 1965; Barnum *et al.*, 1967).

Nonpathogenic *E. coli* are normally found in the intestinal

tract of animals and man along with other microorganisms and

protozoa, and together they comprise the normal intestinal micro-

flora. Consequently, *E. coli* are routinely isolated from the

intestine and feces of calves that are sick or have died from

diseases similar or dissimilar to colibacillosis.

> "Because of this, it is extremely difficult to assess the
> significance of an isolation of *E. coli* from a specimen,
> and there is even greater difficulty in assessing reports
> in the literature dealing with such isolations."
>
> (Gay, 1965).

It became evident that laboratory methods were necessary to provide

for a classification scheme between and within pathogenic and non-

pathogenic groups of *E. coli*. Methods for classification of *E. coli*

organisms into different strains have been developed and are based

on serological typing of the dominant antigens of the organisms,

namely the somatic (O), capsular (K) and flagellar (H) antigens.

Briefly, unknown isolates are reacted separately with typing anti-

sera of known specificity against particular antigenic determinants.

After suitable incubation, test samples are examined for presence

or absence of agglutination (Glantz *et al.*, 1959; Edwards and Ewing,

1962; Sojka, 1965a; Glantz, 1971). The current scheme of serological

typing of *E. coli* is based on the early work of Kauffmann (1947)

and presently includes 150 O, 91 K and 49 H serotypes (Ørskov,

1971). Extensive works by Glantz *et al.* (1959), Sojka (1965a) and Glantz (1971) have provided useful information regarding the nature, specificity and relationship of *E. coli* antigens. Other methods of biotyping *E. coli* include phage typing, colicin production and hemolysin production (Fey, 1972). Fermentation experiments have also been used to achieve a more precise identification of different serotypes of *E. coli* (Fey, 1957b). The serotypes and biotypes remain constant for any given isolate (Fey, 1972). Complete typing of *E. coli* isolates according to the Kauffmann-Knipschildt-Vahlne serological scheme makes it possible to show that certain serotypes of *E. coli* are more often associated with the various forms of colibacillosis. Researchers have found that the septicemic form of colibacillosis is usually associated with an *E. coli* bacteremia and that pure cultures of the invasive strain of the organism can usually be isolated from many internal organs (Gay, 1965), including joint cavities and leptomeninges (Fey, 1972), but excluding intestine. Fey and Margadant (1961a) elucidated the pathogenesis of colisepticemia by studying the distribution of the septicemia-producing organisms in different organs. By culturing selected organs and using the slide agglutination test, they found that most of the organs tested, including urine, were positive for the septicemic strains.

The O antigens, or endotoxins, are not single antigens, but rather are composed of several antigenic components. They are therefore often referred to as O group antigens (Gay, 1965). In *Enterobacteriaceae*, the O antigens are thermostable and include

polysaccharide-phospholipid complexes and protein fractions (Sojka, 1965a). The O antigens are an integral part of the cell wall of many pathogenic and nonpathogenic organisms and can be liberated in soluble form by the use of solvents, heating or rapid freeze thawing (Sojka, 1965a). Going and Kaiser (1969) found that the endotoxin complex can be extracted from the bacteria in the amount of 5 to 10% by weight. The complex was found to contain 45 to 60% polysaccharide, 5 to 15% lipoid A, 15 to 20% protein and 10% lipoid B. The toxic activity was associated with the entire endotoxin complex and also the polysaccharide-lipoid A fraction. Certain antigenic components may be present in more than one O serotype resulting in antigenic relationships between related O serotypes. As a result, certain O specific antisera and O antigens will cross-react either reciprocally or unilaterally (Sojka, 1965a). According to field investigations of colibacillosis in calves in several countries, certain O serotypes have been isolated from serum and internal organs in pure culture and have predominated in cases of colisepticemia. Predominant serotypes included O78, O15, O115, O8, O9, O117, O86, O137, O55 and O101 (Fey, 1972). The O26 serotype was also reported by Bokhari and Ørskov (1952), Willinger and Mathois (1965) and Glantz (1971) as being associated with naturally occurring cases of colisepticemia. In contrast, it was found by Glantz *et al.* (1959) that O26 as well as several other O serotypes caused a typical fatal scours when experimentally fed to colostrum deprived calves. Smith (1962) stated that

"Studies on the *E. coli* strains recovered from the blood
and the results of experimental oral infections indicated
that the essential property of a potentially bacteriaemic
strain was the ability to grow in fresh pre-colostral
serum. Bactericidal substances in the serum of colostrum-
fed calves appeared to play an important part in protect-
ing them against invasion by *E. coli*."

Gay (1965) has also tabulated many of the above serotypes and other

serotypes as being important in association with colisepticemia

but expressed caution in inferring that only a relatively few O

groups are selectively and highly pathogenic for calves. He further

stated that

"...the significance of the *E. coli* isolations from
calves can be truly assessed only by a comparison with
the distribution of these O groups in the normal *E.
coli* environment of the calf."

The K antigens are a class of thermolabile polysaccharides which

occur as envelopes, capsules, or sheaths around the bacterial cell

wall. They also inhibit the agglutination of the live suspensions

of *E. coli* with homologous antiserum (Sojka, 1965a). The K surface

antigens have been subdivided into L, A and B varieties according

to differences in heat stability and agglutinin-binding capacity

(Sojka, 1965a). Normally, serotypes of *E. coli* containing K antigen

have either the L, A or B form present (Sojka, 1965a). However,

Ørskov *et al.* (1961) have shown a new type of antigenic variation

in the K antigens and also that certain *E. coli* strains can possess

more than one form of K antigen. Less information is available

regarding the direct association of the different K serotypes of

E. coli with the various forms of colibacillosis in calves despite

the fact that "The majority of strains of *E. coli* isolated from

cases of colibacillosis possess K antigens" (Gay *et al.*, 1964a).

There has been some confusion in the literature regarding the association of K antigen with pathogenicity. For example, early work by Wramby (1948) showed that 76.1% of *E. coli* strains isolated from calves with colisepticemia had no K antigen as determined by slide agglutination with homologous O serum. Later, Fey (1957a) showed that 98.9% of O groupable strains from calves with colisepticemia had K antigen. He further pointed out that the surest method to show absence of K antigen is if the living strain absorbs out only O antibodies from an homologous OK serum leaving K antibodies unabsorbed. This would explain the results reported by Wramby (1948) as being incorrect when the only criterion used for assuming the presence of K antigen was O inagglutinability. Besides, "...O inagglutinability is quantitatively dependent on the presence of more or less K antigen rather than an all or none-phenomenon" (Fey, 1972). An alternate explanation of Wramby's results was offered by Gay (1965), who stated, "...it is quite possible that calves which died from conditions other than colibacillosis were sent to him for examination." Briggs (1951) reported serological evidence relating pathogenicity of several strains of *E. coli* with K antigens present. Gay (1965) has cited several examples of an increase in virulence of pathogenic strains of *E. coli* with K antigen present when tested *in vivo* in laboratory animals. Strains of *E. coli* with no K antigen were less virulent. Jones and Rutter (1972) reported that K88 positive strains of *E. coli* (Stirm *et al.*, 1967) adhered to the mucosa of the small intestine of gnotobiotic piglets, whereas K88 negative strains did not adhere. The K88 negative strains were

distributed throughout the intestinal lumen. Their conclusion was
that the adhesion activity is essential for the virulence of K88
positive bacteria in conventionally reared piglets. Kauffmann
(1947) concluded in his early work that the K antigens were
important. He found that *E. coli* strains with K antigens were more
toxic than strains without K antigen, especially when the strains
were isolated from pathogenic material. The toxicity of the K
strain was constant and characteristic but differed in the indi-
vidual types of an O group. Furthermore, he found that K antigens
were particularly resistant to the defensive forces of the host and
also to bacteriophages. Overall, there are variations in the patho-
genicity of various strains of O or K serotypes such that some
strains may be pathogenic and others nonpathogenic. Also, some
serotypes may be pathogenic for one host species and nonpathogenic
for another (Gay, 1965).

There have been few reports regarding the development and dis-
tribution of the normal intestinal flora of young calves. In
contrast, a number of studies have been reported on the develop-
ment of the normal intestinal flora of the pig (Gay, 1965). One
study (Smith and Crabb, 1961) showed similarities in the initial
development and various alterations of the fecal flora in all young
animals of the same species studied including calves. Several
factors influence the relationship and distribution of the normal
intestinal flora and they include age, diet, pH, oxidation-reduction
potentials and antagonism and synergism between and within various
bacterial species present (Gay, 1965). Furthermore, near maximum

numbers of bacteria such as *E. coli, Streptococcus* spp., *Clostridium perfringens, Lactobacillus* spp. and *Bacteroides* spp. are known to colonize the intestine by the second day of life. Less information is known about the distribution of pathogenic strains of *E. coli* in the intestinal tract of calves with colisepticemia and particularly colienteritis. A study by Fey (1957b) suggested that infectious strains of *E. coli* are not ubiquitous in the intestinal tract or environment of calves but, when present, they seem to be selectively pathogenic for newborn calves. In studies by Wramby (1948) and Fey and Margadant (1961a) it was found that the strains of *E. coli* isolated from many of the internal organs of calves with colisepticemia differed from the strains isolated from the intestine. In these cases the serotype of *E. coli* causing the septicemia could not be isolated from the feces. The conclusion was that the feces are not always responsible for the spread of infection. Gay (1965) has stated that "There is little significant data on serotypes isolated from the enteric form of colibacillosis..." and, furthermore, "These strains may be part of the normal flora of the calf...." Glantz *et al.* (1959) identified several strains of *E. coli* by bacteriologic methods from naturally occurring cases of colienteritis. They were unable to definitely group the strains as pathogenic or nonpathogenic on the basis of biochemical reactions.

> "The main difficulty in establishing *E. coli* as an enteric pathogen is its presence in the intestine and feces....The establishment of a new coli type as an enteric pathogen is an enormous task, but there is no doubt that additional types are potentially pathogenic, but hitherto not recognized as such....Once statistical and epidemiological evidence for pathogenicity of a given strain seems to be acceptable, one

still has to exercise great caution in branding any
coli type as the cause of enteritis...." (Fey, 1972)

Similarly, Gay (1965) has reported:

"There have been few serological studies on *E. coli*
associated with the enteric forms of colibacillosis
and the serotypes associated with these syndromes
are, therefore, largely unknown. This is partly
owing to the difficulty in determining the etiologi-
cal significance of an isolation of *E. coli* from the
intestine of a scouring calf....However,...there is
at present no means by which the enteric form of
colibacillosis can be differentiated from other
causes of diarrhea in calves. Because of this, no
great significance can be placed on reports of the
isolation of given serotypes of *E. coli* from the
intestine of individual scouring calves. If a suf-
ficient number of such isolations were made, it is
possible that a comparison of the incidence of a given
serotype in this material with its incidence in *E.
coli* isolated from the intestines of healthy calves
might give an indication of its pathogenicity."

On the basis of early work by Carpenter and Woods (1924) and

Smith and Orcutt (1925), it was assumed for many years that there

was an endogenous ascension and proliferation of pathogenic *E. coli*

organisms from the lower small intestine proximally into the upper

small intestine in cases of colienteritis. Smith (1960) and Smith

and Crabb (1961), however, made differential bacterial counts on

the feces of healthy calves and showed that *E. coli* organisms were

prevalent throughout the small intestine. Their studies also indi-

cated a similarity between the fecal flora of calves with colienteritis

and healthy calves but that changes occurred in the dominant serotypes

present during the scouring period. Smith (1962) concluded that

neither *E. coli* nor any other bacteria are directly responsible for

calf scours and that many cases of calf diarrhea are probably of

noninfectious origin and may occur as a result of harsh environmental

factors. Smith (1962) summarized as follows the evidence suggesting that neither pathogenic *E. coli* nor any other intestinal bacteria are responsible for causing scours: 1) the absence of incriminating serological findings, 2) the diversity of phage types of *E. coli* found in the small intestine of scouring calves, 3) the finding of the same types in healthy calves, 4) the phage type of an *E. coli* found in the intestine of a scouring calf may change several times during the scouring period, and 5) negative results in many trans- mission experiments. One exception to the above involved a field study of the enteritis-toxemia form of colibacillosis of calves in which several noninvasive serotypes of *E. coli* with an A-type K antigen were found in large numbers in the large and small intestine and associated lymph nodes, but not in the internal organs (Gay *et al.*, 1964b,c).

Enteric diseases in young calves can also be caused by certain viruses (Amstutz, 1965). The viral enteric pathogens that have been reported include bovine viral diarrhea (Lambert and Fernelius, 1968; Steck *et al.*, 1971; Lambert *et al.*, 1974), infectious bovine rhinotracheitis (Amstutz, 1965), parainfluenza 3 (Steck *et al.*, 1971), rhinoviruses, enteroviruses (Mayr, Kalich and Mehnert, 1964), adenoviruses (Steck *et al.*, 1971), reovirus-like agent (White *et al.*, 1970; Mebus *et al.*, 1971, 1973) and coronavirus-like agent (Mebus *et al.*, 1973). All the viruses mentioned were reported to have caused a primary enteritis. Two factors have been recognized as important in determining whether viruses infect young calves. These factors are age (Amstutz, 1965) and ingestion of protective

colostrum prior to exposure. Steck *et al.* (1971) found that high

colostral antibody titers against bovine virus diarrhea were not

protective for calves. It has been suggested, although not con-

clusively documented, that a virus and *E. coli* may both be involved

in some cases of diarrhea in young calves (Amstutz, 1965; Mebus *et*

al., 1971, 1973).

There is confusion regarding the pathogenesis of colibacillosis

which is compounded by various hypotheses and many forms of the

disease. The pathogenesis of colienteritis seems particularly

unclear because, as Gay reported (1965), there is little evidence

confirming the ability of *E. coli* to produce diarrhea in calves.

Part of the problem in assessing the significance of *E. coli* isolates

from scouring calves is due to the normal habitation of *E. coli* in

the intestinal tract (Gay, 1965). Despite this, certain strains of

E. coli have been reported (Glantz *et al.*, 1959; Gossling *et al.*,

1964) as causing scours in calves. It has been shown that there

is an antagonism or interference effect of the established intestinal

flora to the implantation and multiplication of exogenous bacteria

including enteropathogenic *E. coli*. Experimentally, it was found

necessary to suppress the normal intestinal flora with antibiotics

before infecting with the test organism (Rauss and Ketyi, 1960;

Ashburner and Mushin, 1962). Gay (1965) has suggested that the

enteropathogenic strains of *E. coli* must have an unknown mechanism

for becoming established as a part of the intestinal flora and

later causing an enteritis. The accepted hypothesis to explain the

pathogenesis of colisepticemia has been the following, as reported

by Fey (1972).

"'The infective coli strain is ingested and established
in the intestine, or endogenous colibacteria ascend
from the lower to the upper part of the intestine. For
reasons unknown they begin to proliferate massively,
causing an enteritis. The consequence of inflammation
is an increased permeability of the gut wall, which
enables the colibacteria to spread within the whole
body. Colisepticemia would then be the consequence of
the preceding colienteritis.'"

Fey and Margadant (1961a) and Fey *et al.* (1962) reported evidence

suggesting that virulent strains of *E. coli* associated with septi-

cemia do not necessarily have to be ingested and later invade the

intestinal tract to cause disease. They transected and ligated

the esophagus of 3 calves and then challenged them by the nasal

route with live *E. coli* organisms. All 3 calves died of severe

septicemia and the same strain of challenge organism was isolated

from all organs and the bile or intestine or both. The conclusions

from these studies were that 1) *E. coli* organisms can become septi-

cemic by invading the nasopharynx, 2) septicemia organisms can gain

entrance to the intestine via either the bile or the blood stream,

and 3) the enteritis seen with colisepticemia was a consequence of

the septicemia. Payne and Derbyshire (1963) showed that bacteria

can also enter the body of newborn calves from the tonsils and lungs.

The pathogenesis of the enteritis-toxemia form of colibacillosis as

reported by Gay (1964b) apparently involves a massive proliferation

of the particular strain of *E. coli* which is limited to the small

and large intestine and associated lymph nodes. Bacteremia occurred

in some cases but not with the same strain of organism that caused

the enteritis-toxemia.

Early reports by Smith and Little (1922a, 1923, 1924) and Smith

and Orcutt (1925) showed a direct association between ingestion of

colostrum and protection of calves against white scours and septi-

cemia. Smith and Little (1922b) also found some protective benefit

from the feeding of serum from normal lactating cows to calves.

This and other studies prompted them to conclude that

> "The rapid absorption into the blood of agglutinins
> towards *Brucella abortus* ingested in the colostrum
> indicated that the immunity of the calves receiving
> colostrum is due to the protective antibodies which
> tend to accumulate in the colostrum up to the time of
> parturition."

These early observations regarding the protective immune function

of colostrum were confirmed by Aschaffenburg and co-workers (1949a,b,

1951a). They fed whole colostrum, colostrum containing (non-fat)

fractions or colostrum substitutes to unsuckled newborn calves.

The calves survived when given the diets with the aqueous phase

of the colostrum containing the immune lactoglobulins, whereas the

colostrum-deprived calves either failed to grow well or died. They

also observed a poorer performance in newborn calves that were fed

postpartum lacteal secretions from cows that had been pre-milked

before parturition (Aschaffenburg *et al.*, 1951b). It was originally

shown by Orcutt and Howe (1922) and Howe (1924) and later confirmed

by others (Blakemore *et al.*, 1948; Aschaffenburg, 1949c; Reisinger,

1965; Fey, 1967) that there was a direct association between the

appearance of certain protein fractions (euglobulin and pseudo-

globulin I) in the serum of newborn calves with the simultaneous

absorption from the intestine of agglutinins and globulins of

colostrum. Studies also showed that agglutinins and globulins were

absent from calf serum when only milk was fed. Famulener (1912)
made early and similar observations of the antibody content of
colostrum by studying the hemolysins against sheep red blood cells
in the colostrum from immunized adult goats and the serum of newborn
goats after suckling. Since that time many researchers, including
McEwen (1950), Glantz et al. (1959, 1966), Fey (1962) and Gay et
al. (1964c), have demonstrated difficulty in reproducing colisepti-
cemia in calves previously fed colostrum soon after birth or within
1 or 2 hours after oral challenge. In contrast, they were able to
reproduce colisepticemia in colostrum deprived calves provided
virulent E. coli challenge strains were used. Smith (1962) reported
that diarrhea can occur in young calves that have been fed colostrum
but in such cases there was no bacteremia. Fey (1972) has stated
that

> "If colostrum is given too late it is obvious that the
> virulent colibacteria have time to establish themselves
> in the still unprotected animal and thus 'win the
> race.'"

Gay et al. (1964c) stated that the enteritis-toxemia form of coli-
bacillosis could not be reproduced experimentally regardless of
whether the calves had been given colostrum or not.

The immune lactoglobulins of colostrum and milk whey from
several animal species, including bovine, have been fractionated
and characterized electrophoretically in studies by Smith (1946a,b,
1948), Pierce and Feinstein (1965) and Deutsh (1947). The immune
lactoglobulins normally account for about 10% of milk whey protein
(Smith, 1946b) and about 55% of the total protein of colostrum at
parturition (Smith, 1946a). Smith (1948) reviewed the available

information regarding the biochemical composition and electrophoretic properties of colostral proteins and emphasized the important function of colostrum in enhancing the resistance of the newborn to infections. The transfer of immune globulins from maternal serum to the lacteal secretions begins about 4 weeks before parturition and reaches a maximum a few days before parturition (Larson, 1958). According to Pierce and Feinstein (1965) and Porter (1973a), the mammary gland had a highly selective ability to transport from the blood and concentrate in the lacteal secretions the electrophoretically fastest gamma G globulin. Therefore, the electrophoretically fastest gamma G globulin was found in lower concentration in maternal serum but in highest concentration of all the immune globulins in the colostrum. The other immune globulins of serum with fast or slow electrophoretic mobility were transported into the colostrum at different rates such that the fastest immune globulin was found in higher concentration in colostrum than in serum and the slowest immune globulin was a minor component of colostrum. The immune globulin content of colostrum rapidly decreases to a low level of activity after the first few days of lactation (Larson and Kendal, 1957; Porter, 1973). Larson and Kendal (1957) and Larson (1958) showed that in adult cattle there was a quantitative decrease in the blood serum proteins and subsequent increase in the protein content of lacteal secretions before parturition. These changes in serum were directly associated with the B_2 and the γ_1 globulin fractions and corresponded to the time that colostrum containing the immune lactoglobulins was being formed in the mammary gland. It

has been shown that the immune lactoglobulins of colostrum and the immune globulins in the serum from suckled calves have the same electrophoretic characteristics (Hansen and Phillips, 1947, 1949; Smith and Holm, 1948; Johnson and Pierce, 1959). The immune lactoglobulins of colostrum, with the 3 electrophoretic mobilities, are normally absorbed nonselectively from the intestinal lumen of the newborn suckled calf at equal rates (Pierce and Feinstein, 1965). Further, they are absorbed through the epithelium of the small intestine by the process of pinocytosis (El-Nageh, 1967) and reach the blood primarily via the intestinal lymphatics and thoracic duct (Comline *et al.*, 1951, 1952) rather than by the portal circulation. The nonselective absorption of undegraded proteins and other macromolecules from colostrum by the intestine of the suckled newborn calf normally continues for the first 24 to 36 hours of life (Hansen and Phillips, 1947; Comline *et al.*, 1951; Smith and Erwin, 1959; Gay *et al.*, 1965). Not all the immune globulins are equally absorbed throughout the entire 24 to 36 hour period. As an example, there is a 50% reduction in absorption of antibody against an *E. coli* somatic antigen by 16 hours after birth (Kaeckenbeeck *et al.*, 1961). Similarly, Gay *et al.* (1965) found that the capacity of some calves to absorb immune lactoglobulins from colostrum was lacking by 4 to 6 hours after birth. The absorbed immune lactoglobulins appear in the calf serum in from 1 to 3 hours after ingestion and reach maximum levels in 24 hours (McDiarmid, 1946). Further, they have a mean half-life ranging from 21.5 (McEwan *et al.*, 1970a) to 23 days (Colinet *et al.*, 1961) in the serum of suckled calves and

recede as a logarithmic curve over varying periods depending on the

antigen(s) they are directed against (Smith and Holm, 1948) and

the initial titer in the colostrum (McDiarmid, 1946). McEwan *et*

al. (1970b) described the use of the zinc sulphate turbidity test

on neonatal calf serum as an inexpensive, rapid and simple method

for the relative estimation of serum immune globulins. They found

a highly significant correlation between the zinc sulphate turbidity

reactions and the specific immune globulins when measured singly

and in combination. Generally, it is thought that the value of

the absorbed immune lactoglobulins is to provide rapid and specific

or nonspecific immune protection for the newborn calf against a

variety of infectious organisms. Several studies (Gay *et al.*,

1965; McEwan *et al.*, 1970a; Selman *et al.*, 1971b) have shown a

close association between serum immune globulin levels as measured

by the zinc sulphate turbidity test and death of calves from coli-

septicemia and other causes. The highest death rates were found

in the calves that had zinc sulphate colorimeter readings below

10 indicating very low or negligible serum immune globulin levels.

Calves with low immune globulin levels also seemed to be predisposed

to death from the effects of diarrhea. Other findings included

extreme variations in the average serum globulin concentrations

with different seasons of the year and the environment and manage-

ment factors affecing the newborn calves. There is no active or

passive transplacental transfer of antibodies from the maternal to

the fetal circulation during normal gestation in the bovine species,

and the newborn ruminant is either hypogammaglobulinemic (Hansen

and Phillips, 1949; Johnson and Pierce, 1959; McEwan *et al.*, 1970a)

or agammaglobulinemic (Orcutt and Howe, 1922; McDiarmid, 1946;

Smith and Holm, 1948; Lovell, 1955; Gay, 1965; Penhale *et al.*, 1970).

Therefore, the newborn calf must receive protective antibody from

the colostrum during a critical and limited period of time (Brambell,

1970) before the onset of "closure" and the beginning of selective

absorption by the intestine.

Based on experiments with laboratory animals, researchers

have shown that K antibodies in serum and coloserum prevented infec-

tion with *E. coli* (Gay *et al.*, 1964a; Barnum *et al.*, 1967). On the

basis of other studies in calves (Aschaffenburg *et al.*, 1951a; Briggs,

1951), it has become generally accepted by many that specific agglu-

tinating antibody against the K antigens of *E. coli* is the factor

in colostrum that protects calves against colibacillosis (Fey, 1971).

Gay (1965), however, disagreed with this hypothesis and inferred

that the value of K specific colostral antibody in preventing coli-

bacillosis in calves is not yet clear.

> "It is most unlikely, however, that K agglutinins are
> the factor in colostrum which protects calves against
> colisepticemia, for it has been shown in field studies
> that although calves normally receive in the colostrum
> agglutinins against the somatic antigens of *E. coli*
> associated with colibacillosis, they do not usually
> receive the agglutinins against the K antigens of
> these strains. Furthermore, colostrum-fed calves
> are resistant to experimental infection with serotypes
> of *E. coli* associated with colisepticemia regardless
> of the presence or absence in their serum of specific
> agglutinins against these serotypes. Further, it was
> ...found that the serum bactericidal activity is of
> some importance in protecting calves from colisepti-
> cemia....Although specific agglutinins do not appear
> to be the factor in colostrum which protect calves
> against colisepticemia, there is some evidence that
> the presence of specific agglutinins in the serum of

the calf protects it from the enteric form of colibacil-
losis. And finally...Although there are indications
that the presence of specific agglutinins and bacteri-
cidal activity in the serum of the calf confers resistance
to infection with *E. coli*, it is also apparent that
the full protective nature of colostrum is yet unknown."

Calves are resistant to experimental colisepticemia if they have

absorbed and acquired sufficient colostral antibody to achieve a

certain minimum level of circulating gammaglobulins. These passively

acquired circulating gammaglobulins may be serotype specific (O and

K) or nonspecific. The nonserotype specific antibody in colostrum

is postulated as the more important gammaglobulin associated with

the acquired minimum serum levels in newborn calves and also is

the same gammaglobulin that prevents colisepticemic epizootics in

all ages of calves. In support of this postulation, it has been

argued that if resistance to colisepticemia soon after birth was

totally dependent on serotype specific antibody, newborn calves would

have to passively acquire a great variety of this serotype specific

antibody in the colostrum. However, most natural colostrum probably

does not contain serotype specific antibody against all the pre-

vailing serotypes of *E. coli* which might pose a threat of exposure

to the newborn calves. Alternately, if serotype specific antibody

was not the protective antibody, newborn calves would have to be

exposed to a wide variety of pathogenic strains of *E. coli* at birth

sufficient to stimulate a primary immune response of their own which

would be detectable 10 to 14 days later. It is unlikely that this

exacting set of circumstances of multiple exposures routinely

occurs. Moreover, under such circumstances many calves would

probably die of colisepticemia. In summary, the nonserotype specific

antibody is produced in response to whatever *E. coli* serotypes happen to stimulate the adult cow or colonize the intestinal tract of the newborn calf. The nonserotype specific antibody would have universal protective properties and would normally be available to the calf in the colostrum or through *de novo* production by the calf itself. Preliminary studies showed that this nonserotype specific antibody was an incomplete antibody and seemed to fulfill the criteria for antibody which would protect calves against natural outbreaks of colibacillosis (Gay, 1971).

McEwen (1950) suggested that resistance or immunity enjoyed by calves may not necessarily be attributable to ingestion of colostrum or resistance acquired in other ways, but rather may be due to an immunity comparable to a species resistant animal.

