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Escherichia coli and Neonatal Disease of Calves

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INTRODUCTION

The economic loss occasioned by neonatal disease in young calves has been recognized for many years. It is apparent from postmortem and bacteriological examination of such calves that there are many causes of this loss; however, colibacillosis infection caused by *Escherichia coli* is by far the most common (117, 147). The clinical syndrome believed to be associated with colibacillosis may vary considerably. Calves may be affected with diarrhea for prolonged periods of time, or they may die suddenly with an acute septicemia. There are, however, other diseases of calves which may simulate colibacillosis (i.e., acute septicemias caused by *Streptococcus*, *Diplococcus*, *Pasteurella*, and *Salmonella* spp.). There are also a variety of conditions, both infectious and noninfectious, which may cause diarrhea in young calves. Many of these may be differentiated from colibacillosis on clinical, pathological, or bacteriological grounds, but others, especially those manifest primarily by diarrhea, are more difficult to identify. By nature of its habitat, *E. coli* may be isolated from the feces in all of these diseases; in fact, routine cultural methods tend to select for it. 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. A further problem exists in that a reliable method of classifying *E. coli* was not available until the 1940's. It is not surprising, therefore, that some doubt still exists as to the role played by *E. coli* in neonatal disease in calves, especially its significance in the etiology of diarrhea or "calf scours." In part, this is probably the result of many workers dealing with diarrhea in calves as an entity rather than as a clinical sign.

The literature on scouring and death in calves was last reviewed in 1955, by Lovell in English (119) and by Fey in German (50). The purpose of this review is to summarize literature dealing with the isolation of *E. coli* from calves and to attempt to assess the significance of this bacterium in neonatal disease and death of calves. For this purpose, the syndromes associated with colibacillosis have been divided into three forms on clinical and bacteriological grounds and on the grounds of possible pathogenesis.

(i) The colisepticemic form of colibacillosis usually results in the rapid death of the calf and is associated with an *E. coli* bacteremia. Although many strains of *E. coli* have been isolated from cases of colisepticemia, the isolations from the internal organs of a given case are of a single strain in pure culture.

(ii) The enteric-toxemic form is also associated

with the collapse and rapid death of the calf, but it is associated with the proliferation of certain specific strains of *E. coli* in the small intestine. There is no bacteremia, and death is presumably due to a toxemia. This form is probably analogous to that called isocolibacillosis in some earlier publications.

(iii) The enteric form is part of the syndrome of the scouring calf. Death may or may not occur depending upon the severity of the physiological derangements produced.

IDENTIFICATION AND CLASSIFICATION OF THE *E. COLI* GROUP

It is not intended to review here the literature pertaining to the classification and identification of the *E. coli* group within the family Enterobacteriaceae; however, a brief description of the group and of the nomenclature as used in this text is included here for purposes of clarification. The precise and detailed definition of the *E. coli* group, the required biochemical characteristics, and the methods used for identification may be found in Edwards and Ewing's *Identification of the Enterobacteriaceae* (39) from which much of the following description has been drawn.

The family Enterobacteriaceae is composed of gram-negative rods which may or may not be motile, and it attacks glucose with the production of acid or acid and gas. Nitrates are usually reduced to nitrites. On the basis of further biochemical reactions, the family may be arranged into divisions and then into groups, one of which is *E. coli*. These groups are not distinct, but form dense populations within the family which have certain biochemical properties. Within the *E. coli* group, the individual strains are identified by serological methods. These serological types can be further divided or classified by biochemical characteristics, phage susceptibility (141, 197), and susceptibility to colicines (10, 57, 75, 114, 148).

Early attempts at the classification and identification of individual strains of *E. coli* were of limited value, and it was not until the 1940's that a reliable method of serological typing was developed. In 1947, Kauffmann (101) published an antigenic schema for *E. coli* which was based on his own previous work and that of Knipschildt and Vahlne. This has subsequently been enlarged by other workers [for complete references, see Edwards and Ewing (39)].

There are three main antigens of *E. coli* which are used in its identification: (i) O or somatic antigens, (ii) K antigens which occur as capsules or microcapsules, and (iii) H or flagella antigens. The difficulty encountered by earlier workers in serotyping *E. coli* was apparently due to the K antigen which, when present, prevents the agglutination of the O or somatic antigen by the homologous sera. In earlier work, Smith and Bryant (210) noticed that, under certain conditions, colonies of *E. coli* gave rise to translucent outgrowths which microscopically were found to be devoid of a capsule. This is one method of removing the K antigen to allow for O group identification. The K antigen may also be removed by heat. However, there are differences in heat susceptibility of these K antigens, and it is on this basis that they are further subdivided into three groups.

(i) The L-type K antigens are completely destroyed by heat at 100 C for 1 hr, thus rendering the O antigen agglutinable in O antisera. They retain no antigenicity and lose their ability to combine with homologous K antisera.

(ii) The B-type K antigens are also destroyed by heat at 100 C for 1 hr and lose their antigenicity. However, they retain their ability to bind or combine with their homologous antisera.

(iii) A-type K antigens are not inactivated by heat at 100 C, but require heat at 121 C for 2.5 hr before the O antigen becomes agglutinable.

Recently (146), it was shown that there is some variation with certain K antigens, and that certain *E. coli* strains may possess more than one type of K antigen.

The O antigens are not single antigens, but they are composed of several antigenic components and, therefore, are called O group antigens. Different O groups may share some of these antigenic components; hence, there are many cross-relations between O groups. As might be expected, these antigenic cross-relations are not restricted to within the *E. coli* group, but also occur between it and other groups in the family Enterobacteriaceae with all antigens (44).

Flagella, or H antigens, may or may not be present.

All of these antigens are named numerically, the O group antigen being given first, followed by the K antigen and then the flagella antigen; e.g., O26:K60:H11. Human enteropathogenic *E. coli* all possess B-type K antigens, and, occasionally, these are numbered separately; e.g., O26:B6:H11. When the full antigenic structure of a strain is known, it may be called a serotype. Since it is not always possible to completely define a newly isolated strain in terms of Kauffmann's schema, regional designations are given to these strains, and a formula such as O101:K(A) RVC 118 may be seen. This indicates that the strain belongs in O group 101 and possesses an A-type K antigen serologically indistinguishable from the Royal Veterinary College strain no. 118.

Other strains which have been fully identified may still have the original strain name following to identify their source; e.g., O137:K79:H41 (RVC 1787).

Other antigens, both group-specific and non-specific, have been described for *E. coli* (18, 31, 106, 107, 164, 203, 225, 245), but these are not used for the classification of the group, and, in most cases, their significance is not known.

Certain strains of *E. coli* are hemolytic (120, 203), and, as this property is usually possessed by certain serotypes pathogenic for pigs, it is used to facilitate their isolation and identification.

NORMAL BACTERIAL FLORA OF THE CALF INTESTINE AND ITS ABERRATIONS IN CASES OF COLIBACILLOSIS

Despite the fact that strains of *E. coli* have been incriminated in cases of scours, there is a surprising lack of publications on the development and distribution of the normal intestinal flora of the calf, the relationship between the various bacterial species involved, and the aberrations of these in cases of scours. Of the large animal group, the intestinal flora of the pig has been most extensively studied (36, 84, 103, 121, 122, 124, 151, 152, 162, 246, 248). It is evident from these and other publications (66, 67, 73, 94, 201) that the relationship between various bacterial species in the intestine and their distribution varies greatly in the normal animal and is influenced by such factors as age, diet, *p*H, oxidation-reduction potentials in the various portions of the intestine, and antagonisms and synergisms between and within the bacterial species involved.

Although Hagan and Williams (74, 247) found evidence of *in utero* contamination of the calf intestine, it is evident that the main colonization of the intestine occurs after birth, the bacteria originating from the environment (134, 171, 199, 200, 241, 250). In terms of total fecal flora, the maximal numbers are reached by 24 hr (134, 199, 200, 241). At this time, the flora is comprised mainly of *E. coli*, *Streptococcus* spp., and *Clostridium perfringens*, with *Lactobacillus* and *Bacteroides* species appearing on the second day (199, 200). These various bacterial species persist in high numbers for periods of time which vary with the species, but gradually decrease in numbers to a lower plateau after this initial peak.

There are conflicting reports regarding the distribution of these bacteria in the intestinal tract of calves. Until recently, knowledge of this subject was based entirely on the early studies of Carpenter and Woods (21) and Smith and Orcutt (208). In these studies, which dealt primarily with *E. coli*, the intestinal flora of healthy calves was compared with that of scouring calves. In the healthy calf, *E. coli* and other bacteria were prevalent only in the large intestine and the distal portion of the small intestine; the medial and proximal portions of the small intestine were described as being virtually free of bacteria, except for a few streptococci and lactobacilli. In contrast to this, it was found that, in calves dying of "scours," *E. coli* was present in large numbers in the upper small intestine. The numbers found were such that they were described as carpeting or coating the mucosal surface (208). From these findings it was concluded that scouring in calves was the result of an active invasion and proliferation of *E. coli* strains up into the medial and proximal portions of the small intestine, an area where they were not normally present.

In both of these studies, the methods used to determine these results were insensitive and subject to error. Despite this, these results were generally accepted, and, other than some debate over the significance of the proliferation of *E. coli* in the pathogenesis of scouring (13), they have, until recently, remained unchallenged. Recently, however, Smith (202) examined the intestinal flora of calves using more exacting methods. In contrast to the earlier studies, Smith found that *E. coli* was prevalent throughout the intestinal tract of the healthy calf. Although he found a marked increase in number of *E. coli* from the proximal to the distal portion of the small intestine, the only bacteria which showed a definite selective distribution in the intestine were *Bacteroides* spp. and *C. perfringens*. Recent publications on the intestinal flora of pigs indicated that this distribution may be true for this species also (36, 84, 121, 151). There is little doubt that the superior methods of Smith (202) allowed a more exact demonstration of the intestinal flora than did those methods of Carpenter and Woods (21) and Smith and Orcutt (208), and, therefore, Smith's description of this flora is the more acceptable.

Smith (202) also examined the intestinal flora of scouring calves, and again his findings differed from those of the earlier workers. Smith found no evidence of a proliferation of *E. coli* in the small intestine of the scouring calves and, in fact, could find no evidence for associating *E. coli* with this syndrome at all. This finding has led some people to discount the earlier works of Carpenter and Woods and Smith and Orcutt and to assume that *E. coli* is not of etiological significance in scouring calves. However, these opinions are ill-founded; scouring in calves is not an entity, and it is quite possible that the disease described by Smith was different from that described by the earlier

workers. This is purely conjecture in the case of the disease described by Carpenter and Woods, but, in the case of Smith and Orcutt, there is reasonable evidence for this. The description (210) of the *E. coli* strains isolated from the sick calves in Smith and Orcutt's study led Wramby (251) to conclude that these strains were mucoid *E. coli* possessing A-type K antigens. This type of *E. coli* is associated with a specific form of colibacillosis (the enteric-toxemic form) in which tremendous numbers of *E. coli* are found in the small intestine (60*b*). The clinical and bacteriological syndrome described by Smith and Orcutt (208) fits that of this form of colibacillosis. Therefore, it is apparent that Smith (202) was describing a form of diarrhea in calves in which *E. coli* apparently played no significant role, whereas Smith and Orcutt (208) and perhaps Carpenter and Woods (21) were describing a specific *E. coli* infection. There have been no further studies of this kind, and the deviations from normal, if any, in the intestinal flora of calves with the enteric form of colibacillosis remain to be determined. Thus, there is no knowledge of the mechanism by which *E. coli* might cause diarrhea. The physiological and biochemical derangements associated with diarrhea in calves were studied by Blaxter and Wood (13), McSherry and Grinyer (135), and Roy et al. (179).

Kauffmann and Perch (100) and Wallick and Stuart (243) observed that the *E. coli* strains within the intestinal flora of man were not stable, but were continually changing. Sears et al. (186, 187, 188) confirmed this by showing that the individual strains comprising the *E. coli* flora of healthy men and dogs could be divided into resident and transient strains according to the pattern of their isolation from the feces. The resident strains, of which there were only one or two at any one time, were the predominant strains in terms of numbers and persisted as the dominant strains for several weeks to several months before being displaced by another strain which then became resident; the transient strains, of which there might be three or four at any one time, were few in numbers and persisted at the most for only a few days. A similar relationship was subsequently shown in the *E. coli* intestinal flora of infants (58), horses (180), and calves (197, 199, 200, 250). The factors involved in this dominance or antagonism of one strain to another are not fully known. The production of colicines by strains of *E. coli* may be of some importance in maintaining them as resident strains (15), but, generally, this is not believed to be a major factor in the ability of a strain to establish itself as a resident (163, 186, 226). It was shown that host antibody to the *E. coli* strains involved plays no part in the phenomenon (61, 163, 175). Recently, an attempt was made to study this antagonism between certain strains of *E. coli* in vitro (220).

The few studies which deal with the intestinal flora of the calf have been presented. There is not sufficient information on this subject available at present to allow many conclusions to be drawn. There has been only one exacting study of the distribution of the intestinal flora of calves, and the factors which govern this distribution are unknown. There is evidence that a specific change occurs in this flora associated with the enteric-toxemic form of colibacillosis, but it has also been shown that diarrhea unassociated with *E. coli* may occur without any change in the intestinal flora. Confusion has arisen over the interpretation of these findings, and may occur in the interpretation of the findings of future studies of this kind if due emphasis is not given to determining the etiology of the disease studied.

RELATIONSHIP OF INGESTION OF COLOSTRUM TO SEPTICEMIC INVASION BY *E. COLI*

In a series of papers published in the 1920's (204, 205, 206, 208, 209), Theobald Smith and co-workers observed that calves deprived of colostrum almost invariably died, death being due to the invasion by *E. coli* and a resultant septicemia. They concluded that the serum of the calf deprived of colostrum lacked something which permitted the invasion of these intestinal bacteria. It was subsequently shown that calf serum was devoid of globulins and agglutinins until after the ingestion of colostrum (86, 206), and it was believed that the acquisition of these or, more specifically, of antibodies against *E. coli* (213, 214) was the protective factor. Calves deprived of colostrum which survived often had residual lesions of a septicemia (204, 209).

These observations of Smith pertaining to the significance of colostrum were confirmed shortly thereafter by a series of papers by Aschaffenburg and co-workers (1, 2, 3, 4, 5). They fed unsuckled calves various fractions of colostrum or a colostrum-like substance rich in vitamins. Only those calves receiving the globulin fraction of the colostrum, or fractions containing it, survived and grew well. The majority of the colostrum-deprived calves, including those fed the high vitamin feed, died. The survivors failed to grow as well as the colostrum-fed calves. As little as 80 ml of the aqueous fraction of colostrum or 14 g of immune lactoglobulins protected the majority of calves against colisepticemia, and there was some evidence that, within certain limits, the incidence of scouring decreased, and

the live weight gain improved in proportion to the amount of colostrum fed.

These two sets of experiments established definitely the value of the colostrum to the newborn calf, especially its protective value against colisepticemia, the main protective factor being associated with the immune lactoglobulins.

