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Normal Immunity Reactions of the Cow and the Calf with Reference to Antibody Transmission in the Colostrum

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Normal Immunity Reactions of the Cow and the Calf with Reference to Antibody Transmission in the Colostrum

BY JOHN B. NELSON*

ABSTRACT.—A study was made of the normal immunity reactions to *Bacillus coli* with the cow and the calf. The blood serum of the cow was shown to possess a marked bacteriolytic action towards *Bacillus coli*. With the unsuckled calf the bacteriolytic action was weak and there was a deficiency in complement. After nursing both the bacteriolytic action and the complement titer increased. With calves fed ordinary milk in place of colostrum there was likewise an increase of the lytic property and of the complement. There appeared to be an increase of complement with age and it was thought that this might be associated with the augmented bacteriolytic action. Anti-coli agglutinins were found present both in the blood serum and the colostrum of the cow. Agglutinins were not present in the blood of the newborn calf but appeared upon suckling. There was no immediate appearance of agglutinins in the blood of the milk-fed calves. It was thought that the augmented immunity reaction might be accountable, in part, for the observed resistance of the colostrum-fed calf to invasion by *Bacillus coli*.

INTRODUCTION

The feeding of colostrum (that secretion of the mammary gland prior to and immediately following parturition) appears to be an essential factor for the successful raising of dairy calves. Calves which are removed at birth from their dams and fed the milk of a normal lactating cow, in place of colostrum, show a higher mortality than do calves which are permitted to suckle normally. The calf from which colostrum is withheld is prone to the development of diarrhea, the condition termed "scouring", commonly beginning on the second or third day of life. The condition is progressive with attendant prostration and often terminates fatally, usually within the first week.

Post mortem examination of such calves reveals an enteritis with involvement of the mesenteric lymph nodes and usually marked hyperemia of the intestinal mucosa. Bacteriological examination of the visceral organs and of the blood regularly shows the presence of a strain of *Bacillus coli*, usually in pure culture.

*The writer desires to express his obligation to A. C. Ragsdale, Professor of Dairy Husbandry at the University of Missouri, to whom credit is due for developing the general plan of the project of which this investigation is a part.

Acknowledge is also made to Samuel Brody, who is responsible for the immunological, bacteriological and chemical phases of the Colostrum Project of which this investigation forms a part, under whose direction and with whose collaboration this investigation was conducted.

NOTE.—Also presented to the faculty of the Graduate School of the University of Missouri in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The calf that has received colostrum may show a transitory type of diarrhea, lasting for a few days, but rarely is it of any consequence.

An experiment conducted by Smith and Little¹ shows clearly the result of withholding colostrum from the newborn calf. Twelve calves were separated from their dams before nursing and fed whole milk in place of colostrum. Of the calves which had received only cow's milk, nine died and three survived. Post mortem examination showed intestinal and kidney lesions. From all animals *Bacillus coli* was isolated from spleen, liver, kidneys, and from the blood stream. As a control, ten calves which had nursed from birth were kept under observation. All of the calves in this series survived the first week of life, but at a longer interval three died. None of these, however, showed any evidence of a systemic invasion by *Bacillus coli*.

Ragsdale and Brody² give a mortality rate of 32 per cent for non-colostrum calves from the dairy herd of the University of Missouri. Out of twenty-two calves removed from their dams at birth and fed whole milk in place of colostrum, seven died. The mortality is not as high as that quoted from the preceding work but is much greater than the rate of normally fed calves.

There is commonly ascribed to colostrum the function of serving as a purgative to clear the intestinal tract of meconium in addition to supplying the essentials of nourishment to the calf. Experimental work in immunology has indicated quite a different function for colostrum, that of transmitting antibodies from the blood of the dam to the blood of the nursing calf.

The susceptibility of the newborn calf, that has been deprived of colostrum, to a systemic invasion by *Bacillus coli* suggests an absence or a lack of development of the normal protective mechanism, predisposing to such an invasion by the normal bacteria of the intestinal tract. Rarely is such a condition seen with the calf that has suckled, even though the intestinal tract shows much the same bacterial flora as does that of the milk-fed calf. Apparently the ingestion of colostrum, and subsequent assimilation, results in an increased resistance which is not present at birth or if present is only imperfectly developed.

The transference of antibodies, normally present in the blood stream of the dam, to the colostrum with subsequent carriage to the blood of the calf following ingestion and assimilation, would very well explain the apparent increase in resistance on the part of the calf that has nursed. This presupposes that such antibodies are absent or deficient in the blood of the calf at birth.

The experimental part of this investigation is concerned with a comparative study of normal immunity reactions in blood serum of the

cow and of the calf, immediately at birth and after nursing, as an indication of the transmission of antibodies which might be accountable for the increased resistance of the suckled calf to *Bacillus coli* invasion.