It had been generally accepted that colibacillosis occurred in calves after exposure to a pathogenic strain of *E. coli* because the calves had acquired no protective antibody in the colostrum against that serotype. However, calves were known to have died from colisepticemia despite having been given colostrum soon after birth in the conventional manner. Fey and Margadant (1961b) and Fey (1962) pursued this problem further and discovered that a severe and persistent hypogammaglobulinemia or agammaglobulinemia was present in most colostrum fed calves that died of colisepticemia. In contrast, healthy calves, who were usually agammaglobulinemic at birth, had normal gammaglobulin levels soon after the first feeding of colostrum. These findings have been confirmed (Klaus *et al.*, 1969; McEwan *et al.*, 1970a) and have led Fey and Margadant

(1962), Smith (1962), Gay *et al.* (1965) and Fey (1971) to postulate

that one of the most important predisposing factors in the patho-

genesis of colisepticemia may be the failure of some colostrum-fed

calves to acquire gammaglobulins in their serum from the colostrum

thus resulting in persistent agammaglobulinemia or hypogammaglobulin-

emia. Not all hypogammaglobulinemic calves have died from coli-

septicemia, especially if they were never exposed to a pathogenic

strain of *E. coli* during the first several weeks of life. However,

the results would strongly suggest that hypogammaglobulinemia was

an important predisposing condition for infection with a virulent

coli strain at the critical time. Theories have been proposed

(Fey, 1966, 1971), but attempts (Fey, 1971) have yet failed, to

clearly explain the mechanism of the early loss of absorption

capacity of the intestine resulting in persistent agammaglobulinemia

in newborn calves despite colostrum feeding. In answer to the

problem of agammaglobulinemia, it has been suggested that colostrum

be fed to calves in the first 6 hours or less of life (Gay *et al.*,

1965; Reisinger, 1965; Fisher, 1971), and that at least 2 kg be

fed twice daily for 4 days (Kruse, 1969). Selman *et al.* (1971a)

investigated the feasibility of producing uniform absorption of

immune lactoglobulins in newborn calves by standardizing the condi-

tions of feeding pooled colostrum and also the conditions of calf

maintenance. Results showed a marked uniformity in the 48 hour

serum concentrations of absorbed immune lactoglobulins and that

all of the calves studied were able to absorb immune lactoglobulins.

Friesian-Ayrshire cross calves and mothered calves attained

significantly higher 48 hour serum concentrations of absorbed immune

lactoglobulins than did Ayrshire or unmothered calves, respectively.

Treatment of colibacillosis in calves has resulted in variable

success because of the different forms of the disease, age of the

host and other complicating factors. Treatment of the septicemic

form is complicated by the usual rapid course of the disease plus

the bacteremia, whereas more success has been shown in treatment of

the enteric form. Sulfonamides and a variety of antibiotic compounds

including nitrofurans, neomycin, tetracyclines, dihydrostreptomycin

and chloramphenicol have been extensively used in the past both

therapeutically and prophylactically. Field studies on white scours

in calves by Smith (1958) showed a greater number of drug resistant

strains of *E. coli* in the feces of calves that had been given drugs

therapeutically or prophylactically than in calves from herds in

which chemotherapy had not been practiced. Furthermore, it was

more difficult to establish successful chemotherapeutic control

of white scours in herds where the disease frequently occurred

because of the emergence of multiple resistant strains of *E. coli*

which eventually became the predomidant flora. It was also shown

that drug sensitive cultures of *E. coli* could be made drug resistant

in vivo. Finally, Smith (1958) established the value of basing

the treatment of white scours on prior drug sensitivity tests.

Other studies by Smith (1960) have shown that

> "Antibiotics have a profound effect on the emergence of
> resistant strains of *E. coli* in the faeces of treated
> and in-contact calves. The widespread use of these
> agents is now being reflected in the increased inci-
> dence of resistant strains in the general calf popu-
> lation."

Further,

> "The efficiency of chemotherapeutic agents in elimi-
> nating sensitive strains of *E. coli* is matched by
> the extreme speed at which resistant strains may
> replace them during chemotherapy."

In addition to antibiotics, other supportive treatment measures have

been recommended for affected calves (Radostits, 1965). In many

cases it was found that fluid replacement therapy with balanced

electrolyte solutions and a source of energy (acetate or lactate)

was also essential, particularly in treating the effects of pro-

longed diarrhea.

The successful prevention of many animal diseases often involves

implementing a variety of established methods and practices which

include improved management and sanitation, prophylactic measures

and immunization. It is difficult to place colibacillosis among

the group of diseases that readily responds to the benefits of

preventive methods because of the complex of factors that contribute

to the cause of the disease and the unsettled dispute over the exact

causative organism(s). Barnum *et al.* (1967) and Fey (1972) have

summarized the recommendations of others regarding prevention of

colibacillosis according to husbandry, management and immunization

procedures. Fey (1972) expressed caution in attributing unquali-

fied value and success to disease prevention methods without critical

evaluation by adequately controlled field trials.

Smith (1930) and Smith and Little (1930) were the first to

report the significance of the passive protection of specific hyper-

immune colostrum and adult serum titrated in guinea pigs. They

showed that colostral and serum antibody can be directed against

E. coli by prior active immunization of the dam, although the protective antibody content of serum was below that of colostrum of the same animal. Calves treated with either the specific hyperimmune serum or colostrum from the vaccinated dams withstood challenge with the homologous *E. coli* organism. Calves fed milk alone, even from hyperimmunized dams, were not protected from challenge. According to Gay (1971) there are few reports in the literature and there is still a conflict of opinion as to the value of vaccination of the dam as a means of protection of calves against colibacillosis. The reports cited by Gay (1971) were conflicting because 1) several studies were only partially controlled or had no controls, 2) spontaneous remissions of colibacillosis occurred, and 3) the criteria for establishing a diagnosis of colibacillosis in the herds studied were seldom stated. Another problem may have been that the studies were conducted without the present knowledge of the pathogenesis of the various forms of colibacillosis. Fey and Hunyady (1962) and Fey *et al.* (1963) studied the benefits of passive immunization of calves with preformed antibodies from pooled colostral whey administered parenterally. Ten of 14 calves survived challenge with a septicemic strain of *E. coli* and 4 died from diarrhea without septicemia. These experiments prompted the subsequent production and sale of commercial serotype-specific antisera for parenteral administration in calves against colisepticemia. Gay (1971) stated that this serotype-specific antisera would protect calves only against the homologous serotype of *E. coli* and would probably not be effective against most natural

epizootics involving other serotypes. More recently, Gay *et al.* (1964a) reported that a formalin-killed *E. coli* vaccine, emulsified in oil, caused significantly higher O and K antibody titers in the colostrum of vaccinated dams than in control dams. Likewise, the calves from vaccinated dams had significantly higher levels of O and K serum antibodies to the vaccine strain than did the control calves. A few calves had no demonstrable O or K agglutinins despite receiving high levels of these antibodies in the colostrum and were either hypogammaglobulinemic or agammaglobulinemic. In a subsequent field study in 8 herds, Gay *et al.* (1964b) attempted to show the efficacy of vaccination of the dams with a single strain of *E. coli* isolated from a single epizootic from each of the respective herds as a means of preventing colibacillosis in the calves. It was impossible to evaluate the protective value to the calves of specific colostral antibody for 2 reasons. First, the vaccination of the dams did not alter the incidence pattern of colibacillosis in the herds because deaths continued to occur in vaccinated and control calves of some of the herds due to strains of *E. coli* antigenically distinct from those used in preparation of the vaccines. Secondly, there was a spontaneous remission of colibacillosis in some herds due to factors other than specific immunization. Barnum *et al.* (1967) stated that evaluation of the use of colostral antibody or hyperimmune serum in the newborn against naturally occurring colibacillosis is complicated by several factors. They include: 1) spontaneous cessation of the disease in a given herd, 2) variable morbidity and mortality within and between herds, and 3) variation in pathogenic serotypes of *E. coli* within a herd.

Gay (1971) was one of the first to attempt active oral immuni-
zation of calf fetuses with a single colisepticemic serotype of
E. coli and attempt to show resistance of the same immunized neo-
natal calves to colisepticemia when challenged with other colisepti-
cemic strains of *E. coli*. Calves responded by producing serotype
specific antibody and some also produced heterogenetic antibody
to the other *E. coli* strains tested. The resistance observed to
challenge was mediated by the nonserotype specific antibody. Gay
suggested that fetal immunization without surgery could be developed
into a method of protecting calves against colisepticemia.

General Immunology

Man and animals are continually exposed to a wide variety of
foreign substances and microorganisms which may or may not be
harmful. Resistance of an immunologically competent host to harm-
ful substances or microorganisms may be due either to evoking an
immune response by the specific immune mechanisms or by nonspecific
resistance mechanisms, or both. Nonspecific or innate resistance
is determined by such factors as genetic and hormonal influences,
age and humoral and cellular mechanisms (Humphrey and White, 1971a).
The majority of the harmful substances or microorganisms which gain
entrance into a susceptible host are made harmless and are eliminated
by the phagocytic cells of the nonspecific cellular immune system.
The cell types of this system include the macrophages or the poly-
morphonuclear neutrophilic and eosinophilic leukocytes and the
macrophages represented by connective tissue histiocytes, blood
monocytes, microglial cells of the central nervous system, sinus

lining cells of the spleen, liver and bone marrow and reticulum cells of lymphoreticular tissues (Humphrey and White, 1971a). The specific actively acquired immune system has been divided into the cellular, humoral (Humphrey and White, 1971b) and the secretory immune systems (Tomasi, 1971). Much of what is known about the specific immune systems is based upon hypotheses gained from experimentation with laboratory animals. The cellular and humoral immune systems involve lymphocytic cells found in blood, lymph nodes and in lymphoid tissues. There is evidence that the cellular and the humoral immune systems are functional in the spleen and peripheral lymph nodes. The secretory immune system is confined to external secretory epithelial surfaces such as the intestinal, respiratory and urinary tracts and the lacrimal and salivary glands. It is generally agreed that there are at least 2 functionally distinct populations of lymphocytes found circulating in blood or within tissues depending upon their course of development and maturation (Feldman, 1973; Raff, 1973). These 2 populations of lymphocytes are morphologically indistinguishable, however. The cellular immune system is mediated by so-called T lymphocytes that originate from bone marrow stem cells and have undergone final maturation in the thymus. The T lymphocytes are long-lived and are found in lymphatic organs such as lymph nodes, spleen and gut associated lymphatic tissue (Raff, 1973) as well as recirculating between blood and lymph (Gowans, 1971). The T lymphocytes produce and secrete biologically potent chemical mediators (lymphokines) which affect mainly phagocytes and other lymphocytes (David, 1971; Raff, 1973).

The lymphokines have no specificity for the eliciting antigen

(Williamson, 1972). The T lymphocytes are not progenitors of anti-

body secreting cells (Williamson, 1972) and thus do not secrete

antibody in the usual sense (Raff, 1973). Cellular immunity thus

involves an interaction between sensitized T lymphocytes and macro-

phages and has been recognized by various clinical manifestations

such as delayed hypersensitivity, autoimmune diseases and graft

rejection (David, 1971; Feldman, 1973). The humoral immune system

is mediated by antibodies and involves antibody forming cell pre-

cursors or B lymphocytes (Miller and Sprent, 1971) that also

originate from bone marrow stem cells but have undergone maturation

in some organ other than the thymus (Feldman, 1973; Raff, 1973).

The B lymphocytes are also found in blood, lymph, spleen and lymph

nodes (Williamson, 1972). The humoral immune system also depends

to a certain extent on T lymphocytes as helper cells (Feldman, 1973;

Raff, 1973) and macrophages although certain antigens may stimulate

B lymphocytes directly both *in vivo* and *in vitro*. The B lymphocytes

undergo blast cell transformation and successive divisions and some

eventually differentiate into plasma cells. Plasma cells are morpho-

logically distinct from the B lymphocyte precursors. The plasma

cells are short-lived terminal cells that are found in clones in

tissues and which actively produce and secrete antibody. It is

still not clear whether one plasma cell produces antibody of only

one class or whether it may be capable of producing 2 or more classes

of antibody. Radioiodination studies have shown that both T and B

lymphocytes have antigen-specific receptors bound to their outer

cell membrane surfaces and these receptors are believed to be anti-

body molecules (Uhr and Vitetta, 1973). Further studies have sug-

gested that lymphocytes and plasma cells produce and transport

intracellular immunoglobulin in a similar manner. The question of

whether cell surface immunoglobulin and conventionally secreted

immunoglobulin are produced and transported within the cell by the

same or different processes remains unanswered (Uhr and Vitetta,

1973). It is also believed that the attachment of a critical amount

of specific antigen onto the cell surface receptors is a necessary

step leading to activation and blast cell transformation of both

T and B lymphocytes. This is believed to be important in the pri-

mary immune response because as the clonal selection theory of anti-

body formation suggests, there are many small populations of lympho-

cytes (clones) with antigen specific membrane-bound receptors on their

surface. These cell-bound receptors are present independent of any

previous antigen stimulation. When contact is made between specific

complementary antigen and the cell-surface receptors, there is a

selective proliferation of those cells capable of production of the

required antibody (Williamson, 1972; Raff, 1973). Experimental

evidence strongly supports the clonal selection hypothesis for both

T and B lymphocytes (Raff, 1973), although it has been more clearly

defined for B lymphocytes (Williamson, 1972). The results of *in

vitro* studies of lymphocytes treated with mitogenic substances

indicated that there is more to lymphocyte activation than simple

binding of an antigen to the cell surface receptors (Raff, 1973).

Once T and B lymphocytes have been primarily sensitized with specific

antigen molecules, there develops a subpopulation of both T and B
lymphocytes referred to as memory cells which affect the immune
responses to subsequent exposures to the same antigens (Miller and
Sprent, 1971). For example, the secondary humoral immune response
is often more rapid, of a greater magnitude and sometimes quali-
tatively different than the primary immune response (Raff, 1973).
It has also been suggested that immunologic memory cells may origi-
nate from stem cell precursors in the bone marrow (Miller and
Cudkowicz, 1972). The B lymphocyte memory cells retain the property
of self-regeneration as well as the ability to differentiate into
antibody secreting cells (Williamson, 1972).

Important advances in research have been made during the past
10 to 15 years toward a better understanding of antibodies and
their association with the specific humoral and the secretory immune
systems of man and animals. Knowledge gained from the isolation and
characterization of the immunoglobulins has been particularly help-
ful in explaining the mechanisms of the reactions between antigens
and antibodies (Cebra, 1969). Early work classified the plasma
proteins into the euglobulins and the pseudoglobulins depending
upon their insolubility or solubility, respectively, in distilled
water or in dilute salt solutions at or near their isoelectric
points. Other studies classified the globulin proteins of plasma
into α, b and γ fractions in order of descending mobility in an
electrical field on paper, gel or other support medium. It was
found that the γ globulins had the lowest net negative charge com-
pared to the other serum proteins when examined in an alkaline

medium and thus migrated the shortest distance toward the anode.

Also it was shown that the b and γ globulins carried the antibody

activity and were thus referred to as the immunoglobulins. Modern

techniques presently used for distinguishing the immunoglobulins of

various classes and subclasses include protein analysis and fractiona-

tion procedures such as gel diffusion, density gradient ultracentri-

fugation and chromatography, immunoelectrophoresis, gel filtration

and ion-exchange chromatography (Pierce, 1966). During the rapid

development of information concerning the immunoglobulins, there

developed some confusion regarding the nomenclature to be used when

referring to the various classes of immunoglobulins (Edelman and

Marchalonis, 1967). By subsequent agreement (Bull. W.H.O. Meeting

on Nomenclature of Human Immunoglobulins; Edelman and Marchalonis,

1967) the immunoglobulins were collectively called Ig and the major

classes were subdivided into IgG, IgM and IgA. Other minor classes

included IgE and IgD.

The molecular structures of the immunoglobulins have been

extensively studied because of an increasing interest in a better

understanding of the specificity of the reaction between antibody

molecules and antigens. Chemical degradation with reducing and

alkylating agents and limited hydrolysis with proteolytic enzymes

are 2 methods that were developed to dissociate the immunoglobulin

molecules into constituent polypeptide chains which represent subunits

of the parent molecules. These analytical methods also allowed the

subunits to retain or to be reconstituted and regain their biological

activity (Olins and Edelman, 1964; Edelman and Marchalonis, 1967;

Hammer *et al.*, 1968). Other techniques involving the structural

analysis of immunoglobulin molecules included amino acid sequencing

of single polypeptide chains. According to present understanding,

all immunoglobulins consist of 2 kinds of polypeptide chains, heavy

(H) chains and light (L) chains which are linked together by disulfide

bonds, hydrophobic bonds, and electrostatic forces.

In the bovine species, there are 3 major classes of immuno-

globulins (Butler, 1973) and the molecules of each class consist of

2 identical H and 2 identical L chains or, in some cases, multiples

thereof. In all cases, the L chains are of the same type (K or λ),

whereas the H chains of each class of immunoglobulin are structurally

and antigenically different. The property of structural and anti-

genic differences in H chains of the immunoglobulin classes is helpful

in distinguishing the different classes from one another. Immuno-

globulin G consists of 2 H and 2 L chains symmetrically arranged into

a monomeric unit (Williamson, 1972). The monomer has a sedimentation

coefficient of 7S and a molecular weight of approximately 160,000

Daltons. Two subclasses of IgG have been described in the bovine

species as IgG_1 and IgG_2, which differ in immunoelectrophoretic

mobility, biological reactions (Porter, 1973b) and mean concentrations

in normal serum and colostrum (Butler, 1973). Immunoglobulin G is

the major immunoglobulin found in normal bovine serum, colostrum

(Porter, 1973b) and other fluids that are completely confined within

the body (Tomasi, 1971). Immunoglobulin M consists of a pentamer

of 5 subunits arranged in a starfish-like configuration and linked

together by a polypeptide or J chain. Each subunit of the IgM

pentamer is in the shape of a single IgG monomer. Immunoglobulin M has a sedimentation coefficient of 19 S and a molecular weight of approximately 900,000 Daltons. Immunoglobulin M has an intermediate immunoelectrophoretic mobility and is found primarily in serum and occasionally in external secretions (Allen and Porter, 1970). Immunoglobulin A in serum consists of 1 to 3 subunits linked together by a polypeptide J chain similar to IgM (Tomasi, 1971). Immuno- globulin A is also found in most external secretions as the principal immunoglobulin of the secretory immune system (Duncan et al., 1972) and is in the form of a dimer with an additional nonimmunoglobulin component called secretory piece. The sedimentation coefficients and molecular weights of serum and secretory IgA are 6.9S and 11.4S and 170,000 and 390,000 Daltons, respectively (Tomasi, 1971).

Immunoglobulin molecules contain localized regions in the amino terminal ends of both H and L chains which help to stabilize the structure of the molecule at that site and also participate directly in the specific binding with antigen molecules (Grossberg, 1973). The antibody activity of an immunoglobulin is specified by the antigen binding site. The amino acid sequences of the antigen bind- ing regions of immunoglobulin molecules are similar (conservative region) in some areas and variable in other areas in both H and L chains (Singer and Doolittle, 1966). The contact amino acids of the immunoglobulin binding site are those that are associated directly with the specificity of the site by virtue of their being in close contact with the structure against which the site is directed (Grossberg, 1973). The contact sites of the immunoglobulin molecules

constitute only a small portion of the mass of the entire molecule, however (Singer and Doolittle, 1966). It has been suggested that the reaction between an antibody molecule and an antigen at the binding site may be due to a conformational change at some other locus of the antibody molecule (Pressman, 1973). Naturally occurring antigens consist of a variety of variable and repeating molecular configurations which may result in production of a corresponding heterogeneous population of immunoglobulins of more than one class. Kinetics and thermodynamic studies by such techniques as equilibrium dialysis, fluorescence quenching and electron-spin resonance (Kabat, 1968a) to determine the strength of the bond between antigen and antibody have shown that there is a primary point of attachment of an antigenic determinant to an antibody molecule which involves the antigen grouping that contributes the highest proportion of the binding energy. This determinant group has been called the immuno-dominant group and association constants have been determined for a number of them (Kabat, 1968b). Other studies have attempted to determine the maximum size and shapes of antigen binding sites of immunoglobulin molecules (Kabat, 1968b; Singer and Doolittle, 1966). It has been suggested that the antigen binding activity of an anti-body molecule is probably independent of its other biological func-tions such as complement fixation, reaginic activity and transport across membranes (Pierce, 1966).

Several factors have been reported as important in determining the nature and magnitude of the primary and secondary humoral immune responses to a foreign antigen. These factors include 1) whether

the antigen is living or dead, 2) whether the antigen is immunogenic, 3) the amount of antigen presented to the host, 4) the route of entry into the host, 5) the extent to which the antigen remains in the host (Humphrey and White, 1971b), and 6) the immunocompetence of the host.

Antibody formation has been divided into inductive and productive phases. The inductive phase is that period soon after introduction of an antigen when no antibody can be detected either within immunocompetent cells or in serum. It has been suggested that antibodies cannot be detected either because of the small amount formed at that time which is below the limits of sensitivity of quantitative methods or that the antibody formed is immediately complexed with excess antigen present. The productive phase of antibody production is when antibody can be detected by the usual analytical methods. The inductive phase also differs from the productive phase in that the former is more sensitive to the action of cortisone, prednisolone, X-irradiation and antibiotics (Sterzl, 1963).

In the primary immune response, immunogens will usually elicit a 19S followed by a 7S antibody response during the productive phase. The 19S response is usually more transient and of a lower magnitude than the 7S response. During this time, memory cells are generated so that the result of a second exposure to the same antigen is marked by more rapid 19S and 7S responses. Also, the total 19S and 7S antibody produced is greater than the primary response and the 7S antibody persists for a longer time (Hammer *et al.*, 1968; Humphrey and White, 1971b).

Methods in Immunology

Separation of Macromolecules by Precipitation Techniques, Chromatography on Cellulose Ion Exchangers and Porous Gels and Immunoseparations in Agar-Gel

The proteins of normal serum have been divided into the albumin and the globulin fractions based on electrophoretic mobility. Antibodies most commonly occur in the b and the γ globulin fractions of the serum. For various immunological procedures, it becomes necessary to separate the immunoglobulins from other components of serum or samples of crude biological mixtures by either specific or nonspecific methods. The specific methods of separation are based on the addition of either a soluble or insoluble specific antigen or antiglobulin to a sample resulting in the precipitation, agglutination or binding of the specific antibody. The antibody can be dissociated from the antigen by adjusting the pH or by other means and then separated (Campbell et al., 1970b). Nonspecific methods of separation are based on the physical properties of antibody molecules and usually produce high yields of low purity material (Campbell et al., 1970b). Methods frequently used for nonspecific separation of proteins include salting-out procedures and chromatography on cellulose ion exchangers and porous gels.

It has been found advantageous to initially precipitate out globulins from crude sample mixtures so as to decrease the complexity of the material to be separated later by chromatography (King, 1968). The most versatile precipitant used in salting-out procedures is ammonium sulfate (Chase and Williams, 1968). Serum globulins may be

precipitated at one-half saturation (Chase and Williams, 1968) by

slowly mixing equal volumes of sample with saturated ammonium

sulfate at 25 C (Campbell *et al.*, 1970b). The precipitated globulins

may then be dissolved in a desired buffer and the sulfate eliminated

from the sample by dialysis (Craig, 1968; Campbell *et al.*, 1970a).

Gammaglobulins can be reprecipitated several times in the same

manner to separate them in fairly pure form (Campbell *et al.*, 1970b).

The separation of proteins by cellulose ion exchange chroma-

tography is believed to depend on the electrostatic interactions of

the polyelectrolyte proteins of the sample with the oppositely

charged adsorbent (King, 1968). Proteins can carry positive and/or

negative charges or no charge at all (isoelectric point) depending

on the pH of the solvent. Most proteins have a net negative

(anionic) charge in basic solutions and will migrate in an electric

field or be attracted toward the anode, whereas the opposite is true

in an acidic solution (cations migrating in an electric field or

be attracted toward the cathode) (Pierce, 1966). The relative

affinity of the charged protein molecules for the immunoadsorbent

depends on their charge density. Two cellulose ion exchangers have

been widely used, namely DEAE-cellulose (diethyl amino ethyl linked

to cellulose) and CM-cellulose (carboxy-methyl linked to cellulose).

The DEAE-cellulose has a positive charge at pH values ranging from

neutral to about 9 and is used as an anionic exchanger, whereas

the CM-cellulose has a negative charge at pH values ranging from

neutral to 3 and is used as a cationic exchanger. The ion exchangers

are normally used as chromatographic columns equilibrated with a

selected buffer of known pH and ionic strength (Chase, 1968b)

depending upon the type of ion exchanger used and the stability of

the protein under study. Two methods are used for the sequential

displacement and elution of the sample proteins from the ion exchanger:

first by changing the pH of the solvent buffer and thereby making the

sample proteins isoelectric, and secondly by increasing the ionic

strength of the buffer which will then compete with the sample pro-

teins for binding sites on the exchanger. Gradient changes in pH

and/or ionic strength of elution buffers can be made in a step-wise

or a linear manner depending upon the critical nature of the separ-

ation. The techniques of ion exchange chromatography have been

adequately described (King, 1968).

The separation of proteins based principally on molecular size

and sometimes shape can be carried out on chromatography columns

composed of macromolecular sized beads of cross linked dextran which

is available under the trade name of Sephadex. A high degree of

cross linkage of the dextran beads creates a matrix of low porosity

which can only be penetrated by low molecular weight proteins. Con-

versely, low cross linkage allows penetration by larger molecular

weight proteins. Dextrans of different degrees of cross linkage

are commercially available and are suitable for separation of solutes

with molecular weights ranging from several hundred to several

hundred thousand. The principle behind the separation by this

method is that a dextran preparation is selected which will not

allow the larger molecules to penetrate the gel. These larger

molecules are thus excluded from the gel and are confined to the

exclusion or void volume (fluid outside the gel beads) whereby they

are eluted first. Smaller molecules enter the gel, have a larger

volume of solvent available to them and are eluted more slowly.

The efficiency of separation of solutes by chromatography on

Sephadex is dependent primarily upon the ratio of the column's

height to the diameter, the volume of sample and the elution rate.

High ratio columns operated at slow elution rates and charged with

sample volumes no greater than 1% of the total bed volume result

in the most complete separations. For these reasons, sample elutions

often require 1 to several days and are often carried out in a cold

room to avoid natural fluctuations in ambient temperature and growth

of contaminating microorganisms in the dextran exchanger or eluant.

Generally, chromatography on Sephadex columns is insensitive to

slight changes in pH and ionic strength of the eluant. Proper choice

of an eluant depends on the stability of the sample in the eluant

and the stability of the eluant at the elution temperature (Chase,

1968b). The techniques of chromatography on porous gels have been

adequately described (Pierce, 1966; King, 1968).

It is sometimes difficult to separate the immunoglobulins from

one another or from other contaminating substances in a mixed sample

by nonspecific methods. One example of where it becomes important

to be able to separate a class of immunoglobulin from all other

contaminants is when one wishes to use that class of immunoglobulin

as an antigen for the production of class-specific antiglobulin.

A specific method of separation of a single class of immunoglobulin

from a semipurified sample has been described. This method combined

both immunoelectrophoresis and double diffusion in agar-gel. The

agar-gel was extensively washed to remove any unprecipitated material

and the immunoprecipitate was then cut out of the washed agar-gel.

The material was either injected directly or first emulsified in

adjuvant and then injected into a heterologous species of laboratory

animal for the production of class-specific antiglobulin. It was

concluded that relatively large quantities of class-specific anti-

globulin could be produced conveniently and simply by injecting

small quantities of the agar-gel immunoprecipitate material into

laboratory animals. Furthermore, the antigen (in this case the

immunoglobulin) in the form of an agar-gel immunoprecipitate was

apparently a more powerful antigen than if it had been in a soluble

form, although it was found that various precipitates differed

in their ability to induce antibody production (Smith *et al.*, 1964;

Shivers and James, 1967; Nansen *et al.*, 1971).

Immunoassays in Agar-Gel

Several immunodiffusion methods have been developed in semi-

solid agar-gel to facilitate quantitative and qualitative determina-

tions of immunoglobulins. They include 1) double diffusion in 2

dimensions, 2) single radial diffusion and 3) immunoelectrophoresis.