TRANSFERENCE OF IMMUNITY FROM THE DAM TO THE CALF

For many years, the importance of colostrum to the calf was thought to rest with its costive action (85), although Ehrlich (40), as early as 1892, demonstrated that immunity could be passed from the mother to the young via the milk. Ehrlich's observations and subsequent ones were summarized up to 1912 by Famulener (47) in an extensive review. Famulener, drawing upon the results of these previous experiments and his own, postulated that the young acquired their maternal passive immunity from the ingestion of antibodies contained in the colostrum. After immunizing goats against sheep red blood cells, he demonstrated that the colostrum contained antisheep erythrocyte hemolysins, often in titers two to three times as great as those of maternal blood at the time of parturition. The serum of the offspring remained devoid of hemolysins until after it had been fed colostrum or hemolysin-positive serum. From this, he postulated that the colostrum contained, in addition to normal milk proteins, immune serum proteins derived from the maternal blood and that these were absorbed unchanged through the gut wall of the young animal. He also observed that this absorption did not occur after a few days of life. Subsequent work has tended only to confirm these observations and postulations.

Nature and Origin of Immune Proteins of Bovine Colostrum

Famulener observed that hemolysins were also present in postcolostral milk, but that their presence was variable. Using salt precipitation techniques, Howe (85) was unable to demonstrate the presence of globulins in the milk of cattle, but their presence was subsequently demonstrated by electrophoretic and immunological methods (33, 79, 177, 194). Normally, these immune globulins account for only approximately 10% of the protein of milk whey, but this level may be increased by hyperimmunizing the cow (33). About 1 month before parturition, the globulin concentration in the udder secretion of the cow commences to rise, reaching a maximum approximately 5 days before calving (11, 79, 110); this is evidenced by the high concentration (54 to 55% of total proteins) of globulin found in colostrum at the time of parturition. Globulins also account for a high proportion of precolostral secretion in heifers (11, 132). The relationship of these colostral immune proteins to those of maternal blood serum was studied chemically by Crowther and Raistrick (27) and immunologically by Wells and Osborne (244). No difference between the two could be detected by either method.

Smith (191, 196) fractionated the various protein constituents of maternal serum and colostrum and studied the various components electrophoretically. He found that the immune activity of the colostrum was confined to the two fractions associated with the lactoglobulins which could be separated by dialysis into water-soluble pseudoglobulins and water-insoluble euglobulins. In comparing these with γ- and T-globulins prepared from the maternal serum, he found that the colostral globulin differed markedly from maternal γ-globulin in isoelectric point and electrophoretic motility, but he could not differentiate between the colostral immune globulins and the serum T-globulins by these methods. He also failed to detect any difference between the two by anaphylactic tests but found that, although they were qualitatively similar with regard to carbohydrate and amino acid content, they differed quantitatively with respect to certain amino acids (192), the difference being reflected in their absorption spectra (193). On this basis, he concluded that the immune proteins of bovine sera and colostrum were not identical. Smith's views were not supported by the electrophoretic studies of Polson (161) or of Pierce (153). Other work also indicated that the colostral globulins were the same as those of the maternal serum (6, 11, 12, 59, 108). It remained for Larson (109) to show the association between the immune globulins of colostrum and those of the serum; there was a considerable fall in blood serum proteins during the period just before parturition, and this fall coincided with the time at which the protein content could be shown to be increasing in the udder secretion. This fall in serum proteins was due to a loss of β-2 and γ-1 globulins (γ-1 = Smith's T fraction), there being no loss of γ-2 globulin from the blood (γ-2 = Smith γ). The increase in protein content of the colostrum was primarily due to a tremendous increase in β-2 and γ-1 globulins, and this gain was quantitatively equivalent to the loss from the blood serum.

Acquisition of Immune Proteins by the Calf

By means of salt precipitation, Howe (85) demonstrated that these globulins did not appear

in the serum of the newborn calf until after the ingestion of colostrum, and the acquisition of antibodies was shown subsequently to be entirely dependent upon the acquisition of these globulins (115, 142). The feeding of bovine serum (86, 205) or purified pseudoglobulin (77) also results in the appearance of γ-globulin in the serum of the calf. These principles have been confirmed by other methods (78, 92, 95, 127, 129, 131, 153, 161, 242). The immune proteins of the colostrum and those appearing in the calf serum after its ingestion have the same properties (77, 79, 95, 191, 195) and, therefore, appear unchanged by this transfer. The site of absorption of these proteins is the small intestine and proteins passing through the epithelial cells to reach the lymphatics (8, 25, 26). They may be detected in the thoracic duct 1 to 2 hr after the introduction of the protein into the duodenum (25), a time interval which approximates that observed by Little and Orcutt (115) and by McDiarmid (131) for the appearance of agglutinins in the blood after the ingestion of colostrum.

Famulener's observation, that the absorption of antibodies through the intestinal wall was limited to a short time after birth, has been well substantiated, and it appears that, in ruminants, very little absorption occurs more than 24 to 36 hr after birth (25, 34, 77, 130, 154, 158, 215, 221). The bare statement of these limits, however, gives a false impression, for it has been shown that, in terms of absorption of antibody to a somatic antigen of *E. coli*, this function is reduced almost 50% at 16 hr after birth (98). In some calves, this function may be lost as early as 6 to 8 hr after birth (Gay, *unpublished data*). The factors involved in this cessation of intestinal permeability are unknown, but digestive degradation of the proteins does not appear to play a part (34, 215) as was originally thought (83, 111).

The absorption of protein from the intestine of newborn herbivores is not as selective as it is for some other animal species (176), because proteins of low molecular weight, such as β-lactoglobulins, are absorbed along with the immune lactoglobulins (7, 9, 34, 156, 158, 161). The former are cleared from the blood by the kidneys; this results in a proteinuria which lasts for approximately the same period of time that the intestine is permeable (34, 86, 154, 155, 156, 157, 158, 207). The immune lactoglobulins are largely retained, very little being filtered out via the urine. The principal constituent of the proteinuria is β-lactoglobulins (34, 154, 155, 156, 157, 158). This subject was discussed in some detail in an article by Pierce which was given at the 13th Symposium of the Colston Research Society (159).

The degree and duration of the protection afforded by these colostral antibodies is difficult to assess. For example, Thorpe and Graham (233) noticed great variation in the persistence of the titer to *Brucella abortus* in the sera of calves which had suckled positive dams. McDiarmid (131) believed this variation in persistence of titer to be dependent to a certain extent upon the initial titer in the calf. Electrophoretically the immune components of the sera of calves have a half-life of about 16 days; however, the antibody levels to different antigens do not necessarily decrease in this order, and their half-lives vary considerably (24, 104, 195).

Conclusions on Transference of Immunity

It is now well established that the newborn calf acquires its maternal passive immunity solely via the colostrum. There is little doubt that the immune lactoglobulins of the colostrum are derived directly from the blood serum globulins of the dam, and that they are absorbed, unchanged, through the intestinal wall of the calf. However, there is little known of the mechanism of this absorption and of the factors which control it. Although the period of time after birth during which this absorption can occur has been established, there is almost no knowledge of the relative absorption capacity of the intestine at various intervals during this period of time. The importance of this information is discussed later in this paper.

NATURE OF COLOSTRAL PROTECTION

Briggs (16, 17) studied serologically the *E. coli* strains that were isolated from the dead calves in the previously mentioned experiments of Aschaffenburg and co-workers. By typing with immune sera prepared from five of these strains, he found that he could classify the majority of the other 102 strains isolated. Briggs demonstrated that the majority of his strains possessed K antigens. He found that, to protect mice against lethal doses of these *E. coli*, the antisera given had to contain the relevant K antibody, since O group antibody had little protective value. This was also previously demonstrated by Smith (212). Briggs also found that the K agglutinin content of bovine colostrum was related to its ability to protect mice. By applying this interpretation of the importance of K antibody, he was able to determine that the colostrum fed to 11 calves which had subsequently died of coliseptic emia contained no K agglutinins to the *E. coli* isolated from them. There was also some evidence that the calves which survived had usually received colostrum containing K ag-

glutinins to the strains which had killed both colostrum-fed and colostrum-deprived calves in contact with them.

Because of this suggestive evidence of an immunological protection, further experiments were conducted from 1950 to 1953 to establish this more clearly (89, 90). During this period, 103 colostrum-deprived calves and 225 colostrum-fed calves were under experiment. The *E. coli* strains isolated from those calves which died of colibacillosis were serologically identified, and a record was made of the presence or absence of K agglutinins to these *E. coli* in the colostrum fed to contemporary calves which survived. Ninety-four of the colostrum deprived calves died; 88 with colisepticemia and 6 with localized intestinal *E. coli* infections. Of these deaths, 66 were associated with strains of *E. coli* against which K agglutinins were present in the colostrum fed to "in contact" calves that survived. Of the 225 colostrum-fed calves, a total of 59 died; 29 with colisepticemia and 30 with localized intestinal *E. coli* infections. Of these dead calves, 45 had received colostrum which contained no K agglutinins to the *E. coli* strains associated with their deaths (90, 250). The K agglutinin content of colostrum fed to surviving calves was not given. Specific types of *E. coli* were associated with many of the deaths in these calves. RVC101 [O35:K(B)] and RVC51 [O114:K(B)] were responsible for approximately 30% of the deaths in colostrum-deprived calves; all colostrum-fed calves received K agglutinins against these strains and none died from infection with them. RVC118 [O9:K(A)], RVC330 [O78:K80], and RVC95A [O26:K60] were responsible for the majority of the deaths in colostrum-fed calves. It was stated that the colostrum fed to the calves which died of infection with these *E. coli* types contained no K agglutinins against them. Unfortunately, the K antibody levels against these strains in the colostrum fed to surviving calves was not stated.

As a result of these studies, it has become generally accepted that specific agglutinating antibody against the K antigens of *E. coli* is the factor in colostrum which protects the calf against colisepticemia and other forms of colibacillosis. However, it is doubtful whether the findings in these studies warrant this conclusion. The fact that the colostrum fed to surviving calves contained K agglutinins against approximately 70% of the *E. coli* that killed colostrum-deprived calves is not, by itself, conclusive evidence that the K agglutinins protected these calves. Similarly, the fact that 45 of the 59 colostrum-fed calves which died had received no K agglutinins in the colostrum against the *E. coli* that killed them cannot be fully evaluated without some knowledge of the content of K agglutinin against these strains in the colostrum fed to the surviving calves. It was assumed in these studies that all calves fed colostrum absorbed the antibodies or globulins contained in the colostrum. It should be noted, however, that a total of 42 colostrum-fed calves died with coliseptiemia. In view of present knowledge of the pathogenesis of colisepticemia (vide infra), it is extremely unlikely that these calves did, in fact, absorb globulins from the colostrum. This contributes to even greater difficulty in the interpretation of these studies.

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 agglutinins against the K antigens of these strains (60, 60*a*). 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 (60, 202).

Smith (202) found that the serum bactericidal activity is of some importance in protecting calves from colisepticemia. This was associated with two factors—a heat-labile factor in precolostral serum (complement-properdin system) and a heat-stable factor in the colostrum (antibody). He found a direct relationship between the ability of a strain to grow in precolostral serum and its ability to produce the septicemic form of colibacillosis when fed to a colostrum-deprived calf. Most *E. coli* strains tested did not grow in postcolostral sera and were without effect when fed to colostrum-fed calves. However, two strains which did grow in postcolostral sera were also without effect when fed to these calves. Glantz et al. (64) also compared the bactericidal activity of the sera and the resistance of the calf. No relationship was found.

Although specific agglutinins do not appear to be the factor in colostrum which protects calves against colisepticemia, there is some evidence (60, 64) that the presence of specific agglutinins in the serum of the calf protects it from the enteric form of colibacillosis. There is also some evidence that the vitamin A acquired by the calf via the colostrum determines to some extent its resistance to infection with *E. coli* (56, 76, 222).

Although there are indications that the presence of specific agglutinins and bactericidal activity in the serum of the calf confers resistance to infection with *E. coli*, it is also apparent that these

are not the sole mechanisms involved and that the full protective nature of colostrum is yet unknown.

SEROLOGICAL STUDIES OF *E. COLI* ISOLATED FROM CASES OF COLIBACILLOSIS

The persistent isolation of *E. coli* from certain conditions in animals and man led to the early conviction that certain pathogenic strains were involved in these diseases. However, before the Kauffmann-Knipschildt-Vahlne serological scheme was evolved, studies to substantiate this belief were of little value, because of the lack of a suitable method of identifying and recognizing the isolated strains. Since the publication of this serological scheme, there have been many studies which have shown that specific serological types of *E. coli* are, in fact, associated with certain diseases. From reports published to date, it appears that colibacillosis of calves is one of these diseases; however, there are still many gaps in the knowledge of the serotypes associated with this condition. The majority of the serological studies on *E. coli* isolated from cases of colibacillosis of calves have been on strains isolated from colisepticemia. Although there is now good knowledge of the predominant serotypes of *E. coli* associated with colisepticemia, there is little information available of the serotypes of *E. coli* associated with the enteric forms of colibacillosis. Also, most of these studies have been concerned solely with the serological classification of the strains isolated. Therefore, there is very little knowledge of the epidemiology of outbreaks of colibacillosis in calves.

Serotypes of E. coli Associated with Colibacillosis of Calves

In 1917, Christiansen (23) extended Jensen's (93) earlier work in attempting to classify *E. coli* isolated from scours in calves; he found that he could not distinguish between strains isolated from scouring calves and those isolated from normal calves on the basis of morphological, biochemical, or serological studies. On the basis of biochemical reactions, Smith (204, 208) believed that certain pathogenic races did exist, a view supported in 1937 by the serological examinations by Lovell on *E. coli* isolated from calves that died from colibacillosis (117, 118). Lovell made the first determination of the true nature of the antigens of *E. coli*. He found that he could place 79 of 110 strains (isolated from 45 calves) into 1 of 8 K antigen types, 2 of which accounted for 46 strains. Although there were isolations of the same type from different calves from the same herd, it was also evident that there was often more than one K antigen type associated with a problem within a herd.

Wramby (251) was the first to utilize Kauffmann's scheme of serological typing with *E. coli* isolated from calves. He made an extensive study involving 4,262 strains isolated from 484 "sepsis" calves and 1,699 strains isolated from 492 normal calves, and typed them to antisera made to Kauffmann's O groups 1 to 25 and his own types 26W to 43W which were isolated from calves. These latter types were subsequently classified according to the Kauffmann-Knipschildt-Vahlne scheme by Ørskov (155), and this nomenclature will be used here. Wramby found little difference between the frequency of occurrence of the various O groups in the intestine of "sepsis" calves and of normal calves, with the exception of O group 9 which occurred with a significantly greater frequency in the intestine of "sepsis" calves. In view of the suspected pathogenesis of colisepticemia (vide infra), this finding is not surprising. When the frequency of O groups in the organs (spleen, liver, brain, lymph nodes) and intestine of the "sepsis" material was examined, the same O groups as those found in the intestine of normal calves tended to predominate, but in a different order; some (e.g., group 78) were markedly more predominant. The more predominant O groups of this "sepsis" material are shown in Table 1.

This first report of the use of Kauffmann's scheme in typing serologically strains of *E. coli* from colibacillosis did not lend much support to the view that certain pathogenic strains of *E. coli* were involved in colibacillosis. However, Wramby found that 76.1% of the strains from the "sepsis" material possessed no K antigen. Most authors dealing with isolations of *E. coli* from colibacillosis of calves and certain conditions in other animal species have stressed the importance of the K antigen as an index of pathogenicity and have found that the vast majority of their isolations possessed K antigens. In view of the high incidence of K negative strains in Wramby's "sepsis" material, it is quite possible that calves which had died from conditions other than colibacillosis were sent to him for examination.