REVIEW OF LITERATURE

There is an abundance of experimental proof for the transmission of the antibodies of an actively or passively immunized mother to her young in the colostrum. It was very early recognized that the offspring of parents immune to certain diseases were born possessing a temporary resistance to that disease. Thus, Burckhardt,³ in 1879, found that infants of mothers inoculated with cow pox vaccine during the last weeks of pregnancy were born possessing an immunity. Rickert,⁴ in the same year, observed that lambs born of ewes immune to sheep pox likewise possessed immunity. Chauveau,⁵ in 1880, found such a transmitted immunity with the lambs of ewes immunized against anthrax.

The early workers in immunity transmission had not regarded the possibility of such a transfer through suckling. The acquired resistance of the young was regarded as due either to an intra-uterine immunization or to a germ cell transmission, a true inheritance of the factor of immunity possessed by one or both of the parents.

The work of Ehrlich,⁶ in 1892, marked the real beginning of organized, experimental investigation of immunity transmission. His studies were made with the plant toxins abrin and ricin, and the transfer of an antitoxic immunity to them in mice. Ehrlich proved conclusively that immunity was not transferred from parent to offspring as an inherited character, either by the spermatozoon or the ovum. The young of immune females regularly displayed an immunity. At first, Ehrlich believed that the immunity of the young was acquired during intra-uterine life. Later he was able to show that while intra-uterine transfer of antitoxin did occur, there was also a transfer of antitoxin to the young in the milk. Passively as well as actively immunized females transmitted antitoxin to their suckling young in the milk. He was able to show the same suckling immunity with the young of mice immune to tetanus antitoxin.

To explain the appearance of antibodies in the blood of nurslings, following the ingestion of the milk of an immune mother, it was necessary to assume that antibodies were absorbed directly from the intestinal tract in an unchanged condition. Such an assumption was an apparent contradiction of a previously accepted dictum that antitoxin administered by mouth was rendered functionless by reason of enzymatic action.

Romer⁷ was able to show that there were no properties peculiar to milk responsible for the absorption of antibodies from the intestinal tract of nurslings, and that the age of the animal was the limiting factor. He found that the antitoxin content in the blood of a foal, born of an

immune dam, declined faster after the twelfth day of life than did the antitoxin content of the milk. There was an indication that conditions in the intestinal tract were no longer favorable for the absorption of antitoxin. Romer concluded that antitoxin and hence protein could be absorbed in an unaltered state from the intestinal tract but only during the first days of life. He thought that anatomical and physiological differences in the intestinal tract of the young and of the mature animals would account for the difference in absorption.

Ganghofner and Langers⁸ gave further proof that protein was absorbed unchanged only during the first few days of life. They fed egg white to kittens, puppies, kids, and rabbits of different ages and by means of anti-egg-white serum tested the blood for egg white. They found absorption of unaltered egg white up to the eighth day. After that, absorption occurred only when very large amounts were fed or when some injury to the intestinal tract existed.

Antibodies present in the blood stream of an animal, by reason of natural or artificial immunization are found associated with the serum globulins. Removal of globulin by appropriate means as "salting out" will result in a removal of the antibodies. Brieger and Ehrlich⁹ had been able to prepare highly active, dry preparations of tetanus antitoxin from the milk of immune animals by precipitating the whey protein with a 30 per cent concentration of ammonium sulphate.

The whey proteins of milk and colostrum are lactalbumin and lactoglobulin, the latter in a concentration of 0.03 per cent in milk and from 6 to 12 per cent in colostrum. The association of antibodies with globulin both in blood serum and in milk gave evidence of a close relationship between serum globulin and lactoglobulin, and evidence of the manner by which milk acquired its antibody content.

Hamburger,¹⁰ by means of specific precipitating sera prepared against milk, lactalbumin, casein, and ox serum was able to show a close relationship between the protein constituents of milk and of ox blood. Thus, anti-milk serum gave a precipitate with ox serum, anti-lactalbumin serum likewise gave a precipitate with ox serum.

Schlossman and Moro¹¹ confirmed the findings of Hamburger. They also employed the precipitin test. In addition they prepared an anti-lactoglobulin serum and obtained a precipitate with ox serum. They also showed a strong specificity for the proteins of blood serum and milk.

The work of Bauer¹² was not entirely in accord with that of the previous investigators. Bauer by means of the complement fixation test was unable to show any common antigenic constituents in milk and ox serum. That is, an anti-milk serum did not fix complement in the presence of ox serum as antigen. Anti-colostrum serum, however, did fix

complement in the presence of ox serum as antigen. He believed that the soluble proteins of colostrum were derived directly from the blood, while the proteins of milk were a product of the mammary gland.