The immunodiffusion methods are similar in several ways and basically

they allow soluble antigen(s) and antibodies to react at optimal

concentrations by diffusion in agar-gel. The result is the forma-

tion of visible single or multiple precipitates depending upon the

number of antigen-antibody systems that are present. It has been

reported, however, that in some cases a single antigen-antibody

system may give rise to more than one band in gel diffusion. The immunodiffusion methods differ with respect to the different combinations of reactants used, the patterns cut in the agar-gel and the physical forces used to cause a reaction. The most commonly used stabilizing substance for immunodiffusion analyses has been agar-gel, although other materials such as pectin, alginate and cellulose acetate have also been used. Preparations ranging in concentrations of agar-gel from 1.3 to 1.5% (w/v) in 0.85% saline or buffered saline (pH variable) (Chase, 1968) have been used and will not interfere with normal diffusion of immunological reactants, antibodies and antigens of moderate molecular size and shape. Tests are usually incubated at a constant temperature of either 4, 18 or 37 C and in a humid atmosphere to avoid excessive drying of the agar-gel and possible distortion of the immunoprecipitates. Preservatives have been added to the agar-gel buffer mixtures to prevent the growth of contaminating microorganisms. Once the immunoprecipitates have developed to maximum size and intensity, they may be photographed using special equipment, lighting and film. If the immunoprecipitate preparations are to be preserved, it has been recommended that they be washed in several changes of 2% (w/v) sodium chloride followed by deionized distilled water for 24 to 48 hours each to remove any unprecipitated materials from the agar-gel. The washed preparations may then be slowly dried at 25 C by placing moistened filter paper cut to size onto the agar-gel surface. The dried, permanently mounted immunoprecipitates can be stained with one of several stains. The stained preparations are washed in

several changes of 5% (v/v) glacial acetic acid to remove excess stain and then air dried. The stained immunoprecipitates can also be photographed using special methods and equipment (Ouchterlony, 1968; Campbell *et al.*, 1970c).

Three methods of double diffusion in 2 dimensions have been described; 2 of them are tube methods and the third is carried out either on agar-gel layered onto glass plates or microscope slides (micromethod). In either the plate or the micromethod, various patterns of wells (or basins) are cut into the agar-gel depending upon the amount and concentration of the reactants, the thickness of the agar-gel layer, the size of the wells and the desired distance between wells. The wells are loaded with different antigens, antibody preparations or serial dilutions of either depending upon the purpose of the test. The preparations are then incubated until the immunoprecipitates form. The main advantages of this method are that it can detect more than one antigen-antibody system and its sensitivity, being able to detect 40 µg/ml of antibody (Ouchterlony, 1968; Campbell *et al.*, 1970c; Gill, 1970).

Numerous methods have been described for quantitating individual immunoglobulins by immunodiffusion in agar-gel (Fahey and McKelvey, 1965). Mancini *et al.* (1965) have developed a system of incorporating a monospecific antiglobulin throughout heated agar-gel and pouring the mixture into a special mold so as to obtain an agar-gel layer of uniform thickness. Test samples of antigen of known (standard) and unknown immunoglobulin concentrations are placed into small wells cut in the agar-gel and allowed to diffuse. An antigen-

antibody precipitin ring forms around the well and the diameter of

the ring reflects the concentration of the antigen. The diameters

of the rings formed by the standard and the unknown samples can be

measured directly and compared. Sometimes the rings formed are not

perfectly round, in which case the images of either the unstained

or stained rings can be projected onto white paper. The images are

then traced, cut out individually, weighed and compared. This

quantitative method has the advantages of a simple test suitable

for testing large numbers of samples, small amounts of reactants

are needed, and the least amount of immunoglobulin that can be

detected is from 3 to 10 µg/ml (Fahey and McKelvey, 1965; Gill,

1970). More recently a modified technique for quantitating immuno-

globulins has been developed which combines the principles of

double diffusion in 2 dimensions and single radial immunodiffusion

(Kim, 1970). It was found that tests were simple to perform,

required small amounts of reactants and were more sensitive than

the qualitative double diffusion method (Koller et al., 1974).

Immunoelectrophoresis is a technique used to study antigens

and antibodies which involves the principle of immunodiffusion in

agar-gel and the mobility of proteins in an electrical field. The

procedure as originally described was modified by one of the earlier

investigators (Scheidegger, 1955) into a microtechnique using glass

microscope slides. Immunoelectrophoresis has several useful func-

tions such as 1) detecting and identifying individual components in

a multiple-component system, 2) determining the purity of a one

component substance, 3) determining the efficacy of a fractionation

procedure, and 4) recognizing atypical proteins (Campbell *et al.*, 1970c). The antigen sample is first placed in a small well cut in the agar-gel and separated into its component parts by electrophoresis. Antiserum is then placed in a trough cut in the agar-gel parallel to the path of electrophoretic migration. The separated components of the antigen and the antiserum are allowed to diffuse until immunoprecipitates form. The principles associated with the electromigration and diffusion of proteins in agar-gel in immunoelectrophoresis have been described (Campbell *et al.*, 1970c). Several factors are important to the successful operation of the technique. They include: 1) the size and shape of the well, 2) the distance between the well and trough, 3) the concentrations of antigen and antibody, 4) the purity, type and concentration of agar-gel, 5) the pH and ionic strength of the buffer, 6) the strength and the amount of time of the applied current, and 7) temperature (Campbell *et al.*, 1970c). Variations of the basic technique such as quantitative, qualitative and comparative immunoelectrophoresis, electrosyneresis (Ouchterlony, 1968) and a technique for facilitating the identification of specific precipitin arcs (Osserman, 1960) have been described. Immunoelectrophoresis can detect antibody in a sample at a concentration of 100 μg/ml or more (Gill, 1970).

Another technique has been described (Jerne *et al.*, 1963) which combines immunodiffusion of antibody in gel and a hemolytic indicator system to detect and enumerate antibody producing cells from host tissues after *in vivo* stimulation with antigen. Modifications have been described (Bernovska *et al.*, 1963; Cunningham and Szenberg,

1968) to improve the original procedure. Basically, the technique involves harvesting viable antibody producing cells from lymphoid or other tissues of a host that had previously been injected with a test antigen. The concentration of cells is standardized and they are then added to a mixture of gel material and red blood cells. Agarose gel material and sheep red blood cells are most often used. The tissue cells-agarose-sheep red blood cells mixture is then poured onto a Petri dish or a glass slide and incubated at 37 C. During incubation, antibody is secreted by the antibody producing cells, diffuses into the agarose and reacts with the antigen. Antibodies to red blood cell surface antigens or other antigens that can be absorbed onto the surface of these cells will be detected (Bernovska et al., 1963). After incubation, diluted guinea pig complement is added and allowed to diffuse into the gel resulting in lysis of the sheep red blood cells coated with the antigen-antibody complexes. The zone of diffusing antibodies surrounding each antibody producing cell is recognized as a hemolytic plaque midst a uniform sheet of nonlysed sheep red blood cells. Each hemolytic plaque observed represents a single antibody producing cell which can most probably be classified within the plasma cell series. Hemolytic plaques may vary in number and size depending upon a number of factors (Jerne et al., 1963). The procedure described above is the direct method and has been interpreted as useful in detecting cells capable of producing and secreting IgM antibody. The indirect method for the detection of other classes of antibody requires the addition of a monospecific antiglobulin to the agarose after the initial

incubation and before the addition of complement. Hemolytic plaques

may be counted with low-power magnifying equipment either directly

or as stained preparations. The hemolytic plaque assay has been

used to determine the plaque forming activity during the inductive

phase of antibody formation (Sterzl and Mandel, 1964) and also

activity from various organs such as spleen, lymph nodes, thymus

and blood (Schultz et al., 1971); lung (Kaltreider et al., 1974);

and intestine (Robertson and Cooper, 1972).

Other Assays and Procedures Commonly Used in Immunology

Other immunological assays have been developed in which the

antigen is reacted directly with the antibody in an aqueous medium

or with the use of an insoluble matrix. The primary reaction of

antibody with a soluble homologous antigen results in the secondary

formation of a precipitate, whereas combination of antibody with

a particulate antigen can result in the formation of an agglutinate.

A theoretical lattice hypothesis has been proposed for precipitin

reactions to explain the interactions between optimal concentra-

tions of antigen and antibody molecules resulting in a macromolecular

lattice network and eventual formation of a precipitate. The same

principle more or less holds true for the agglutination reaction

(Humphrey and White, 1971c). The hemagglutination test involving

red blood cells has been divided into direct and indirect methods.

In humans, the direct method provides the basis for typing of blood

into the A, B, O and AB groups by using specific antisera. The

indirect (or passive) hemagglutination method involves adsorption

of an antigen onto the surface of red blood cells and then reacting
the modified cells with heat-inactivated antibody directed against
the adsorbed antigen. Bacterial polysaccharide antigens (Westphal
et al., 1964) may be directly adsorbed onto red blood cells by one
of several alternate methods (Neter *et al.*, 1956; Buxton, 1959;
Sharpe, 1965; Wilson and Svendsen, 1971), whereas cells must first
be pretreated with a dilute solution of tannic acid before adsorbing
protein antigens (Boyden, 1951). Both direct and indirect hemag-
glutination tests can be conducted by a standard tube method
(Campbell *et al.*, 1970d) or by a microtitration method (Sever, 1962).
The advantages of the microtechnique include an 8-fold saving of
reagents, rapid performance of microdilutions and reliability and
validity of the system compared to other standard systems (Sever,
1962). The indirect hemagglutination test has been reported as a
sensitive assay and can be used to detect as little as 0.02 to
0.04 µg/ml of antibody (Campbell *et al.*, 1970d; Gill, 1970).

Antigens and antisera are important reagents that are routinely
used in immunologic studies. Several procedures have been described
for the preparation and use of antigens and the production of anti-
sera in laboratory animals depending on the type of antigens, the
immunization procedures employed and the uses intended for the
antisera (Williams and Chase, 1967).

Ontogeny of the Immune System

Many advances have been made during the past 2 decades toward
a better understanding of the immune mechanisms and responses of
man and animals. Part of this development in knowledge has come

from studies on the ontogenesis of the immune systems. Ontogenetic

studies have also increased the understanding of congenital and

neonatal diseases commonly encountered (Osburn, 1973). For many

years it was thought that the mammalian fetus was incapable of

responding in a normal manner to any antigenic stimulus (the so-

called immunologic null state) (Silverstein and Kraner, 1965). This

theory was disproven because of numerous studies in fetuses and

neonates of many species showing that the development of immunologic

competence is an important feature of embryonic development (Ingram

and Smith, 1965; Thorbeck and Van Furth, 1967; Sterzl and Silver-

stein, 1967; Schultz, 1973b). Recent surgical techniques have been

developed for the study of immune responses of intrauterine fetal

animals (Kraner and Parshall, 1968). The attainment of immunologic

competence appears to be a complex series of events depending on

the host species and the suitability of the antigen being considered.

Evidence has been reported that fetuses of various species are

capable of both humoral and cellular immune responses (Silverstein

and Kraner, 1965). Silverstein (1967) indicated that there is a

wide range of developmental ages at which different species attain

immunologic competence. Depending on the antigen, a developing

fetus is incapable of responding to antigenic stimulus prior to a

critical age. A deleterious consequence of exposure to an antigen

during the early period of immunologic unresponsiveness may be the

development of tolerance by the fetus to the antigen. Early studies

in fetal monkeys and lambs (Silverstein, 1967), however, showed that

the earliest immunological responses to antigens are in no way

immature responses, but are characteristic of those found in the normal adult animal. More recently, Schultz *et al.* (1971) and Schultz (1973a) have reported on the times at which different lymphoid tissues developed, the times at which immunoglobulins appeared and the sequence of immunoglobulin development in the bovine fetus. Quantitative determinations of immunoglobulins by single radial immunodiffusion from fetal and precolostral calf sera provided practical detection methods of *in utero* infections and further showed that IgM and IgG_1 are the most reliable indicators of antigenic stimulation of the fetal host (Schultz, 1973a).

Gay (1971) was one of the first researchers to investigate the possible practical application of immunization of bovine fetuses by parenteral and oral injections of *E. coli* antigen. Other similar studies in fetal lambs and calves by surgical methods have been reported by Richardson and Conner (1972) and Conner *et al.* (1973). Recently, a nonsurgical technique has been developed that permits oral fetal immunization with antigen and eliminates the costly and time consuming requirements of surgery (Conner, G. H., Michigan State University, personal communications). Schultz (1973a,b) has suggested that similar studies should be continued and expanded to investigate the feasibility of the procedure to control or prevent diseases of the fetus and the newborn calf.

Summary

Colibacillosis continues to be a common and serious disease causing great economic losses of young animals. It is a complex disease syndrome and many factors have been reported as contributing

to the cause. Numerous attempts at prevention and control of coli-
bacillosis based on the vast amount of information already known about
the disease have only resulted in temporary, limited or no success,
and in many cases the disease has still reached epizootic proportions.

Significant advances in research have been made during the past
2 decades in studies of the immune systems of man and animals. Much
has been learned about the mechanisms of specific immunity and nonspeci-
fic resistance, both normal and abnormal. Ontogenetic studies have shown
that fetuses and neonates of several animal species are immunocompe-
tent to a variety of antigenic substances. Further, the evidence
from these studies suggests that fetal immunizations may provide an
effective and suitable method for the prevention of colibacillosis.

MATERIALS AND METHODS

Animals

Thirty commercial Hereford cows bred by artificial insemination or natural service to either Aberdeen Angus or Beef Friesian bulls, were obtained as a source of calves.[a] Reproductive examinations showed that the pregnant cows ranged from 7 to 8.5 months of gestation. The cows were confined to a pole barn and an adjoining lot where they were placed under 24 hour observation after arrival of the first calf and for the duration of the calving period. The diet consisted of brome-alfalfa hay fed twice daily and water.

The calves were born by natural delivery and were removed from the cows as soon after birth as possible and before suckling. The calves were transported to an enclosed calf barn where each was dried, weighed and placed in a separate pen. Daily observations were made of the calves to note appetites and evidence of clinical signs. Blood samples were taken from the jugular vein within one-half to two hours after birth and before oral inoculation or first feeding. Serum was derived by the procedures described elsewhere. Blood samples were also taken at death. The calves were divided into principal and control groups depending on whether they were given *in utero* injections of *E. coli* 026:K60:NM bacterin or saline,

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[a]Kindly supplied by Premier Corp., Fowlerville, Michigan.

respectively. The principal and control groups were further subdivided depending on the procedure followed after birth. The experimental design is summarized in Figure 1. Calves were reimmunized at birth by oral inoculation of 5 x 10^{10} killed, washed *E. coli* organisms (1 ml), whereas challenge of calves consisted of an oral inoculation of live, unwashed *E. coli* organisms. The challenge doses were 1.5 x 10^{10}, 1.0 x 10^{11} or 1.5 x 10^{11} organisms (5, 30 or 50 ml, respectively). The calves were fed 1/2 cupful of a milk replacer diet[a] in 1/2 quart of warm water twice daily. Feed was withheld for 1 hour after oral reimmunization or challenge.

Laboratory animals were used for the production of antisera and consisted of 12 male Dutch Belted rabbits averaging 2.36 kg and 18 male Hartley-White guinea pigs averaging 600 gm each. Preliminary control tests on sera from these animals by the Ouchterlony method were negative for antibody activity against bovine serum.

Six additional crossbred cows were used as a source of whole bovine serum (WBS). Whole blood was collected into sterile, 250 ml vacuum flasks[b] and allowed to clot overnight at 4 C. The serum from the donor animals and calves was separated by centrifugation at 100 g at 4 C for 20 minutes. The serum from the donor animals was pooled, divided into 2 ml and 40 ml aliquots in sterile vials and stored at -70 C. The serum from the calves was stored in individual vials as above.

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[a] MMPA Premium Calf Milk Replacer, Michigan Milk Prod. Assoc., Detroit, Michigan.

[b] Abbo-Vac single dose containers, Abbott Labs, North Chicago, Illinois.
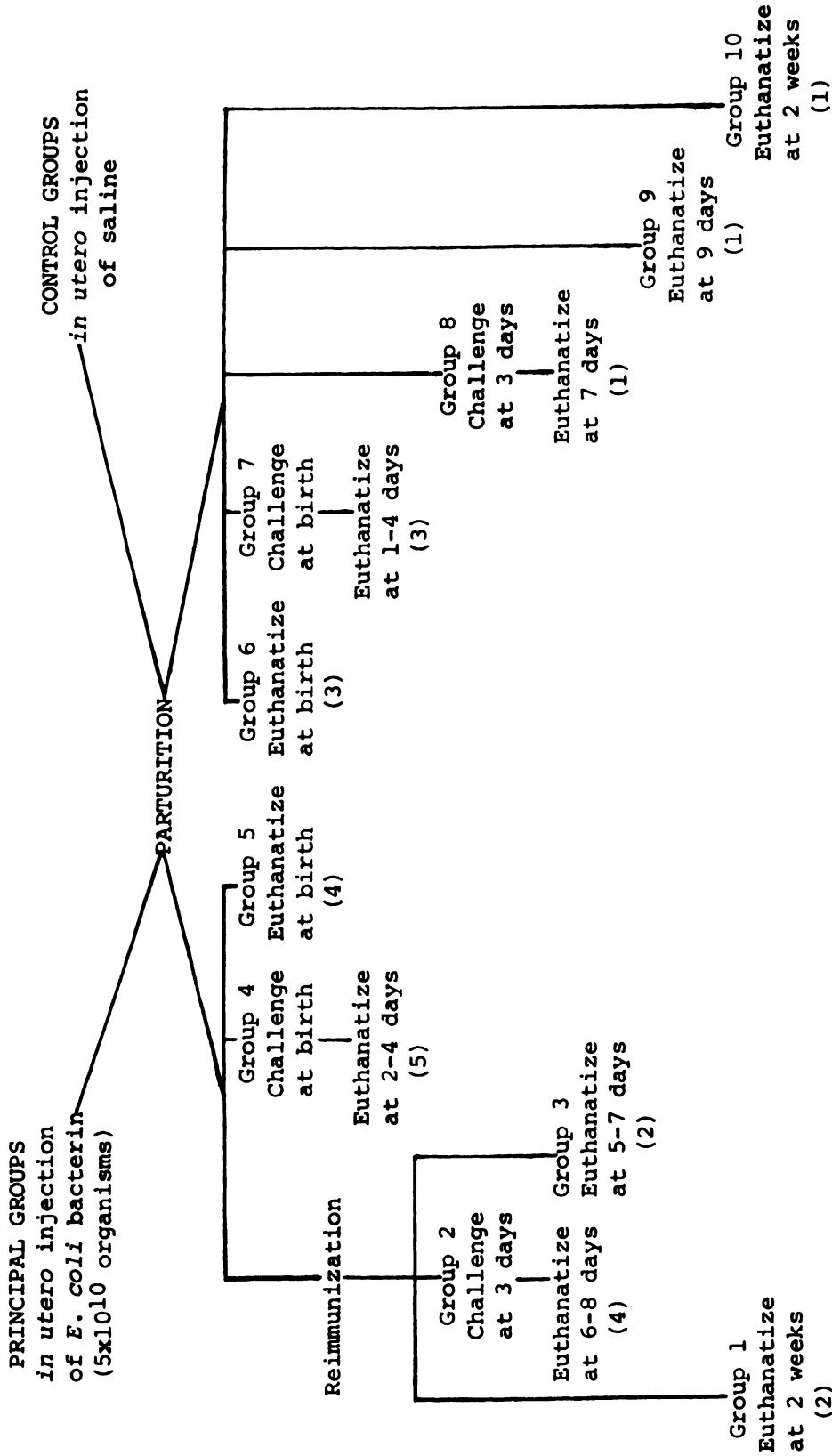
Figure 1. Experimental groups of calves and ages at reimmunization, challenge and euthanasia. The number of animals in each group is indicated in parentheses.

Fetal Injection

The technique used for injection into the amniotic fluid was
one developed by Dr. G. H. Conner of Michigan State University
(unpublished data) and was accomplished without surgery. Briefly,
a cow was confined in a standing position in a cattle chute which
allowed working access to the right flank. Ropes were wrapped and
tied around the flank and also used to tie the right rear leg and
the tail. The fetus was located through the lower right abdominal
wall by deep external palpation and gentle ballottment, and the
exact position was noted. Generally, fetuses were located in an
area approximately 2.5 to 5 cm dorsal to the fold of the flank and
30 cm anterior to the division between the front and rear quarters
of the udder. The area was clipped and cleansed with a preparative
scrub.[a] A small area of skin, muscle layers and peritoneum was
then anesthetized.[b] The area was given a final scrub with a sani-
tizing solution[a] and sterile gauze. All needles and syringes used
in the procedure were either disposable and packaged in sterile
dispensers or nondisposable and prepared in a sterilized pack. The
operator making the fetal injections wore sterile rubber gloves.
A small skin incision was made over the anesthetized area. A 12 G,
5 cm cannula needle was then inserted through the abdominal wall
including the peritoneum. The cannula needle was stabilized with

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[a]Betadine Scrub and Solution, The Purdue Frederick Co.,
Norwalk, Connecticut.

[b]Procaine HCl Solution, 2.5%, Bio-ceutic Lab, Inc., St.
Joseph, Missouri.

one hand in a position pointing straight in while another needle
(16 G, 30 cm) with an empty 10 ml syringe attached was used to
complete the procedure. The longer needle was passed through the
cannula and slowly directed inward toward the fetus. The gravid
uterus was usually located 2 to 4 cm within the abdominal cavity.
The operator usually met slight resistance as the needle was passed
through the uterine wall. The needle was carefully inserted a short
distance further until the tip struck a part of the body of the fetus
which was noted by a much firmer resistance. Often the fetus moved
violently in response to the needle stimulus. The needle was
withdrawn slightly and negative pressure was applied with the
attached syringe in an attempt to withdraw a sample of amniotic
fluid. If amniotic fluid could not be withdrawn or if the fluid
that appeared resembled allantoic fluid, the longer needle was then
redirected until amniotic fluid could be retrieved. Once amniotic
fluid was obtained, the syringe was exchanged for another syringe
containing 1 ml of *E. coli* bacterin (5 x 10^{10} organisms) which was
then injected. The needles were withdrawn and the site of injection
was treated with an antibiotic powder. Occasionally it was difficult,
and in one case it was impossible, to locate the fetus with the long
needle. In the latter instance, the fetus was injected successfully
several days later. Effort was made to randomly divide the bacterin
and saline fetal injections according to the length of time before
expected date of calving. The cows were routinely inspected for
2 to 3 days after fetal injections and rectal temperatures were
recorded after 2 days. A portion of each amniocentesis sample was

cultured for bacterial growth and the remainder was then placed in sterile vials. The samples were analyzed later for physical and bio-chemical properties as described elsewhere.

Collection of Specimens

Calves were euthanatized by exposure for 30 seconds to 110 v of electricity. They were placed in dorsal recumbency and the sternum and ventral abdominal wall were incised and reflected caudally exposing the abdominal viscera. Blood was collected into sterile vials by severing the brachial artery at the time the carcass was opened. Sections of liver, spleen, kidney and mesenteric lymph nodes were taken by aseptic methods for bacteriologic culturing from certain calves. The small intestine was divided into segments, each approximately 40 cm long, by incising along the mesenteric attachment. Samples were taken at each of 4 locations. The first segment was taken from the duodenum starting 5.0 cm from the pylorus and progressing caudally and the last was taken from the ileum starting at the ileocecal valve and progressing anteriorly. The middle segments (upper and lower jejunum) were taken 1/3 and 2/3, respectively, of the distance from the pylorus to the ileocecal valve. Each segment was clamped with a surgical forceps at the distal end and the proximal end was incised. Twenty milliliters of a chilled mixture of phosphate buffered saline (PBS) (0.01M phosphate, pH 7.2; 0.15M NaCl) and dithiothrietol[a] (0.001M final concentration) was pipetted into the lumen of each segment and the proximal end was clamped. The

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[a]DTT, Sigma Chemical Corp., St. Louis, Missouri.

fluid was mixed with the contents of the intestinal lumen by gentle

massage and then thoroughly drained into a stoppered flask after

removing the clamps. The intestinal washings were kept on ice until

further processing. Each segment of intestine was removed and the

mucosal surface was exposed by cutting along the mesenteric border.

Paired, adjacent full-thickness sections were cut; one was prepared

for histopathologic examinati‌on and the other, approximately 12 cm

long, was placed in a beaker containing chilled Hanks' balanced

salt solution (HBSS) (Kalter, 1963) and kept on ice until further

processing. Samples of mesenteric lymph node, adjacent to the

intestinal segments, were also taken. Cut sections of lymph nodes

were prepared for histopathologic examination and samples of whole

nodes, approximately 3 gm total wet weight, were placed in the same

sample beakers containing the corresponding sections of intestine

and HBSS. The spleen was removed and sections were prepared for

histopathologic examination. Six triangular sections of spleen,

approximately 12 gm total wet weight, were also cut from the edge

at equal intervals around the circumference of the organ, placed in

HBSS and kept on ice until further processing. Sections of thymus

were prepared for histopathologic examination. Serum was derived

by the procedure described elsewhere. The intestinal washings were

centrifuged at 10,000 g for 10 minutes at 4 C. The supernates were

dialyzed in 6 liters of PBS at 4 C for 48 hours, concentrated[a] to

2 to 4 ml and stored at -70 C.

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[a]Polyethylene Glycol 20,000, Fisher Scientific Co., Fairlawn,
New Jersey.

Sheep red blood cells were aseptically collected from a donor

and stored for 1 week at 4 C in sterile Alsevers' solution

(Campbell *et al.*, 1970e) before use.

Immunologic Procedures

Immunoprecipitation in Agar-Gel

Immunoelectrophoresis. Immunoelectrophoresis (IEP) was carried

out according to the micromethod of Scheidegger (1955). The equip-

ment used[a] included a buffer chamber, power source, slide frames,

agar pattern and trough cutters, frame holders, moist chambers,

buffer wicks and filter paper strips. Barbital buffer (Chase, 1968)

(0.05M barbital, pH 8.2) was used in the buffer chamber and also to

make the agar-gel.[b] One percent agar-gel (w/v) was prepared by melting

the dried agar in the barbital buffer and boiling the mixture until

all the agar had melted. Merthiolate (1:10,000) was added to a

concentration of 1% (v/v) as a preservative. Fourteen milliliters

of melted agar in buffer were pipetted into stoppered tubes which

were stored at 4 C until used. Six microscope slides (25 mm x 75 mm)

were placed end to end on a slide frame by forming 2 rows of 3

slides each. Two tubes of melted agar-gel were poured onto each

slide frame and allowed to harden in a moist chamber for 30 to 45

minutes before the wells and troughs were cut. Samples were placed

in the wells with capillary pipettes and the material was

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[a] Gelman Instruments Co., Ann Arbor, Michigan.

[b] Noble Agar, Difco Laboratories, Detroit, Michigan.

electrophoresed using 12 ma of current per frame for 90 minutes. Afterwards, antiserum was placed in the troughs and the slides were incubated at 25 C for 24 to 48 hours in a moist chamber depending on the rate of development of the immunoprecipitates. Preparations to be preserved and stained were washed in 2% (w/v) sodium chloride and in deionized distilled water for 48 hours each. Changes of washing solutions were made every 12 hours. The preparations were removed from the final wash and dried at 25 C by placing moistened filter paper strips cut to size onto the agar-gel. The dried preparations were stained[a] for 10 minutes, washed in 3 changes of 5% (v/v) glacial acetic acid to remove excess stain and air dried.

Double diffusion in 2 dimensions (Ouchterlony method). The same 1% agar-gel preparation was used on microscope slides for the Ouchterlony method as was used for IEP. The agar wells were 3 mm in diameter and were spaced 6 mm apart, center to center. The well pattern used was 1 center well and 4 outside wells evenly spaced. Two well patterns were cut on each microscope slide and slides were either prepared singly or in groups of 6 on IEP slide frames. Incubation conditions and preservation by drying and staining were the same as described previously for IEP.