Subsequent studies (Table 1) have given more positive evidence of the association between specific serogroups and serotypes of *E. coli* and colibacillosis of calves (14, 28, 51, 97, 166, 240). The number of isolates studied, the number of these which were serogrouped, and the number of O group sera used, are given for each study. The serogrouped isolates are presented to show the distribution of the individual O groups within the total number serogrouped.

Where it is possible, these figures are expressed as number of cases, each case representing a calf.

TABLE 1. *Distribution of Escherichia coli O groups in cases or strains which could be serotyped*

Reference	No. of strains or cases studied	No. of strains or cases O grouped	No. of O group sera used	Type of colibacillosis	O group	No. of strains or cases*	Total	
							O group	Cases or strains*
Wramby (251)	Unknown	1,167	43	Colisepticemia ?	15	*214*	40	*1,167*
					8	*137*		
					9	*109*		
					117	*97*		
					78	*54*		
					45	*53*		
					115	*49*		
					18	*29*		
					25	*26*		
					20	*24*		
					30 other O groups	*371*		
Bokhari and Orskov (14)	155	91	129	Colisepticemia	78	18	24	91
					15	15		
					115	10		
					8	6		
					9	6		
					117	6		
					45	5		
					26	4		
					16 other O groups	21		
Ulbrich (240)	78	41	17	Colisepticemia and enteric colibacillosis	9	9	13	41
					8	7		
					78	6		
					4	4		
					7	4		
					2	1		
					26	1		
					55	1		
					64	1		
					4 other O groups	7		
Fey (51)	105	91	130	Colisepticemia	78	*29*	20	*91*
					115	*15*		
					86	*10*		
					15	*8*		
					117	*8*		
					88	*3*		
					7	*2*		
					8	*2*		
					20	*2*		
					24	*2*		
					10 other O groups	*10*		
Rees (166)	129	40	14	Colisepticemia and enteric colibacillosis	78	15	10	40
					137	7		
					9	4		
					35	4		
					15	2		
					86	2		
					103	2		
					117	1		
					2 other O groups	3		

TABLE 1—*Cont.*

Reference	No. of strains or cases studied	No. of strains or cases O grouped	No. of O group sera used	Type of colibacillosis	O group	No. of strains or cases*	Total	
							O group	Cases or strains*
Kaeckenbeeck and Thomas (97)	278	221	136	Colisepticemia	78	*55*	41	*220*
					55	*50*		
					86	*28*		
					15	*20*		
					8	*9*		
					23	*6*		
					20	*5*		
					32	*3*		
					45	*3*		
					101	*3*		
					102	*3*		
					30 other O groups	*35*		
Dam (28)	431	328	16	Colisepticemia	78	185	15	328
					3-115	61		
					15	20		
					33-88	12		
					45	11		
					107-117	10		
					114	7		
					8-93	5		
					140-34	5		
					7-116-68	5		
					131	3		
					4 other O groups	4		

* Cases appear in Roman numerals and strains are in italics.

This is permissible as most workers have emphasized that the *E. coli* isolated from the internal organs (excluding intestines) of a given case of colisepticemia belong to a single O group. However, in the studies of Wramby (251), Fey (51), and Kaeckenbeeck and Thomas (97), the presentation of their results did not allow this, and these results have been tabulated as number of strains.

The majority of these studies were on *E. coli* isolated from the septicemic form of colibacillosis. The studies of Ulbrich (240) and Rees (166), however, include some *E. coli* isolated from the enteric form of colibacillosis. Rees (166) has given some epidemiological data of the *E. coli* in his study and states that O78:K80, O137:K79, and O35:K? (RVC 101) were usually associated with a rapidly fatal septicemia, whereas the other serotypes and O groups were more commonly associated with diarrhea, although they were occasionally found to be bacteremic.

Certain O groups occur quite commonly in most of these studies (Table 1). This is even more apparent in Table 2 in which the more frequently occurring O groups have been listed with the frequency of their occurrence in the various studies. For example, if the approximate percentage distribution in the total number of strains isolated or case studies of O groups 78, 45, 86, 115, and 117 is determined, the following figures are derived: Bokhari and Ørskov, 59%; Fey, 58%; Rees, 15.5%; Kaeckeenbeeck and Thomas, 29%; Dam, 65.5%. A recent publication by Dam (29) showed that the three O groups most common in his material (O groups 78, 115, and 15) have remained so from 1957 through 1961–1962, accounting for 44 to 63% of the total strains isolated in the various years. However, there is also considerable variation in O groups isolated in the studies and in their individual percentage occurrences (Table 1 and 2); i.e., O group 55 would appear quite important from the study of Kaeckeenbeeck and Thomas, yet it features in few of the other studies. Similarly, although O78:K80 is the most frequent isolate in the above-mentioned studies, Gossling, McKay, and Barnum (67*a*), in a study of *E. coli* isolated from colibacillosis of calves in Ontario, Canada, failed to find this serotype once, despite the fact that it has been isolated from cases in Pennsylvania by Glantz (64). Kramer (105) also gives brief mention of its iso-

TABLE 2. *Percentage distribution of the more common O groups in cases (or strains) O grouped and in total cases (or strains) studied**

O group	Wramby (251)† SO	Bokhari and Orskov (14)		Ulbrich (240)		Fey (51)		Rees (166)		Kaeckenbeeck and Thomas (97)		Dam (28)	
		CO	CS	CO	CS	SO	SS	CO	CS	SO	SS	CO	CS
	%	%	%	%	%	%	%	%	%	%	%	%	%
7	0.7	1.1	0.6	9.8	5.1	2.2	1.9					1.5	1.2
8	11.7	6.6	3.9	17.1	9.0	2.2	1.9			4.1	3.2	1.5	1.2
9	9.3	6.6	3.9	22.0	11.5	1.1	0.95	10	3.1				
15	18.3	16.5	9.7			8.8	7.6	5	1.5	9.1	7.2	6.1	4.6
20	2.1	1.1	0.6			2.2	1.9			2.3	1.8		
26		4.4	2.6	2.4	1.3	0	0			0.5	0.4		
35		1.1	0.6			0	0	10	3.1	0.9	0.7	0.3	0.2
45	4.5	5.5	3.2			1.1	0.95			1.4	1.1	3.4	2.6
55		0	0	2.1	1.3	0	0			22.7	18.0		
78	4.6	19.8	11.6	14.6	7.7	31.9	27.6	37.5	11.5	25.0	19.8	56.4	42.9
86		0	0			11.0	9.5	5.0	1.5	12.7	10.1		
88		1.1	0.6			3.3	2.9			0.5	0.4	3.7	2.8
114	1.5	1.1	0.6			0	0					2.1	1.6
115	4.2	11.0	6.5			16.5	14.3			0.5	0.4	18.6	14.2
117	8.3	6.6	3.9			8.8	7.6	2.5	0.8	0.9	0.7	3.0	2.3
No. of strains or cases studied...	—†	155		78		105		129		278		431	
No. of strains or cases O grouped	1167	91		41		91		40		221		328	
No. of O group sera used.....	43	129		17		130		14		136		16	

* SO = strains O grouped; SS = total strains studied; CO = cases O grouped; CS = total cases studied.

† Total number of strains could not be determined.

lation from cases of calf colibacillosis in Western Canada. Gossling's study involved strains from enteric colibacillosis as well as colisepticemia, and the most frequent isolations in her study were of mucoid *E. coli* belonging to O groups 9 and 101 possessing A-type K antigens. As previously mentioned, this type of *E. coli* is associated with a typical clinical and bacteriological syndrome—the enteric-toxemic form of colibacillosis in which colisepticemia is an uncommon finding.

Table 3 shows the strains encountered by Wood (250) and Glantz et al. (64) in natural outbreaks under experimental conditions. Many of the O groups are the same as those encountered in other studies.

The inference drawn from the results of all these serological studies is that the occurrence of relatively few O groups in a high proportion of the cases studied is evidence of their primary pathogenicity for the calf. That this is not necessarily true has been shown recently in a study of *E. coli* strains from human sources (237) where it was found that, although O groups 6, 75, 4, and 1 accounted for approximately 60% of *E. coli* isolated from urinary infections, these same O groups accounted for approximately the same percentage of *E. coli* isolated from extraurinary sources (e.g., purulent foci-pulmonary infections), for 72% of *E. coli* contaminants of urine, and for approximately 50% of the fecal flora *E. coli*. Thus, this apparent selective pathogenicity was purely a reflection of the prevalence of these O groups in the environment. Therefore, 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. Unfortunately, there are few studies of this kind in the literature; those that are available are presented in tabular form (Table 4). The study of Sakazaki and Namioka (181) involved isolations from abattoir material; the age of the animals is unknown, and it is presumed that the animals were healthy although isolations are reported from the mesenteric lymph nodes and liver as well as the intestines. Only those from the intestine have been tabulated. Wramby's study (251) of 1,699 strains of *E. coli* isolated from the intestines of healthy calves cannot be included in this table, as Wramby gave no details of the distribution of O groups in this

material. The frequency of occurrence of the O groups considered significant in calf colisepticemia is generally quite low in this normal material (Table 4).

Fey's studies with the *E. coli* serotype O78:K80 emphasized this point. In his first report (51) of the isolation of *E. coli* from cases of colisepticemia, Fey found that O78:K80 accounted for 29 (27.6%) of the 105 *E. coli* strains examined (Table 1). In a subsequent report (52), the number of strains was enlarged to 145 of which 37.5% were O78:K80. Despite this frequent isolation of O78:K80 from calves dying of colisepticemia, in an examination of 8,630 strains of *E. coli* isolated from the intestines of healthy calves and cows and sick calves (other than colibacillosis), this serotype was only found eight times, and none of these isolations was from calves (52). However, it could be found in the environment where calves were dying from infection with this serotype (54).

There is little significant data on serotypes isolated from the enteric form of colibacillosis; therefore, such a comparison cannot be made for these strains. These strains may be part of the normal flora of the calf as is true for *E. coli* strains considered significant in gut edema and hemorrhagic gastroenteritis in the pig, and for *Clostridium perfringens* type D in sheep. There is, however, another limitation to the form of the investigations presented above. It is evident from the studies of Fey (51, 52) that several serotypes or biotypes were represented within the few predominant O groups isolated from cases of colisepticemia. From the data presented, the main variation occurred with the H antigens or the biochemical reactions; the K antigens of strains within an O group appeared identical. The importance of such complete serological identification has been emphasized by Ewing (46) because, of the very many serotypes of *E. coli* isolated from animals which are of the same O group as strains isolated from infant epidemic diarrhea, only a very few possess completely identical antigenic structure. In applying this to isolations of *E. coli* from calves, the isolation of the same O group from normal calves, as well as calves with colibacillosis, may mean very little since completely different serotypes might be involved.

TABLE 3. *Escherichia coli O groups isolated by Wood (250) and by Glantz et al. (64) from naturally occurring cases of colibacillosis in experimental calves*

O group	Wood		Glantz et al.	
	No. of isolations made	Per cent of total isolations	No. of isolations made	Per cent of total isolations
3 rel.			9	4.0
8			30	13.2
9	29(+4)*	24.6	10	4.4
15	1	0.7	11	4.9
17			15	6.6
20			15	6.6
21			11	4.9
26	6	4.4	2	0.8
35	18	13.4		
40			9	4.0
45			7	3.1
78	12(+5)*	12.7	9	4.0
114	13	9.7	1	0.4
119			20	8.8
Others	13	9.7	78	34.4
Untypable	33	24.6	—	—
Total	134	(100%)	227	(100%)

* Number in association with other bacteria.

Serotypes of E. coli in E. coli Infections of Other Animal Species

The association of specific serotypes and O groups of *E. coli* with colibacillosis has been described above. Specific serotypes and serogroups of *E. coli* are also associated with other diseases in

TABLE 4. *Percentage distribution of the O groups most frequently associated with colibacillosis in Escherichia coli isolated from healthy calves*

Reference no.	No. of strains tested	No. of strains O grouped	No. of O group sera used for classification	O group															
				7	8	9	15	20	26	35	45	55	78	86	88	114	115	117	Others
185	1,095	79	15	NT*	0.36	0.55	0.7	1.3	0.7	NT	NT	0.36	0.91	0.18	NT	0.36	0.27	0.55	0.91
240	36	6	17	2.8	2.8	2.8	NT	NT	0	NT	NT	0	0	NT	NT	NT	NT	0	11.1
181	330	115	128	0	6.6	1.2	1.2	0	0	0.6	0	0.3	0	0	0	0	0	3.0	22.4

* NT = not tested for. The numbers express the percentage of total strains of each O group

which *E. coli* is believed to play a significant role. In general, the serotypes isolated from these conditions are host- and disease-specific; that is, they are seldom isolated from other "*E. coli* diseases" in other animal species. There are a few notable exceptions to this statement. However, much of the apparent nonspecificity reported in earlier studies has subsequently been shown to be false and the result of incomplete serological typing.

From a limited number of reports of colisepticemia in lambs, the O groups and serotypes involved appear much the same as in calves and include O78:K80 (82, 99, 167, 172, 173, 240). O78:K80 is also of some importance in salpyngitis and colisepticemia in poultry along with other serotypes in O groups 78, 1, and 2 (38, 65, 72, 218), the latter two being of little significance in calves. Mucoid *E. coli* strains belonging to O group 9 and possessing A-type K antigens have been isolated from Hjärres granuloma (240, 251); similar strains are associated with the enteric toxemic form of colibacillosis in calves.

In pigs, in addition to colibacillosis of piglets, *E. coli* is associated with two other conditions, gut edema and coliform gastroenteritis, which generally occur in the 8- to 12-week age group and generally follow some change in management such as weaning. Of the three diseases, gut edema and, to a lesser extent, coliform gastroenteritis have received most attention, but it is not the purpose of this paper to review fully these two conditions. Briefly, however, there appears to be more than a casual relationship between these two conditions; although they are manifest in different clinical syndromes, they share the same age incidence, occur under similar management and environmental conditions, and often the same serotypes are isolated from both diseases.

Shortly after it became apparent that hemolytic *E. coli* might play a part in edema disease, Sojka, Erskine, and Lloyd in 1957 (216) made the first serological examination of such strains and found that the vast majority could be serotyped with only two OK sera. Numerous reports of serological typing of *E. coli* from both gut edema and gastroenteritis followed (71, 91, 102, 105, 125, 149, 168, 170, 174, 217, 219, 249). From these studies, it is evident that the majority of *E. coli* isolated from these two diseases fall into one of four main O groups, O138, O139, O141, and O8, in which there are several subgroups. The typing of these strains according to the Kauffmann-Knipschildt-Vahlne scheme has been mainly carried out by Ewing, Tatum, and Davis (45), F. Ørskov et al. (145), and I. Ørskov et al. (146), and the serotypes identified so far in these O groups are O138:K81(B):H14 or 4 or 8 or NM; O139:K82(B):H1; O141:K85ab – ac(B):H4, O141:K85ac(B):NM; O141:K85ab(B)K88(L):H4; O8:K87(B?)K88(L):H19; and a related strain, O?:K87(B?):H45. There are considerable antigenic relationships among these strains which are explained in the publication of I. Ørskov (146). The serotype in O group 8 is not commonly associated with edema disease, but, with this one possible exception, these serotypes are found to be common to both edema disease and coliform gastroenteritis; the predominance of any one serotype appears to vary with the country or area of isolation (249).