Bauereisen¹³ by means of specific precipitating sera was able to show a species specificity of the proteins of milk and blood serum. His work was in agreement with that of the investigators other than Bauer as to a close relationship between the proteins of milk and blood. He also showed relationship between the proteins of colostrum and those of the blood of the newborn infant and concluded that the blood of the infant, before nursing, contained the same protein constituents as did the milk but in smaller amounts.

The work of the above mentioned investigators was somewhat conflicting but in general there was agreement that milk, colostrum, and blood serum of the same animal species possessed a common, antigenic constituent indicating the identity of a protein present in the three fluids. The particular protein appeared to be albumin or globulin or a mixture of the two.

More recent work has shown conclusively that globulin is the common protein constituent of milk, colostrum, and blood serum. Crowther and Raistrick¹⁴ isolated and purified the proteins of milk, colostrum, and blood serum. The composition was compared on the basis of the nitrogen distribution between the nitrogenous constituents by the method of Van Slyke. The analysis showed casein, lactoglobulin, and lactalbumin to be distinct proteins. The composition of lactalbumin from either colostrum or milk was different from that of serum albumin. The composition of lactoglobulin, of either colostrum or milk, was practically identical with that of serum globulin.

Wells and Osborne¹⁵ made a comparative study of the proteins of milk on the basis of their anaphylactic reactions. The protein constituents were found to be immunologically distinct. Globulin was the only protein of milk which would produce anaphylactic shock in an animal sensitized with ox blood serum.

The identity of lactoglobulin and serum globulin indicates a direct passage of the globulin of the circulating blood, by way of the mammary gland to the colostrum and the milk. Such antibodies as may be present in the blood likewise appear to pass directly to the colostrum and milk, by reason of their association with the globulin.

The effect on antibody absorption of the feeding of heterologous antitoxin as compared with homologous antitoxin was studied by a number of workers. By heterologous antitoxin is meant that antitoxin associated with a globulin foreign to that of the animal to which it is

administered. By homologous antitoxin is meant that antitoxin associated with the native globulin of the animal.

The work of Salge¹⁶ seemed to indicate that heterologous antitoxin was not absorbed. He immunized goats with diphtheria toxin and with typhoid bacilli, and fed the milk to nursing infants. In no case was he able to demonstrate the presence of diphtheria antitoxin or of typhoid agglutinin in the blood of the infants.

Romer and Much¹⁷ were able to show that absorption of heterologous antitoxin occurred with calves. They fed the calf of a non-immune dam its mother's milk to which had been added measured amounts of tetanus antitoxin and demonstrated the presence of antitoxin in the blood stream. The amount absorbed was approximately 1/10 that absorbed by calves allowed to nurse tetanus immune dams. Romer and Much believed that the tetanus antitoxin after its introduction into the cow underwent a change of substrate, a transference from the globulin of the horse serum, and that the change was accountable for the increased degree of absorption. A single experiment gave presumptive evidence that such a change occurred. The whey of milk from a cow passively immunized with tetanus antitoxin from a horse gave no reaction with an anti-horse precipitating serum. Antitoxin was present but apparently had become dissociated from the horse serum globulin which was absent from the milk.

The work of Hamburger¹⁸ did not confirm the theory of Romer and Much regarding the alteration of passively introduced antitoxin. He was able to demonstrate the presence of horse serum protein by means of the precipitin test, in the milk of rabbits injected with tetanus antitoxin. The blood serum of the young, after nursing, also showed horse serum protein as well as antitoxin. The milk of goats immunized with the same antitoxin likewise gave a precipitate with an anti-horse serum. Hamburger believed there was a firm union of antitoxin with the precipitable protein.

Much and Happich¹⁹ confirmed the previous work of Romer and Much. They fed the suckling child of a non-immune woman its mother's milk to which had been added measured amounts of tetanus antitoxin. The heterologous antitoxin was absorbed but the amount was much less than that absorbed by infants allowed to nurse tetanus immune mothers. Much and Happich were unable to demonstrate the presence of horse serum in the milk of the immune women, either by the precipitin or complement fixation tests. Moreover, they were unable to show horse serum protein or human serum protein in the milk of a lactating rabbit that had received an injection of milk from the tetanus immune woman.

They believed that antitoxin, associated with a foreign globulin, underwent a profound change in passing through the system of an animal.