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[a]Analine Blue Black, Matheson, Coleman and Bell, Norwood, Ohio.

Separation of Immunoglobulins and the
Production of Antiglobulins

Some of the tests used to determine the quantitative and quali-
tative immune responses of calves required the use of antiglobulins.
The antiglobulins, produced in laboratory animals, reacted mono-
specifically against one class of bovine immunoglobulin. The
classes of immunoglobulins that were tested for in the calves were
IgM, IgG_1 and IgG_2. Antiwhole bovine serum (AWBS) was also used to
test for the composition, purity and monospecificity of samples by
the IEP and Ouchterlony methods.

The three classes of immunoglobulins (IgM, IgG_1 and IgG_2) were
separated from WBS into semipurified fractions by nonspecific methods
and finally into a pure immunoprecipitate form by a specific immuno-
diffusion method. The final products were injected into laboratory
animals for the production of monospecific antiglobulins. Whole
bovine serum was used as a crude source of immunoglobulins M, G_1
and G_2. All three immunoglobulins were first precipitated from WBS
by salting-out procedures. Immunoglobulin M was semipurified by gel
filtration chromatography and IgG_1 and IgG_2 were semipurified first
by ion exchange and then gel filtration chromatography.

Salting-out procedure. Thirty milliliters of saturated ammonium
sulfate were slowly added to an equal volume of WBS with stirring.
Stirring was continued for 3 hours at 25 C and the mixture was then
centrifuged at 10,000 g for 30 minutes at 4 C. The supernatant
was decanted and the precipitate was dissolved in PBS to 1/3 the
starting volume. The dissolved precipitate was dialyzed with

stirring in PBS for 24 hours at 4 C. The dialyzed protein was repre-
cipitated with saturated ammonium sulfate and dissolved in the
starting buffer used for column chromatography. The sample was then
dialyzed in 4 to 6 liters of starting buffer for 48 hours at 4 C.
A small volume of the final material was tested for the presence of
sulfate by adding 1 to 2 drops of 10% barium chloride (w/v). Absence
of a precipitate meant that no sulfate was present. Finally, the
dialyzed protein was filtered[a] (0.45 μm) before further separation
by column chromatography.

Ion exchange chromatography. Ion exchange chromatography was
carried out at 25 C with samples applied to glass columns[b] (1.5 cm
x 60 cm) packed with the microgranular form of the ion exchanger.[c]
The procedure followed for preparative washing of the ion exchanger
for chromatographic separations has been previously described (King,
1968). Phosphate buffered saline was used to equilibrate the ion
exchanger and for step-wise elution of the samples. Buffer concen-
trations of phosphate and pH values used for step-wise elution were
0.005M, 0.01M, 0.05M, pH 7.9 and 0.05M and 0.1M, pH 5.8. Two molar
sodium chloride was used to elute any remaining material from the
ion exchanger. Fractions of eluate were collected in 3 ml volumes
in a fraction collector[d] at a flow rate of 40 ml/hr and monitored at

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[a]Metricel GA-6, 25mm, Gelman Instrument Co., Ann Arbor, Michigan.

[b]Glenco Scientific Inc., Houston, Texas.

[c]Whatman DE 52, Reeve Angel, Clifton, New Jersey.

[d]Volumetric Fractionator, Model V-10, Gilson Medical Elect.,
Middleton, Wisconsin.

280 mμ of ultraviolet light in a spectrophotometer.[a] Individual tubes from selected peaks were examined by IEP to determine the protein composition and were held at 4 C. The sample was placed in one well, WBS in the other well for comparison and AWBS in the trough after electrophoresis. Individual tubes containing the highest concentrations of immunoglobulin desired were concentrated by ultrafiltration in a vacuum (Craig, 1968) and then dialyzed for 48 hours at 4 C in 4 to 6 liters of the buffer used for gel filtration chromatography.

Gel filtration chromatography. Gel filtration chromatography was carried out in a cold room (4 C) on glass columns[b] (2.5 cm x 90 cm) packed with a fine grade of porous gel.[c] Dried gel was weighed out in an amount estimated to be 15 to 20% in excess of that required to fill a column and then swelled by boiling in distilled water for 3 hours. The gel was allowed to settle and to cool to room temperature, the supernatant was decanted and the gel was resuspended in chilled Tris-HCl buffer[d] (0.1M Trizma, pH 8.2) to which sodium azide (400 mg/l) had been added as a preservative. The gel was degassed in a vacuum for 3 hours at 4 C with gentle stirring. After settling, the gel was resuspended in 1.5 times the

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[a]Model DB, Beckman Instruments Co., Fullerton, California.

[b]Glenco Scientific Inc., Houston, Texas.

[c]Sephadex G 200, Pharmacia Fine Chemicals, Piscataway, New Jersey.

[d]Sigma Technical Bulletin No. 106B, Sigma Chemical Co., St. Louis, Missouri.

settled volume with chilled Tris-HCl buffer and poured into a column
as previously described (King, 1968) using an attached packing
reservoir. After packing, the flow rate of buffer was adjusted and
maintained throughout (9 ml/hr) to that used during sample elutions
and at least 2 total bed volumes of buffer were passed through before
use. Gel filtration columns were calibrated[a] before application of
samples. Once the columns were stabilized and calibrated, buffer was
passed through continuously whether the colums were in use or not.
Samples were filtered and sucrose was dissolved in the filtrate to
a concentration of 3% (w/v) before being applied onto the columns.
The sample-sucrose mixture (total volume did not exceed 1% of the
total bed volume) was carefully layered onto the surface of the gel
with a Pasteur pipette and sample fractions were collected in 3 ml
volumes with a collector and monitored as previously described.
Selected fractions were examined for composition by IEP and the
Ouchterlony methods, dialyzed in PBS, concentrated, filtered and
stored at 4 C as previously described. Additional samples were
applied to the gel filtration columns after the spectrophotometric
optical density readings returned to zero.

Preparation of antigens and production of antisera. Six rabbits
were used for the production of monospecific antiglobulin against
bovine IgM. Each rabbit was injected with 36 immunoprecipitate bands
which were formed on 18 IEP slides by reacting semipurified bovine

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[a]Calibration Kit, Pharmacia Fine Chemicals, Piscataway,
New Jersey.

IgM with rabbit antiserum monospecific for bovine IgM.[a] The bands

were extensively washed as previously described and cut out of agar-

gel. They were then minced in a small volume of PBS and used as

antigen for injection.

Four rabbits were used for the production of AWBS by injection

of 2.15 mg of WBS total protein diluted in PBS into each rabbit.

Nine guinea pigs each were used for the production of mono-

specific antiglobulin against bovine IgG_1 and IgG_2. Immunoprecipitate

bands were produced by IEP using semipurified IgG_1 and IgG_2 and

guinea pig antisera monospecific for bovine IgG_1 and IgG_2, respec-

tively.[a] Each animal was injected with 24 bands (12 slides on IEP)

of washed and minced material as a primary dose and 12 bands (6

slides on IEP) for the second injection.

All minced immunoprecipitate band material was emulsified with

an equal volume of a repository adjuvant[b] before injection into

laboratory animals. Bilateral injections of 1/2 the volume of

material were made intramuscularly in the thigh and the rest was

injected subcutaneously at several sites in the anterior thoracic

region. Animals were bled after 2 weeks and sera were tested by

the Ouchterlony and IEP methods for monospecificity of the antiglobulin

activity. Animals with a negative or poor serologic response after

primary injection were reinjected and retested in 10 days. Blood

from rabbits showing a strong serologic response was collected from

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[a]Kindly supplied by Dr. R. D. Schultz, Cornell University,
Ithaca, New York.

[b]Freund Complete Adjuvant, Difco Laboratories, Detroit,
Michigan.

the marginal ear vein whereas guinea pigs showing a strong serologic

response were anesthetized with ether and exsanguinated from the

heart. All unpooled antiglobulin sera were divided into 2 ml aliquots

in sterile vials and stored at -70 C. Animals failing to show a

strong response after 2 injections of antigen were discontinued from

the experiment. Antisera that were not monospecific were absorbed

to remove activity against light chains or other globulins that may

have contaminated the original antigen.

Qualitative and Quantitative Procedures

Agar plaque technique. The agar plaque technique as originally

described by Jerne *et al.* (1963) was used with modifications to

quantitate the number of cells and qualitate the class of antibody

produced by the cells from spleen, intestinal sections and correspond-

ing lymph nodes. A microtechnique on microscope slides and agarose[a]

gel was used for the procedure. Tissue cell suspensions of the

surface epithelium and lamina propria of the intestinal segments were

made by scraping the mucosa with a razor blade and mixing the coarse

suspension of cells in chilled HBSS. Similar cell suspensions of

lymph nodes and spleen were made by teasing the cells from the tissue

with a scalpel and forceps. All coarse cell suspensions were forced

once through a No. 120 fine mesh stainless steel screen, suspended in

chilled HBSS and centrifuged (750 g for 10 minutes at 4 C). Spleen

cells were washed and centrifuged first in 0.83% (w/v) ammonium

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[a]Agarose A 45, Fisher Scientific, Livonia, Michigan.

chloride (Schultz *et al.*, 1971) to lyse the red blood cells present and then washed again in HBSS. The washed packed cells were resuspended in HBSS to obtain the desired concentration and held on ice until used. Examination of cells by the trypan blue exclusion test showed 85 to 90% viability and that a majority of the cells were suspended as single cells. Cells were counted with a hemacytometer before dilution and the average number/mm^3 was 1.7×10^7 for intestinal sections, 1.3×10^8 for mesenteric lymph nodes and 2.6×10^8 for spleen. Agarose was diluted to a final concentration of 0.5% (w/v) with HBSS and 0.4 ml aliquots were pipetted into tubes and held in a 60 C water bath. Tanned (Boyden, 1951) sheep red blood cells (SRBC) were coated with 026 antigen of *E. coli* 026:K60:NM as previously described (Wilson and Svendsen, 1971) and were diluted to a 20% (v/v) suspension in saline. A 20% suspension of uncoated SRBC used as control cells was also prepared. Microscope slides were coated in advance by dipping in hot 0.1% (w/v) agarose in distilled water and air dried. Coated slides were placed in 2 rows on 4 slide frames constructed so that the ends of the slides were supported by strips of tape placed lengthwise on the frame and the center of the slides were raised off the frame surface. For conducting the test, 0.05 ml of 20% coated SRBC were pipetted into a tube containing the agarose-HBSS mixture. Then 0.1 ml of the tissue cell suspension was pipetted into the mixture, lightly shaken to avoid bubble formation and quickly poured onto a slide. Duplicate tests were made of each tissue suspension with coated SRBC and single tests were made with each tissue suspension and uncoated SRBC as controls.

After hardening, all slides were turned over, agarose side down, and held for 1 hour in a humid (37 C) CO_2 incubator. Guinea pig complement[a] diluted 1:10 in HBSS was added to one frame used for the direct plaque assay (IgM) and the frame containing the control test samples by pipetting the material into the space between the agarose and the frame surface. Heat inactivated (56 C for 30 minutes) monospecific antiglobulin (anti-IgG_1 or -IgG_2) diluted 1:100 in HBSS was pipetted onto the remaining 2 frames (one per frame) for the indirect plaque assay and reincubated as above. After the second incubation of the indirect plaque assays, excess anti-globulin was removed and complement was added. All frames with complement added were reincubated for 1 hour, then held overnight at 4 C. Unstained hemolytic plaques were counted with either a dissecting microscope or a regular microscope under a low power objective lens. Any detectable clearing (hemolysis) around an identifiable cell was counted as a plaque. The direct (IgM) plaque counts were recorded by subtracting the average count from each tissue sample from the control count. The indirect counts (IgG_1 and IgG_2) were recorded by subtracting the average count for each sample from the average direct count and the control count.

Single radial immunodiffusion. The technique of Mancini et al. (1965) was used for quantitating immunoglobulins M and G_2 from calf sera by single radial immunodiffusion. Immunoglobulin G_1 was

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[a]Difco Laboratories, Inc., Detroit, Michigan.

not quantitated in calf sera because of difficulty in obtaining IgG_1 in pure form for use as a standard in the test. A 2% (w/v) agar-gel was made with barbital buffer (0.1M barbital, pH 8.6) to which sodium azide (0.05%, w/v) had been added as a preservative. Equal volumes of 2% agar-gel and diluted monospecific antiglobulin were mixed and pipetted into the frames as described. Preliminary tests with purified IgM[a] and IgG_2 used as standard antigens of known concentrations were run to determine the optimal dilutions of mono-specific antiglobulin to incorporate into the agar-gel and the serial dilutions of standard antigen to be made. Five serial dilu-tions of standard antigen were placed in wells (2.4 mm) spaced 12 mm apart across the top of each plate and unknown samples of calf sera were placed in the remaining wells. Plates were incubated for 48 hours in a moist chamber and stained as previously described for IEP. Stained immunoprecipitates were recorded by projecting the ring images with an overhead projector onto white paper. The images were traced, cut out and weighed and compared to the standards on graph paper.

Qualitative immunoelectrophoresis. Immunoelectrophoresis was used to qualitate the electrophoretic profile of sera from calves. Calf serum was placed in the upper well, WBS in the lower well for comparison and AWBS in the trough. The technique of IEP has been previously described.

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[a]Kindly supplied by Dr. B. I. Osburn, University of California, Davis, California.

Direct bacterial agglutination. The method described by Conner

et al. (1973) was used for the direct bacterial agglutination test.

The macrotitration (tube method) and the microtitration[a] (Sever,

1962) methods were used to test duplicate samples of calf sera and

intestinal washings for agglutinin activity against 026 or K60 *E.*

coli antigens. Control tests included a suspension of bacterial cells

in saline.

Passive hemagglutination assay. Calf sera and intestinal wash-

ings were tested by the passive hemagglutination (PHA) assay (micro-

titration method) as described by Wilson and Svendsen (1971).

Untanned SRBC were coated with 026 or K60 antigen of *E. coli* and

used as a 0.25% cell suspension in PBS. The method used for the

isolation of K60 antigen from *E. coli* has been previously described

(Nowotny, 1969). All calf sera and intestinal washings were heat

inactivated (56 C for 30 minutes) before being tested. Serial 2-fold

dilutions of sample, in 0.025 ml amounts, were made in duplicate in

saline starting with 1:2 and through 1:1024. Control tests included

coated and uncoated SRBC with saline and uncoated SRBC and test

samples. Tests were incubated for 1 hour at 25 C, then overnight

at 4 C before recording the results. A separate series of PHA assays

was used to test for cross reactivity, only this time by testing all

calf sera with 4 different *E. coli* serotypes.[b] Those used contained

the following serotypes: 04, 035, 068 and 0117. The PHA titers were

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[a]Microtiter, Cooke Engineering Co., Alexandria, Virginia.

[b]Kindly supplied by Dr. P. Glantz, The Pennsylvania State
University, College Station, Pennsylvania.

determined as the highest dilution of sample in which the coated SRBC

formed a broad disc with a serrated margin across the bottom of the

well. A negative reading was recorded when the coated SRBC formed

a dense compact button at the bottom of the well.

Radioimmunoassay. Preliminary attempts at reacting different

combinations of calf sera with 026 antigen directly in a precipitin

tube test or an immunoprecipitation test in agar-gel, including

radioimmunoelectrophoresis, resulted in no visible evidence that

an antigen-antibody reaction had occurred. The intent was to develop

a qualitative and semiquantitative assay to demonstrate what class(es)

of immunoglobulin and their quantity in calf sera had activity against

the *E. coli* 026 antigen. Preliminary tests on selected calf sera

by the passive hemagglutination inhibition assay indicated that a

dilution of at least 1:16 of 026 antigen was still sufficient to

cause complete inhibition of PHA. The results also gave indirect

evidence that anti-026 antibodies in calf sera were complexing with

026 antigen but that the complex remained in soluble form. A radio-

immunoassay tube method was developed as a modification of the radio-

immunoelectrophoresis assay in agar-gel (Campbell *et al.*, 1970c).

Briefly, 5 ml of soluble *E. coli* 026 antigen was radiolabeled by

incubation with 0.5 ml of $Na_2Cr^{51}O_4$[a] (11.7 x 10^6 CPM total -

diluted in saline) at 37 C with stirring for 48 hours (Braude *et*

al., 1955). The mixture was extensively dialyzed in several changes

of distilled water until there was no further significant loss of

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[a]New England Nuclear, Boston, Massachusetts.

radioactivity into the dialysate. The preparation was centrifuged (5,000 g for 10 minutes at 4 C) and the Cr^{51}-labeled supernatant was monitored on a gamma scintillation spectrophotometer[a] for specific activity before use in the test. All monospecific anti-globulins used in the test were first adsorbed with Cr^{51}-labeled 026 antigen to remove any nonspecific activity. Initially, 3 0.25 ml aliquots of selected calf sera were mixed in tubes with 0.1 ml of undiluted Cr^{51}-labeled 026 antigen. Similarly, 3 aliquots of 2 selected calf sera were mixed with 0.1 ml of Cr^{51}-labeled 026 antigen diluted 1:5 in saline. The mixtures were incubated at 37 C for 30 minutes and then at 4 C for 72 hours. Adsorbed antiglobulin, previously centrifuged, was added (0.15 ml/tube) to different tubes and the mixtures were reincubated as above. The precipitates formed were washed and centrifuged (10,000 g for 10 minutes at 4 C) 5 times in saline until there was no further radio-activity in the supernatants and until the radioactivity of the precipitates had stabilized. The precipitates were drained dry and monitored as before. A control tube consisted of saline and Cr^{51}-labeled 026 antigen.

Bacteriologic Procedures

Stock cultures of *E. coli* 026:K60:NM[b] were stored in the dark at ambient room temperature in sealed slant tubes containing

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[a]Packard Gamma Scintillation Spectrometer, Model 578, Packard Instrument Co., Inc., Downers Grove, Illinois.

[b]Kindly supplied by Dr. G. H. Conner, Michigan State University, East Lansing, Michigan.

trypticase soy agar with no dextrose. Subcultures were prepared at
regular intervals, incubated overnight at 37 C, sealed and stored
as above. Bacterin, used for injecting fetuses and neonates and
suspensions of live challenge organisms were prepared according to
the procedures described by Conner *et al.* (1973). The formalinized
bacterin was washed 3 times in saline, resuspended in sterile saline
to a concentration of 5 x 10^{10} organisms/ml and stored at 4 C in
a sterile vial. Merthiolate (0.02%, w/v) was added as a preservative.
Fresh 100 ml suspensions of 24 hour broth cultures containing unwashed
challenge organisms were prepared every 48 hours and stored in the
dark at 25 C. The viable count of challenge organisms in the broth
after incubation was approximately 3 x 10^9/ml.

Organ tissues and amniocentesis samples were cultured for the
presence of bacterial organisms by streaking the material onto blood
agar plates and incubating at 37 C for 24 hours. Isolates were
identified by standard biochemical tests. No attempts were made
to identify *E. coli* isolates according to serotype.

Histopathologic Technique

Sections were fixed in 10% (v/v) formalin-sodium acetate solu-
tion and stained with hematoxylin and eosin according to established
procedures (Luna, 1968).

Protein Analysis

The concentration of total protein in WBS and fractions from
chromatographic separations was determined either by the biuret

(Coles, 1974a) or the Lowry (Lowry *et al.*, 1951) chemical methods of analysis or by a refractometer.[a]

Photographic Recording

Unstained immunoprecipitates in agar-gel were photographed by the use of a multipurpose industrial view camera[b] and black and white film.[c]

Analysis of Amniotic Fluids

Amniotic fluid samples were analyzed for pH,[d] relative viscosity[e] (constant temperature water bath maintained at 38 C) and glucose (Cole, 1974b). The refractive index of each sample was also measured[a] to calculate the specific gravity.

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[a]American Optical Company, Buffalo, New York.

[b]Polaroid MP-3 Land Camera, Polaroid Corp., Cambridge, Massachusetts.

[c]Polaroid Land Roll Film Type 47, Polaroid Corp., Cambridge, Massachusetts.

[d]Beckman Expanded Scale pH Meter, Beckman Instruments, Inc., Fullerton, California.

[e]The Cannon-Ubbelohde Semi-Micro Viscometer, Cannon Instrument Co., State College, Pennsylvania.

RESULTS

Production of Monospecific Antiglobulins

The preliminary phase of this study involved the production

of monospecific antiglobulin for use in the immunoassays of various

samples from experimental calves. Gamma globulins IgG_1 and IgG_2,

previously precipitated and concentrated from WBS, were fractionated

in semipurified form with different elution buffers on an ion

exchanger. Generally, IgG_2 was eluted in highest concentration in

the ascending portion of the first peak with the starting buffer

(Figure 2). Semipurified immunoglobulin G_1 was eluted in highest

concentration in the descending portion of the third peak with the

0.01M buffer (Figure 3). Chromatographic separation of immuno-

globulin by filtration on porous gel resulted in elution of semi-

purified IgM in the ascending portion of the first (fall through)

peak (Figure 4), whereas semipurified IgG_1 and IgG_2 eluted in the

second peak. The semipurified eluates were reacted with the

respective antiglobulins by the Ouchterlony and IEP methods to

identify and determine the purity of the immunoglobulin fractions

obtained (Figures 5 through 9). Immunoglobulin G_2 was fractionated

in apparently pure form (Figures 2 and 6), whereas IgG_1 was con-

taminated with IgG_2 (Figures 3 and 8) and IgM was contaminated with

α2 macroglobulin (Figure 4). In most cases, the antiglobulin sera

81
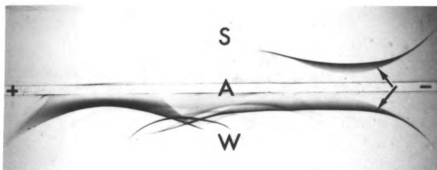
Figure 2. Immunoelectrophoretogram of a semi-purified fraction of bovine IgG_2 obtained by ion-exchange chromatography. Semipurified sample (S), WBS (W), AWBS (A) and IgG_2 (arrows). Notice the single IgG_2 immunoprecipitate band which has the same electrophoretic mobility as that of IgG_2 of WBS. Analine blue black stain.
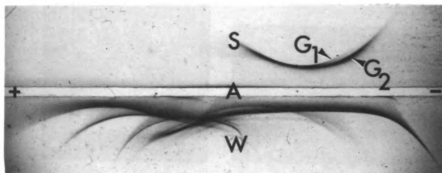
Figure 3. Immunoelectrophoretogram of a semi-purified fraction of bovine IgG_1 obtained by ion-exchange chromatography. Semipurified sample (S), WBS (W), AWBS (A), IgG_1 (G_1) and IgG_2 (G_2). Notice that the IgG_1 sample was heavily contaminated with IgG_2. Analine blue black stain.
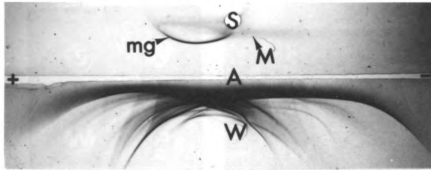
Figure 4. Immunoelectrophoretogram of a semi-purified fraction of bovine IgM obtained by porous gel filtration. Semipurified sample (S), WBS (W), AWBS (A), IgM (M). Notice that the IgM sample is contaminated with $\alpha 2$ macroglobulin (mg). Analine blue black stain.
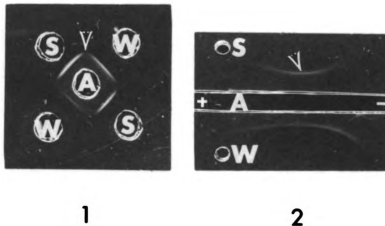
Figure 5. Photographs of Ochterlony (1) and immunoelectrophoresis (2) assays for the identification of semipurified bovine IgG_2. Semipurified sample (S), WBS (W) and monospecific antibovine IgG_2 (A). Notice that the sample IgG_2 forms a single immunoprecipitate band of identity and has a similar immunoelectrophoretic mobility (arrows) to that of IgG_2 of WBS. Unstained.

1   **2**
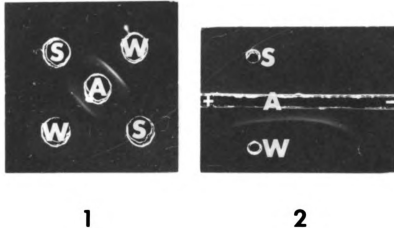
Figure 6. Photographs of Ouchterlony (1) and immunoelectrophoresis (2) assays to determine the purity of a bovine IgG$_2$ fraction. IgG$_2$ sample (S), WBS (W) and monospecific antibovine IgG$_1$ (A). Notice that there is no immunoprecipitate band formation between the sample and the monospecific anti-IgG$_1$ in either assay indicating the absence of IgG$_1$ as a contaminant. Unstained.

1   **2**
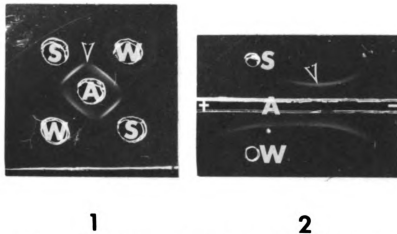
Figure 7. Photographs of Ouchterlony (1) and immunoelectrophoresis (2) assays for the identification of semipurified bovine IgG$_1$. IgG$_1$ sample (S), WBS (W) and monospecific antibovine IgG$_1$ (A). Notice that the sample IgG$_1$ forms a single immunoprecipitate band of identity and has a similar immunoelectrophoretic mobility (arrows) to that of IgG$_1$ of WBS. Unstained.
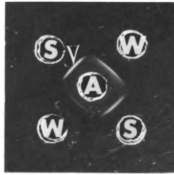
Figure 8. Photograph of an Ouchterlony assay to determine the purity of a fraction of bovine IgG_1. IgG_1 sample (S), WBS (W) and monospecific antibovine IgG_2 (A). Notice the immunoprecipitate band formation (arrow) between the sample and monospecific anti-IgG_2 indicating contamination of the sample with IgG_2. Unstained.
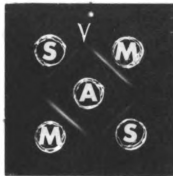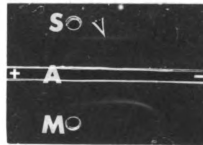
Figure 9. Photographs of Ouchterlony (1) and immunoelectrophoresis assays (2) for the identification of semipurified bovine IgM. IgM sample (S), IgM standard (M) and monospecific antibovine IgM (A). Notice that the sample IgM forms a single immunoprecipitate band of identity and has a similar immunoelectrophoretic mobility (arrows) to that of the IgM standard. Unstained.

produced in laboratory animals had monospecific activity when tested

by the Ouchterlony and the IEP methods (Figures 10 through 12).

These antiglobulins met the criteria for monospecificity by forming

only single immunoprecipitate bands with the respective immunoglobulin

M, G_1 and G_2. Antiglobulins produced in some animals were not mono-

specific, however, and required absorption to eliminate the undesirable

activity (Figure 13).

Clinical Responses to Fetal and Neonatal Injections

Two calves died at parturition; one (No. 392) was stillborn and

the other (No. 2170) died as a result of dystocia. A third fetus

(No. 1762) was aborted 3 days after *in utero* injection, apparently

due to injury to the cow and the fetus on the day of injection. Of

the reamining 27 calves, 21 were born unassisted and 6 required manual

assistance because of delayed delivery or dystocia. Fetal membranes

were retained in the first 8 cows that calved but were an infrequent

problem in the remaining cows. There was difficulty in correlating

the available breeding data with the physical findings on rectal

palpation. Consequently, it was not always possible to determine

the exact stage of gestation at the time of fetal injections. The

results of the clinical responses to fetal injections and the chal-

lenge of neonates are summarized in Table 1. Breeding data were not

available for 5 cows and birth weights were not recorded for 4 calves.