Although these serotypes are usually isolated in pure culture from the intestines of pigs dead of edema disease or coliform gastroenteritis, they may also be found in low numbers in the feces of normal pigs as part of the normal *E. coli* flora (20, 126, 149, 174, 216, 217). They are often found in high percentage in the *E. coli* of the fecal flora of litter mates of pigs dead of edema disease (116, 174), which is probably a reflection of the normal increase in their percentage numbers which occurs at weaning (19, 226). However, this increase in number is not limited to only these serotypes; a temporary increase in the total *E. coli* flora also occurs at this time (152, 246). Since these serotypes form part of the normal fecal flora, it is not surprising that these diseases cannot be reproduced by oral dosing with them (41, 116, 149). However, edema disease can be reproduced by the intravenous inoculation into normal pigs of a supernatant fluid of the lower intestinal contents of pigs with edema disease, or by extracts of these serotypes grown in vitro (42, 62, 68, 69, 70, 225, 234, 235, 236); the supernatant fluid of bowel contents of normal pigs or extracts made from *E. coli* other than these serotypes do not produce this syndrome. The toxic factor has been studied chemically and immunologically (31, 70, 190, 225), but its exact nature is as yet unknown. Buxton and Thomlinson (19, 231, 232) are of the opinion that these two conditions are not the result of a direct toxic action, but are mediated by an anaphylactic reaction to the toxic factor.

There is not as much in the literature regarding serotypes isolated from piglet colibacillosis; however, it is apparent that some of the serotypes associated with gut edema and coliform gastroenteritis are involved to some extent, because the serotypes O8:K87(B?)K88L:H19, O138:K-81(B), O139:K82(B), O141:K85ab(B)K88(L):H4, and also O133:K88(L):H19 have been isolated from septicemia, or enteritis, or both in piglets (126, 174, 182, 219). However, this emphasis on the importance of these serotypes may be somewhat biased, because, apart from the study of Saunders et al. (182), these studies dealt purely

with hemolytic *E. coli* and, hence, might tend to exaggerate the importance of these serotypes. *E. coli* strains belonging to O group 8 have also been isolated by Ulbrich from piglets (240) and by Leece and Reep (112) from colostrum-deprived piglets. Ulbrich also found O group 9 of some importance as did Pesti (150); this O group along with O groups 21 and 28 was most predominant in enteritis and septicemia, whereas they were seldom found in the fecal flora of normal piglets.

In man, an analogous condition to enteric colibacillosis of calves occurs in infants under 1 year of age from which various subgroups of the serotypes O26:K60(B6), O55:K59(B5), O111:K58(B4), O127:K63(B8), and O128:K67(B12) are commonly isolated (39, 43, 228). Other serotypes not listed here are less commonly incriminated [for a complete list of serotypes involved, see Edwards and Ewing (39)]. Many of these serotypes, or serotypes of similar antigenic structure (46), have been isolated on occasion from cases of calf colibacillosis (22, 63, 143, 165, 169, 239, 240, 250), but, except for O26:K60(B6), O86:K61(B7), and possibly O55:K59(B5), they do not appear to be significant pathogens for calves. The disease in man is restricted to enteritis, bacteremia, or septicemia, being extremely rare in these cases.

Epidemiology of Colibacillosis in Calves

Epidemiological studies have shown that many of the human serotypes mentioned above are capable of spreading and actually causing epidemics of diarrhea in nurseries and hospitals (80, 223, 224). If evidence of this kind were available from field outbreaks of colibacillosis, it would provide convincing evidence of the pathogenicity of certain strains of *E. coli* for calves. Although there are numerous reports of the epidemic and contagious quality of field outbreaks of colibacillosis, there are few in which these observations are confirmed by serological typing.

The first studies of an epidemiological nature (88, 89, 90, 178, 250) were conducted on the experimental calves used in the previously mentioned studies of Aschaffenburg and co-workers. Calves deprived of colostrum were obtained from approximately 30 farms in the surrounding area. They were brought into the calf house and placed in 1 of 13 individual pens where they stayed until they died or were taken off the experiment at 21 days of age. The pens were disinfected between succeeding calves, and the experiment was discontinued over the summer of each year. On arrival, the calves were either fed colostrum or left deprived of it, the basic feed being a synthetic milk (1). It was found that "white scour infections" developed spontaneously, but their incidence and the incidence of deaths increased as the period of occupation of the calf house increased. It was believed that this was due to a natural selection of virulent strains conditioned by the calves' lack of antibody to these strains, resulting in a buildup of infection. This could be shown by the pattern of the serotypes isolated from the dead calves and feces of the live calves; at the beginning of the experiment a heterologous group of *E. coli* were present, but, as the occupation time increased, certain serotypes became dominant. The serotypes which became predominant were not the same every year, and, even within the same year, different serotypes would predominate during different periods of time. This buildup of virulent strains could be halted by leaving the calf house empty for a period of time after which a heterologous group of *E. coli* were once again present.

Glantz and co-workers (64) have since reported on another "natural" outbreak of scours in experimental calves. This was also conditioned by colostrum-deprived calves. The majority of the colostrum-deprived calves in this study scoured and died, and the same strains were isolated from the organs of these dead calves as had been isolated at some time from their feces while they were scouring, although other strains were also isolated from the feces. The majority of colostrum-fed calves scoured but survived. Although there were several serotypes isolated from both sets of calves, two serotypes predominated—O8:K?:NM and O119:K69(B):H9. It was also found possible to reproduce this disease experimentally in both colostrum-deprived and colostrum-fed calves by oral feeding of these serotypes, which generally resulted in scouring and death, although the deaths occurred mainly in the colostrum-deprived group.

Both of these experimental studies suggest that the occurrence of field outbreaks of colibacillosis due to a single serotype is quite feasible, and that the isolation of the same serotype from a number of different calves would be a significant indication of its importance as an etiological agent in the outbreak. This is true, providing it can be first established that the repeated isolation of a given serotype from an outbreak of colibacillosis is truly a reflection of its selective pathogenicity and not merely a reflection of the natural or normal dominance of that serotype in the fecal flora of the calves at that particular time. There are two sets of evidence for the belief that these isolations would be indicative of the selective pathogenicity of the serotype. The first is the frequency with which isolations are made of certain serogroups and serotypes of *E. coli* from cases

of colibacillosis in comparison to the frequency of their isolation from normal calves, as has been previously shown. The second results from the study of Smith and Crabb (197) on the normal fecal flora of calves and the relationship between the fecal flora of healthy calves in close contact in the same environment. In this study, it was found that the dominant phage type of *E. coli* in the intestinal flora of any one calf was not usually the dominant type in the flora of other calves in the same environment.

Thus, the significance of a single serotype field outbreak of colibacillosis would seem quite credible; however, there is little in the literature either to confirm or refute this.

Lovell (118) reported the isolation of *E. coli* possessing the same K antigen from several cases of colibacillosis in a single herd; however, other *E. coli* types were also responsible for some of the calf deaths in this herd. Rees (166) was able to isolate the same serotype of *E. coli* from two calves in each of five herds. The time interval between the deaths of the two calves in a herd varied up to 6 weeks. In contrast to this, Kaeckenbeeck and Thomas (97), who studied isolations made from two to four calves received from each of five herds during periods varying up to 14 months, found little evidence that a single serotype of *E. coli* was associated with the deaths in a herd. It was not stated whether each isolation represented a separate outbreak or whether the problem had continued over the interim period of several months. Ulbrich (239) reported the isolation of O55:B5 from several calves dead of colisepticemia which came from a single herd. The results of a study involving two or more calves from each of 35 farms have been reported by Dam (28) and give some support to the occurrence of single strain outbreaks of colisepticemia. In herds where calves were submitted for examination within 50 days of each other, the strains isolated generally belonged to the same O group, whereas isolations made at longer periods more often fell into different O groups. The majority of the single O group outbreaks were associatd with O group 78.

The isolations of *E. coli* in the studies mentioned above were primarily from calves dead with colisepticemia, and few data are presented regarding the morbidity or mortality in the herds from which they were received. Recently, I attempted to classify serologically all strains of *E. coli* isolated from cases of colibacillosis, both enteric and septicemic, that occurred during approximately a 12-month period in several herds which had a previous history of colibacillosis (60*b*). Unfortunately, the incidence of this disease in most of the herds during the period was low; however, it did appear in this study that an individual strain of *E. coli* was responsible for only a limited outbreak of colibacillosis involving only a few calves over a few weeks at any one time; subsequent other cases or outbreaks in the same herd were associated with a different strain. The factor limiting these outbreaks could not be determined; however, it was not associated with the presence or emergence of any demonstrable antibody to the strain involved in the sera of subsequent calves which remained healthy.

In contrast to the evidence in the studies above, Smith and Crabb (197) could find no evidence of the association of an individual strain of *E. coli* with outbreaks of "calf scours." Using a set of 16 bacteriophage to identify *E. coli* strains isolated, they studied intensively an outbreak of calf scours. They isolated 70 phage types from the feces of healthy calves and 23 phage types from the feces of a smaller number of scouring calves of which only one type was not found in the types from healthy calves. They could find no significant difference in the number of types found in the feces of a scouring calf compared with those in a normal calf, and showed that the type could even change during the scour period. The type most predominant during the scour period was often present before scouring and was invariably present for some time after. On examining specimens from other herds, they could find no evidence that any one type was associated with scours in any herd at a particular time. On the basis of these results, they stated that less emphasis should be placed on scours being caused by specific strains of *E. coli*. They believed it more likely due to an upset of host-parasite relationship as was postulated by Lovell (119). Unfortunately, there has been no correlation of the phage types of Smith with the serotypes of Kauffmann. Although there are often several bacteriophage types among strains of the same serological type, it would be expected that a true single strain outbreak would be associated with a single phage type. However, Smith has since reported the isolation of a single phage type from an outbreak (198).

Conclusions on Serological Investigations

Specific serotypes of *E. coli* are associated with certain diseases in animals and man. The evidence of this association in colibacillosis of calves has been presented. The majority of serological studies on *E. coli* isolated from calves has been on strains isolated from the septicemic form of colibacillosis. It is now evident that certain serotypes and O groups of *E. coli* are commonly associated with this syndrome. There have been few sero-

logical 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 etiological significance of an isolation of *E. coli* from the intestine of a scouring calf. The clinical and bacteriological syndrome associated with the enteric-toxemic form of colibacillosis is sufficiently distinctive to allow its differentiation from other causes of calf death. Thus, it is probable that future studies will establish more clearly the serotypes of *E. coli* associated with this syndrome. 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 sufficient 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.

It is probable that the serotype of *E. coli* associated with the enteric form of colibacillosis will be more easily determined by epidemiological studies of outbreaks of calf diarrhea. There is some evidence to suggest that the isolation of a single serotype of *E. coli* from multiple cases of scouring within the same herd would be an indication that it is the causative agent of the diarrhea. Further knowledge is required of the relationship between *E. coli* in the intestines of healthy calves kept in the same environment before such studies can be fully evaluated.

The surest method of determining the pathogenicity of a serotype of *E. coli* is to test, by experimental infection, its ability to reproduce diarrhea in calves.

FACTORS WHICH INFLUENCE PATHOGENICITY OF STRAINS OF *E. COLI*

It is evident that the disease colibacillosis, especially colisepticemia, cannot be accounted for entirely by the suggestion that it is caused by a few pathogenic strains (Table 2). This does not negate the possibility of pathogenic strains as yet unidentified in the untypable material nor the fact that the pathogenicity of a strain is probably not absolute and that "non-pathogenic strains" may achieve a degree of virulence because of a lack of "immunity" to them. Unfortunately, the factors associated with virulence of a strain are unknown, and there is no in vitro test that will detect these strains. For example, the susceptibility to serum bactericidal activity (128) or to phagocytin (138) cannot be directly correlated with pathogenicity. Similarly, routine in vivo tests, such as pathogenicity for laboratory animals (16, 35, 113, 137, 189, 210, 211, 240) or chick embryo (87), appear to be of little value in this respect, because there is a wide variation and overlapping in lethal dose between serotypes considered to be pathogens and normal strains from healthy sources. These tests do show, however, that the presence of K antigen is associated with a markedly increased virulence over the K negative form. Individually, the O and K antigens of a pathogenic serotype have no relationship with virulence—that is, if they are in combination with different antigens, the different serotype is not necessarily virulent. A possible exception to this statement is the K antigens of the serotypes isolated from gut edema and coliform gastroenteritis of pigs. There are even variations within a serotype; some strains may be highly pathogenic and others are virtually nonpathogenic (46, 228). Similarly, serotypes which are associated with certain diseases in one type of animal are, with some exceptions, not of importance in other animal species. The toxicity of the group is, in part, associated with its endotoxin; however, this is qualitatively common to all *E. coli* (81, 230).

Recently, an in vivo test was described which held some promise for the identification of enteropathogenic strains (32, 227, 229). This involved the injection of living cultures of *E. coli* into an isolated loop of rabbit small intestine. The rabbits were killed 24 hr later, and the enteropathogenicity of the strains was assessed according to the severity of the reaction of the gut. There was considered to be a good correlation between enteropathogenicity and severity of reaction with respect to strains isolated from cases of infant epidemic diarrhea, because strains from urinary infections and strains which possessed the same antigenic structure as the enteropathogenic strains, but which were isolated from healthy babies or well-water, gave negative results to the test. The correlation with respect to strains from colibacillosis, however, is not so definite, although the serotypes O78:K80:NM, O26:K60:H11 and O137:K79:H41 were positive to this test, O9:K?:H19 and four other strains from the isolations of Rees (166) were negative. In this study, strains from cases of gut edema of pigs were also negative; however, more recent studies (136, 138) have shown positive results with gut edema strains. Furthermore it has been shown that strains isolated from the small intestine of pigs dead of transmissible gastroenteritis (a viral disease) were positive to this test (136, 138). In view of these findings, the competence of this test ap-

pears dubious as a means of assessing the pathogenic importance of strains of *E. coli* isolated from colibacillosis in calves.

EXPERIMENTAL REPRODUCTION OF COLIBACILLOSIS IN CALVES

The obvious test for pathogenicity of a strain of *E. coli* is its ability to reproduce the same disease in the animal species from which it was isolated. Limited studies in man have shown that strains isolated from infant epidemic diarrhea have the ability to cause diarrhea, when fed in sufficient numbers, in adults (48, 49, 96) as well as infants (139). In adults, as in infants, infection is usually followed by a rise in titer against the somatic antigen of the strain (40, 49, 140, 224). The feeding of nonpathogenic strains has no effect and causes no rise in antibody titer.

The first report of the reproduction of colibacillosis in young calves was by Jensen (93), who produced a mild diarrhea by feeding them milk containing cultures of *E. coli* isolated from dead calves. The severity of the diarrhea could be markedly increased by feeding intestinal irritants. In contrast, Poels (160), Smith and Orcutt (208), McEwan (133), and Van Pelt et al. (241) failed in their attempts to reproduce this condition. Williams et al. (247) also failed to reproduce calf scours and have criticized Jensen's work, because no data concerning the feeding of the calves was given—their "colostrum status" is unknown—and the controls also scoured. The serological identities of the strains used in the above experiments are not known.