In addition to the work with antitoxins an equal amount of investigation had been carried out with other antibodies, particularly the agglutinins, bacteriolysins, and hemolysins. Much of the early work gave conflicting results and was not productive of definite conclusions. The work of Famulener,²⁰ in 1913, brought clarity to the transmission of such antibodies, those produced against bacteria and foreign cells. He studied the transmission of anti-sheep hemolysin in goats immunized against sheep cells. Kids born of immune mothers showed anti-sheep hemolysin after nursing. Before nursing, the kids of highly immune mothers showed little if any hemolysin. Practically all of the hemolysin was transmitted through the colostrum which at parturition showed a titer frequently higher than that of the dam's blood. Immediately after parturition the antibody content of colostrum rapidly declined. If the immunization of the dam was carried out soon after the birth of the kid there was no demonstrable transmission of antibody.

More recent studies in immunity transmission, particularly on the transfer of agglutinins have been made by a number of investigators. Reymann²¹ studied the transfer of normal typhoid and coli agglutinins and hemagglutinins in the goat. The agglutinins were regularly present in the blood of the dams but appeared in the blood of the kids only after nursing. The agglutinins were demonstrated in the milk; usually the titer was higher than that of the dam's blood. There was a rapid decline in the agglutinin titer of the milk after parturition and likewise in the blood of the young. After several months the agglutinins reappeared probably as the result of colon-immunization from the digestive tract. There seemed to be a relation between the reappearance of the bacterial agglutinins and the hemagglutinins. An experiment showed that the introduction of either antigen would stimulate to the production of both types of antibody.

Little and Orcutt²² investigated the transmission of agglutinins of *Bacillus abortus* from cow to calf in the colostrum. A study of maternal and foetal blood showed that even when the blood of the dam had a high agglutinin titer little or no agglutinin was present in the blood of the foetus. The colostrum of reacting cows regularly contained agglutinin. In only one instance was agglutinin found in the blood of calves before nursing. Agglutinins appeared after nursing and reached nearly the maximum concentration in five hours. If colostrum was withheld and milk of a low or negative titer fed agglutinins failed to appear.

Orcutt and Howe²³ studied the relation between the appearance of agglutinins and the accumulation of globulin in the blood of newborn

calves. Howe,²⁴ in a previous work, had shown that proteins precipitated by sodium sulphate in concentrations up to 17.4 per cent were present only in negligible amounts in the blood of the unsuckled calf. After nursing, relatively large amounts of these proteins appeared. The work of Orcutt and Howe was done with the agglutinins of *Bacillus abortus*. The appearance of agglutinins in the blood serum of calves after suckling was accompanied by an increase in globulin. If colostrum was withheld and ordinary milk substituted neither globulins nor agglutinins appeared in any appreciable amount.

Orcutt and Howe further studied colostrum and blood serum to determine the protein fractions with which the agglutinins were associated. It was found that the protein mixtures precipitated up to and including 16.4 per cent of sodium sulphate carried with them the agglutinins. This fraction was absent from the blood of the newborn kid and was absorbed directly from the colostrum.

In general it may be said that an immune mother transfers to her young a passive type of immunity. The mechanism of the transmission varies with different animals. With some there is undoubtedly a direct transfer of antibodies from the maternal to the foetal circulation during intra-uterine life. With other animals the transmission occurs after the birth of the young, through suckling. At parturition antibodies are found accumulated in the colostrum, associated with the lactoglobulin. There appears to be a direct passage of serum globulin with associated antibodies from the maternal blood to the colostrum. The intestinal mucosa of the young is permeable to protein during the first days of life, and following the ingestion of colostrum globulin and antibodies are absorbed and appear unchanged in the blood. The immunity thus acquired by the young is usually of short duration. There is a rapid decline in the antibody content of colostrum after parturition and nursing should take place soon after birth for the maximum transmission of antibody. The greatest absorption of antibody by the young occurs when colostrum of the same species is fed. Absorption of antibody from colostrum of a different species occurs but is relatively meager.

Presentation of Data

I.—THE TRANSMISSION OF HEMOLYSIN FROM THE DAIRY COW TO THE CALF IN COLOSTRUM

To show experimentally the transmission of actively acquired antibodies to the calf through the milk of its dam two cows were immunized late in the gestation period with horse blood cells. At parturition the blood of the calf, the blood of the dam, and the colostrum were tested for the presence of hemolytic amboceptor. After suckling the blood of the calf was again tested.

The cows were injected with horse blood cells which had been washed repeatedly with normal saline solution to remove all trace of serum. The dose employed was 20 cc. of a packed suspension of cells and it was administered intra-venously. Five injections were given, spaced three days apart. The immunization was so timed that there was a 14-day rest between the last injection and the calculated date of calving. During the last week of gestation a protector was placed over the udder of the cow as a precaution against nursing should the calf be dropped prematurely.

Immediately after birth, and before the calf had suckled, a sample of blood was drawn from the jugular vein. At the same time blood was drawn from the dam and a sample of colostrum taken. The blood sera and colostrum were tested for the presence of anti-horse hemolysin using the customary method of titration.