Newborn calves were assigned to groups according to the kind of

(bacterin or saline) *in utero* injection made, the number of days

between *in utero* injection and birth and the clinical responses

observed in calves previously challenged. Calf No. 1939 was not
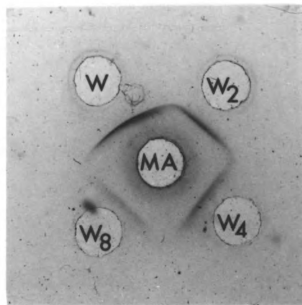
1
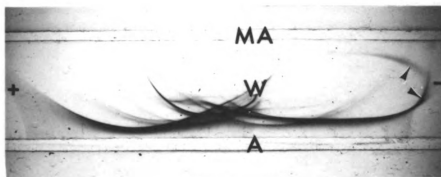
2

Figure 10. Photograph of the Ouchterlony
assay (1) and immunoelectrophoretogram (2) of mono-
specific antibovine IgG$_2$ produced in guinea pigs.
Monospecific antiglobulin (MA); WBS undiluted (W),
1:2 (W$_2$), 1:4 (W$_4$) and 1:8 (W$_8$) and AWBS (A).
Notice that the antiglobulin is monospecific and
forms only a single immunoprecipitate band with
IgG$_2$ of WBS (arrows). Analine blue black stain.
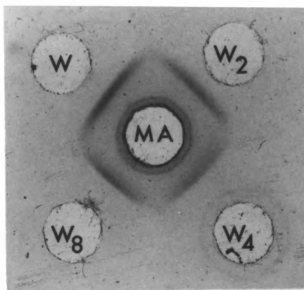
1
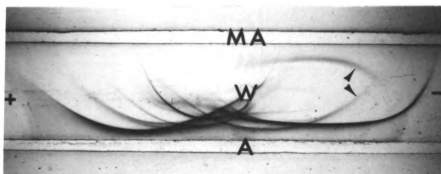
2

Figure 11. Photograph of the Ouchterlony assay (1) and immunoelectrophoretogram (2) of monospecific antibovine IgG$_1$ produced in guinea pigs. Monospecific antiglobulin (MA); WBS undiluted (W), 1:2 (W$_2$), 1:4 (W$_4$) and 1:8 (W$_8$) and AWBS (A). Notice that the antiglobulin is monospecific and forms only a single immunoprecipitate band with IgG$_1$ of WBS (arrows). Analine blue black stain.
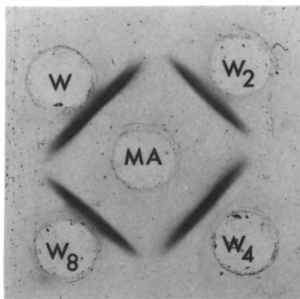
1
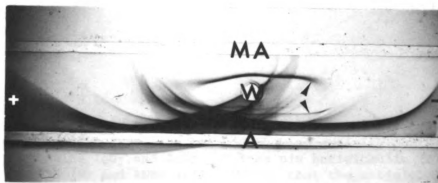
2

Figure 12. Photograph of the Ouchterlony assay (1) and immunoelectrophoretogram (2) of monospecific antibovine IgM produced in rabbits. Monospecific antiglobulin (MA); WBS undiluted (W), 1:2 (W_2), 1:4 (W_4) and 1:8 (W_8) and AWBS (A). Notice that the antiglobulin is monospecific and forms only a single immunoprecipitate band with IgM of WBS (arrows). Analine blue black stain.
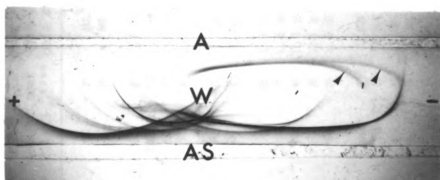
Figure 13. Immunoelectrophoretogram of anti-
globulin from a guinea pig showing activity against
bovine IgG$_1$ and IgG$_2$. Guinea pig antiglobulin (A),
WBS (W) and AWBS (AS). Notice that the antiglobulin
is not monospecific and forms at least 2 immunopre-
cipitate bands (arrows). Analine blue black stain.

Table 1. Details of *in utero* injections of bovine fetuses with *E. coli* 026:K60:NM bacterin or saline and responses in neonatal calves

Group no.*	Cow and calf no.	Type of in utero injection**	Time of injection (days before birth)	Gestation period (days)***	Birth weight (lbs.)	Sex ****	Oral challenge dose (viable E. coli org.)	Signs of clinical dis. after oral chal.δ	Time of euthanasia (age or days post-challenge)
1	1429	B	44	282	60	F	NA††	NA	14
	2042	B	49	281	70	M	NA	NA	14
2	1322	B	8	265	DNA†	F	1.5×10^{10}	--	4 PC^II
	67	B	8	270	DNA	M	1.5×10^{10}	--	5 PC
	1214	B	15	284	62	F	1.0×10^{11}	--	3 PC
	2036	B	47	275	70	F	1.5×10^{11}	--	4 PC
3	2116	B	11	278	55	F	NA	NA	5
	4	B	44	DNA	62	F	NA	NA	7
4	1459	B	10	274	72	M	1.5×10^{10}	--	3 PC
	2510	B	11	272	DNA	F	1.5×10^{10}	--	3 PC
	1377	B	18	282	64	M	1.0×10^{11}	+	2 PC
	2459	B	34	DNA	72	M	1.5×10^{11}	--	4 PC
	1534	B	28	282	64	M	1.5×10^{11}	--	4 PC
5	2290	B	10	281	33	F	NA	NA	at birth
	1690	B	20	273	58	F	NA	NA	at birth
	1518	B	20	DNA	44	M	NA	NA	at birth
	2452	B	28	281	67	M	NA	NA	at birth
6	334	S	34	274	50	M	NA	NA	at birth
	2816	S	36	DNA	64	F	NA	NA	at birth
	1717	S	62	287	66	M	NA	NA	at birth

Table 1 (continued)

Group no.*	Cow and calf no.	Type of in utero injection**	Time of injection (days before birth)	Gestation period (days)***	Birth weight (lbs.)	Sex ****	Oral challenge dose (viable E. coli org.)	Signs of clinical dis. after oral chal.δ	Time of euthanasia (age or days post-challenge)
7	4657	S	12	280	68	M	1.5×10^{10}	--	3 PC
	299	S	25	278	89	M	1.0×10^{11}	--	4 PC
	1912	S	26	276	82	M	1.5×10^{11}	+	1.5 PC
8	1567	S	39	280	84	F	1.5×10^{11}	--	4 PC
9	2085	S	5	268	DNA	F	NA	NA	9
10	2201	S	45	DNA	72	F	NA	NA	14

* See Figure 1.

** B=bacterin and S=saline.

*** Normal gestation period assumed to be 280 days.

**** F=female and M=male.

† Data not available.

‡ Not applicable.

δ -- = no clinical disease and + = clinical disease.

μ Postchallenge.

included in any group because 2 attempts were necessary before suc-
cessful fetal injection of bacterin was achieved and also because
the calf was born only 2 days after injection.

Six fetuses (Nos. 1939, 1322, 67, 1459, 2510 and 2085) were
injected between 2 and 11 days before birth. These fetuses were
born from 6 to 15 days earlier than expected. The gestation period
was not affected, however, for 3 other cows (Nos. 2116, 2290 and
4657), whose fetuses were injected between 10 and 12 days before
birth. All other fetuses, with known calving dates and injected
more than 12 days before birth, were born within the normal expected
range of gestation (Table 1).

The dose of unwashed viable *E. coli* organisms used for oral
challenge varied from 1.5 x 10^{10} (5 ml) to 1.5 x 10^{11} (50 ml).
Initially, the lower challenge dose given to newborn calves, pre-
viously injected *in utero* with bacterin (Nos. 1459 and 2510) or
saline (No. 4657) failed to produce clinical disease. The same low
challenge dose also failed to produce clinical disease in 2 other
principal calves reimmunized at birth (Nos. 1322 and 67) and chal-
lenged at 5 days of age. An intermediate challenge dose (1.0 x 10^{11})
produced clinical disease in one newborn principal calf (No. 1377)
but not in an older reimmunized principal calf (No. 1214) or a control
calf (No. 299). Two newborn principal calves (Nos. 2459 and 1534),
one reimmunized principal calf (No. 2036) and one 3-day-old control
calf (No. 1567) withstood the high challenge dose whereas clinical
disease was produced with the high challenge dose in one newborn
control calf (No. 1912) (Table 1).

The clinical signs observed in challenged calves were those associated with colisepticemia and toxemia and included anorexia, depression, dehydration and generalized weakness. A mild diarrhea with mucous- and blood-covered feces was observed in association with the adjustment to the liquid milk replacer diet and also in some cases of clinical disease. At birth, one calf (No. 2116) had severe respiratory distress which persisted until euthanasia. The appetite of this animal was normal, although the calf remained recumbent at all times. There were no apparent clinical signs of other infectious diseases observed in the calves.

The gross lesions in calves with clinical disease were mild and consisted of edema and swelling of mesenteric lymph nodes, congestion of mesenteric blood vessels, petechiation of the spleen and thymus and occasional swelling of the liver. The histopathologic lesions observed in calves with clinical disease were confined primarily to the lamina propria of the intestinal villi and included edema beneath the mucosal epithelium, congestion of capillaries in the lamina propria, dilatation of central lacteals and infiltration with inflammatory cells (Figure 14A and B). There were no apparent differences in the histologic appearance of tissues of calves injected *in utero* with bacterin and reinjected at birth compared to control calves. The normal histologic appearance of tissues from unexposed principal and control calves changed with age. For example, there was an increase in the formation and size of lymphoid follicles in the mesenteric lymph nodes (Figures 15 and 16). Also, intra-epithelial lymphocytes and goblet cells were observed in the mucosa of the intestinal villi of older calves (Figure 17).

Figure 14A and B. Photomicrographs of the lower jejunum of
principal calf No. 1377 (Group 4) previously challenged at birth
with *E. coli* 026:K60:NM and euthanatized at 2 days of age. Notice
the edema beneath the mucosal epithelium (1), congestion of capil-
laries in the lamina propria (2) and dilatation of central lac-
teals (3). H&E stain; X 135 (A) and 540 (B).
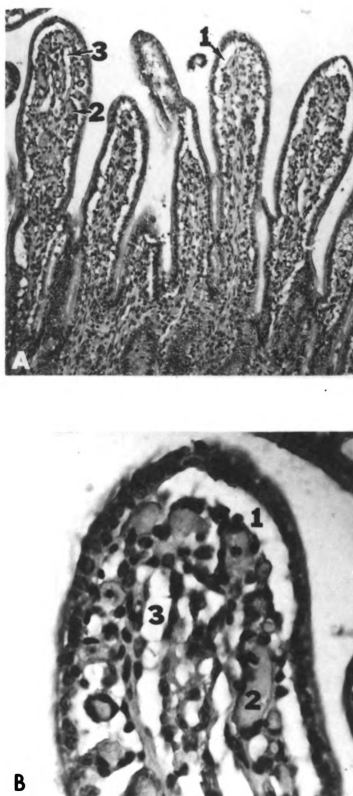
Figure 14

Figure 15. Photomicrograph of the mesenteric lymph node in the region of the ileum from principal calf No. 2290 (Group 5) euthanatized at birth. Notice the random distribution of lymphocytes in the cortical area (arrows) and also the sinusoidal structures in the medulla (S). H&E stain; X 135.

Figure 16. Photomicrograph of the mesenteric lymph node in the region of the ileum from principal calf No. 4 (Group 4) euthanatized at 7 days of age. Notice the lymphoid follicles in the cortical area (arrows). H&E stain; X 135.
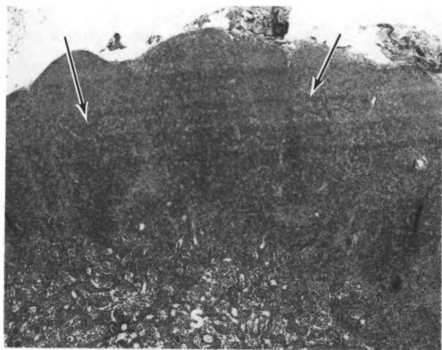
Figure 15
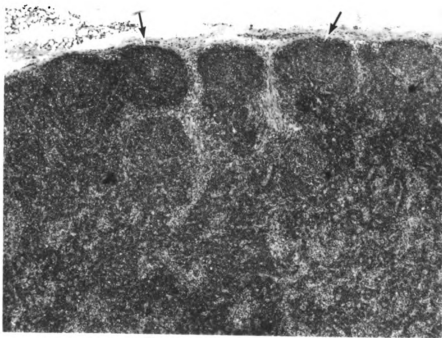
Figure 16
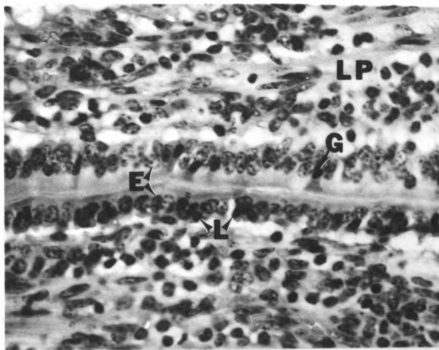
Figure 17. Photomicrograph of the lower
jejunum of principal calf No. 2042 (Group 1)
euthanatized at 2 weeks of age. Notice the goblet
cell (G) and occasional lymphocytes (L) positioned
between columnar epithelial cells (E) of the
mucosae of 2 adjoining intestinal villi; lamina
propria (LP). H&E stain; X 540.

The results of the bacteriologic examination of calves infected

orally with an unwashed suspension of live *E. coli* organisms 026:K60:NM

are summarized in Table 2. Calves selected for examination were those

that showed clinical signs of disease after challenge or those chal-

lenged with the moderate or high challenge dose of *E. coli*. Bacterio-

logic examination of amniocentesis samples showed either no bacterial

growth or very light growth of *Micrococcus* sp., *Bacillus* sp. or

Enterobacter sp., which probably represented contamination of the

samples by saprophytic organisms.

Table 2. Results of bacteriologic examination of neonatal calves
previously injected *in utero* with *E. coli* 026:K60:NM
bacterin or saline and orally challenged with the
homologous organism

Group no.	Calf no.	Oral challenge dose (viable *E. coli* org.)	Tissues examined			
			Heart	Liver	Spleen	Kidney
4	1377	1.0×10^{11}	LGNH* *E. coli*	LGNH *E. coli*	LGNH *E. coli*	LGNH *E. coli* *Strep.*sp.
	2459	1.5×10^{11}	NG**	NG	NG	NG
	1534	1.5×10^{11}	NG	NG	NG	NG
7	299	1.0×10^{11}	LGNH *E. coli* *Strep.*sp. *Prot.* sp.	LGNH *E. coli* *Strep.*sp.	LGNH *E. coli* *Strep.*sp. *Prot.* sp.	LGNH *E. coli* *Strep.*sp.
	1912	1.5×10^{11}	LGNH *E. coli*	LGNH *E. coli*	LGNH *E. coli*	LGNH *E. coli*
8	1567	1.5×10^{11}	NG	NG	NG	NG

*Light growth of nonhemolytic.

**No growth.

Immunologic Responses to Fetal and Neonatal Injections

Direct Bacterial Agglutination and Passive Hemagglutination Assays

The results of the direct bacterial agglutination and PHA tests on calf sera with O26 and K60 *E. coli* antigens are summarized in Table 3. Preliminary testing by the PHA method showed that serum from 7 calves caused hemagglutination of uncoated SRBC. These samples were subsequently absorbed with uncoated SRBC to remove the nonspecific hemagglutinating activity and then retested. Three serum samples, with known hemagglutinating titers but no nonspecific activity against uncoated SRBC, were also absorbed in the same manner. Results of the latter absorption control tests showed that absorption with uncoated SRBC had no effect on the O26 hemagglutination titers. There was general agreement between the direct agglutination and the PHA assays in detecting O26 agglutinins in serum. However, the passive O26 hemagglutinin titers were generally greater than the direct O26 agglutinin titers and in some cases there was a 5-fold difference. There was little K60 agglutinin or hemagglutinin activity as measured by either the direct or the PHA methods. All control calves had negative O26 agglutinin titers at birth with the exception of Nos. 299 and 1567.

Only the serum from calf No. 299 had passive hemagglutination titers with the 4 *E. coli* O serotypes other than O26. The titers of serum taken at birth ranged from 4 to 16, whereas the titers of serum taken at necropsy were either 64 or 128. The serum from all other calves showed either incomplete PHA or were negative.

Table 3. Direct bacterial agglutination and passive hemagglutination titers* of serum from neonatal calves previously injected *in utero* with *E. coli* 026:K60:NM bacterin (Groups 1-5) or saline (Groups 6-10)

Group no.	Calf no.	026 Antigen				K60 Antigen			
		Direct		Passive		Direct		Passive	
		b**	n**	b	n	b	n	b	n
1	1429	32	32	512	64	2[††]	4	-	-
	2042	-	4	2	4	-	-	-	-
2	1322	-	-	4	4	-	2	-	-
	67	2	16	4	32	-	-	-	-
	1214	-	128	-	256	-	-	-	-
	2036	-	-	-	2	-	-	-	-
3	2116	-	4	-	4	2	2	2	2
	4	8	128	64	128	-	2	-	-
4	1459	2	4	2	-	-	-	-	-
	2510	-	2	2	2	2	2	2	2
	1377	-	2	16	16	-	-	-	-
	2459	16	32	64	64	2	4	-	-
	1534	4	64	64	1024	-	-	-	-
5	2290	-		32		-		-	
	1690	-		4		-		-	
	1518	-		32		-		-	
	2452	2		8		-		-	
6	334	-		-		-		-	
	2816	-		-		-		-	
	1717	-		-		-		-	
7	4657	-	-	-		-	-	-	-
	299	4	32	16	64	-	4	2	-
	1912	-	2	-	64	-	-	-	-
8	1567	2	8	2	32	-	2	-	2
9	2085	NT†	-	NT	-	NT	-	NT	-
10	2201	-	2	-	4	-	-	-	-

* Titers are expressed as the reciprocal of the highest dilution showing agglutination. Numbers represent the highest titers observed by either macro- or microtitration methods.

** b=sample taken at birth, n=sample taken at necropsy.

† not tested

†† negative

The results of the PHA assay of intestinal washings with 026

antigen of *E. coli* are summarized in Table 4. Several intestinal

washing samples initially caused either a rapid hemolysis of antigen

coated and uncoated SRBC or nonspecific agglutination of uncoated

SRBC. It was necessary to absorb these samples from 1 to 4 times

with uncoated SRBC to remove the hemolytic and the nonspecific

agglutinin activity. Also, during the testing, several intestinal

washing samples became cloudy. Microscopic examination of stained

smears of this material showed the presence of large numbers of rod-

shaped microorganisms. The PHA test with duplicate samples of

intestinal washings was rerun later and the 026 hemagglutinin titers

were either decreased or negative.

All intestinal washing samples were negative, with the exception

of calf No. 67 when tested by the PHA assay with K60 antigen of *E.*

coli. The poor responses of intestinal washings to the K60 antigen

of *E. coli* could have resulted from the following causes: 1) the

K60 antigen was destroyed during the preparation of the bacterin or

2) the possible enzymatic activity of the intestinal washings could

have denatured the antigen. To test the former possibility, direct

bacterial agglutination tests (macro and micro) were run in duplicate

by reacting serial 2-fold dilutions of bacterin, left over from fetal

injections, with monospecific anti-026 or anti-K60 typing sera

diluted 1:2.[a] A live suspension of homologous *E. coli* organisms was

also tested in the same manner as a control. The results indicated

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[a]Kindly supplied by Dr. P. Glantz, The Pennsylvania State
University, College Station, Pennsylvania.

Table 4. Passive hemagglutination titers* of intestinal washings from neonatal calves previously injected *in utero* with *E. coli* 026:K60:NM bacterin (Groups 1-5) or saline (Groups 6-10)

Group no.	Calf no.	Source of Intestinal Washings**			
		D	UJ	LJ	I
1	1429	4	4	16	4
	2042	-	-††	-	-
2	1322	-	-	-	-
	67	-	2	8	-
	1214	-	16	-	4
	2036	-	-	-	-
3	2116	-	-	-	-
	4	4	16	8	64
4	1459	2	-	-	-
	2510	2	-	-	-
	1377	-	-	-	-
	2459	-	4	32	8
	1534	NT†	NT	NT	NT
5	2290	-	2	2	-
	1690	-	-	-	-
	1518	-	-	-	-
	2452	-	-	-	-
6	334	-	-	-	-
	2816	-	-	-	-
	1717	-	-	-	-
7	4657	-	-	-	NT
	299	-	-	-	-
	1912	-	-	-	-
8	1567	-	4	2	2
9	2085	2	-	-	-
10	2201	-	-	-	-

*Titers are expressed as the reciprocal of the highest dilution showing hemagglutination of SRBC coated with 026 antigen of *E. coli*.

**D=duodenum, UJ=upper jejunum, LJ=lower jejunum and I=ileum.

†Not tested

††Negative

the presence of K60 antigen in the bacterin (1:64) and also in the suspension of live organisms (1:128). Preparation methods apparently caused only a 2-fold decrease in the K60 antigen content of bacterin. The 026 antigen was also present (1:32) in both the bacterin and the live bacterial suspension.

Agar Plaque Assay

The agar plaque assay was conducted on single cell suspensions of selected tissues from 21 calves. The results of the tests are summarized in Table 5. The number of calves with plaque forming responses in the various tissues tested is summarized in Table 6. The hemolytic plaques were identified as a clearing within a uniform suspension of coated SRBC in the agar-gel (Figure 18). Often there was a structure identifiable as a cell within the plaque area (Figure 19). The IgM producing cells predominated, were found in the tissues of a greater number of calves and were nearly evenly distributed throughout the intestinal tissues and corresponding mesenteric lymph nodes (Table 6). Indirect plaque forming cells were also observed in tissues from all but 5 calves. Immunoglobulin G_1 plaque forming cells were observed in tissues with intermediate frequency and IgG_2 plaque forming cells were observed with the least frequency (Table 6). There were also direct plaque forming cells in the control samples tested from the intestine of many calves. In many cases, where there was a direct (IgM) plaque response, there were also indirect (IgG_1 and IgG_2) responses. The plaque forming responses observed in the spleen were, in some cases, inconsistent

Table 5. Results of the agar plaque assay of selected tissues from 21 neonatal calves previously injected *in utero* with *E. coli* 026:K60:NM bacterin (Groups 1-5) or saline (Groups 6-10)

Group no.	Calf no.	Total Number of Plaques from Selected Tissues								
		Small Intestine (no./10^8 cells)			Mesenteric L.N. (no./10^8 cells)			Spleen (no./10^8 cells)		
		IgM	IgG_1	IgG_2	IgM	IgG_1	IgG_2	IgM	IgG_1	IgG_2
1	1429	10,800	--*	--	--	--	--	--	--	--
	2042	12,600	18,500	300	--	--	--	--	--	--
2	1322	250	--	--	--	--	--	--	--	--
	67	3,750	250	500	225	--	125	325	--	--
	1214	950	850	--	15	--	--	--	--	--
	2036	53,350	--	--	--	--	--	--	--	--
3	4	16,900	--	--	--	--	--	--	--	--
4	1459	8,250	250	--	50	--	25	--	--	25
	1377	39,050	16,100	6,350	1,465	530	--	550	--	--
	2459	113,000	--	--	15	1,100	45	--	630	30
	1534	152,650	850	--	--	--	--	--	--	--
5	2290	5,500	500	2,250	225	--	50	--	--	25
	1690	1,000	--	--	--	--	75	50	--	--
	1518	1,950	350	--	65	15	--	15	--	15
	2452	6,100	3,850	700	345	--	--	50	65	--
6	334	18,600	--	--	215	--	--	230	--	--
	2816	--	1,100	950	--	30	30	--	--	--
	1717	--	5,950	2,800	--	--	--	--	--	--
7	299	10,450	--	--	530	65	15	--	--	--
8	1567	53,100	25,150	4,000	--	--	--	--	--	--
10	2201	10,150	--	--	--	--	--	--	--	--

*Either no plaque formation or the number of plaques formed did not exceed the number of control plaques.

Figure 18. Photograph of an agar plaque assay of a single cell suspension of tissue from a neonatal calf. A hemolytic plaque can be seen as a clear area (arrow) within a uniform suspension of coated SRBC in agar-gel. Unstained.

Figure 19. Photograph of an agar plaque assay of a single cell suspension of tissue from a neonatal calf showing a cell (arrow) in the center of a single plaque. Unstained.
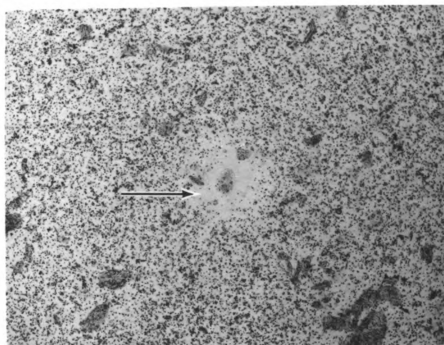
Figure 18
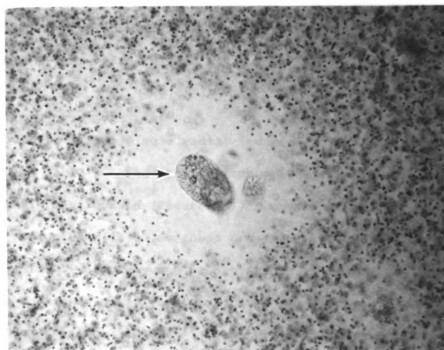
Figure 19

Table 6. Results of the plaque forming responses of the intestine and mesenteric lymph nodes of 21 neonatal calves previously injected *in utero* with *E. coli* 026:K60:NM bacterin (Groups 1-5) or saline (Groups 6-10)

| | Tissue Samples** | | | | | | |
| | Intestine | | | | Mesenteric lymph nodes | | |
Plaque Assay	D	UJ	LJ	I	UJ	LJ	I
Direct (IgM)	17	12	12	10	7	7	9
Indirect (IgG$_1$)	7	6	5	2	4	3	1
Indirect (IgG$_2$)	2	2	5	1	4	5	6

* Figures represent the total number of calves with responses in the respective tissues tested.

** D=duodenum, UJ=upper jejunum, LJ=lower jejunum and I=ileum.

with the responses observed in the intestine and mesenteric lymph nodes from the same animal (Table 5).

Single Radial Immunodiffusion

The results of the single radial immunodiffusion assay are summarized in Table 7. The stained immunoprecipitate rings formed were not uniformly rounded (Figure 20), thus requiring that the images be projected with an overhead projector, traced onto paper and then cut out. Samples with no detectable ring formation were assumed to be either negative or to contain quantities of immuno-globulin less than the limits of sensitivity of the assay. The points made on graph paper for the standard antigens assumed nearly a straight line. Therefore, a straight line curve was drawn free hand to best fit the data rather than to determine the line by

Table 7. Results of single radial immunodiffusion of serum from neonatal calves previously injected *in utero* with *E. coli* 026:K60:NM bacterin (Groups 1-5) or saline (Groups 6-10)

Group no.	Calf no.	Quantity of Immunoglobulins* IgM b**	IgM n**	IgG$_2$ b	IgG$_2$ n
1	1429	-	0.52	0.50	0.65
	2042	0.51	2.02	-††	0.85
2	1322	0.37	0.45	-	-
	67	0.38	0.75	-	-
	1214	-	0.79	-	-
	2036	-	0.58	-	-
3	2116	0.67	0.79	-	-
	4	-	1.23	-	-
4	1459	-	0.31	1.28	1.44
	2510	0.89	0.56	-	-
	1377	0.37	0.40	-	-
	2459	-	0.35	-	-
	1534	-	0.77	-	-
5	2290	1.90		0.80	
	1690	-		-	
	1518	1.06		0.64	
	2452	0.29		-	
6	334	0.43		0.55	
	2816	-		-	
	1717	-		-	
7	4657	-	0.28	-	-
	299	0.35	1.36	0.85	2.90
	1912	-	-	-	-
8	1567	0.35	1.55	-	1.40
9	2085	NT†	1.08	NT	0.81
10	2201	-	0.61	-	-

*Quantities of immunoglobulins are expressed as mg/ml.