In 1959, Glantz et al. (64) reported an intensive study of the experimental reproduction of the disease. The strains of *E. coli* used were isolated from a natural outbreak of colibacillosis in experimental calves. During this outbreak and subsequent experiments, it was possible to demonstrate the transmission of many of these strains from scouring calves to in-contact calves which resulted in scouring. This transmissibility was most evident for the two types, O8:K:NM(Ps56) and O119:K69(B):H9. Further experiments with the strain O8:K:NM(Ps56) showed that it was possible to infect experimentally both colostrum-deprived and colostrum-fed calves; contact transmission to colostrum-fed calves was also demonstrated. Six other strains were fed to colostrum-deprived calves, but only two of these, Ps219G (related to O group 3) and O119:K69(B):H9, caused scours and death. None of these strains had the ability to reproduce the condition in colostrum-fed calves. Colostrum-deprived calves which were left as unchallenged controls remained normal. From an examination of the dam's sera and colostrum and calf sera for agglutinins against the O antigen of the respective strains, it was stated that there was a direct relationship between the presence of agglutinins and the resistance of the calf. No such relationship existed between the presence of bactericidal activity in the calf sera to a strain and the susceptibility of the calves to infection with that strain. There were no determinations made of antibody against the K antigens. In an earlier paper, these authors (37) reported the reproduction of colibacillosis with scouring and death in both colostrum-deprived and colostrum-fed calves with the strain Ps125M [O26:K60(B)].

Similar results have been published by Schoenaers and Kaekenbeeck (183), who were able to reproduce colisepticemia in calves deprived of colostrum by feeding them the serotype O137:K79:K41 (RVC1787) plus crude endotoxin. The colostrum from cows vaccinated with this serotype protected the majority of the calves to which it was fed against infection with this serotype (184). It was stated that the colostrum-fed calves which died of colisepticemia had received the least amount of antibody; however, this is not evident from the table in the paper, and the antibody titers shown do not include those against the K antigen. Unfortunately, the resistance of calves fed a "nonimmune" colostrum to infection with this serotype was not determined.

Fey (56) was unsuccessful in his efforts to reproduce the disease by feeding colostrum-fed calves with O78:K80, except for the appearance of a temporary dysentery, although this serotype would easily kill colostrum-deprived calves. Greater success was achieved by infecting the calf as soon as possible after birth and withholding the colostrum until several hours later. Dam (30) was also able to produce colisepticemia in colostrum-deprived calves with this serotype; however, only two of seven calves fed colostrum and exposed to this serotype died with colisepticemia. Dam also had some success in infecting colostrum-deprived calves with a strain belonging to O group 115, which is also frequently isolated from field cases of colisepticemia, but he was not able to kill colostrum-fed calves with this strain.

An attempt was made (60c) to reproduce colibacillosis both in calves deprived of colostrum and in calves fed colostrum, the latter being either from "normal" cows or from cows vaccinated with an oil-adjuvant vaccine prepared from the challenge strains of *E. coli*. The first attempts were with the strains O9:K(A)RVC118, O101:K(A)RVC118 and O9:K(A)Ps274, which are strains isolated from the enteric-toxemic form of colibacillosis, and were unsuccessful. Further at-

tempts were made with the serotype O137:K79:H49 (RVC1787). All colostrum-deprived calves challenged orally with this strain died with colisepticemia within 24 to 48 hr. RVC1787 was isolated in pure culture from the internal organs of these calves. Contact transmission from a colostrum-fed calf fed this strain and scouring with it to colostrum-deprived calves which died of colisepticemia was also demonstrated. Colostrum-fed calves were also infected and scoured with this strain. However, no cases of colisepticemia occurred in these calves. Some of these colostrum-fed calves were exposed to infection by placing them in an environment contaminated with this strain. The most important factor in the susceptibility, or resistance, of these calves to infection was the ability, or inability, of the strain to establish itself in the intestinal flora. Once established, its ability to cause scouring appeared to rest on the presence or absence of specific agglutinins to this strain, although this relationship was not definitely established.

Smith (202) also reproduced colisepticemia in calves deprived of colostrum. However, in contrast to Glantz et al., he found the bactericidal activity of the serum of some importance, for he was able to reproduce colisepticemia only with phage types of *E. coli* which grew in precolostral sera, whereas those which failed to grow in this serum were without effect when challenged to colostrum-deprived calves. He was unable to produce colisepticemia or scouring in colostrum-fed calves challenged with several phage types of *E. coli* isolated from field and laboratory cases of scours.

PATHOGENESIS OF COLIBACILLOSIS IN CALVES

From these publications, it is obvious that there is no difficulty in experimentally reproducing colisepticemia in calves deprived of colostrum, providing they are challenged with the invasive strains of *E. coli*. In view of this, perhaps one of the more significant publications on colibacillosis published recently is that of Fey and Margadant (55), who found from an examination of 22 calves dying of colisepticemia that all 22 were either agammaglobulinemic or markedly hypogammaglobulinemic. This prompted a study of healthy calves of the same age group (1 week); approximately 11% of these calves were found deficient in γ-globulins. Fey (56) believed this deficiency in γ-globulins to be a major factor in the incidence and pathogenesis of colisepticemia in calves, because such calves would be susceptible to infection with the more invasive strains of *E. coli*. This hypothesis is extremely attractive in view of the age incidence of colisepticemia and of the relative ease with which colisepticemia can be reproduced in calves deprived of colostrum in comparison with the general inability to reproduce it in calves fed colostrum. Smith (202) has also observed that some calves thought to have been fed colostrum possessed very low levels of γ-globulin, and variations have been found in the γ-globulin levels of calves fed colostrum (60). Some of those with low levels died of colibacillosis.

The reason for such variation in the γ-globulin content of various sera cannot be fully explained. As mentioned previously, the mechanism allowing, or inhibiting, the absorption of proteins through the intestinal wall of the young calf is unknown, and, although it is known that such absorption ceases 24 to 36 hr after birth, there is little knowledge of the relative absorptive capacity of the intestine at any given time during this 24 to 36 hr. Fey (56) believed the deficiency of γ-globulins in a calf was due to the early failure of the intestine to absorb globulins, although mention was made of the practice in Switzerland of withholding the colostrum from the calf until after the dam had passed the placenta. Whatever the cause of the deficiency, it is probably quite significant in the pathogenesis of many cases of colisepticemia. However, Fey has also shown that calves, from which colostrum was withheld for several hours following oral challenge with *E. coli* O78:K80, developed and died of colisepticemia despite having subsequently acquired normal serum levels of γ-globulin. It should be stressed that the majority of the serological studies on *E. coli* isolated from cases of colibacillosis presented earlier in this review (Table 2) were on isolations made from cases of colisepticemia. The majority of these isolations fell into comparatively few O groups or serotypes; these serotypes predominate probably because of their capacity to invade, and because they are resistant to the bactericidal effect of precolostral sera. There is very little bactericidal activity in precolostral calf sera against smooth gram-negative organisms (238). Outbreaks of colisepticemia caused by one of these strains are probably the result of early infection of the calf, while it is still agammaglobulinemic, from a contaminated environment. From bacteriological examination of cases of colisepticemia, it is evident that invasion need not necessarily occur from the intestinal flora, but may occur via the navel or even from other areas such as the nasal or pharyngeal mucosae (28, 53, 56). The factors associated with invasiveness in *E. coli* and the mechanisms by which they invade are as yet unknown. However, this does account for many of the cases of colisepticemia in which the septicemic strain cannot be found as part of the intestinal flora. The con-

tamination of the environment in these cases occurs via the urine (56).

Experimental calves that have been fed colostrum and have absorbed γ-globulins are resistant to "septicemic invasion" by *E. coli*, but, as mentioned previously, if infection and bacteremia precede the feeding of colostrum, the calf may die of colisepticemia despite its absorbing γ-globulins to normal or near-normal levels. The factors associated with resistance to the septicemic form of colibacillosis in the colostrum-fed calf are unknown. Resistance does not appear to be associated with agglutinating antibody, since many healthy calves in the field are devoid of demonstrable agglutinins against *E. coli* strains associated with colibacillosis. Those calves that do possess agglutinins against these *E. coli* strains generally only possess agglutinins against the somatic antigens, for although most calves receive antibody against these antigens in the colostrum, few receive agglutinins against the K antigens of these bacteria (60). Furthermore, *E. coli* strains which produce colisepticemia when fed to colostrum-deprived calves do not produce colisepticemia when fed to colostrum-fed calves, regardless of the presence or absence of O and K agglutinins in the calves' sera (60, 202). The serum bactericidal activity also appears to play no part in this resistance (64, 202).

Calves fed colostrum are susceptible to the enteric forms of colibacillosis; however, almost nothing is known of the pathogenesis of these infections. In the enteric-toxemic form of colibacillosis, which is associated with one or other of several strains of mucoid *E. coli* possessing A-type K antigens and which generally belong to O groups 8, 9, and 101, the clinical course of the disease is very short; death occurs within a few hours of initial symptoms and appears to be associated with a massive proliferation of these strains in the small intestine. It is not unlikely that this syndrome is a result of absorbed endotoxin, as it resembles in several features that seen when purified endotoxin is given intravenously to calves. This syndrome has not been reproduced by oral feeding of these strains to calves. They may form part of the intestinal flora without ill effect. The factor which allows or initiates their sudden multiplication in cases of colibacillosis is unknown.

With respect to the enteric form of colibacillosis, there is not much in the literature which confirms the ability of *E. coli* to produce diarrhea in calves. Undoubtedly, there are many causes, both known and unknown, of scouring in calves, of which *E. coli* is only one. The problems associated with assessing the significance of an isolation of *E. coli* from a scouring calf have been dealt with earlier. Also, most of the serological studies on *E. coli* isolated from calves have been concerned with colisepticemia. However, there is evidence that certain strains of *E. coli* are capable of causing scouring in the calf (60, 64, 198, 67*a*), which is not without precedent, as it is well known that certain strains of *E. coli* can produce this syndrome of enteric colibacillosis in infants. Probably one of the more important factors in determining the susceptibility or resistance of the calf to infection with these strains is the ability, or inability, of the strain to establish itself in the intestinal flora (60*c*). Once established as part of the intestinal flora, these strains, through some mechanism, are able to initiate an enteritis. There is also some evidence that specific antibody will protect the calf against this action of these strains.

Although these three types of colibacillosis have been dealt with as separate entities, they are not mutually exclusive. With the enteric-toxemic form, infection and depression often occur shortly after birth so that the calf remains deficient or poor in γ-globulins. In these circumstances, invasion by a different strain of *E. coli* with resultant colisepticemia often occurs which may confuse or obscure the actual cause of the calf's death. Similarly, early infection by strains capable of producing the enteric form of colibacillosis, or other agents causing scouring, may result in a deficient absorption of γ-globulins predisposing the calf to colisepticemia at the same or a later time.

SUMMARY

An attempt has been made to examine the role played by *E. coli* in neonatal disease of calves. This disease, colibacillosis, has been divided into three syndromes which, on the basis of probable pathogenesis, can be considered as three distinct entities. There is still very little knowledge of the mechanisms by which *E. coli* produces these syndromes in calves or of the mechanisms by which the calf is protected from them.

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LITERATURE CITED

1. ASCHAFFENBURG, R., S. BARTLETT, S. K. KON, P. TERRY, S. Y. THOMPSON, D. M. WALKER, C. BRIGGS, E. COTCHIN, AND R. LOVELL. 1949. The nutritive value of colos-

trum for the calf. I. The effect of different fractions of colostrum. Brit. J. Nutr. **3**:187–196.

2. ASCHAFFENBURG, R., S. BARTLETT, S. K. KON, D. M. WALKER, C. BRIGGS, E. COTCHIN, AND R. LOVELL. 1949. The nutritive value of colostrum for the calf. II. The effect of small quantities of the non-fatty fraction. Brit. J. Nutr. **3**:196–200.
3. ASCHAFFENBURG, R. 1949. The nutritive value of colostrum for the calf. III. Changes in the serum protein of the newborn calf following the ingestion of small quantities of the non-fatty fraction. Brit. J. Nutr. **3**:200–204.
4. ASCHAFFENBURG, R., S. BARTLETT, S. K. KON, J. H. B. ROY, D. M. WALKER, C. BRIGGS, AND R. LOVELL. 1951. The nutritive value of colostrum for the calf. IV. The effect of small quantities of colostral whey, dialysed whey and 'immune lactoglobulins.' Brit. J. Nutr. **5**:171–176.
5. ASCHAFFENBURG, R., S. BARTLETT, S. K. KON, J. H. B. ROY, D. M. WALKER, C. BRIGGS, AND R. LOVELL. 1951. The nutritive value of colostrum for the calf. V. The effect of prepartum milking. Brit. J. Nutr. **5**:343–349.
6. ASKONAS, B. A., P. N. CAMPBELL, J. J. HUMPHREY, AND R. S. WORK. 1954. The source of antibody globulin in rabbit milk and goat colostrum. Biochem. J. **56**:597–601.
7. BALFOUR, E. W., AND R. S. COMLINE. 1959. The specificity of the intestinal absorption of large molecules by the newborn calf. J. Physiol. **148**(2):77P.
8. BALFOUR, W. E., AND R. S. COMLINE. 1962. Acceleration of the absorption of unchanged globulin in the newborn calf by factors in colostrum. J. Physiol. **160**:243–257.
9. BANGHAM, D. R., P. L. INGRAM, J. H. B. ROY, K. W. G. SHILLAM, AND R. J. TERRY. 1958. The absorption of ^{131}I-labelled serum and colostral proteins from the gut of the young calf. Proc. Roy. Soc. (London) Ser. B **149**:184–191.
10. BARRY, G. T., V. D. ABBOT, D. L. EVERHART, R. J. LEFFLER, AND E. MYNANT. 1962. Relationship of serotype to colicine type in *Escherichia coli*. Bacteriol. Proc., p. 51.
11. BLAKEMORE, F. 1951. Maternal transfer of antibody in the bovine. Vet. Rec. **63**:397.
12. BLAKEMORE, F., AND R. J. GARNER. 1956. The maternal transference of antibody in the bovine. J. Comp. Pathol. Therap. **66**:287–289.
13. BLAXTER, K. L., AND W. A. WOOD. 1953. Some observations on the biochemical and physiological events associated with diarrhoea of calves. Vet. Rec. **65**:889–892.
14. BOKHARI, S. M. H., AND F. ØRSKOV. 1952. O grouping of *E. coli* strains isolated from cases of white scours. Acta Pathol. Microbiol. Scand. **30**:87–89.
15. BRANCHE, W. C., V. M. YOUNGE, H. C. GILLEM, AND E. D. MASSEY. 1960. Observations on *Escherichia coli* as a component of the normal intestinal flora: effect of colicine production. Bacteriol. Proc., p. 134.
16. BRIGGS, C. 1951. Nutritive value of colostrum for the calf. VI. The 'K' antigens of *Bacterium coli*. Brit. J. Nutr. **5**:349–355.
17. BRIGGS, C., R. LOVELL, R. ASCHAFFENBURG, S. BARTLETT, S. K. KON, J. H. B. ROY, S. Y. THOMPSON, AND D. M. WALKER. 1951. Nutritive value of colostrum for the calf. VII. Observations on the nature of the protective properties of colostrum. Brit. J. Nutr. **5**:356–362.
18. BRINTON, C. C. 1959. Non flagellar appendages of bacteria. Nature **183**:782–786.
19. BUXTON, A., AND J. R. THOMLINSON. 1961. The detection of tissue sensitizing antibodies to *Escherichia coli* in oedema disease, haemorrhagic gastroenteritis and in normal pigs. Res. Vet. Sci. **2**:73–88.
20. CAMPBELL, S. G. 1959. Studies on strains of haemolytic *Escherichia coli* isolated from normal swine after weaning. Vet. Rec. **71**:909–911.
21. CARPENTER, C. M., AND B. A. WOODS. 1924. The distribution of the colon-aerogenes group of bacteria in the alimentary tract of calves. Cornell Vet. **14**:218–225.
22. CHARTER, R. E. 1956. *E. coli* type O114 isolated from infantile diarrhoea and calf scours. J. Pathol. Bacteriol. **72**:33–38.
23. CHRISTIANSEN, M. 1917. Bakterien der Typhus-coli-Gruppe in Darm von gerunden spankälbern und bei deren Darminfektionen. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **79**:196–248.
24. COLINET, G., A. KAECKENBEECK, AND F. SCHOENAERS. 1961. Elimination et production d'anticorps colibacillaires chez le veau. Ann. Med. Vet., **105**:254–257.
25. COMLINE, R. S., H. E. ROBERTS, AND D. A. TITCHEN. 1951. Route of absorption of colostrum globulin in the newborn animal. Nature **167**:561–562.
26. COMLINE, R. S., H. E. ROBERTS, AND D. A. TITCHEN. 1952. Histological changes in the epithelium of the small intestine during protein absorption in the newborn. Nature **168**:84–85.
27. CROWTHER, C., AND H. RAISTRICK. 1916. A comparative study of the proteins of the colostrum and milk of the cow and their relation to serum proteins. Biochem. J. **10**:434–451.
28. DAM, A. 1960. Serological O grouping of strains of *E. coli* isolated from cases of colisepticaemia in calves. Nord. Veterinarmed. **12**:321–342.
29. DAM, A. 1962. Betydningen af de i forbindelse med colibacillose hos kalve i Danmark hyppigst isolerede typer af *Escherichia coli*. Communication no. 377. State Veterinary Serum Laboratory, Copenhagen, Denmark.