The blood serum was heated to 56° C. to remove native complement. Colostrum serum, or whey, was used in place of whole colostrum and was not heated. The whey which contains the soluble colostrum protein, and hence the antibodies, was separated by the addition of rennet. The cell suspension was prepared from oxalated horse blood from which serum had been removed by centrifugation and washing. The packed and serum free erythrocytes were diluted with physiological saline solution to a concentration of 2.5 per cent. The suspension was kept uniform by comparing the intensity of color of a 0.5 c.c. portion hemolyzed by the addition of 1.5 cc. of water with a standard maintained from day to day, and further diluted if necessary. The complement was a guinea pig serum and was used in a concentration of 10 per cent. Fresh complement was always employed and was titrated each day against an amboceptor of known strength and the dilution altered if necessary.

Guinea pig serum frequently contains native anti-horse hemolysin sufficient to cause partial hemolysis of horse cells in the dilution used.

As a precaution, the native amboceptor was removed from each lot of guinea pig serum by adding 0.05 cc. of packed cells to each 1 cc. of serum, keeping the tube in an ice mixture for 30 minutes and centrifuging to recover the amboceptor free serum.

In the test proper the reagents were used in the following amounts: cell suspension —0.5 cc., complement —0.5 cc., unknown serum—decreasing amounts of a stock dilution, saline—sufficient to bring the total volume to 2 cc. Incubation was carried out in a water bath at 37° C. and readings made at the end of an hour of incubation.

■ Cow No. 160 showed a high titer of anti-horse hemolysin in the blood and colostrum. To show the decline in antibody content samples of blood serum from the calf and of colostrum were titrated on successive days after parturition. The results of the titration of the several samples are presented in Tables 1 and 2. The titration figure represents that dilution of serum just causing complete hemolysis of the cell suspension in the presence of excess complement.

The blood serum of cow No. 160 at parturition gave complete hemolysis of the cell suspension in dilution 1 — 5,000. No further titrations were made.

TABLE 1.—THE HEMOLYSIN TITER OF COLOSTRUM OF COW No. 160, FOLLOWING PARTURITION

Time of collection	Dilution of colostrum giving complete hemolysis
Immediate	1 — 5,000
1st day after parturition	1 — 1,500
2nd day after parturition	1 — 640
3rd day after parturition	1 — 320
4th day after parturition	1 — 160
5th day after parturition	1 — 80
6th day after parturition	1 — 80
12th day after parturition	1 — 40
18th day after parturition	No hemolysis undiluted

TABLE 2.—THE HEMOLYSIN TITER OF THE BLOOD SERUM OF CALF No. 160, BEFORE AND AFTER NURSING

Time of collection	Dilution of serum giving complete hemolysis
Before nursing	No hemolysis undiluted
12 hours after	1 — 2,000
24 hours after	1 — 2,500
48 hours after	1 — 2,200
3rd day after	1 — 2,200
4th day after	1 — 1,700
5th day after	1 — 1,700
6th day after	1 — 1,200
7th day after	1 — 800
13th day after	1 — 400
19th day after	1 — 100
25th day after	1 — 100
31st day after	1 — 50

The second cow, No. 285, showed a relatively low concentration of anti-horse hemolysin in the blood and colostrum. The blood serum of the cow at parturition gave complete hemolysis in dilution 1—1000. The colostrum titer was likewise 1—1000. The blood serum of the calf at birth contained no anti-horse hemolysin. The hemolysin titer twelve hours after nursing was 1—300. The immunity reaction of the two cows affords a striking example of the difference in response to immunization of animals injected under identical conditions.

The preceding experiment indicates the manner in which actively acquired antibodies are transmitted to the calf in the colostrum. At parturition the concentration of antibody in the colostrum parallels that of the maternal blood, or as cited from previous work, may even be higher. The newborn calf, before suckling, shows no hemolysin in the blood, an indication that antibody is not transmitted to the calf during intra-uterine life. Immediately after nursing, antibodies appear in the blood. In this instance the maximum concentration of antibody occurred twenty-four hours after nursing. There is a gradual decline of antibody until at the end of a month nearly complete exhaustion is reached. The antibody content of the colostrum shows a rapid decline during the first twenty-four hours after parturition and then continues to decline more slowly, indicating that the hemolysin is transmitted passively. At the end of two weeks the antibody content is practically exhausted.