**b=birth and n=necropsy

†not tested

††Either negative or present at a concentration less than the sensitivity of the assay.
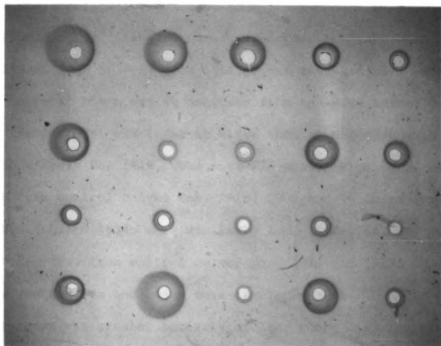
Figure 20. Results of the quantitative determination of bovine IgM in calf sera by single radial immunodiffusion. Standard antigen (IgM) was placed in the row of wells across the top and calf sera were placed in the remaining wells. The concentrations of standard antigen from left to right in mg/ml were 1.75, 1.35, 0.95, 0.55 and 0.15, respectively. Analine blue black stain.

regression methods. Of the two immunoglobulin classes quantitated,

the major immunoglobulin present in the serum of most calves was IgM.

Radioimmunoassay

The results of the radioimmunoassay of serum from selected calves

are summarized in Table 8. The results showed in part that the

response was quantitative but also that the response was dependent

on the amount of Cr^{51}-labeled 026 antigen used to react with calf

serum (1534[n] and 1534-1:5[n]; 1912[n] and 1912-1:5[n]). Where

samples taken at birth and at necropsy from the same animal were

tested, the response was lower at birth than at necropsy with the

exception of calf No. 1429. The anti-026 activity of serum taken

at birth from control calves Nos. 2816, 299b and 1912b and principal

calves Nos. 4b and 1534b were similarly low. Likewise, the serum

taken at necropsy from control calves Nos. 299n and 1912n and prin-

cipal calves Nos. 4n and 1534n were similarly high. In most cases,

wherever there was greater activity of IgM, there was also increased

activity of either IgG_1 or IgG_2 or both.

Qualitative Immunoelectrophoresis

The immunoelectrophoretograms of serum from selected calves

are shown in Figures 21 and 22. The IEP profiles of normal WBS and

serum from selected calves were similar, and the albumin and globulin

fractions were readily identifiable in all samples tested. The

globulin proteins of serum from calves tested at birth were generally

not as prominent in the immunoelectrophoretograms as the globulin

proteins in serum from older calves and in adult serum, although

Table 8. Results of the radioimmunoassay of serum from selected
calves previously injected *in utero* with *E. coli*
026:K60:NM bacterin (Groups 1-5) or saline (Groups 6-10)

Group no.	Calf no.†	Gamma Radiation Counts of Precipitates Formed by the Respective Antiglobulins**		
		IgM	IgG_1	IgG_2
1	1429(b)	1,631	1,373	900
	1429(n)	1,986	777	834
2	67(n)	2,916	1,001	1,077
	1214(n)	3,194	1,593	1,548
3	4(b)	433	488	423
	4(n)	3,263	1,206	2,430
4	2459(b)	587	572	768
	2459(n)	2,008	1,489	1,926
	1534(b)	250	423	471
	1534(n)	2,849	2,115	6,396
	1534-1:5(n)	695	450	1,827
5	2290(n-b)	1,237	446	1,659
	1518(n-b)	1,598	450	660
6	2816(n-b)	238	152	708
7	299(b)	594	353	588
	299(n)	3,199	1,828	3,237
	1912(b)	256	195	735
	1912(n)	2,844	1,166	2,112
	1912-1:5(n)	630	299	527
8	1567(n)	5,777	728	1,308

*Cr^{51}-labeled 026 antigen was used as the primary antigen in the test.

**Figures represent CPM of Cr^{51} gamma radiation.

†b=birth, n=necropsy, n-b=necropsy at birth.
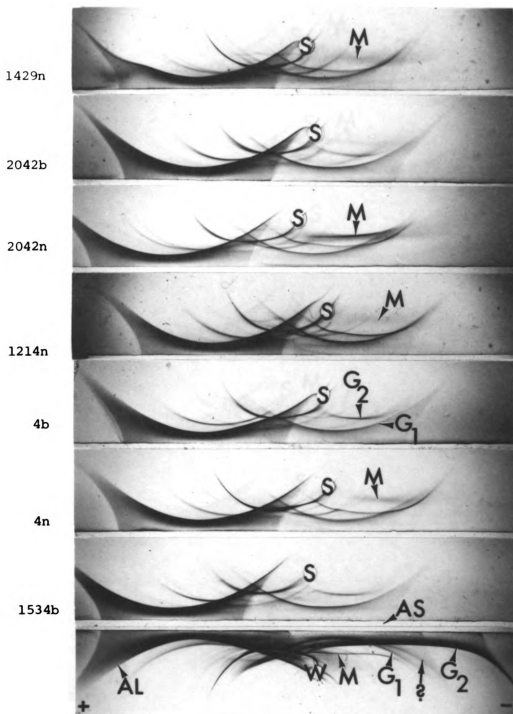
Figure 21. Immunoelectrophoretograms of sera taken at birth or at death from selected calves; Calf serum sample (S), WBS (W), AWBS (AS), IgM (M), IgG$_1$ (G$_1$), IgG$_2$ (G$_2$) and Albumin (AL). Notice that the IEP profiles of calves at birth are not as prominent as in older calves or adult serum although IgG$_1$ and IgG$_2$ are present in all sera. Also notice the immunoprecipitate band between IgG$_1$ and IgG$_2$ (?). Analine blue black stain.

Figure 22. Immunoelectrophoretograms of sera taken at birth or at death from selected calves; Calf serum sample (S), WBS (W), AWBS (AS), IgM (M), IgG_1 (G_1), IgG_2 (G_2), IgA (A) and Albumin (AL). Notice the similarity of the IEP profiles of calves No. 299 and 1567 at death to adult serum. Also, the IgM immunoprecipitate band is especially prominent in calf No. 2290. Notice the immunoprecipitate band between IgG_1 and IgG_2 (?). Analine blue black stain.

immunoglobulins G_1 and G_2 were always detectable at birth. The IEP profiles of the serum gammaglobulins of calves tested at necropsy were not necessarily identical to those of adult serum, with the exception of control calves Nos. 299 and 1567 (Figure 22). The serum of most calves tested at necropsy reflected an apparent increase in concentration of immunoglobulins M, G_1 and G_2 over what was observed at birth as shown by the appearance, size and intensity of the respective immunoprecipitate bands. Serum immunoglobulin A was also observed in control calf No. 299 when tested at necropsy (Figure 22). A third immunoprecipitate band in the gamma region between IgG_1 and IgG_2 was consistently observed in the immunoelectrophoretograms of normal adult WBS (Figures 21 and 22).

Analysis of Amniotic Fluids

The results of the analysis of the amniotic fluid samples are summarized in Table 9.

Table 9. Results of the analysis of amniotic fluid samples obtained
from 30 cows at the time of *in utero* injection of the
fetuses

Test	Results*
Specific gravity	1.0095 + .1985
pH	8.065 + 1.116
Relative viscosity	2.258 + 1.038
Glucose	2.28 + 0.09 mg/ml
Refractive index	1.3366 + 0.0019

*Mean + s.e.

DISCUSSION

Production of Monospecific Antiglobulins

Several immunological and analytical procedures were used to attempt to fulfill the objectives of the present study. Conventional immunological laboratory procedures were used to characterize the humoral and local immune responses of bovine fetuses and neonates to *E. coli* antigen.

Some of the assays used in the present study to qualitate and quantitate the immune responses of bovine fetuses and neonates required the use of antiglobulins which were special immunological reagents that reacted with or "recognized" only one class of immuno-globulin protein from whole calf serum. The laboratory procedures involved in the nonspecific isolation of semipurified and purified globulin fractions from WBS were technically complicated and did not always yield a pure product (Figures 3, 4 and 8). The monospecificity of the final antiglobulin produced in laboratory animals was only as good as the purity of the globulin antigens used for primary and/or secondary immunization (Figures 10 through 12). The method used for finally obtaining purified globulins as antigens by immunopre-cipitation in agar-gel with known monospecific antiglobulin represented one of the most direct methods for antigen purification. Additional advantages of this final purification procedure were 1) the agar-gel

was a convenient medium for emulsification of the immunoprecipitates
with adjuvant, 2) agar-gel had adjuvant properties of its own (Chase,
1968a) and 3) the laboratory animals produced antibody primarily
against the bovine globulin component of the immunoprecipitate since
the known antiglobulin portion of the immunoprecipitate was from the
same animal species. There may, however, have been allotypes of the
antiglobulin which could have caused an additional immune response.
Preparation of a purified globulin fraction is important for conduct-
ing the single radial immunodiffusion test since serial dilutions of
a purified globulin must be used as a standard antigen. Nonspecific
methods for purification of IgM or IgG_1 often result in products
contaminated with $\alpha 2$ macroglobulin (Figure 4) or IgG_2 (Figure 3),
respectively. Alternate methods of obtaining either of these 2
globulins in pure form from semipurified preparations would have
been either affinity chromatography or electrophoresis in Sephadex
G-25 on Perspex plates as described by Porter (1972). Sufficient
quantities of antiglobulins of known monospecificity to absorb out
the contaminating materials from a semipurified sample were not
available for use with affinity chromatography.

Experimental Design and Procedures

The original experimental plan was to divide the calves such
that there would have been 4 calves in each principal group and 2
calves in each control group and then to subject all the calves in
the same group to the same respective treatment. This planned
distribution of calves and like treatment within groups would have
resulted in sufficient data to make a statistical comparison between

similar principal and control groups according to the clinical responses to challenge and other immunological responses. Due to the limited number of calves originally available and variations in treatments given to calves within the same group, necessitated by variations in observed clinical responses, it was necessary to deviate from the original plan and divide the calves into unequally sized groups. As a result, the data generated from the small numbers of calves subjected to the same treatment could not be applied to a statistical comparison. Nonetheless, differences and similarities in clinical and immunological responses were observed and are noted later.

Experimental injection and immunization of bovine fetuses have not progressed to the same degree of sophistication that has been achieved in other animal species (Sterzl and Silverstein, 1967). This is due in part to the ease of handling of smaller animal species in the surgical techniques involved and the costs incurred in using cattle for experimentation. Limited studies with fetal immunization have shown that the bovine fetus is immunocompetent to a variety of antigenic substances (Osburn, 1973; Schultz, 1973a) and that clinical application can possibly be made of these findings. Furthermore, Porter (1973a) has stated that

"...in the local immune system the mechanisms of active synthesis and secretion of antibody in the intestinal mucosa start functioning in the first week of life, although the high levels of passively-acquired maternal antibodies produce a potentially suppressive effect. This suggests that there are opportunities for oral immunization while the young animal is suckled, with the attractive prospect of increasing the protective characteristics of the local epithelial defence mechanisms before weaning."

Proper selection of a combination of immunizing substances used to immunize fetuses could result in newborn calves which are resistant to the respective diseases that often affect neonates. Also, Gay (1971) found that neonatal calves, previously immunized *in utero,* had cross immunity when exposed to nonhomologous challenge organisms.

The nonsurgical technique of fetal immunization required that fetal development had progressed well into the third trimester of pregnancy to assure the proper positioning and size of the fetus for accurate placement of the antigen. In addition, near-mature fetuses are more likely to be immunocompetent than younger fetuses (Osburn, 1973; Schultz, 1973a). The original plan was to obtain females that had advanced to the same stage of pregnancy so that fetal injections could be made as close as possible to the beginning of the 8th month of gestation. As noted in the Results, there was difficulty in assessing the exact stage of pregnancy at the time fetal injections were made. As a result, data indicate (Table 1) that the time of *in utero* injection before birth varied from 5 to 62 days. This wide variation may have affected the immune responses observed in neonates. It is doubtful, however, that the injections made in the younger fetuses resulted in tolerance of those fetuses to the antigen.

The requirement of advanced pregnancy for fetal injections poses some practical problems with beef breeding cattle maintained under range conditions because the majority of these animals are pasture bred by natural service and precise breeding data are usually not available. Furthermore, it is often difficult to accurately estimate the time of gestation in advanced pregnancies by rectal palpation.

This problem could be lessened by maintaining beef breeding cattle under dry lot management systems or by the use of artificial insemination. Neither of these management practices is currently used to any great extent by either commercial or purebred beef cattle producers.

R. D. Schultz (personal communications, 1974) indicated that fetal injections made during the advanced stages of gestation may result in the shortening of the normal gestation period by as much as 2 weeks. With the exception of 3 animals (67, 1322 and 2085), results (Table 1) indicated that the cows calved within the limits of the normal range expected for gestation regardless of the type of injections made and when they were made during the gestation period. Although fetal injection may in some circumstances have an effect on the normal bovine gestation, the data from the present study do not support this hypothesis. The fetal injections were made several days after the cattle had been shipped a long distance.

Colibacillosis most commonly occurs as an enteric or septicemic disease of young calves. Limited data indicate that the disease is more prevalent in dairy than in beef or dual-purpose breeds of calves (Olson, D. P., unpublished data, 1975). One reason for the greater incidence of colibacillosis in dairy calves may be management practices which result in forced confinement and a greater concentration of neonatal calves in a small area and thus an increased opportunity for exposure to pathogenic strains of *E. coli*. Beef calves are often raised in less confined areas and it is presumed have less opportunity for concentrated exposure to pathogenic *E. coli*. A beef breed was

chosen in the present study because colibacillosis nonetheless does pose as a threat of disease to young beef calves and because of experimental interest in determining the immune responses of the beef-type animal to the antigen.

In a recent study of fetal immunization in dairy calves, Conner et al. (1973) injected *E. coli* bacterin into the amniotic fluid and later demonstrated an immune response to the homologous antigen in the serum of neonates. It was assumed that immunization occurred as a result of oral ingestion of the antigen by the fetus with subsequent development of either local and/or humoral immunity. The exact fate and distribution of the ingested antigen in the fetus is unclear. Apparently, some of the antigenic material remains in the intestine and the rest is nonselectively absorbed by the intestine resulting in the potential for stimulation of both the local and humoral immune systems. The nonsurgical method of fetal immunization into amniotic fluid seems more desirable than surgical intervention and parenteral injection of antigen into the fetus (Schultz et al., 1971) partly because of uncertainty of where the parenteral injection is actually being made. Further, there is the potential danger with parenteral injection of injuring a vital fetal organ such as the eye. By carefully following the procedures of *in utero* injection by nonsurgical methods, as described in the Materials and Methods section, one should be reasonably assured of avoiding injury to vital fetal organs and of placement of the antigen into the amniotic fluid.

Data regarding the compositional analysis of the amniocentesis samples obtained (Table 9) agreed fairly well with the values reported by Needham (1931). However, it was not clear how the values reported by the latter were determined. The purpose of analyzing amniotic fluids was to obtain additional evidence beyond visual evaluation of the material that the fetal injections were actually made into the amniotic fluid as intended. There would have been little or no chance for oral ingestion by the fetus to have occurred if the injections had been made into the allantoic fluid because of no direct contact between this material and the fetus. It is possible that the negative PHA results on serum from principal calves Nos. 1214, 2036 and 2116 could have been due to injection into the allantoic cavity although records kept during the time that fetal injections were made indicated that the material aspirated resembled amniotic more than allantoic fluid. Based primarily on the appearance, character and consistency of the material and secondarily on the compositional analysis, it can only be concluded that the samples obtained in the present study were amniotic fluid.

Clinical Responses to Fetal and Neonatal Injections

The original dose of *E. coli* used to challenge certain neonatal calves (1.5×10^{10} live organisms/ml) was determined on the basis of an earlier report by Conner *et al.* (1973) which involved dairy calves. The unexpected failure of this challenge dose to produce clinical disease when given at birth to the first control calf born (No. 4657) resulted in the final adjustment of the challenge dose given to calf No. 1912 to 1.5×10^{11} unwashed organisms/ml. This

was 10 times the dose used initially. It was possible that bacterial endotoxins present in the unwashed challenge inoculum may have also contributed to the clinical disease observed. The calves were maintained under environmental conditions almost identical to those used by Conner *et al.* (1973). These results and the data summarized from the Missouri Veterinary Diagnostic Laboratory support the hypothesis that there are breed differences in natural susceptibility (or resistance) to *E. coli* infection in neonatal calves. The clinical responses of control calf No. 299 to an intermediate challenge dose given at birth represents a special case and will be discussed later in light of other data. The clinical responses of principal calves 1459 and 2510 challenged at birth with the low dose of challenge organisms were meaningless since the same dose failed to cause clinical disease in a comparable control calf (No. 4657). The apparent resistance of principal calves Nos. 2459 and 1534 to the high challenge dose was more consistent with expected results and was likely enhanced by an artificially acquired immunity resulting from fetal injection (Table 3). Principal calf No. 1377 did not survive an intermediate challenge dose primarily because of a poorer immune response to fetal injection (Table 3). The results of the oral challenge of 3-day-old control and principal calves indicated an association between age and resistance. Further, these results suggested that older calves (3 days and older) may have been naturally more resistant to the strain of *E. coli* used than were younger calves. This conclusion becomes more valid when one considers that the calves were raised without colostrum.

The results of the bacteriologic examination of neonatal calves challenged with live unwashed *E. coli* organisms (Table 2) indicated that *E. coli* were the predominant organisms isolated and identified. Only a light growth of *E. coli* was noted, however. This is not what one would expect from calves suffering from colisepticemia. Nonetheless, calves with clinical disease (Nos. 1377 and 1912) did show signs consistent with septicemia and/or toxemia. It was assumed that the *E. coli* isolated from these calves were of the same serotype as that of the challenge organisms, although this was not proven since isolates were not serotyped. Young calves may be naturally exposed to high concentrations of pathogenic *E. coli* organisms and endotoxins together, and it can be reasoned that the method of challenge used approximated these natural conditions.

Immunologic Responses to Fetal and Neonatal Injections

The procedures used to prepare the antigen substances for *in vitro* immunological tests may result in changes of the antigens such that they are structurally different than the antigens used for immunization. As a result, the *in vivo* immune responses may be different than the responses that are measured by *in vitro* methods because the antibody will not react optimally to the altered antigen. This becomes more important when experimental procedures are designed to measure specific antigen and antibody interactions as in hapten-antihapten systems. It was assumed that this was not a problem since the methods used to prepare *E. coli* antigens as immunological reagents were standard and accepted procedures even though they probably resulted in rather crude preparations of antigen.

Tables 3, 7 and 8 and Figures 21 and 22 contain data which

quantitate and qualitate the humoral responses of the calves.

Although the results of the direct bacterial agglutination and the

PHA assays are expressed as titers of antibody present in serum,

these assays are usually not considered as actual quantitative

measures of antibody. Rather, the single radial immunodiffusion

and the radioimmunoassay tests, by convention, represent more direct

methods of quantitating antibody. There was an association between

the direct bacterial agglutination, PHA and the radioimmunoassays

in that all 3 tests measured direct activity of antibody present

against the *E. coli* test antigen. In contrast, the single radial

immunodiffusion and IEP assays quantitated and qualitated, respectively,

the total antibody present in serum which had activity against all

antigenic substances, including *E. coli*. Despite these differences,

it can be concluded that the assays were interrelated and in many

cases supportive of one another.

The radioimmunoassay used was especially useful as both a quali-

tative and quantitative test of immune responses against the 026

antigen of *E. coli*. Chromium[51]-labeled 026 antigen and monospecific

antiglobulins both were used to measure the induced antibody

responses in calf sera. The radioactivity of the precipitates formed

was interpreted as a direct reflection of the antibody activity

against *E. coli* 026 antigen, and the response measured was dependent

on the amount of 026 antigen used in the test. One of the purposes

of the preliminary PHA inhibition test was to approximate the amount

of 026 antigen to use in order to work with a system of antigen

excess. Although the excess of antigen in the test system may not

have allowed for an optimal interaction of antigen and antibody

within the zone of equivalence, it did circumvent the possibility

of an antibody excess and did assure that sufficient antigen was

initially present to interact with all the homologous antibody that

may have been present for the formation of soluble antigen-antibody

complexes. The absorbed antiglobulin also contained Cr^{51}-labeled

026 antigen which also contributed an additional small amount of

antigen to the test system. It is assumed that the radioactivity

measured in the final precipitates came from 3 sources: 1) the

Cr^{51}-labeled 026 antigen, 2) Cr^{51} unbound and nonspecifically bound

to the antiglobulin molecules, and 3) nonspecifically "trapped"

Cr^{51} in the precipitates. It was further assumed that the amount

of radioactivity from the latter 2 sources was constant in all

samples and that any major differences in radioactivity observed

between control and principal sera or serum taken at birth and at

death from the same animal were due to the primary interactions

between Cr^{51}-labeled 026 antigen and antibody in the calf sera.

Likewise, it was assumed that the low counts observed in serum

taken at birth from control calves Nos. 2816 and 1912 represented

nonspecific or "background" radioactivity. The figures reported in

Table 8 represent the total radioactivity observed in each sample

and include the radioactivity resulting from antigen-antibody inter-

actions plus "background."

The results of the single radial immunodiffusion tests (Table

7) are incomplete in that IgG_1 was not quantitated in calf sera for

reasons discussed elsewhere. In most cases, there was agreement between the quantities of IgM measured and the appearance of IgM on IEP, except that IgM bands were not observed on IEP when serum IgM values were less than approximately 0.50 mg/ml (Figures 21 and 22). This discrepancy probably reflects the differences in sensitivity of the 2 assays. In contrast, there was not consistent agreement between the quantities of IgG_2 measured and the appearance of IgG_2 on IEP. Immunoglobulin G_2 was observed as either a light or a dense immunoprecipitate band in all the sera tested (Figures 21 and 22). The reason for this discrepancy is not clear, except for the possibility that the monospecific antiglobulin used in the single radial immunodiffusion assay may have had a low antigen binding capacity (low avidity).

To facilitate the interpretation and conclusions made from tests run on serum from individual animals or groups of animals, it was felt that comparisons should be made of the results obtained by the different assays. Factors which may have influenced the results obtained included 1) the kind of *in utero* injection and the time before birth that the *in utero* injections were made, 2) dose of challenge organisms, 3) ages of the animals, and 4) individual variations in responses.

Regarding the calves in Groups 5 and 6 euthanatized at birth (Figure 1), all the principal calves in Group 5 had demonstrable PHA titers whereas none of the control calves in Group 6 had demonstrable PHA titers against 026 antigen. The principal calves with PHA titers of 32 (Nos. 2290 and 1518) had high levels of IgM (Tables

7 and 8; Figure 22). The radioimmunoassays of calves Nos. 2290 and 1518 were similar, except that calf No. 2290 had greater IgG_2 activity than did No. 1518 (Tables 7 and 8; Figure 22). It was assumed that these were primary immune responses since the calves had been injected *in utero* either 10 or 20 days earlier (Table 1), respectively. The other principal calves in Group 5 (Nos. 1690 and 2452) had weak immune responses (Tables 3 and 7) for reasons that are unclear.

The calves that were challenged at birth (Groups 4 and 7) had similar differences in PHA titers against 026 antigen at birth and at death (Table 3) with the exception of control calf No. 299. The data on calf No. 299 from Tables 3, 7 and 8 and Figure 22 provide considerable evidence suggesting that this animal may have suckled before it was removed from its dam. The PHA titers (Table 3) and the quantities of immunoglobulins M and G_2 (Table 7) at birth and at death were comparable to or greater than other principal calves. Similarly, there was anti-026 antigen activity, somewhat higher than "background", of immunoglobulins M, G_1 and G_2 at birth. These developed into much higher levels at death (Table 8). If serum had been taken several hours after birth, it is probable that the PHA titer and the other tests would have been even higher due to more complete absorption of colostral lactoglobulins. The IEP profiles also showed the presence of strong immunoglobulin bands not normally seen at birth and a serum profile at death which was very similar to adult serum. Also, a strong IgA band, which could only have come from colostrum, was present at death (Figure 22). Final evidence

for colostrum intake by calf No. 299 is that the animal withstood an

intermediate challenge dose of *E. coli* (Table 1), and serum from this

calf was the only one which had activity against 4 other serotypes

of *E. coli* tested. It is presumed that the passively acquired

colostral antibodies were primarily responsible for the resistance

of the calf to the challenge and also the source of humoral antibody

activity against the other *E. coli* serotypes. Data do not support

that ingestion of colostrum occurred in any of the remaining calves.

Control calf No. 1912 had an unexpected and rapid immune response

(Tables 3 and 8) to a high challenge dose of *E. coli* (Table 1)

despite the fact that the animal was near death and had to be euthana-

tized at only 1.5 days of age. This suggests either that calves

have the capacity to mount rapid immune responses at a very young

age or that there was hemoconcentration of serum protein, including

gamma globulins, due to rapid dehydration. There was a direct rela-

tionship between the PHA responses of principal calves in Group 4

tested at birth and the time between *in utero* injection and birth.

There was also a direct relationship between the challenge dose

given to these calves and the PHA titers at death (Tables 1 and 3).

The radioimmunoassays and immunoelectrophoretograms of sera from

calves Nos. 2459 and 1534 of Group 4 were similar and showed anti-026

antigen activity above "background" at birth and increased responses

at death (Table 8 and Figures 21 and 22). Immunoglobulin M in calf

No. 1534 was below detectable levels in serum taken at birth (Table

7; Figure 21) and was barely above detectable levels in serum taken

at death (Table 7; Figure 22). In contrast, IgG_2 in calf No. 1534

appeared to account for the majority of the response at death (Tables 3 and 8), although this was not confirmed by single radial immuno-diffusion (Table 7). There was a discrepancy between the immune responses, as measured by the PHA and radioimmunoassay methods, in serum from calf No. 2459 at birth and at death (Tables 3 and 8). There may have been a dilution error with the PHA assay since the direct bacterial agglutination test did show an increase in titer. With the exception of principal calf No. 1377, it appeared that the immune responses observed in the serum of principal calves Nos. 2459 and 1534 and control calf No. 1912 at death were dependent on the challenge dose given. The 2 principal calves were immune from previous *in utero* injection with bacterin and probably reacted to the challenge organisms with a secondary immune response.

The purpose of Group 2 was to determine the resistance of calves revaccinated at birth and later challenged whereas Group 3 was to determine the immune responses resulting only from revaccination at birth. Group 8 was to serve as a control for revaccination and chal-lenge of older principal calves. Considering the response of all the calves in Groups 2, 3 and 8, and especially control calf No. 1567, it is apparent that there was an age dependence factor with regard to challenge. Consequently, it was not possible to evaluate the resist-ance of calves revaccinated at birth and challenged at 3 days of age regardless of the challenge dose used in the present study. It appeared that control calf No. 1567 reacted to challenge in a way similar to a normal primary immune response (Tables 3, 7 and 8; Figure 22), whereas principal calves Nos. 67 and 1214 probably

responded with a secondary immune response. The IgM and IgG$_2$

responses of calf No. 1567 were particularly noticeable (Tables 7

and 8; Figure 22). The PHA responses of calves in Groups 2 and 3

tested at birth were highly variable (Table 3) and were most likely

influenced by the time lapse between *in utero* injection and birth

(Tables 1, 3, 7 and 8). Revaccination at birth caused either a con-

version of serum from negative to sero-positive or a 2-fold increase

in the PHA titer (Group 3). Revaccination at birth and challenge

at 3 days of age also caused a conversion of serum from sero-negative

to sero-positive or an increase in the PHA titers in Group 2 depend-

ing on the challenge dose used. Calves Nos. 1322 and 2036 (Group 2)

seemed refractory to immunization and challenge perhaps because of

individual differences in primary immune responsiveness.