30. DAM, A. 1962. Infektionsforsøg på kalve med forskellige typer af *Escherichia coli*. Communication no. 378. State Veterinary Serum Laboratory, Copenhagen, Denmark.
31. DAVIS, J. W., R. C. ALLEN, AND R. M. SMIBERT. 1961. Studies on hemolytic *Escherichia coli*. Amer. J. Vet. Res. **22**:736–740.
32. DE, S. N., K. BHATTACHARYA, AND J. K. SARKAR. 1956. A study of the pathogenicity of strains of *Bacterium coli* from acute and chronic enteritis. J. Pathol. Bacteriol. **71**:201–209.
33. DEUTSH, H. F. 1947. A study of whey protein from the milk of various animals. J. Biol. Chem. **169**:437–448.
34. DEUTSH, H. F., AND V. R. SMITH. 1957. Intestinal permeability to protein in the newborn herbivore. Amer. J. Physiol. **191**: 271–276.
35. DE WITT, C. W., AND P. M. ALLEN. 1961. The relationship of virulence and K antigen in *Escherichia coli*. Bacteriol. Proc., p. 119.
36. DICKINSON, A. B., AND G. MOCQUOT. 1961. Studies on the bacterial flora of the alimentary tract of pigs. I. Enterobacteriaceae and other gram-negative bacteria. J. Appl. Bacteriol. **24**:252–284.
37. DUNNE, H. W., P. J. GLANTZ, J. F. HOKANSON, AND A. L. BORTREE. 1956. *Escherichia coli* as a cause of diarrhea in calves. Ann. N.Y. Acad. Sci. **66**:129–135.
38. EDWARDS, P. R., AND W. H. EWING. 1954. Studies on a coliform type isolated from the organs of fowls. Cornell Vet. **44**:50–56.
39. EDWARDS, P. R., AND W. H. EWING. 1962. Identification of Enterobacteriaceae. Burgess Publishing Co., Minneapolis.
40. ERHLICH, P. 1892. Über Immunität durch Vererbung und Säugung. Z. Hyg. Infektionskrankh. **12**:183.
41. ERSKINE, R. G., M. K. LLOYD, AND W. J. SOJKA. 1956. Unpublished results, cited in Sojka et al. (217).
42. ERSKINE, R. G., W. J. SOJKA, AND M. K. LLOYD. 1957. The experimental reproduction of a syndrome indistinguishable from oedema disease. Vet. Rec. **69**:301–303.
43. EWING, W. H. 1956. Enteropathogenic *Escherichia coli* serotypes. Ann. N.Y. Acad. Sci. **66**:61–70.
44. EWING, W. H., H. W. TATUM, B. R. DAVIS, AND R. W. REAVIS. 1956. Studies on the serology of the *Escherichia coli* group. Communicable Disease Center, Atlanta, Ga.
45. EWING, W. H., H. W. TATUM, AND B. R. DAVIS. 1958. *Escherichia coli* types associated with edema disease of swine. Cornell Vet. **48**:201–206.
46. EWING, W. H. 1962. Sources of *Escherichia coli* cultures that belonged to O antigen groups associated with infantile diarrhoeal disease. J. Infect. Dis. **110**:114–120.
47. FAMULENER, L. W. 1912. On the transmission of immunity from mother to offspring. A study on serum haemolysins in goats. J. Infect. Dis. **10**:332–368.
48. FERGUSON, W. W., AND R. C. JUNE. 1952. Experiments on feeding adult volunteers with *E. coli* 111, B4, a coliform organism associated with infant diarrhea. Amer. J. Hyg. **55**:155–169.
49. FERGUSON, W. W. 1956. Experimental diarrheal disease of human volunteers due to *Escherichia coli*. Ann. N.Y. Acad. Sci. **66**:71–77.
50. FEY, H. 1955. Coliprobleme bei Mensch und Tier. Schweiz. Arch. Tierheilk. **97**:118–132.
51. FEY, H. 1957. Bakteriologie und Serologie der Colisepsis des Kalbes. I. Serologische und biochemische Untersuchungen. Zentr. Veterinaermed. **4**:309–318.
52. FEY, H. 1957. Bakteriologie und Serologie der Colisepsis des Kalbes. II. Die Bedeutung des Colityps 78:80B für die Kalberruhr. Zentr. Veterinaermed. **4**:447–458.
53. FEY, H., AND A. MARGADANT. 1961. Zur Pathogenese der Kälber-Colisepsis. I. Verteilung des Sepsistyps in den Organen. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **182**:71–79.
54. FEY, H., AND A. MARGADANT. 1961. Zur Pathogenese der Kälber-Colisepsis. II. Umgebungsuntersuchungen in Sepsisbeständen. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **182**:465–472.
55. FEY, H., AND A. MARGADANT. 1961. Hypogammablobulinämie bei der Colisepsis des Kalbes. Pathol. Microbiol. **24**:970–976.
56. FEY, H. 1962. Neuere Untersuchungen über die Colisepsis des Kalbes. Schweiz. Arch. Tierheilk. **104**:1–12.
57. FREDERICQ, P., M. BETZ BAREAU, AND P. NICHOLLE. 1956. Typage des souches d'*Escherichia coli* de gastro-enterite infantile par reserche de livres proprietes colicinogenes. Compt. Rend. Soc. Biol. **150**: 2039–2042.
58. GAGE, P., C. B. GUNTHER, AND E. H. SPAULDING. 1961. Persistence of *E. coli* serotypes in the stools of infants. Bacteriol. Proc., p. 117.
59. GARNER, R. J., AND W. CRAWLEY. 1958. Further observations on the maternal transference of antibody in the bovine. J. Comp. Pathol. Therap. **68**:112–114.
60. GAY, C. C. 1962. Bacteriological and serological studies of colibacillosis in calves with special reference to vaccination as a means of prevention. M.V.Sc. Thesis, University of Toronto, Toronto, Canada.
60*a*. GAY, C. C., K. A. MCKAY, AND D. A. BARNUM. 1964. Studies on colibacillosis of calves. I. The antibody acquired by calves as the result of vaccination of the dam. Can. Vet. J. **5**:248–261.
60*b*. GAY, C. C., K. A. MCKAY, AND D. A. BARNUM. 1964. Studies on colibacillosis of calves. II. A clinical evaluation of the efficiency of vaccination of the dam as a

means of preventing colibacillosis of the calf. Can. Vet. J. **5**:297–308.

60*c*. GAY, C. C., K. A. MCKAY, AND D. A. BARNUM. 1964. Studies on colibacillosis of calves. III. The experimental reproduction of colibacillosis. Can. Vet. J. **5**:314–325.

61. GILLEM, H. C., V. M. YOUNG, E. D. MASSEY, AND W. C. BRANCHE. 1960. Observations on *Escherichia coli* as a component of the normal intestinal flora: relationship of host antibody levels to autologous serotypes present in the intestine. Bacteriol. Proc., p. 85.

62. GITTER, M., AND M. K. LLOYD. 1957. Haemolytic *Bact. coli* in the 'Bowel Oedema' syndrome. II. Transmission and protection experiments. Brit. Vet. J. **113**:212–218.

63. GLANTZ, P. J., AND H. W. DUNNE. 1955. Isolation of anaerogenic *Escherichia coli* O26B6 serotype from cases of calf scours. Science **121**:902.

64. GLANTZ, P. J., H. W. DUNNE, C. E. HEIST, AND J. F. HOKANSON. 1959. Bacteriological and serological studies of *Escherichia coli* serotypes associated with calf scours. Penn. State Univ. Agr. Exp. Sta. Bull. 645, p. 22.

65. GLANTZ, P. J., S. NAROTSKY, AND G. BUBASH. 1962. *Escherichia coli* serotypes isolated from salpingitis and chronic respiratory disease of poultry. Avian Dis. **6**:322–328.

66. GOLDMAN, A. 1924. Studies in intestinal bacteriology. J. Infect. Dis. **34**:459–501.

67. GOLDMAN, A. 1924. Studies in intestinal bacteriology. II. The effect of special feeding on the intestinal flora. J. Infect. Dis. **34**: 502–508.

67*a*. GOSSLING, J., K. MCKAY, AND D. A. BARNUM. 1964. Colibacillosis of calves in Ontario. 1. A serological study of *Escherichia coli* strains. Can. Vet. J. **5**:151–159.

68. GREGORY, D. W. 1955. Role of beta hemolytic coliform organisms in edema disease of swine. Vet. Med. **50**:609–610.

69. GREGORY, D. W. 1960. Experimental edema disease (hemolytic *Escherichia coli* toxemia) in pigs and mice. Amer. J. Vet. Res. **21**:88–94.

70. GREGORY, D. W. 1960. The (oedema disease) neuro-oedema toxin of haemolytic *Escherichia coli*. Vet. Rec. **72**:1208–1209.

71. GREGORY, D. W. 1962. Hemolytic coliform enteritis of weanling pigs. J. Amer. Vet. Med. Assoc. **141**:947–949.

72. GROSS, W. B. 1958. Symposium on chronic respiratory diseases of poultry. II. The role of *E. coli* in the cause of chronic respiratory disease and certain other respiratory diseases. Amer. J. Vet. Res. **19**:448–452.

73. HAENEL, H. 1961. Some rules in the ecology of the intestinal micro-flora of man. J. Appl. Bacteriol. **24**:242–251.

74. HAGAN, W. A. 1917. The etiology and mode of infection of white scours. Cornell Vet. **7**:263–283.

75. HAMON, Y. 1959. Recherche et identification de la propriete colicinogene parmi les differents lysotypes d'*Escherichia coli* pathogenes pour le nourrison. Ann. Inst. Pasteur **96**:614–629.

76. HANSEN, R. G., P. H. PHILLIP, AND I. W. RUPEL. 1946. The effect of vitamin supplements upon survival of newborn calves. J. Dairy Sci. **29**:761–766.

77. HANSEN, R. G., AND P. H. PHILLIP. 1947. Studies on protein from bovine colostrum. I. Electrophoretic studies on the blood serum proteins of colostrum-free calves and calves fed colostrum at various ages. J. Biol. Chem. **171**:223–227.

78. HANSEN, R. G., AND P. H. PHILLIP. 1947. Studies on the proteins of bovine colostrum. II. The absorption of globulins by the young calf. J. Dairy Sci. **30**:560.

79. HANSEN, R. G., AND P. H. PHILLIP. 1949. Studies on protein from bovine colostrum. III. The homologous and heterologous transfer of protein to the blood stream of young animals. J. Biol. Chem. **179**:523–527.

80. HARRIS, A. H., A. YANKAUER, D. CROSBY GREEN, M. B. COLEMAN, AND M. Y. PHANEUF. 1956. Control of epidemic diarrhea of the newborn in hospital nurseries and pediatric wards. Ann. N.Y. Acad. Sci. **66**:118–128.

81. HARVEY, D. G., AND P. CARNE. 1960. Studies on some chemical aspects of the pathological activities of strains of *Escherichia coli* of bovine origin. J. Comp. Pathol. Therap. **70**:84–108.

82. HEATH, G. B. S., AND M. BARR. 1962. Disease associated with *E. coli* in lambs. Vet. Rec. **74**:350–351.

83. HILL, K. J. 1956. Gastric development and antibody transference in the lamb with some observations on the rat and guinea pig. Quart. J. Exp. Physiol. **42**:72–95.

84. HORVATH, D. J., H. W. SEELEY, R. G. WARNER, AND J. K. LOOSLI. 1958. Microflora of intestinal contents and faeces of pigs fed different diets including pigs showing parakeratosis. J. Animal Sci. **17**:714–722.

85. HOWE, P. E. 1921. An effect of the ingestion of colostrum upon the composition of the blood in newborn calves. J. Biol. Chem. **49**:115–118.

86. HOWE, P. E. 1924. The relation between the ingestion of colostrum or blood serum and the appearance of globulins and albumen in the blood and urine of the newborn calf. J. Exp. Med. **39**:313–320.

87. HUGHES, B. A., AND R. LOVELL. 1962. Virulence of *Escherichia coli* for chick embryos. Intern. Cong. Microbiol. 8th, Montreal, Abstr. no. E33B. **1**:114.

88. INGRAM, P. L., R. LOVELL, P. C. WOOD, R. ASCHAFFENBURG, S. BARTLETT, S. K. KON, J. H. B. ROY, AND H. F. SEARS. 1953. Further observations of the significance of colostrum for the calf. I. White scours of calves in an enclosed community. Intern. Dairy Cong. 13th, The Hague, Proc. p. 1365–1368.