II.—THE BACTERIOLYTIC ACTION OF NORMAL BLOOD SERUM OF THE DAIRY COW FOR *BACILLUS COLI*

A study was made of the development of *Bacillus coli* in fresh, normal serum of the dairy cow as an indication of any growth-inhibiting property normally possessed by the serum. Blood was drawn from the jugular vein of a cow, observing aseptic precautions. After clotting, the serum was removed and cleared of blood cells by centrifugation. A 2.5-cc. portion of serum was pipetted into each of a series of small tubes and increasing amounts of a 12-hour broth culture of *Bacillus coli* added to the series. After a thorough mixing of the serum and culture the tubes were incubated at 37° C. At intervals of 6, 12, 24, and 48 hours a 0.5-cc. portion was removed from each tube, suitable dilutions made, and agar plates prepared. The plates were incubated at 37° C. for 48 hours and counts made. The counts are presented in Table 3. The figure given denotes the number of bacteria present in each cubic centimeter of the serum-culture mixture. Counts made on the blood serum of additional cows are given in Table 4, but only for that amount of culture first showing survival and growth of the introduced bacteria.

TABLE 3.—THE GROWTH OF *BACILLUS COLI* IN NORMAL BLOOD SERUM

Amount of culture	Bacteria per c.c. after incubation			
	6 hours	12 hours	24 hours	48 hours
One loopfull.....	*—	—	—	—
0.01 c.c.-----	—	—	—	—
0.02 c.c.-----	—	—	—	—
0.04 c.c.-----	—	—	—	—
0.06 c.c.-----	—	—	—	—
0.08 c.c.-----	—	—	—	—
0.1 c.c.-----	—	—	—	—
0.2 c.c.-----	—	—	—	—
0.4 c.c.-----	—	—	—	—
0.6 c.c.-----	200	—	—	—
0.8 c.c.-----	1,200	1,500	3,000	7,000,000
1.0 c.c.-----	2,000	2,000	6,000	13,000,000

*No growth is indicated by —.

TABLE 4.—THE GROWTH OF *BACILLUS COLI* IN ADDITIONAL LOTS OF NORMAL BLOOD SERUM

Number of cow	Amount of culture	Bacteria per c.c. after incubation		
		6 hours	12 hours	24 hours
328	0.6 c.c.	6,000	9,000	22,000
329	0.8 c.c.	17,000	80,000	320,000
517	0.5 c.c.	500	1,000	4,000
516	0.8 c.c.	3,000	5,000	30,000
288	0.6 c.c.	—	200	1,500

All the lots of blood serum displayed a marked inhibitory action. Only after the introduction of relatively large amounts of culture was there survival and later growth of the bacteria. The addition of smaller amounts of culture was not attended by growth, even after a prolonged incubation. The counts are not quite an accurate index of the actual growth of *Bacillus coli* in blood serum because of agglutination of the bacteria by the serum.

For the purpose of comparison, the effect of fresh blood serum on the growth of other micro-organisms was studied. *Staphylococcus aureus* and *Bacillus subtilis* were used as the test bacteria. Increasing amounts of a 12-hour broth culture were added to tubes containing 2.5 cc. of fresh serum. The tubes were incubated at 37° C. and at intervals of 6, 12, and 24 hours a .5 cc. portion removed from each tube and plated. With both bacteria there was growth in the serum, even in the portion inoculated with one loopful of culture. *Bacillus subtilis* showed a much lower count than did *Staphylococcus aureus* which may be attributed largely to its manner of growth in a fluid medium. Plate counts are presented in Table 5. The growth inhibiting property of fresh blood serum apparently is not displayed against bacteria in general but has some selective action.

TABLE 5.—THE GROWTH OF *BACILLUS COLI*, *STAPHYLOCOCCUS AUREUS*, AND *BACILLUS SUBTILIS* IN FRESH, NORMAL BLOOD SERUM

Amount of culture	Bacteria per c.c. after incubation		
	6 hours	12 hours	24 hours
<i>Bacillus coli</i> , one loopful -----	—	—	—
<i>Staphylococcus aureus</i> , one loopful -----	1,500,000	20,000,000	60,000,000
<i>Bacillus subtilis</i> , one loopful -----	85,000	650,000	1,500,000

The failure of *Bacillus coli* to grow in fresh blood serum, except when present in large numbers, might be attributed to an inhibitory effect of the serum on growth or to actual destruction, that is, lysis of the bacteria. Lysis, in the immunological sense, is a reaction involving the union of three components: antigen—foreign cell or bacterium; amboceptor—an antibody specific for it; and complement—a non-specific constituent of fresh, normal, blood. The union of the three components is followed by partial or complete dissolution of the foreign cell or bacterium. A more detailed study of blood serum was made to determine whether there was actual lysis of *Bacillus coli*.