The PHA responses of calves Nos. 1429 and 2042 in Group 1 were

not similar (Table 3) in spite of the fact that both calves had been

given *in utero* injections about the same time before birth (Table 1).

The unusually high PHA titer observed in calf No. 1429 at birth

(Table 3) was in contrast to the results obtained by single radial

immunodiffusion and radioimmunoassay (Tables 7 and 8). Further, the

high PHA titer observed at birth in calf No. 1429 would suggest a

stronger than expected fetal immune response that was not influenced

by revaccination and continued to decline with age.

The results of the PHA assay with K60 antigen indicated poor

humoral (Table 3) and local responses. This could have been caused

by nonspecific enzymatic destruction of the K60 antigen in the

intestinal lumen resulting in no immunological stimulation.

Apparently, the 026 antigen remained intact and appeared to be the
dominant antigen of the test organism although this may not actually
be the case when both antigenic components are naturally present.
It is possible that parenteral injection of the bacterin into fetuses
or neonates would have induced a greater immune response to the K60
antigen. As a result of the poor PHA responses to K60 antigen, no
attempt was made to conduct the radioimmunoassay to qualitate and
quantitate the immune responses in serum and intestinal washings to
the same antigen.

Discussion of the results of the PHA assay of intestinal wash-
ings and the agar plaque assay of intestinal segments and mesenteric
lymph nodes is considered separately since these tests were intended
to assess the immune responses of the local immune system of calves
for the production of immunoglobulins M, G_1 and G_2. It is widely
accepted that IgA is the principal protective immunoglobulin normally
produced and secreted from the intestine, although recent work by
Porter *et al.* (1970) in pigs indicated that a common mechanism may
be operative for the secretion of IgM and IgA in the intestine of
very young animals. Whereas IgA is primarily produced and secreted
locally, it has been postulated that immunoglobulins M, G_1 and G_2 are
also produced locally in small quantities and either remain in the
intestinal tissues or are secreted. Immunoglobulin M, however, has
also been found in the intestinal secretions in significant amounts
(Porter *et al.*, 1970).

The results of the PHA assay of intestinal washings indicated
a poor local immune response to 026 antigen compared to the humoral

response. However, the results were subject to variations, possibly
due in part to the experimental procedures, and suggest that further
refinements be made of the techniques employed. The experimental
variables included: 1) the amount of dithiothrietol used as a reduc-
ing agent may not have been sufficient to interact with the intestinal
surface-bound immunoglobulin molecules and free them into the intes-
tinal lumen, 2) the volume of dialysate used to dialyze the intestinal
washing samples may not have been sufficient; thus the undialyzed
dithiothrietol may have reduced and degraded the immunoglobulin
molecules further, 3) the contamination of some samples with a heavy
growth of bacteria may have caused a breakdown of proteins in the
sample, and 4) the samples, after dialysis, may not have been con-
centrated enough even though the final volume of concentrate was
approximately that of the original fluid from the intestinal lumen.
Another possibility for explaining the low level of antibody activity
in intestinal secretions has nothing to do with experimental procedures
but is associated with the fact that IgM, unlike IgA, is released
into the intestinal secretions but is not bound to the mucous coating
on the surface of the intestinal epithelium (Porter et al., 1972).
Instead, IgM is degraded by proteolytic intestinal enzymes similar
to those that may have caused the rapid nonspecific hemolysis of SRBC
with the PHA assay of intestinal washings. The PHA assay of intes-
tinal washings measured total antibody activity and did not qualitate
the local immune response. Therefore, it was not possible to determine
the class(es) of antibody present in the intestinal washings from the
tests conducted. In comparing the PHA assays of serum (Table 3) and

intestinal washings (Table 4), with few exceptions it was found that the calves with the highest serum O26 hemagglutinin titers at death also had demonstrable titers in the intestinal washings. This would suggest either that high levels of humoral antibody may have "spilled over" into the local intestinal secretions or that antigenic stimulation resulted in the responses of 2 separate immune systems that operated independently of one another. There was no direct relationship between the PHA titers of intestinal washings (Table 4) and the agar plaque assay (Table 5). This finding was unexpected since it was assumed that the PHA assay of intestinal washings and the agar plaque assay from intestinal segments together would reflect the responsiveness of the local immune system. A more direct relationship between the 2 assays may have been observed if the plaque formation assay had been conducted to test for the presence of IgA producing cells although development of the IgA system usually requires 1 to 2 weeks in young animals. Thus the PHA titers in the intestinal washings most likely represented IgA and/or IgM from the early developing local secretory immune system. On the other hand, the agar plaque assays, as conducted in the present study, represented immunoglobulin M, G_1 and G_2 producing cells whose secreted antibody either remained in the local intestinal tissues or was transported and found in diluted concentrations in the intestinal secretions (IgM).

The agar plaque assay is usually conducted on spleen, lymph node or bone marrow since these tissues are normally rich in lymphoid tissue and antibody producing plasma cells. By contrast, the intestinal tract has concentrations of lymphocytes in the form of Peyer's

patches located primarily in the ileum. The remainder of the small intestine has only scattered lymphocytes and plasma cells in the lamina propria of the villi and in the submucosa and contains a heterogeneous population of cell types which is not dominated by immunocytes. Any major change in the number of plaque-forming cells in a tissue that is not ordinarily as rich in immunocompetent cells as lymph nodes are, for example, would indicate that a significant antigenic stimulation had occurred. Other than for a recent study by Robertson and Cooper (1972), there have been few other reports which have used the agar plaque assay to test scrapings from the intestinal mucosa under similar experimental conditionas as were used in the present study.

The results of the agar plaque assays of intestine and mesenteric lymph node tissues are a direct measure of the responses to the test antigen of the IgM, IgG_1 and IgG_2 antibody forming cells present as a part of the local immune system (Table 5). Revaccination with bacterin at birth had some stimulatory effect on the plaque forming responses. For example, the plaque forming responses of tissues from revaccinated calves Nos. 1429, 2042 and 4 were greater than the responses observed in calves injected with bacterin *in utero* and killed at birth (Group 5) and also greater in control calves killed at birth with the exception of calf No. 334 (Group 6). The plaque forming responses recorded for tissues from principal calves in Group 2 also included the responses that may have resulted from revaccination at birth. With the exception of calf No. 2036, the remainder of the calves in Group 2 had a comparatively poor plaque

forming tissue response in spite of revaccination or challenge. The large number of calves with tissues that had plaque forming activity against uncoated SRBC in the control tests was indicative of "background" or nonspecific activity against heterologous antigens by antibody secreted from plaque forming cells. In contrast, the humoral antibody of all the calves, except control calf No. 299, was quite specific, and did not cross react with the 4 other O serotypes of *E. coli* tested.

The major plaque forming responses of tissues in some principal and control calves seemed to be dependent on the challenge dose used such that the higher challenge doses stimulated greater plaque forming responses in tissues. There was also either an inverse or a direct relationship between the plaque forming responses of intestinal and mesenteric lymph node tissues and the PHA responses of serum depending on the challenge dose. A comparison of the agar plaque and the PHA assays including the challenge doses used and the clinical responses observed to oral challenge of selected neonatal calves are summarized in Table 10. Assuming that the live challenge organisms were infectious and also resulted in a stimulatory effect on the antibody immune system in general, it would appear that the intermediate or low challenge doses primarily stimulated either the plaque forming or the humoral immune responses but not both. The high challenge dose, however, stimulated both plaque forming and humoral immune responses (Table 10). In either case, one or both immune systems were involved which affected the immune status of the animals and/or contributed to resistance to challenge.

Table 10. Summary and comparison of the immune responses of selected neonatal calves to immunization and oral challenge with *E. coli* 026:K60:NM as determined by the PHA and agar plaque assays

Group no.	Calf no.	Immunological Assays* PHA	Agar Plaque	Oral challenge dose	Signs of clinical dis. after oral challenge†
1	1429	high	low	NA**	NA
	2042	low	high	NA	NA
2	67	high	low	1.5×10^{10}	--
	1214	high	low	1.5×10^{11}	--
	2036	low	high	1.5×10^{11}	--
3	4	high	low	NA	NA
4	1377	intermed.	high	1.0×10^{11}	CD
	2459	high	high	1.5×10^{11}	--
	1534	high	high	1.5×10^{11}	--
7	299	high	low	1.0×10^{11}	--
8	1567	high	high	1.5×10^{11}	--

*Subjective evaluation of the immune responses.

**Not applicable.

†-- = no clinical disease; CD=clinical disease.

The plaque forming responses of the spleen were weak and inconsistent compared to those of the intestinal and mesenteric lymph node tissues. This may have occurred because of sampling error since only a fraction of the entire organ was tested. However, considering the results of the bacteriologic examination of certain calves (Table 2) and the gross lesions observed, it appeared that the antigenic material from the bacterin and the live challenge organisms rarely got beyond the intestine or mesenteric lymph nodes to any great degree. This suggests that the primary target organs of immune stimulation were the intestine and mesenteric lymph nodes and that these organs were primarily responsible for the local and humoral immune responses observed.

Recent evidence in young pigs (Allen and Porter, 1973) and calves (Porter et al., 1972) has shown that IgM producing cells in the lamina propria of the intestine and free IgM molecules in the intestinal tissues and secretions apparently play an important role in the early development of the local immune system prior to and during the eventual switchover to the IgA secretory system (Porter, 1973a,b). Since the early maturation of the local intestinal immune system is dependent upon antigenic stimulation from the growing bacterial flora of the gut (Porter, 1973b), the tendency of man (van Oss, 1973) and animals (Berman, 1973) to respond immunologically to bacterial lipopolysaccharide by the production of IgM provides a possible explanation for the "'homing' of primed IgM immunocytes to the gut" (Berman, 1973) during early life. Also,

"It is interesting that in the secretory immune system
in the early period of its development IgM predominates
in the lamina propria and IgA predominates in the
intestinal secretions."   (Porter, 1973a)

The results of the hemolytic plaque assay (Table 5) clearly showed a

predominance of IgM producing cells in the intestine of immunized and

challenged calves and provide further supportive evidence of the

important functional role of IgM in the primary immune response of

the external secretory immune system of the young calf as has been

suggested by others (Porter *et al.*, 1972; Porter, 1973b; Allen and

Porter, 1973). A consequence of having IgM predominate in the early

development of the secretory immune system is that IgM in combina-

tion with antigen is an efficient activator of complement (Berman,

1973). Activated complement may result in undesirable side effects

in local tissues and could play a role in the pathogenesis of neo-

natal enteric diseases. Unfortunately, "adequate data on the level

and functional activity of the complement system in the neonatal

calf are not available and are badly needed" (Berman, 1973).

In a study of the early development of the local immune system

in 2- to 6-week-old preruminant calves, Porter *et al.* (1972) found

IgG_2 in higher concentrations in the intestinal secretions than

IgG_1. This suggests that both IgG_1 and IgG_2 are present and may be

secondarily involved in early local immune defense. The data from

the hemolytic plaque assay in intestinal tissues (Table 5) are not

consistent or conclusive in support of the predominance of one γG

antibody class of producing cells over another. The fact that the

calves used were younger than those studied by Porter *et al.* (1972)

could explain this lack of supportive evidence.

Schultz (1973a) recently reported on testing fetal bovine sera by single radial immunodiffusion as a means of detecting *in utero* infections. It was found that immunoglobulins M and G_1 were the most reliable indicators of antigenic stimulation and that IgG_2 and IgA were present either in low quantities or were absent from fetal sera. The results of the radioimmunoassays of calf sera taken at birth (Table 8) indicated that fetal injections with bacterin did result in an immune response but did not consistently cause a predominance of production of one class of immunoglobulin G over another. However, revaccination and/or challenge of neonates did cause greater quantities of IgG_2 than IgG_1 in 10 of the 12 serum samples taken at death although many of the observed differences did not appear to be significant (Table 8). A comparison of immuno-globulin production in bovine fetuses in the present study and the results reported by Schultz (1973a) cannot be made since the latter involved serum from fetuses infected with live organisms whereas the present study involved fetuses immunized with killed bacterin. Like-wise, it is probable that the naturally-infected fetuses (Schultz, 1973a) were septicemic and underwent a greater immune stimulation than did the calves in the present study. Further, it has been stated that "The response of an animal to an inactivated microbial agent may be quite different from that to the infectious microorganism" (Kaeberle, 1973). The predominance of IgG_2 over IgG_1 in revaccinated or challenged calves in the present study is similar to findings in the intestinal secretions of older calves as reported by Porter *et al.* (1972). This similarity suggests that IgG_2 may also be important in both the local and the humoral immune responses of young calves.

The immunoelectrophoretograms of control calves tested at birth (2816 n-b and 1567b, Figure 22) showed the presence of immunoprecipitate bands resembling immunoglobulins G_1 and G_2 and suggests that these calves were not agammaglobulinemic at birth. Based on the size and intensity of these immunoprecipitate bands (Figure 22), however, the quantity of these immunoglobulins must have been low. Immunoglobulins present in low levels at birth have been considered as nonspecific antibody, incomplete immunoglobulin or natural antibody (Sterzl and Silverstein, 1967). This natural or incomplete antibody may be important in nonspecific immunity of neonates to certain diseases (Gay, 1971).

The immunoprecipitate band which consistently appeared between immunoglobulins G_1 and G_2 in immunoelectrophoretograms of WBS (Figures 21 and 22) was not specifically identifiable. The immunoelectrophoretic mobility of the substance was somewhat greater than IgG_2 and only slightly less than IgG_1. This suggests that the substance could be a subclass of either IgG_1 or IgG_2 and could represent a finding which has not previously been reported. Attempts at further characterization and identification of the unidentified immunoprecipitate band were beyond the scope of the present study. However, an effort should be made to characterize the physicochemical and biological properties of this substance since it may represent an important element in the bovine immune system.

SUMMARY

The humoral and local intestinal responses of fetuses and neo-
nates to *Escherichia coli* O26:K60:NM were studied in 26 crossbred

beef-type calves. *Escherichia coli* bacterin (5.0 x 10^{10} organisms)

was injected *in utero* by a nonsurgical technique into the amniotic

fluid of 17, 7- to 8.5-month-old fetuses (principals). Saline was

injected in the same manner into 9 control fetuses. The colostrum-

free newborn calves were removed from their dams after natural

delivery and were maintained in separate pens and fed a milk replacer

diet. Calves were divided into 10 unequally sized groups. Calves

in one principal and one control group were each given an oral

challenge inoculum at birth. Similarly, calves in one principal

group (reimmunized at birth) and one control group each were chal-

lenged at 3 days of age. The challenge doses were 1.5 x 10^{10},

1.0 x 10^{11} or 1.5 x 10^{11} live, unwashed homologous *E. coli* organisms.

Calves were euthanatized at 1.5 to 5 days after challenge. Calves

of the remaining 3 principal groups were either euthanatized at birth

or were reimmunized at birth and euthanatized at 5 to 7 days or at

2 weeks of age. Control calves (3 groups) were also euthanatized

at the above corresponding times.

Immunologic tests included direct bacterial and passive hemag-

glutination (PHA) of sera and intestinal washings; hemolytic plaque

assays of intestine, mesenteric lymph nodes and spleen; and single radial immunodiffusion, radioimmunoassay and qualitative immunoelectrophoresis of sera. Antiglobulin, monospecific against bovine IgM, IgG$_1$ or IgG$_2$ and antiglobulin against whole bovine serum were produced in guinea pigs or rabbits and used in the appropriate immunologic tests. Other tests included histologic and bacteriologic examination of tissues and partial compositional analysis of amniotic fluids.

The resistance of calves to challenge was a function of previous *in utero* injection of bacterin, age when challenged and the dose of challenge organisms used. Clinical disease was observed after inoculation of newborn calves with the intermediate or the high challenge dose in one principal and one control calf, respectively. The low challenge dose failed to produce clinical disease in either the newborn principal or the control calves. Clinical disease was not observed after giving the high challenge dose to 2 other newborn principal calves or in the 3-day-old principal calves, previously reimmunized at birth, or in the 3-day-old control calves. Clinical signs after challenge were those associated with colisepticemia and toxemia. The gross and histologic lesions, however, were mild and not consistent with those usually associated with septicemia. Bacteriologic examination of tissues from the challenged calves revealed a light growth of *E. coli*.

Principal calves, tested at birth, had serum PHA titers against the 026 antigen of *E. coli* ranging from 0 to 512, whereas all but 2 control calves were negative. It was suspected that one of the

control calves had suckled and the passively acquired colostral antibody was the most probable cause for the PHA response to the test antigen. The PHA titers of intestinal washings against the same antigen were either low or negative. Similarly, the PHA titers of serum and intestinal washings against the K60 antigen of *E. coli* were either low or negative. Qualitative and quantitative analysis by radioimmunoassay of sera from selected calves revealed that the antibody activity against the 026 antigen was primarily IgM although there was also increased activity of either IgG_1 or IgG_2 or both. Immunoelectrophoretograms confirmed the presence of these classes of antibody when compared to whole bovine serum.

Reimmunization with bacterin and use of the high challenge dose given to both principal and control calves caused either a conversion of serum from negative to sero-positive or as much as a 4-fold increase in the PHA titers. The intermediate or low challenge doses caused little or no increase in the PHA serum titers. Sera from all but one of the calves was serotype specific for the 026 antigen and did not react by the PHA test with 4 other O serotypes.

Results of the hemolytic plaque assay provided evidence of the important role of IgM in the early development of the local intestinal immune system. IgM producing cells predominated and were nearly evenly distributed throughout the intestinal tissues and corresponding mesenteric lymph nodes of the majority of the calves tested. The plaque forming response in the spleens tested was weak and inconsistent compared to the intestinal tissues. There was no direct relationship between the PHA titers of intestinal washings and the

plaque forming assays from intestinal segments. Reimmunization with bacterin at birth had some stimulatory effect on the plaque forming responses whereas the major increases in plaque forming activity in the intestine occurred after challenge with the high dose. The intermediate or low challenge doses stimulated either the plaque forming or the humoral immune responses whereas the high challenge dose stimulated both responses. The principal target organs for antigenic stimulation by the test organisms were the intestine and the mesenteric lymph nodes, and these immunocompetent organs were primarily responsible for the immune responses observed.

Immunoelectrophoretograms of serum from all control calves tested at birth showed the presence of low levels of IgG_1 and/or IgG_2. This gamma globulin probably represented natural or nonspecific antibody. Immunoelectrophoretograms also showed the presence of an unidentified gamma globulin in whole bovine serum which may represent a subclass of either IgG_1 or IgG_2.

BIBLIOGRAPHY

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BIBLIOGRAPHY

Allen, W. D. and Porter, P.: The demonstration of immunoglobulins
    in porcine intestinal tissue by immunofluorescence with
    observations on the effect of fixation. Immunology, 18,
    (1970): 799-806.

Allen, W. D. and Porter, P.: The relative distribution of IgM and
    IgA cells in intestinal mucosa and lymphoid tissue of the
    young unweaned pig and their significance in ontogenesis of
    secretory immunity. Immunology, 24, (1973): 493-501.

Amstutz, H. E.: Occurrence and etiology of infectious calf diarrhea.
    In Symposium on Infectious Diarrhea in Calves. J.A.V.M.A.,
    147, (1965): 1360-1363.

Aschaffenburg, R.: The nutritive value of colostrum for the calf.
    3. Changes in the serum protein of the newborn calf following
    the ingestion of small quantities of the non-fatty fraction.
    Br. J. Nutr., 3, (1949): 200-204.

Aschaffenburg, R., Bartlett, S., Kon, S. K., Terr, P., Thompson,
    S. Y. and Walker, D. M.: The nutritive value of colostrum
    for the calf. 1. The effect of different fractions of
    colostrum. Br. J. Nutr., 3, (1949a): 187-196.

Aschaffenburg, R., Bartlett, S., Kon, S. K. and Walker, D. M.: The
    nutritive value of colostrum for the calf. 2. The effect of
    small quantities of the non-fatty fraction. Br. J. Nutr., 3,
    (1949b): 196-200.

Aschaffenburg, R., Bartlett, S., Kon, S. K., Roy, J. H. B. and
    Walker, D. M.: The nutritive value of colostrum for the
    calf. 4. The effect of small quantities of colostral whey,
    dialysed whey and 'immune lactoglobulins.' Br. J. Nutr.,
    5, (1951a): 171-176.

Aschaffenburg, R., Bartlett, S., Kon, S. K., Roy, J. H. B. and
    Walker, D. M.: The nutritive value of colostrum for the
    calf. 5. The effect of prepartum milking. Br. J. Nutr.,
    5, (1951b): 343-349.

Ashburner, F. M. and Mushin, R.: Experimental intestinal coliform
    infections in mice. J. Hyg. Camb., 60, (1962): 175-181.

147

Barnum, D. A., Glantz, P. J. and Moon, H. W.: *Colibacillosis*. Ciba Veterinary Monograph Series - 2, Ciba Pharmaceutical Co., Summit, N.J. (1967).

Berman, D. T.: Comments on the bovine immune system and immunoglobulins. In Proceedings of a Colloquium on Immunity to Selected Infectious Diseases of Cattle. J.A.V.M.A., 163, (1973): 798-800.

Bernovska, J., Kostka, J. and Sterzl, J.: Hemolytic reaction in gels. Folia Microbiol., 8, (1963): 376-377.

Blakemore, F., Davies, A., Eylenburg, E., Moore, T., Sellers, K. C. and Worden, A. N.: The relative importance of antibodies and vitamin A in preventing diseases in young calves. Biochem. J., 42, (1948): XXX.

Bokhari, S. M. H. and Ørskov, F.: O-grouping of *E. coli* strains isolated from cases of white scours. Acta Path. et Micro. Scand., 30, (1952): 87-89.

Boyden, S. V.: The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera. J. Exptl. Med., 93, (1951): 107-120.

Brambell, F. W. R.: *The Transmission of Passive Immunity from Mother to Young*. American Elsevier Publishing Co., New York, N.Y. (1970).

Braude, A. I., Carey, F. J., Sutherland, D. and Zalesky, M.: Studies with radioactive endotoxin: I. The use of Cr^{51} to label endotoxin of *Escherichia coli*. J. Clin. Invest., 34, (1955): 850-857.

Briggs, C.: The nutritive value of colostrum for the calf. 6. The 'K' antigens of *Bacterium coli*. Br. J. Nutr., 6, (1951): 349-355.

Bulletin, W.H.O. Meeting on Nomenclature of Human Immunoglobulins, 30, (1964): 447. Cited by Pierce, A. E.: In Modern trends in animal health and husbandry. Recent developments in the isolation and characterization of proteins and their application to the immune globulins. Br. Vet. J., 122, (1966): 3-17.

Butler, J. E.: Synthesis and distribution of immunoglobulins. In Proceedings of a Colloquium on Immunity to Selected Infectious Diseases of Cattle. J.A.V.M.A., 163, (1973): 795-798.

Buxton, A.: The *in vivo* sensitization of avian erythrocytes with *Salmonella gallinarum* polysaccharide. Immunology, 2, (1959): 203-210.

Campbell, D. H., Garvey, J. S., Cremer, N. E. and Sussdorf, D. H.:
     Methods in Immunology, 2nd ed. W. A. Benjamin, New York,
     N.Y. (1970a): 45-48.

Campbell, D. H., Garvey, J. S., Cremer, N. E. and Sussdorf, D. H.:
     Methods in Immunology, 2nd ed. W. A. Benjamin, New York,
     N.Y. (1970b): 183-191.

Campbell, D. H., Garvey, J. S., Cremer, N. E. and Sussdorf, D. H.:
     Methods in Immunology, 2nd ed. W. A. Benjamin, New York,
     N.Y. (1970c): 250-271.

Campbell, D. H., Garvey, J. S., Cremer, N. E. and Sussdorf, D. H.:
     Methods in Immunology, 2nd ed. W. A. Benjamin, New York,
     N.Y. (1970d): 271-292.

Campbell, D. H., Garvey, J. S., Cremer, N. E. and Sussdorf, D. H.:
     Methods in Immunology, 2nd ed. W. A. Benjamin, New York,
     N.Y. (1970e): 425.

Carpenter, C. M. and Woods, B. A.: The distribution of the colon-
     aerogenes group of bacteria in the alimentary tract of
     calves. Cornell Vet., 14, (1924): 218-225.

Cebra, J. J.: Immunoglobulins and immunocytes. Bact. Rev., 33,
     (1969): 159-171.

Chase, M. W.: Adjuvants. In *Methods in Immunology and Immuno-
     chemistry*, I. Edited by C. A. Williams and M. W. Chase,
     Academic Press, New York, N.Y. (1968a): 96-236.

Chase, M. W.: Buffers. Appendix II. In *Methods in Immunology and
     Immunochemistry*, II. Edited by C. A. Williams and M. W.
     Chase, Academic Press, New York, N.Y. (1968b): 365-408.

Chase, M.W. and Williams, C. A.: Chemical analyses. A. Protein
     analysis. In *Methods in Immunology and Immunochemistry*, II.
     Edited by C. A. Williams and M. W. Chase, Academic Press,
     New York, N.Y. (1968): 249-282.

Coles, E. H.: *Veterinary Clinical Pathology*, 2nd ed. W. B. Saunders
     Co., Philadelphia, Pa. (1974a): 558-559.

Coles, E. H.: *Veterinary Clinical Pathology*, 2nd ed. W. B. Saunders
     Co., Philadelphia, Pa. (1974b): 575-576.

Colinet, G. A., Kaeckenbeeck, A. and Schoenaers, F.: Elimination
     et production d'anticorps colibacillaires chez le veau.
     Ann. Med. Vet., 105, (1961): 245-257.

Comline, R. S., Roberts, H. E. and Titchen, D. A.: Route of absorp-
     tion of colostrum globulin in the newborn animal. Nature,
     167, (1951): 561-562.

Comline, R. S., Roberts, H. E. and Titchen, D. A.: Histological changes in the epithelium of the small intestine during protein absorption in the newborn. Nature, 168, (1951): 84-85.

Conner, G. H., Richardson, M. and Carter, G. R.: Prenatal immunization and protection of the newborn: Ovine and bovine fetuses vaccinated with *Escherichia coli* antigen by the oral route and exposed to challenge inoculum at birth. Am. J. Vet. Res., 34, (1973): 737-741.

Craig, L. C.: Dialysis and ultrafiltration. In *Methods in Immunology and Immunochemistry*, II. Edited by C. A. Williams and M. W. Chase, Academic Press, New York, N.Y. (1968): 119-133.

Cunningham, A. J. and Szenberg, A.: Further improvements in the plaque technique for detecting single antibody forming cells. Immunology, 14, (1968): 599-600.

David, J. R.: Lymphocyte factors in cellular hypersensitivity. In *Immunobiology*. Edited by R. A. Good and D. W. Fisher, Sinauer Associates, Inc., Stamford, Conn. (1971): 146-156.

Deutsh, H. F.: A study of whey protein from the milk of various animals. J. Biol. Chem., 169, (1947): 437-448.

Duncan, J. R., Wilkie, B. M., Hiestand, R. and Winter, A. J.: The serum and secretory immunoglobulins of cattle: Characterization and quantitation. J. Imm., 108, (1972): 965-976.

Dunne, H. W., Glantz, P. J., Hokanson, J. F. and Bortree, A. L.: *Escherichia coli* as a cause of diarrhea in calves. In Epidemic and Endemic Diarrheal Diseases of the Infant. Ann. N.Y. Acad. Sci., 66, (1956): 129-135.

Edelman, G. M. and Marchalonis, J. J.: Methods used in studies of immunoglobulins. In *Methods in Immunology and Immunochemistry*, I. Edited by C. A. Williams and M. W. Chase, Academic Press, New York, N.Y. (1967): 405-424.

Edwards, P. R. and Ewing, W. H.: *Identification of Enterobacteriaceae.* Burgess Publishing Co., Minneapolis, Minn. (1962).

El-Nageh, M. M.: Voies d'absorption des gammaglobines du colostrum au niveau de l'intestin grêle du veau nouveau-ne. Ann. de Med. Vet., 111, (1967): 384-390.