89. INGRAM, P. L., R. LOVELL, P. C. WOOD, R. ASCHAFFENBURG, S. BARTLETT, S. K. KON, J. H. B. ROY, AND H. J. SEARS. 1953. Further observations of the significance of colostrum for the calf. II. The immunological importance of colostrum. Intern. Dairy Cong. 13th, The Hague, Proc. p. 1369–1371.
90. INGRAM, P. L., R. LOVELL, P. C. WOOD, R. ASCHAFFENBURG, S. BARTLETT, S. K. KON, J. PALMER, J. H. B. ROY, AND K. W. G. SHILLAM. 1956. *Bacterium coli* antibodies in colostrum and their relation to calf survival. J. Pathol. Bacteriol. **72**:561–568.
91. ITO, A. 1960. Studies on hemolytic *Escherichia coli* strains isolated from edema disease of swine in Japan. Japan. J. Vet. Res. **8**:215–216.
92. JAMESON, E., C. ALVAREX-TOSTADO, AND H. H. SARTOR. 1942. Electrophoretic studies on newborn calf serum. Proc. Soc. Exp. Biol. Med. **51**:163–165.
93. JENSEN, C. O. 1913. Kälberruhr. Handbuck der pathogenen Mikroorganismen. **6**:131, as cited by Dunne et al. (37).
94. JOHANSON, K. R., AND W. B. SARLES. 1949. Some consideration of the biological importance of intestinal microorganisms. Bacteriol. Rev. **13**:25–45.
95. JOHNSON, P., AND A. E. PIERCE. 1959. Ultracentrifugal and electrophoretic studies on neonatal calf sera and maternal colostrum. J. Hyg. **57**:309–320.
96. JUNE, R. C., W. W. FERGUSON, AND M. T. WORFEL. 1953. Experiments on feeding adult volunteers with *E. coli* 55, B5, a coliform organism associated with infant diarrhea. Amer. J. Hyg. **57**:222–236.
97. KAECKENBEECK, A., AND J. THOMAS. 1960. A propos des serotypes colibacillaires dans la diarrhee des veaux. Determination des antigenes somatiques (O). Ann. Med. Vet. **104**:232–239.
98. KAECKENBEECK, A., G. COLINET, AND F. SCHOENAERS. 1961. Evolution de l'aptitude de l'intestine du veaux nouveau-ne a resorber les anticorps apportes par le colostrum. Ann. Med. Vet. **105**:197–205.
99. KATER, J. C., E. A. DAVIS, K. C. HAUGHEY, AND W. J. HARTLEY. 1963. *Escherichia coli* infection in lambs. New Zealand Vet. J. **11**:32–38.
100. KAUFFMANN, F., AND B. PERCH. 1943. Über die Coliflora des gesunden Menschen. Acta Pathol. Microbiol. Scand. **20**:201–220.
101. KAUFFMANN, F. 1947. The serology of the Coli group. J. Immunol. **57**:71–100.
102. KELEN, A. E., S. G. CAMPBELL, AND D. A. BARNUM. 1959. Studies on hemolytic *Escherichia coli* strains associated with edema disease of swine. Can. J. Comp. Med. Vet. Sci. **23**:216–228.
103. KENWORTHY, R., AND W. E. CRABB. 1936. The intestinal flora of young pigs with reference to early weaning, *Escherichia coli* and scours. J. Comp. Pathol. Therap. **73**: 215–228.
104. KERR, W. R., AND M. ROBERTSON. 1954. Passively and actively acquired antibodies for *Trichomonas foetus* in very young calves. J. Hyg. **52**:253–263.
105. KRAMER, T. 1960. Edema disease of swine. I. Study of hemolytic *Escherichia coli* strains isolated from cases of edema disease of swine in Western Canada. Can. J. Comp. Med. Vet. Sci. **24**:289–294.
106. KUNIN, C. M., M. V. BEARD, AND N. E. HALMAGYI. 1962. Evidence for a common hapten associated with endotoxin fractions of *E. coli* and other Enterobacteriaceae. Proc. Soc. Exp. Biol. Med. **111**:160–166.
107. KUNIN, C. M. 1963. Separation, characterization and biological significance of a common antigen in Enterobacteriaceae. J. Exp. Med. **118**:565–586.
108. LARSON, B. L., AND D. C. GILLESPIE. 1957. Origin of the major specific proteins of milk. J. Biol. Chem. **227**:565–573.
109. LARSON, B. L., AND K. A. KENDAL. 1957. Changes in specific blood protein levels associated with parturition in the bovine. J. Dairy Sci. **40**:659–666.
110. LARSON, B. L. 1958. Transfer of specific blood serum proteins to lacteal secretions near parturition. J. Dairy Sci. **41**:1033–1044.
111. LASKOWSKI, M., AND M. LASKOWSKI. 1951. Crystalline trypsin inhibitor from colostrum. J. Biol. Chem. **190**:563–573.
112. LEECE, J. G., AND B. R. REEP. 1962. *Escherichia coli* associated with colostrum free neonatal pigs raised in isolation. J. Exp. Med. **115**:491–501.
113. LINDBERG, R. B., AND V. M. YOUNG. 1956. Observations on enteropathogenic *Escherichia coli*. Ann. N.Y. Acad. Sci. **66**:100–107.
114. LINTON, K. B. 1960. The colicine typing of coliform bacillae in the study of cross infection in urology. J. Clin. Pathol. **13**:168–172.
115. LITTLE, R. B., AND M. L. ORCUTT. 1922. The transmission of agglutinins of *Bacillus abortus* from cow to calf in the colostrum. J. Exp. Med. **35**:161–171.
116. LLOYD, M. K. 1957. Discussion of Oedema disease of swine, by J. F. Timoney. Vet. Rec. **69**:1160–1175.
117. LOVELL, R., AND D. L. HUGHES. 1935. Diseases of young calves: a bacteriological examination of 100 cases. J. Comp. Pathol. Therap. **48**:267–284.
118. LOVELL, R. 1937. Classification of *Bacterium coli* from diseased calves. J. Pathol. Bacteriol. **44**:125–139.
119. LOVELL, R. 1955. Intestinal diseases of young calves with special reference to infection with *Bacterium coli*. Vet. Rev. Annot. **1**:1–32.
120. LOVELL, R., AND T. A. REES. 1960. A filterable haemolysin from *Escherichia coli*. Nature **188**:755–756.
121. MANSSON, I., AND B. OLSON. 1961. The intestinal flora of pigs. I. Quantitative studies of coliforms, enterococci and clostridia in the faeces of pigs self-fed a high-

protein and high-calcium diet. Acta Agr. Scand. **11**:197–210.
122. MANSSON, I., AND B. OLSON. 1961. The intestinal flora of pigs. II. Further quantitative studies of coliforms, enterococci and clostridia in the faeces of pigs self-fed a high-protein and high-calcium diet. Acta Agr. Scand. **11**:257–264.
123. MANSSON, I., AND B. OLSON. 1961. The intestinal flora of pigs. III. The effect of dietary zinc on the number of coliforms, enterococci and clostridia in the faeces of pigs self-fed a high-protein and high-calcium diet. Acta Agr. Scand. **11**:265–269.
124. MANSSON, I., AND B. OLSON. 1962. The intestinal flora of pigs. IV. The effect of dietary citric acid on the number of coliforms, enterococci and clostridia in the faeces of pigs self-fed a high-protein and high-calcium diet. Acta Agr. Scand. **12**:3–8.
125. MANSSON, I. 1962. Haemolytic *Escherichia coli* serotypes isolated from pigs, cattle, dogs and cats. Acta Vet. Scand. **3**:65–78.
126. MANSSON, I. 1962. Isolations of haemolytic *Escherichia coli* from diseased and healthy animals on a particular premises. Acta Vet. Scand. **3**:163–173.
127. MASON, J. W., T. DALLING, AND W. S. GORDON. 1930. Transmission of maternal immunity. J. Pathol. Bacteriol. **33**:783–797.
128. MUSCHEL, L. H. 1960. Bactericidal activity of normal serum against bacterial cultures. II. Activity against *Escherichia coli* strains. Proc. Soc. Exp. Biol. Med. **103**:632–636.
129. MCALPINE, J. G., AND L. F. RETGER. 1925. Serological studies on bovine infectious abortion. J. Immunol. **10**:811–828.
130. MCCARTHY, E. F., AND E. I. MCDOUGAL. 1949. Absorption of immune globulins by the young lamb after ingestion of colostrum. Nature **164**:354.
131. MCDIARMID, A. 1946. The transfer of agglutinins for *Brucella abortus* from cow to calf and their persistence in the calf's blood. Vet. Rec. **58**:146–149.
132. MCDOUGAL, E. I. 1949. An immune globulin fraction from bovine pre-colostrum. Biochem. J. **44**:531–541.
133. MCEWAN, A. D. 1950. The resistance of the young calf to disease. Vet. Rec. **62**:83–93.
134. MCKAY, K. A. 1950. The normal and diarrheal bacterial flora of newborn calves. Ph.D. Thesis, University of Toronto, Toronto, Canada.
135. MCSHERRY, B. J., AND J. GRINYER. 1954. Disturbances in acid-base balance and electrolyte in calf diarrhea and their treatment. A report of 18 cases. Amer. J. Vet. Res. **15**:535–541.
136. NAMIOKA, S., M. URUSHIDO, AND R. SAKAZAKI. 1958. *Escherichia coli* isolated from transmissible gastro-enteritis of pigs. Japan. J. Med. Sci. Biol. **11**:141–151.
137. NAMIOKA, S., M. MURATA, AND R. SAKAZAKI. 1961. Studies on pathogenicity of *Escherichia coli*: an observation on virulence of *Escherichia coli* for mice treated with a wetting agent. Japan. J. Med. Sci. Biol. **14**:11–20.
138. NAMIOKA, S., AND M. MURATA. 1962. Studies on the pathogenicity of *Escherichia coli*. II. The effects of a substance obtained from pseudo-eosinophils of rabbits on the organism. Cornell Vet. **52**:289–296.
139. NETER, E., AND C. N. SHUMWAY. 1950. *E. coli* serotype D433: occurrence in intestinal and respiratory tracts, cultural characteristics, pathogenicity, sensitivity to antibiotics. Proc. Soc. Exp. Biol. Med. **75**:504–507.
140. NETER, E., O. WESTPHAL, O. LÜDERITZ, AND E. A. GORZYMSKI. 1956. The bacterial hemagglutination test for the demonstration of antibodies to Enterobacteriaceae. Ann. N.Y. Acad. Sci. **66**:141–156.
141. NICHOLLE, P., L. LE MINOR, R. BUTTEAUX, AND P. DUCREST. 1952. Lysotypie des *Escherichia coli* isoles dans les gastro-enterites infantiles. I. Schemas des types actuellement individualises. Bull. Acad. Nat. Med. **136**:480–483.
142. ORCUTT, M. L., AND P. E. HOWE. 1922. The relationship between the accumulation of globulins and the appearance of agglutinins in the blood of newborn calves. J. Exp. Med. **36**:291–308.
143. ØRSKOV, F. 1951. On the occurrence of *E. coli* O26B6 in cases of infantile diarrhoea and white scours. Acta Pathol. Microbiol. Scand. **29**:373–378.
144. ØRSKOV, F. 1952. Antigenic relationships between O groups 1-112 of *E. coli* and Wramby's O groups 26W-43W. 10 new O groups 114–123. Acta Pathol. Microbiol. Scand. **31**:51–56.
145. ØRSKOV, F., I. ØRSKOV, T. A. REES, AND K. SAHAB. 1960. Two new *E. coli* O antigens O141 and O142 and two new *E. coli* K antigens K85 and K86. Acta Pathol. Microbiol. Scand. **48**:48–50.
146. ØRSKOV, I., F. ØRSKOV, W. J. SOJKA, AND J. M. LEACH. 1961. 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 Pathol. Microbiol. Scand. **53**:404–422.
147. OTTOSEN, H. E. 1959. Some statistics on calf mortality. Nord. Veterinaermed. **11**:493–512.
148. PARR, L. W., N. N. EL SHAWI, AND M. L. ROBBINS. 1960. Colicine susceptibility patterns of enteropathogenic *Escherichia coli*. J. Bacteriol. **80**:417–418.
149. PASCHERTZ, H. 1959. Ein Beitrag zur sogenannten Ödemkrankheit des Schweines, p. 51. Dissertation, Munich.
150. PESTI, L. 1960. Classification of colibacteria occurring in suckling pigs. Acta Vet. Acad. Sci. Hung. **10**:365–374.
151. PESTI, L. 1962. Qualitative and quantitative examination of the bacterial flora in the intestines of healthy pigs. I. Intestinal

bacterial flora of pigs of different ages. Acta Vet. Acad. Sci. Hung. **12**:299–310.
152. PESTI, L. 1962. Qualitative and quantitative examination of the bacterial flora in the intestines of healthy pigs. II. Changes in intestinal flora during the life time of the animals. Acta Vet. Acad. Sci. Hung. **12**: 311–318.
153. PIERCE, A. E. 1955. Electrophoretic and immunological studies on sera from calves from birth to weaning. J. Hyg. **53**:247–275.
154. PIERCE, A. E. 1959. Studies on the proteinuria of the newborn calf. J. Physiol. **148**:469–488.
155. PIERCE, A. E. 1960. Beta lactoglobulins in the urine of the newborn calf. Nature **188**:940–941.
156. PIERCE, A. E., AND P. JOHNSON. 1960. Ultracentrifuge and electrophoretic studies on the proteinuria in the newborn calf. J. Hyg. **58**:247–260.
157. PIERCE, A. E. 1961. Proteinuria in the newly born. Proc. Roy. Soc. Med. **54**:996–999.
158. PIERCE, A. E. 1961. Further studies on proteinuria in the newborn calf. J. Physiol. **156**:136–149.
159. PIERCE, A. E. 1962. Antigens and antibodies in the newly born. Proc. Symp. Colston Res. Soc. No. 13.
160. POELS, J. As cited by Jensen (93).
161. POLSON, A. 1952. Comparative electrophoretic studies of bovine and human colostrum in relation to neonatal immunity. Ondeerstepoort J. Vet. Res. **25**:7–12.
162. RAIBAUD, P., M. CAULET, J. V. GALPIN, AND G. MOCQUOT. 1961. Studies on the bacterial flora of the alimentary tract of pigs. II. Streptococci: selective enumeration and differentiation of the dominant group. J. Appl. Bacteriol. **24**:285–306.
163. RAUSS, K., AND I. KETYI. 1960. Beitrage zur antagonistischen Wirkung der Coli-flora des Darmkanals. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **177**:161–175.
164. REES, T. A. 1957. The mucus antigen of *Escherichia coli*. Brit. Vet. J. **113**:171–175.
165. REES, T. A. 1958. Isolation of *E. coli* O86B7 and 103B from diseased calves. J. Pathol. Bacteriol. **74**:441–444.
166. REES, T. A. 1958. Studies on *Escherichia coli* of animal origin. I. *E. coli* from natural outbreaks of colibacillosis of calves. J. Comp. Pathol. Therap. **68**:388.
167. REES, T. A. 1958. Studies on *Escherichia coli* of animal origin. II. *Escherichia coli* from young lambs. J. Comp. Pathol. Therap. **68**:399–401.
168. REES, T. A. 1959. Studies on *Escherichia coli* of animal origin. II. *E. coli* serotypes from pigs. J. Comp. Pathol. Therap. **69**:334–338.
169. REES, T. A. 1959. The isolation of *Escherichia coli* serotype O128B12 from a case of gastroenteritis in the calf. J. Pathol. Bacteriol. **79**:203–204.
170. RICHARDS, W. P. C., AND C. M. FRASER. 1961. Coliform enteritis of weaned pigs. A description of the disease and its association with hemolytic *Escherichia coli*. Cornell Vet. **51**:245–257.
171. ROBERTS, H. E., A. N. WORDEN, AND E. T. REES-EVANS. 1954. Observations on some effects of colostrum deprivation in the calf. J. Comp. Pathol. Therap. **64**:283–305.
172. ROBERTS, D. S. 1957. *E. coli* infection in lambs. Australian Vet. J. **33**:43–45.
173. ROBERTS, D. S. 1958. Further observations of *E. coli* disease in lambs. Australian Vet. J. **34**:152–156.
174. ROBERTS, H. E., AND T. F. VALLELY. 1959. An investigation into the relationship of hemolytic *Escherichia coli* to disease in pigs. Vet. Rec. **71**:846–850.
175. ROBINET, H. G. 1962. Relationship of host antibody to fluctuations of *Escherichia coli* serotypes in the human intestine. J. Bacteriol. **84**:896–901.
176. ROGERS-BRAMBELL, F. W. 1958. The passive immunity of the young mammal. Biol. Rev. Cambridge Phil. Soc. **33**:488–557.
177. ROWLANDS, S. J., J. H. B. ROY, H. J. SEARS, AND S. Y. THOMPSON. 1949. The effect of prepartum milking on the composition of the prepartum and postpartum secretion of the cow. J. Dairy Res. **20**:16–28.
178. ROY, J. H. B., J. PALMER, K. W. G. SHILLAM, P. L. INGRAM, AND P. C. WOOD. 1955. The nutritive value of colostrum for the calf. X. The relationship between the period that a calf house has been occupied and the incidence of scouring and mortality in young calves. Brit. J. Nutr. **9**:11–20.
179. ROY, J. H. B., K. W. G. SHILLAM, G. M. HAWKINS, J. M. LANG, AND P. L. INGRAM. 1959. The effect of white scours on the sodium and potassium concentration in the serum of newborn calves. Brit. J. Nutr. **13**:219–226.
180. SAKAZAKI, R., AND S. MIURA. 1956. The enteric bacterial flora of the intestinal tract of the healthy horse. Japan. J. Vet. Res. **4**:57–63.
181. SAKAZAKI, R., AND S. NAMIOKA. 1956. Serological studies on *Escherichia coli* isolated from animals. Japan. J. Exp. Med. **26**: 29–36.
182. SAUNDERS, C. N., A. J. STEVENS, J. B. SPENCE, AND W. J. SOJKA. 1960. *Escherichia coli* infection in piglets. Res. Vet. Sci. **1**:28–35.
183. SCHOENAERS, F., AND A. KAECKENBEECK. 1958. Etudes sur la colibacillosis du veau. I. Realisation experimentale de la maladie. Ann. Med. Vet. **102**:211–221.
184. SCHOENAERS, F., AND A. KAECKENBEECK. 1960. Etudes sur la colibacillosis du veau. III. Essae d'immunoprophylaxie. Ann. Med. Vet. **104**:117–120.
185. SCHOENAERS, F., AND A. KAECKENBEECK. 1960. Les colibacillosis enteropathogenes de la flore intestinale du veau normal. Ann. Med. Vet. **104**:240–245.
186. SEARS, H. J., I. BROWNLEE, AND J. H. UCHIYAMA. 1949. Persistence of individual