Bacteriolysis is attended by a progressive alteration in the morphology and staining property of the bacterium prior to actual dissolution. The effect of fresh serum on the morphology of *Bacillus coli* was determined. A heavy suspension of *coli* was prepared by removing the growth from a 12-hour agar slant with a small amount of physiological saline solution. One-tenth of a cubic centimeter of the suspension was added to 2 cc. of fresh serum and the tube incubated at 37° C. in a water bath. There was a rapid agglutination of the bacteria, which was complete in 15 minutes with the formation of coarse aggregates. The tube was shaken frequently to keep the clumps in suspension. At intervals, a loopful of serum was removed, a smear made and stained with methylene blue. Microscopic examination of the smears showed an early agglutination of practically all the bacteria. The clumps were large and composed of densely packed bacteria. The outlines of individual cells were distinct and the staining was uniform. After an hour of incubation, individual bacteria within the clumps had begun to lose their rod shape and to assume a spherical form with a decreased ability to take the stain. Finally, dissolution of the bacterium occurred and the cell outline was completely lost. After six hours of incubation a smear made from sediment in the bottom of the tube showed outlines of what had been clumps of bacteria with only an occasional cell intact. Other clumps showed a moderate proportion of intact bacteria together with swollen cells and empty spaces. Microscopically there was evidence that lysis of the bacteria had occurred.

For further evidence of lysis, the relationship of complement to the inhibiting action of serum was studied. Complement is a thermolabile constituent of serum and is completely destroyed at temperatures of 50° C. and above. In addition, there is a gradual decline in the titer of complement in serum which is allowed to stand either at low temperature, room temperature, or body temperature. The effect on the inhibitory action of blood serum of the removal of complement both by heating and by deterioration was determined. A portion of fresh blood serum was heated at a temperature of 56° C. for 30 minutes. A second lot of serum had been kept at room temperature, making daily determinations of the complement titer, until undiluted serum gave only a trace of hemolysis when tested with a hemolytic system. The growth of *Bacillus coli* in the two complement deficient sera was compared with the growth in fresh serum. The plate counts are presented in Table 6. The two sera which were deficient in complement did not inhibit the growth of *Bacillus coli*.

TABLE 6.—THE GROWTH OF *BACILLUS COLI* IN FRESH BLOOD SERUM AND IN SERUM DEFICIENT IN COMPLEMENT

	Bacteria per c.c. after incubation		
	6 hours	12 hours	24 hours
Fresh serum plus one loopful of <i>B. coli</i> ..	—	—	—
Heated serum plus one loopful of <i>B. coli</i> ..	3,000,000	35,000,000	80,000,000
Deteriorated serum plus one loopful of <i>B. coli</i> ..	500,000	15,000,000	40,000,000

The union of an antigen with its specific amboceptor forms a complex which is able to fix complement, that is, remove it from solution. The addition of a suspension of *Bacillus coli* to fresh serum should result in the formation of such a complex and the fixation of the native complement of the serum. A small volume of a heavy saline suspension of *Bacillus coli* was added to a portion of fresh serum and the mixture incubated at 37° C. for 30 minutes, with frequent shaking. At the end of the incubation period the tube was centrifuged and the clear serum removed. The complement titer of the treated serum was determined, using an anti-horse hemolytic system. The cell suspension was made from washed horse blood cells, in a concentration of 2.5 per cent. The amboceptor was a standardized anti-horse hemolysin. The amounts of the reagents used in the titration were as follows: cell suspension, 0.5 c.c.; anti-horse hemolysin, .5 c.c., unknown serum-varying amounts; saline, sufficient to bring the total volume to 2 c.c. The tubes were incubated at 37° C. for an hour and reading made. The titer of the treated serum, compared with that of the same serum untreated, is given in Table 7. Fresh blood serum, in dilution 1—2.5, gave complete hemolysis

of the cell suspension in the presence of excess hemolysin. The treated serum in the same dilution gave only slight hemolysis. There had been nearly complete removal of the native serum complement through fixation.

TABLE 7.—THE COMPLEMENT TITER OF FRESH BLOOD SERUM AND OF SERUM TREATED WITH *BACILLUS COLI* AT BODY TEMPERATURE

	Amount of serum causing hemolysis			
	.5 c.c.	.4 c.c.	.3 c.c.	.2 c.c.
Fresh serum diluted 1 — 2.5.....	++	++	++	+
Serum treated with <i>B. coli</i> , diluted 1 — 52. .	+	±	—	—

Legend. ++ = complete hemolysis; + = partial hemolysis; ± = trace of hemolysis; — = no hemolysis.