Fahey, J. L. and McKelvey, E. M.: Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Imm., 94, (1965): 84-90.

Famulener, L. W.: On the transmission of immunity from mother to offspring. A study upon serum haemolysins in goats. J. Infect. Dis., 10, (1912): 332-368.

Feldman, M.: Cellular components of the immune system and their cooperation (T and B cells). Transplant. Proc. 5, No. 1, (1973): 43-48.

Fey, H.: Bakteriologie und serologie der colisepsis des kalbes. I. Seroligische und biochemische untersuchungen. Zent. für Veter., 4, (1957a): 309-318.

Fey, H.: Bakteriologie und serologie der colisepsis des kalbes. II. Die bedeutung des colityps 78:80B für die kalberruhr. Zent. für Veter., 4, (1957b): 447-458.

Fey, H.: Neuere untersuchungen über die colisepsis des kalbes. Schweizer Arch. für Tier., 104, (1962): 1-12.

Fey, H.: Entstehung und verhütung der coliinfektionen der kälber. Zent. für Veter., Reihe B, 13, (1966): 175-179.

Fey, H.: Die bedeutung des gammaglobulins fur das neugeborene kalb. Int. Tier. Arbeit. für Tier, Symposium Salzburg, (1967): 17-28.

Fey, H.: Immunology of the newborn calf: Its relationship to coli-septicemia. In Neonatal Enteric Infections Caused by *Escherichia coli*. Ann. N.Y. Acad. Sci., 176, (1971): 49-63.

Fey, H.: *Colibacillosis in Calves*. Verlag Hans Huber, Bern, Switzerland (1972).

Fey, H. and Margadant, A.: Zur pathogenese der kälber-colisepsis. I. Verteilung des sepsestyps in den organen. Zent. für Bakt., Parasit.,Inf. and Hyg., Abt. I, Originale, 182, (1961a): 71-76.

Fey, H. and Margadant, A.: Hypogammaglobulinämie bei der colisepsis des kalbes. Path. Micro., 24, (1961b): 970-976.

Fey, H. and Hunyady, G.: Zur substitutionsprophylaxe mit colostrum-poolserum bei agammaglobulinämischen kälben. Berliner und München Tier. Woch, 75, (1962): 466-467.

Fey, H. and Margadant, A.: Zur pathogenese der kälber-colisepsis. IV. Agammaglobulinämie als disponierender faktor. Zent. für Veter., 9, (1962): 653-663.

Fey, H., Lanz, E., Margadant, A. and Nicolet, J.: Zur pathogenese der kälbercolisepsis. VI. Experimentelle infektion zum beweis der parenteralen genese. Deutsch Tier. Woch., 69, (1962): 581-586.

Fey, H., Margadant, A., Nicolet, J. and Hunyady, G.: Prophylaxe
der experimentellen colisepsis des kalbes mit einem
colosdrum-serumpool. Schweizer Arch. für Tier., 105,
(1963): 361-370.

Fisher, E. W.: Discussion remarks to the paper by Fey, H. on the
immunology of the newborn calf. In Neonatal Enteric Infec-
tions Caused by *Escherichia coli*. Ann. N.Y. Acad. Sci.,
176, (1971): 64.

Gay, C. C.: *Escherichia coli* and neonatal diseases of calves.
Bact. Rev., 29, (1965): 75-101.

Gay, C. C.: Problems of immunization in the control of *Escherichia
coli* infection. In Perspectives in the Control of Neonatal
Enteric Disease, IV, Ann. N.Y. Acad. Sci., 176, (1971):
336-349.

Gay, C. C., McKay, K. A. and Barnum, D. A.: Studies on colibacil-
losis of calves. I. The antibody acquired by calves as
the result of vaccination of the dam. Can. Vet. J., 5,
(1964a): 248-261.

Gay, C. C., McKay, K. A. and Barnum, D. A.: Studies on colibacil-
losis of calves. II. A clinical evaluation of the efficiency
of vaccination of the dam as a means of preventing colibacil-
losis of the calf. Can. Vet. J., 5, (1964b): 297-308.

Gay, C. C., McKay, K. A. and Barnum, D. A.: Studies on colibacil-
losis of calves. III. The experimental reproduction of
colibacillosis. Can. Vet. J., 5, (1964c): 314-325.

Gay, C. C., Anderson, N., Fisher, E. W. and McEwan, A. D.: Gamma
globulin levels and neonatal mortality in market calves.
Vet. Rec., 77, (1965): 148-149.

Gill, T. J., III: Methods for detecting antibody. Immunochem.,
7, (1970): 997-999.

Glantz, P. J.: Serotypes of *Escherichia coli* associated with coli-
bacillosis in neonatal animals. In Neonatal Gastrointestinal
Disease: Epidemiological and Microbiological Aspects. Ann.
N.Y. Acad. Sci., 176, (1971): 67-79.

Glantz, P. J., Dunne, H. W., Heist, C. E. and Hokanson, J. F.:
Bacteriological and serological studies of *Escherichia coli*
serotypes associated with calf scours. The Penn. St. Univ.,
Coll. of Agr., Agr. Expt. St., Univ. Park, Pa., Bull. 645,
(1959): 1-22.

Glantz, P. J., Kradel, D. C. and Hokanson, J. F.: *Escherichia coli*
serogroup 115 isolated from animals: Experimental infection
of calves. Am. J. Vet. Res., 27, (1966): 1205-1209.

Going, H. and Kaiser, P.: Endotoxine. In "Die Infektionskrankheiten des Menschen und ihre Erreger." Edited by A. Grumbach and W. Kikuth, Georg Thieme Verlag, Stuttgart, Ger. (1969): 225-248.

Gossling, J., McKay, K. A. and Barnum, D. A.: Colibacillosis of calves in Ontario. II. The association of certain serotypes of *Escherichia coli* with calf scours. Can. Vet. J., 5, (1964): 220-228.

Gowans, J. L.: Immunobiology of the small lymphocyte. In *Immunobiology*. Edited by R. A. Good and D. W. Fisher, Sinauer Assoc., Inc., Stamford, Conn. (1971): 18-27.

Grossberg, A. L.: The determination of contact amino acid residues in antibody-combining sites by chemical modification of antibodies. In *Specific Receptors of Antibodies, Antigens and Cells*. 3rd Int. Conv. on Immunology. Edited by D. Pressman, T. B. Tomasi, Jr., A. L. Grossberg and N. R. Rose, S. Karger, Basel, Switzerland (1973): 176-183.

Hammer, D. K., Kickhofen, B. and Henning, G.: Molecular classes and properties of antibodies in cattle serum and colostrum synthesized during the primary and secondary response to protein antigens. Europ. J. Biochem., 6, (1968): 443-454.

Hansen, R. G. and Phillip, P. H.: Studies on the proteins of bovine colostrum. II. The absorption of globulins by the young calf. J. Dy. Sci., 30, (1947): 560.

Hansen, R. G. and Phillips, P. H.: Studies on proteins from bovine colostrum. III. The homologous and heterologous transfer of ingested protein to the blood stream of the young animal. J. Biol. Chem., 179, (1949): 523-527.

Howe, P. E.: The relation between the ingestion of colostrum or blood serum and the appearance of globulin and albumin in the blood and urine of the newborn calf. J. Exp. Med., 39, (1924): 313-320.

Humphrey, J. H. and White, R. G.: Non-specific mechanisms of immunity: Innate immunity. In *Immunology for Students of Medicine*, 3rd ed. Blackwell Scientific Publications, Oxford and Edinburgh, England (1971a): 35-83.

Humphrey, J. H. and White, R. G.: The specific immunological response. In *Immunology for Students of Medicine*, 3rd ed. Blackwell Scientific Publications, Oxford and Edinburgh, England (1971b): 84-147.

Humphrey, J. H. and White, R. G.: Serological aspects of the antigen-antibody reaction. In *Immunology for Students of Medicine*, 3rd ed. Blackwell Scientific Publications, Oxford and Edinburgh, England (1971c): 348-407.

Ingram, D. G. and Smith, A. M.: Immunological responses of young animals. 1. Review of the literature. Can. Vet. J., 6, (1965): 194-204.

Jerne, N. K., Nordin, A. A. and Henry, C.: The agar plaque technique for recognizing antibody-producing cells. In *Cell-Bound Antibodies*. Conf. Nat. Acad. Sci., Nat. Res. Council. Edited by B. Amos and H. Koprowski, The Wistar Inst. Press, Philadelphia, Pa. (1963): 109-125.

Johnson, P. and Pierce, A. E.: Ultracentrifugal and electrophoretic studies of neonatal calf sera and maternal colostrum. J. Hyg., 57, (1959): 309-320.

Jones, G. W. and Rutter, J. M.: Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. Inf. and Imm., 6, (1972): 918-927.

Kabat, E. A.: Precise measurement of hapten-antibody interaction. In *Structural Concepts in Immunology and Immunochemistry*. Holt, Rinehart and Winston, Inc., New York, N.Y. (1968a): 66-81.

Kabat, E. A.: Antigenic determinants and the size of the antibody combining site. In *Structural Concepts in Immunology and Immunochemistry*. Holt, Rinehart and Winston, Inc., New York, N.Y. (1968b): 82-111.

Kaeberle, M. L.: Immune responses to antigens of inactivated microbial agents. In Proceedings of a Colloquium on Immunity to Selected Infectious Diseases of Cattle. J.A.V.M.A., 163, (1973): 810-815.

Kaeckenbeeck, A., Colinet, G. and Schoenaers, F.: Evolution de L'aptitude de L'intestin du veau nouveau-ne a resorber les anticorps apportes par colostrum. Ann. de Med. Vet., 105, (1961): 197-205.

Kalter, S. S.: *Procedures for Routine Laboratory Diagnosis in Virus and Rickettsial Diseases*, 1st ed. Burgess Publishing Co., Minneapolis, Minn. (1963): 81.

Kaltreider, H. B., Kyselka, L. and Salmon, S. E.: Immunology of the lower respiratory tract. II. The plaque-forming response of canine lymphoid tissues to sheep erythrocytes after intrapulmonary or intravenous immunization. J. Clin. Invest., 54, (1974): 263-270.

Kauffman, F.: The serology of the coli group. J. Immunol., 57, (1947): 71-100.

Kim, C. Y.: An immunochemical method for serum γglobulin quantitative determination by micro-Ouchterlony double diffusion. Lab. Invest., 23, (1970): 79-85.

King, T. P.: Chromatographic separations of macromolecules on porous gels and cellulose ion exchangers. In *Methods in Immunology and Immunochemistry*, II. Edited by C. A. Williams and M. W. Chase, Academic Press, New York, N.Y. (1968): 135-162.

Klaus, G. G. B., Bennett, A. and Jones, E. W.: A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. Immunology, 16, (1969): 293-299.

Koller, L. D., Barthold, S. W. and Olson, C.: Quantitation of bovine papilloma virus and serum antibody by immunodiffusion. Am. J. Vet. Res., 35, (1974): 121-124.

Kraner, K. L. and Parshall, C. J., Jr.: Experimental procedures and surgical techniques performed on intrauterine fetal animals. In *Models of Animal Experimentation*, 3rd ed. Edited by W. I. Gay, Academic Press, New York, N.Y. (1968): 211-239.

Kruse, V.: Personal communications. Cited by Fey, H. In *Colibacillosis in Calves*. Verlag Hans Huber, Bern, Switzerland (1972).

Lambert, G. and Fernelius, A. L.: Bovine viral diarrhea virus and *Escherichia coli* in neonatal calf enteritis. Can. J. Comp. Med., 32, (1968): 440-446.

Lambert, G., McClurkin, A. W. and Fernelius, A. L.: Bovine viral diarrhea in the neonatal calf. J.A.V.M.A., 164, (1974): 287-289.

Larson, B. L.: Transfer of specific blood serum proteins to lacteal secretions near parturition. J. Dy. Sci., 41, (1958): 1033-1044.

Larson, B. L. and Kendall, K. A.: Changes in specific blood protein levels associated with parturition in the bovine. J. Dy. Sci., 40, (1957): 659-666.

Lovell, R.: Intestinal diseases of young calves with special reference to infection with *Bacterium coli*. Vet. Rev. and Annot., 1, (1955): 1-32.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem., 193, (1951): 265-275.

Luna, L. G.: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed. McGraw-Hill Book Co., New York, N.Y. (1968): 32-46.

Mancini, G., Carbonara, A. O. and Heremans, J. F.: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2, (1965): 235-254.

Mayr, A., Kalich, I. and Mehnert, B.: Kälber krankheiten. Wiener Tier. Monat., 51, (1964): 74-92.

McDiarmid, A.: The transfer of agglutinins for *Brucella abortus* from cow to calf and their persistence in the calf's blood. Vet. Rec., 58, (1946): 146-149.

McEwan, A. D.: The resistance of the young calf to disease. Vet. Rec., 62, (1950): 83-93.

McEwan, A. D., Fisher, E. W. and Selman, I. E.: Observations on the immune globulin levels of neonatal calves and their relationship to disease. J. Comp. Path., 80, (1970a): 259-265.

McEwan, A. D., Fisher, E. W., Selman, I. E. and Penhale, W. J.: A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clin. Chim. Acta, 27, (1970b): 155-163.

Mebus, C. A., Stair, E. L., Underdahl, N. R. and Twiehaus, M. J.: Pathology of neonatal calf diarrhea induced by a Reo-like virus. Vet. Path., 8, (1971): 490-505.

Mebus, C. A., Stair, E. L., Rhodes, M. B., Underdahl, N. R. and Twiehaus, M. J.: Calf diarrhea of viral etiology. Ann. Rech. Vet., 4, (1973): 71-78.

Miller, H. C. and Cudkowicz, G.: Immunologic memory cells of bone marrow origin. Increased burst size of immunocyte precursors. J. Exptl. Med., 135, (1972): 1028-1036.

Miller, J. F. A. P. and Sprent, J.: Cell-to-cell interaction in the immune response. VI. Contribution of thymus-derived cells to antibody-forming cell precursors to immunological memory. J. Exptl. Med., 134, (1971): 66-82.

Nansen, P., Flagstad, T. and Pedersen, K. B.: Preparation of antisera to bovine immunoglobulin classes by immunization with agar-gel precipitates. Acta Path. Micro. Scand., 79B, (1971): 459-465.

Needham, J.: *Chemical Embryology*, III. The Macmillan Co., New York, N.Y. (1931): 1534-1564.

Neter, E., Gorzynski, E. A., Gino, R. M., Westphal, O. and Luderitz, O.: The enterobacterial hemagglutination test and its diagnostic potentialities. Can. J. Micro., 2, (1956): 232-244.

Nisonoff, A.: Molecules of immunity. In *Immunobiology*. Edited by R. A. Good and D. W. Fisher, Sinauer Associates, Stamford, Conn. (1971): 65-74.

Nowotny, A.: Isolation of capsular bacterial polysaccharides, K antigens. In *Basic Exercises in Immunochemistry*. Springer-Verlag, New York, N.Y. (1969): 28.

Olins, D. E. and Edelman, G. M.: Reconstitution of 7 S molecules from L and H polypeptide chains of antibodies and γ-globulins. J. Exptl. Med., 119, (1964): 789-815.

Orcutt, M. L. and Howe, P. E.: The relationship between the accumulation of globulins and the appearance of agglutinins in the blood of newborn calves. J. Exptl. Med., 36, (1922): 291-308.

Ørskov, F.: Personal communications. Cited by Fey, H. In *Colibacillosis in Calves*. Verlag Hans Huber, Bern, Switzerland (1972): 17.

Ørskov, I., Ørskov, F., Sojka, W. J. and Leach, J. M.: Simultaneous occurrence of *E. coli* B and L antigens in strains from diseased swine. Influence of cultivation temperature. Two new *E. coli* K antigens K87 and K88. Acta Path. et Micro. Scand., 53, (1961): 404-422.

Osborne, J. C.: *Escherichia coli* serotypes orally. I. Induced gastroenteritis and pathological changes in neonatal calves. Cornell Vet., 57, (1967): 204-216.

Osburn, B. I.: Immune responsiveness of the fetus and neonate. J.A.V.M.A., 163, (1973): 801-803.

Osserman, E. F.: A modified technique for immunoelectrophoresis facilitating the identification of specific precipitin arcs. J. Imm., 84, (1960): 93-97.

Ouchterlony, O.: *Handbook of Immunodiffusion and Immunoelectrophoresis*. Ann Arbor Science Publ., Inc., Ann Arbor, Mich. (1968).

Payne, J. M. and Derbyshire, J. B.: Portals of entry for bacterial infection in calves and piglets with particular reference to the tonsil. J. Path. Bact., 85, (1963): 171-178.

Penhale, W. J., Christie, G., McEwan, A. D., Fisher, E. W. and Selman, I. E.: Quantitative studies on bovine immunoglobulins. II. Plasma immunoglobulin levels in market calves and their relationship to neonatal infection. Br. Vet. J., 126, (1970): 30-37.

Pierce, A. E.: Modern trends in animal health and husbandry. Recent developments in the isolation and characterization of proteins and their application to the immune globulins. Br. Vet. J., 122, (1966): 3-17.

Pierce, A. E. and Feinstein, A.: Biophysical and immunological studies on bovine immune globulins with evidence for selective transport within the mammary gland from maternal plasma to colostrum. Immunology, 8, (1965): 106-123.

Porter, P.: The functional role of secretory antibody systems in early development of the pig and the calf. In Symposium on Nutrition of the Young Farm Animal. Nutr. Soc., 32, (1973a): 217-222.

Porter, P.: Functional heterogeneity of the bovine immune system. In Proceedings of a Colloquium on Immunity to Selected Infectious Diseases of Cattle. J.A.V.M.A., 163, (1973b): 789-794.

Porter, P., Noakes, D. E. and Allen, W. D.: Intestinal secretion of immunoglobulins and antibodies to Escherichia coli in the pig. Immunology, 18, (1970): 909-920.

Porter, P., Noakes, D. E. and Allen, W. D.: Intestinal secretion of immunoglobulins in the preruminant calf. Immunology, 23, (1972): 299-312.

Pressman, D.: Introductory remarks: The antigen binding sites of antibody molecules. In Specific Receptors of Antibodies, Antigens and Cells. 3rd Intl. Convoc. Immunol., S. Karger, Basel, Switzerland (1973): 172-175.

Radostits, O. M.: Clinical management of neonatal diarrhea in calves with special reference to pathogenesis and diagnosis. J.A.V.M.A., 147, (1965): 1367-1376.

Raff, M. C.: T and B lymphocytes and immune responses. Nature, 242, (1973): 19-23.

Rauss, K. and Ketyi, I.: Beitroge zur antagonistischen wirkung der coli-flora des darmkanals. Zentr. Bakt. Parasit., Abt. I, Orig., 177, (1960): 161-175.

Reisinger, R. C.: Pathogenesis and prevention of infectious diarrhea (scours) of newborn calves. J.A.V.M.A., 147, (1965): 1377-1386.

Richardson, M. and Conner, G. H.: Prenatal immunization by the oral route: Stimulation of Brucella antibody in fetal lambs. Inf. and Imm., 5, (1972): 454-460.

Robertson, P. W. and Cooper, G. N.: Immune responses in intestinal tissues to particulate antigens. Plaque-forming and rosette-forming cell responses in rats. Aust. J. Exptl. Biol. Med. Sci., 50, (1972): 703-714.

Scheidegger, J. J.: Une micro-methode de l'immuno-electrophorese. Int. Arch. Allergy, 6, (1955): 103-110.

Schultz, R. D.: Comments on the immune response of the fetus and neonate. In Proceedings of a Colloquium on Immunity to Selected Infectious Diseases of Cattle. J.A.V.M.A., 163, (1973a): 804-806.

Schultz, R. D.: Developmental aspects of the fetal bovine immune response. A review. Cornell Vet., 63, (1973b): 507-535.

Schultz, R. D., Dunne, H. W. and Heist, C. E.: Breed difference in the immune response of newborn calves. Am. J. Vet. Res., 32, (1971): 1337-1341.

Selman, I. E., McEwan, A. D. and Fisher, E. W.: Absorption of the immune lactoglobulin by newborn dairy calves. Attempts to produce consistent immune lactoglobulin adsorptions in newborn dairy calves using standardized methods of colostrum feeding and management. Res. Vet. Sci., 12, (1971a): 205-210.

Selman, I. E., de la Fuente, G. H., Fisher, E. W. and McEwan, A. D.: The serum immune globulin concentrations of newborn dairy heifer calves: A farm survey. Vet. Rec., 88, (1971b): 460-464.

Sever, J. L.: Application of a microtechnique to viral serological investigations. J. Imm., 88, (1962): 320-329.

Sharpe, H. B.: A study of antibody levels in the young pig. Res. Vet. Sci., 6, (1965): 490-497.

Shivers, C. A. and James, J. M.: Specific antibodies produced against antigens of agar-gel precipitates. Immunology, 13, (1967): 547-554.

Silverstein, A. M.: Ontogenesis of the immune response. In Comparative Aspects of Reproductive Failure. Edited by K. Benirschke, Springer-Verlag, New York, N.Y. (1967): 392-412.

Silverstein, A. M. and Kraner, K. L.: Studies on the ontogenesis of the immune response. In Mechanisms of Antibody Formation. Edited by J. Sterzl, Czechoslovak Acad. of Sci., Prague, Czechoslovakia (1965): 392-412.

Singer, S. J. and Doolittle, R. F.: Antibody active sites and immunoglobulin molecules. Science, 153, (1966): 13-25.

Smith, E. L.: The immune proteins of bovine colostrum and plasma. J. Biol. Chem., 164, (1946a): 345-358.

Smith, E. L.: Isolation and properties of immune lactoglobulins from bovine whey. J. Biol. Chem., 165, (1946b): 665-676.

Smith, E. L.: The isolation and properties of the immune proteins of bovine milk and colostrum and their role in immunity: A review. J. Dy. Sci., 31, (1948): 127-138.

Smith, E. L. and Holm, A.: Transference of immunity to the newborn from colostrum. J. Biol. Chem., 175, (1948): 349-357.

Smith, H., Gallop, R. C. and Tozer, B. T.: The production of specific rabbit antibodies by injecting individual antigen-antibody complexes separated from mixed antigens. Immunology, 7, (1964): 111-117.

Smith, H. W.: Further observations on the effect of chemotherapy on the presence of drug-resistant *Bacterium coli* in the intestinal tract of calves. Vet. Rec., 70, (1958): 575-580.

Smith, H. W.: The ecology of the intestinal bacteria of the calf with particular regard to *Escherichia coli*. Vet. Rec., 72, (1960): 1178-1183.

Smith, H. W.: Observations on the aetiology of neonatal diarrhoea (scours) in calves. J. Path. Bact., 84, (1962): 147-168.

Smith, H. W. and Crabb, W. E.: The bacterial flora of the faeces of animals and man. Its development in the young. J. Path. Bact., 82, (1961): 53-66.

Smith, T.: The immunological significance of colostrum. I. The relation between colostrum, serum, and the milk of cows normal and immunized towards *B. coli*. J. Exptl. Med., 51, (1930): 473-481.

Smith, T. and Little, R. B.: The significance of colostrum to the newborn calf. J. Exptl. Med., 136, (1922a): 181-198.

Smith, T. and Little, R. B.: Cow serum as a substitute for colostrum in newborn calves. J. Exptl. Med., 36, (1922b): 453-468.

Smith, T. and Little, R. B.: Absorption of specific agglutinins in homologous serum fed to calves during the early hours of life. J. Exptl. Med., 37, (1923): 671-683.

Smith, T. and Little, R. B.: Proteinuria in newborn calves following the feeding of colostrum. J. Exptl. Med., 39, (1924): 303-321.

Smith, T. and Little, R. B.: The immunological significance of colostrum. II. The initial feeding of serum from normal cows and cows immunized towards *B. coli* in place of colostrum. J. Exptl. Med., 51, (1930): 483-492.

Smith, T. and Orcutt, M. L.: The bacteriology of the intestinal tract of young calves with special reference to early diarrhoea "scours." J. Exptl. Med., 41, (1925): 89-106.

Smith, V. R. and Erwin, E. S.: Absorption of colostrum globulins introduced directly into the duodenum. J. Dy. Sci., 42, (1959): 364-365.

Sojka, W. J.: Serological behaviour of *E. coli*. In *Escherichia coli in Domestic Animals and Poultry*. Commonwealth Agricultural Bureaux, Farnham Royal Bucks, England (1965a): 18-51.

Sojka, W. J.: *Escherichia coli* infections in cattle. In *Escherichia coli in Domestic Animals and Poultry*. Commonwealth Agricultural Bureaux, Farnham Royal Bucks, England (1965b): 65-96.

Steck, F., Nicolet, F. and Schipper, E.: Aetiologische untersuchungen über virale und bakterielle infektionen in kälber-und rindermastbetrieben. Berliner und Munchener Tier. Woch., 84, (1971): 21-24.

Sterzl, J.: Quantitative and qualitative aspect of the inductive phase of antibody formation. J. Hyg. Epid., 7, (1963): 301-318.

Sterzl, J. and Mandel, L.: Estimation of the inductive phase of antibody formation by plaque technique. Folia Micro., 9, (1964): 173-176.

Sterzl, J. and Silverstein, A. M.: Developmental aspects of immunity. In *Advances in Immunology*. Academic Press, New York, N.Y. (1967): 337-459.

Stirm, S., Ørskov, F., Ørskov, I. and Mansa, B.: Episome-carried surface antigen K88 of *Escherichia coli*. J. Bact., 93, (1967): 731-739.

Thorbecke, G. J. and Van Furth, R.: Ontogeny of immunoglobulin synthesis in various mammalian species. In *Ontogeny of Immunity*. Edited by R. T. Smith, R. A. Good and P. A. Miescher, Univ. of Florida Press, Gainesville, Fla. (1967): 173-179.

Tomasi, T. B.: The gamma A globulins: First line of defense. In *Immunobiology*. Edited by R. A. Good and D. W. Fisher, Sinauer Associates, Stamford, Conn. (1971): 76-83.

Uhr, J. W. and Vitetta, E. S.: Synthesis, biochemistry and dynamics of cell surface immunoglobulin on lymphocytes. Fed. Proc., 32, No. 1, (1973): 35-40.

van Oss, C. J.: The immunoglobulins. In *Principles of Immunology*. Edited by N. R. Rose, F. Milgrom and C. J. van Oss, Macmillan Publ. Co., Inc. (1973): 122-140.

Westphal, O., Beckman, I., Hammerling, U., Jann, B., Jann, K. and Luderitz, O.: Recent investigations on the polysaccharide component of enterobacterial endotoxins (S and R forms). In *Bacterial Endotoxins*. Edited by M. Landel and W. Braun, Quinn and Boden Co., Inc., Rahway, N.J. (1964): 1-15.

White, R. G. Mebus, C. A. and Twiehaus, M. J.: Incidence of herds infected with a neonatal calf diarrhea virus (NCDV). Vet. Med./ Sm. An. Cl., 65, (1970): 487-489.

Williams, C. A. and Chase, M.W.: *Methods in Immunology and Immuno-chemistry*, I. Edited by C. A. Williams and M. W. Chase, Academic Press, New York, N.Y. (1967): 1-385.

Williamson, A. R.: Clones of antibody-forming cells: natural and experimental selection. Endeavour, 31, No. 114, (1972): 118-122.

Willinger, H. and Mathois, H.: Serologische typisierung von coli-sepsisstammen beim kalb. Wiener Tier. Monat., 52, (1965): 155-159.

Wilson, M. R. and Svendsen, J.: Immunity to *Escherichia coli* in pigs: Serologic response of sows given formalin-treated live *Escherichia coli* vaccine. Am. J. Vet. Res., 32, (1971): 891-898.

Wramby, G.: Investigations into the antigenic structure of *Bacterium coli* isolated from calves with special reference to coli-septicemia (white scours). Acta Path. Micro. Scand., 76, (1948): 168.

VITA

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