strains of Escherichia coli in the intestinal tract of man. J. Bacteriol. **59**:293–301.
187. SEARS, H. J., AND I. BROWNLEE. 1952. Further observations on the persistence of individual strains of Escherichia coli in the intestinal tract of man. J. Bacteriol. **63**:47–57.
188. SEARS, H. J., H. JONES, R. SALOUM, I. BROWNLEE, AND L. F. LAMOUREAUX. 1956. Persistence of individual strains of *Escherichia coli* in man and dog under varying conditions. J. Bacteriol. **71**:370–372.
189. SJOSTEDT, S. 1946. Pathogenicity of certain serological types of *B. coli*. Acta Pathol. Microbiol. Scand. Suppl. **63**:1–148.
190. SMIBERT, R. M., J. C. DAVIS, AND R. C. ALLEN. 1962. Studies on hemolytic *Escherichia coli* associated with edema disease of swine. II. An immunologic study of the toxic factor. Amer. J. Vet. Res. **23**:460–463.
191. SMITH, E. L. 1946. The immune proteins of bovine colostrum and plasma. J. Biol. Chem. **164**:345–358.
192. SMITH, E. L., R. D. GREENE, AND E. BARTNER. 1946. Amino acid and carbohydrate analyses of some immune proteins. J. Biol. Chem. **164**:359–366.
193. SMITH, E. L., AND N. H. COY. 1946. The absorption spectra of immune proteins. J. Biol. Chem. **164**:367–370.
194. SMITH, E. L. 1946. Isolation and properties of immune lactoglobulins from bovine whey. J. Biol. Chem. **165**:665–676.
195. SMITH, E. L., AND A. HOLM. 1948. Transference of immunity to the newborn from colostrum. J. Biol. Chem. **175**:349–357.
196. SMITH, E. L. 1948. The isolation and properties of the immune proteins of bovine milk and colostrum and their role in immunity. A review. J. Dairy Sci. **31**:127–138.
197. SMITH, H. W., AND W. E. CRABB. 1956. The typing of *E. coli* by bacteriophage, its application to the study of the *E. coli* population of the intestinal tract of healthy calves and of calves suffering from white scours. J. Gen. Microbiol. **15**:556–574.
198. SMITH, H. W. 1958. Further observations on the effect of chemotherapy on the presence of drug-resistant *Bacterium coli* in the intestinal tract of calves. Vet. Res. **50**:575–580.
199. SMITH, H. W. 1960. The ecology of the intestinal bacteria of the calf with particular reference to *Escherichia coli*. Vet. Rec. **72**:1178–1183.
200. SMITH, H. W., AND W. E. CRABB. 1961. The bacterial flora of the faeces of animals and man. Its development in the young. J. Pathol. Bacteriol. **82**:53–66.
201. SMITH, H. W. 1961. The development of the bacterial flora of the faeces of animals and man: the changes that occur during ageing. J. Appl. Bacteriol. **24**:235–241.
202. SMITH, H. W. 1963. Observations on the aetiology of neonatal diarrhoea (scours) in calves. J. Pathol. Bacteriol. **84**:147–168.
203. SMITH, H. W. 1963. The haemolysins of *Escherichia coli*. J. Pathol. Bacteriol. **85**: 197–211.
204. SMITH, T., AND R. B. LITTLE. 1922. The significance of colostrum to the newborn calf. J. Exp. Med. **36**:181–192.
205. SMITH, T., AND R. B. LITTLE. 1922. Cow serum as a substitute for colostrum in newborn calves. J. Exp. Med. **36**:453–468.
206. SMITH, T., AND R. B. LITTLE. 1922. Absorption of specific agglutinins in homologous serum fed to calves during the early hours of life. J. Exp. Med. **37**:671–683.
207. SMITH, T., AND R. B. LITTLE. 1924. Proteinuria in newborn calves following the feeding of colostrum. J. Exp. Med. **39**:303–312.
208. SMITH, T., AND M. L. ORCUTT. 1925. The bacteriology of the intestinal tract of young calves with special reference to early diarrhoea. J. Exp. Med. **41**:89–106.
209. SMITH, T. 1925. Focal interstitial nephritis in the calf following interference with the normal intake of colostrum. J. Exp. Med. **41**:413–425.
210. SMITH, T., AND G. BRYANT. 1927. Studies on pathogenic *B. coli* from bovine sources. II. Mutations and their immunological significance. J. Exp. Med. **46**:133–140.
211. SMITH, T. 1927. Studies on pathogenic *B. coli* from bovine sources. III. Normal and serologically induced resistance to *B. coli* and its mutant. J. Exp. Med. **46**:141–154.
212. SMITH, T. 1928. The relationship of the capsular substance of *B. coli* to antibody production. J. Exp. Med. **48**:351–361.
213. SMITH, T. 1930. The immunological significance of colostrum. I. The relationship between colostrum, serum, and the milk of cows normal and immunized towards *B. coli*. J. Exp. Med. **51**:473–481.
214. SMITH, T., AND R. B. LITTLE. 1930. 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. Exp. Med. **51**:483–492.
215. SMITH, V. R., AND E. S. ERWIN. 1959. Absorption of colostrum globulin introduced directly into the duodenum. J. Dairy Sci. **42**:364–365.
216. SOJKA, W. J., R. G. ERSKINE, AND M. K. LLOYD. 1957. Haemolytic *Escherichia coli* and 'oedema disease' of pigs. Vet. Rec. **69**:293–301.
217. SOJKA, W. J., M. K. LLOYD, AND E. J. SWEENEY. 1960. *Escherichia coli* serotypes associated with certain pig diseases. Res. Vet. Sci. **1**:17–27.
218. SOJKA, W. J., AND R. B. A. CARNAGHAN. 1961. *Escherichia coli* infection in poultry. Res. Vet. Sci. **2**:340–352.
219. SØRUM, L. 1962. Undersøkelse av en del hemolytiske *Escherichia coli* stammer isolert fra enteritis-ødemsyken hos gris. Nord. Veterinaermed. **14**:356–359.
220. SPAULDING, E. H., C. B. GUNTHER, AND E. T. DEUTSCH. 1961. Competitive antago-

nisms among *E. coli* strains. Bacteriol. Proc., p. 117.

221. STECK, F. 1961. Untersuchungen über die Gammaglobulinresorption des neugeborenen Kalbes mit Hilfe von Immunpräzipitationsmethoden. Pathol. Microbiol. **24**:963–969.
222. STEWART, J., AND J. W. MCCALLUM. 1938. White scour in calves and related infections. I. The significance of the vitamin A content of the colostrum as a predisposing factor in the causation of such conditions. J. Comp. Pathol. Therap. **51**:290–295.
223. STOCK, A. H., AND M. E. SHUMAN. 1956. Epidemiological studies on enteropathogenic *Escherichia coli* diarrhea. Ann. N.Y. Acad. Sci. **66**:108–111.
224. STULBERG, C. S., AND W. W. ZUELZER. 1956. Infantile diarrhea due to *Escherichia coli*. Ann. N.Y. Acad. Sci. **66**:90–99.
225. SWEENEY, E. J., W. J. SOJKA, AND M. K. LLOYD. 1960. Demonstrable antigens and a toxic factor in oedema disease of swine. Res. Vet. Sci. **1**:260–268.
226. TADD, A. D., AND A. HURST. 1961. The effect of feeding colicinogenic *Escherichia coli* on the intestinal *E. coli* of early weaned pigs. J. Appl. Bacteriol. **24**:222–228.
227. TAYLOR, J., M. P. MALTBY, AND J. M. PAYNE. 1958. Factors influencing the response of ligated rabbit gut segments to injected *Escherichia coli*. J. Pathol. Bacteriol. **76**: 491–499.
228. TAYLOR, J. 1959. *Escherichia coli* as an enteropathogen. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **174**:357–363.
229. TAYLOR, J., M. P. WILKINS, AND J. M. PAYNE. 1961. Relation of rabbit gut reaction to enteropathogenic *Escherichia coli*. Brit. J. Exp. Pathol. **42**:43–52.
230. THOMAS, L. 1954. The physiological disturbances produced by endotoxins. Ann. Rev. Physiol. **16**:467–490.
231. THOMLINSON, J. R., AND A. BUXTON. 1962. A comparison of experimental anaphylactic shock in guinea pigs with naturally-occurring oedema disease and haemorrhagic gastro-enteritis in pigs. Res. Vet. Sci. **3**: 186–202.
232. THOMLINSON, J. R., AND A. BUXTON. 1963. Anaphylaxis in pigs and its relationship to the pathogenesis of oedema disease and gastro-enteritis associated with *Escherichia coli*. Immunology **6**:129–139.
233. THORPE, F., AND R. GRAHAM. 1933. The persistence of Brucella agglutinins in calves of reactor cows. J. Amer. Vet. Med. Assoc. **82**:871–874.
234. TIMONEY, J. F. 1950. Oedema disease of swine. Vet. Rec. **62**:748.
235. TIMONEY, J. F. 1957. Oedema disease of swine. Vet. Rec. **69**:1160–1171.
236. TIMONEY, J. F. 1960. An alternative method to repeated freezing and thawing for the preparation of an extract from a strain of haemolytic *Escherichia coli* which on intravenous injection in pigs mimics the syndrome of oedema disease. Vet. Rec. **72**:1252.
237. TURCK, M., R. G. PETERSDORF, AND M. R. FOURNIER. 1962. The epidemiology of nonenteric *Escherichia coli* infections: prevalence of serological groups. J. Clin. Invest. **41**:1760.
238. TURK, J. L. 1959. Immunological activity of precolostral calf serum. Nature **183**:838.
239. ULBRICH, F. 1953. Escherichia coli Typ O55:B5:H6 als Erreger der Kälberruhr. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **160**:506–507.
240. ULBRICH, F. 1954. Serologische Typendifferenzierung und Prüfung toxisher und immunisierender Eigenschaften von *Escherichia coli* stämmen, die von gerunden und von an Coliruhr erkrankten Jungtieren isoliert wurden. Zentr. Veterinaermed. **1**:603–659.
241. VAN PELT, M. D., R. E. JOHNSON, AND W. M. PLASTRIDGE. 1953. Studies on the faecal flora of calves and its relation to calf scours. Bull. Conn. Agr. Exp. Sta. 306, p. 29.
242. VARNELL, T. R., E. S. ERWIN, V. R. SMITH, AND J. FLEMING. 1960. Changes in serum glyco and lipoproteins in normal and colostrum-deprived calves. J. Dairy Sci. **43**:1006–1010.
243. WALLICK, H., AND C. A. STUART. 1943. Antigenic relationships of *Escherichia coli* isolated from one individual. J. Bacteriol. **45**:121–126.
244. WELLS, H. G., AND T. B. OSBOURNE. 1921. Anaphylaxis reactions with purified proteins of milk. J. Infect. Dis. **29**:200.
245. WHANG, H. Y., AND E. NETER. 1962. Immunological studies of a heterogenetic enterobacterial antigen (Kunin). J. Bacteriol. **84**:1245–1250.
246. WILBUR, R. D., D. V. CATRON, L. Y. QUINN, V. C. SPEER, AND V. W. HAYES. 1960. Intestinal flora of the pig as influenced by diet and age. J. Nutr. **71**:168–175.
247. WILLIAMS, W. L., W. A. HAGAN, AND C. M. CARPENTER. 1920. White or calf scours. J. Amer. Vet. Med. Assoc. **57**:124–146.
248. WILLINGALE, J. M., AND C. A. E. BRIGGS. 1955. The normal intestinal flora of the pig. II. Quantitative bacteriological studies. J. Appl. Bacteriol. **18**:284–293.
249. WITTIG, W. 1963. Untersuchungen über das Vorkommen verschiedener *Escherichia coli* —Serogruppen bei der Coli-Enterotoxämie des Schweines. Arch. Exp. Veterinäermed. **14**:461–482.
250. WOOD, P. C. 1955. The epidemiology of white scours among calves kept under experimental conditions. J. Pathol. Bacteriol. **70**:179–193.
251. WRAMBY, G. 1948. Investigations into the antigenic structure of *Bact. coli* isolated from calves with special reference to colisepticaemia (white scours). Acta Pathol. Microbiol. Scand. Suppl. **76**:168.