At a low temperature, antigen and amboceptor unite but complement is not fixed, or only to a slight extent. In the preceding experiment it was possible that the removal of the complement was due to a mechanical absorption by the agglutinated bacteria. If this was the case then the agglutination of bacteria at a low temperature, which occurs but more slowly than at body temperature, should likewise result in the removal of complement. If the removal of complement at 37° C. was due to a union with the bacteria sensitized by a normal component of the serum, a specific fixation, then at low temperature the sensitization of the bacteria by the serum should cause no appreciable fixation of complement. A small volume of a heavy saline suspension of *Bacillus coli* was added to a portion of fresh serum and the mixture placed in an ice bath at 4° C. Agglutination of the introduced bacteria was nearly complete at the end of two hours. At that time the tube was centrifuged, the clear serum removed and the complement titer determined. The titer of the serum, compared with that of fresh, untreated serum, is given in Table 8. There was only a slight removal of complement by the bacteria, sensitized by the serum at low temperature.

TABLE 8.—THE COMPLEMENT TITER OF FRESH BLOOD SERUM AND OF BLOOD SERUM TREATED WITH *BACILLUS COLI* IN THE COLD

	Amount of serum causing hemolysis			
	.5 c.c.	.4 c.c.	.3 c.c.	.2 c.c.
Fresh serum diluted 1 — 2.5.....	++	++	++	+
Treated serum diluted 1 — 2.5.....	++	++	+	±

The growth of *Bacillus coli* was studied in fresh blood serum, from which the complement had been removed by specific fixation. The addition of a heavy suspension of coli resulted in a marked decrease in the complement titer of the serum. There was not complete removal of

complement since the serum was able to cause some hemolysis of a horse cell suspension in the presence of the specific amboceptor. The bacilli were quickly agglutinated by the serum and apparently did not remain in suspension for a sufficient length of time to effect complete fixation. The procedure was repeated several times. A suspension of coli was added to the serum and as soon as agglutination occurred the mixture centrifuged, the clear serum removed, and a fresh suspension added. The agglutinating action of the serum was markedly decreased by the successive removal of sensitized bacteria. The complement was completely removed after three additions of the suspension. A 0.5 cc. portion of the undiluted serum caused no hemolysis of the cell suspension in the presence of the specific amboceptor. The growth of *Bacillus coli* in the complement-free serum was compared with the growth of fresh, untreated serum. The plate counts are presented in Table 9. The serum from which complement had been removed by specific fixation did not inhibit the growth of *Bacillus coli*.

TABLE 9.—THE GROWTH OF *BACILLUS COLI* IN COMPLEMENT-FREE BLOOD SERUM

	Bacteria per c.c. after incubation		
	6 hours	12 hours	24 hours
Fresh serum plus one loopful of <i>B. coli</i>	—	—	—
Complement free serum plus one loopful of <i>B. coli</i>	800,000	15,000,000	50,000,000

DISCUSSION

Fresh, normal blood serum of the dairy cow markedly inhibits the growth of *Bacillus coli*. The inhibitory action is selective, that is, is not displayed against bacteria in general. Cultures of *Staphylococcus aureus* and of *Bacillus subtilis* grow almost unhindered in the serum. *Bacillus coli* regularly fails to grow except when a large amount of culture is present. The inhibitory action of the serum is attended by actual lysis of the colon bacilli. There is first an agglutination of the bacteria, followed by morphological changes, and terminated by complete dissolution. The presence of complement appears to be essential for the reaction. Fresh serum, from which complement has been removed either by heating, by deterioration, or by specific fixation, does support the growth of *Bacillus coli*.

The inhibitory action of ox serum is regarded as a true bacteriolysis involving complement and an immune body normally present in the serum. It is difficult to demonstrate directly the presence of an anti-bacterial, immune body. Indirect proof is seen in the ability of *Bacillus coli*, sensitized by ox serum, to fix the native complement.

Other types of native immune bodies can be directly demonstrated in normal blood. Thus in working with the transfer of actively acquired, anti-horse hemolysins from cow to calf it was found that the serum of the cow, before immunization, possessed a weak hemolytic action for horse cells. The hemolytic action was dependent on the presence of a hemolytic amboceptor which could be removed from the serum by specific absorption. The manner in which normal antibodies are acquired is not clear. It is easy to say, in the case of anti-coli immune bodies, that they are acquired through an immunization with the normal bacteria of the intestinal tract. If they do occur as the result of immunization it is difficult to see how horse cells could normally become incorporated in the system of the cow in such a manner as to exert an antigenic action. It may be, as Reymann²¹ has shown for kids, that immunization with any one antigen stimulates the production of unrelated immune bodies.

III.—THE BACTERIOLYTIC ACTION OF NORMAL CALF BLOOD SERUM FOR BACILLUS COLI

1. **The Reaction of the Calf Blood Serum Before Nursing.**—A study was made of the growth of *Bacillus coli* in the fresh blood serum of the newborn calf. Blood was drawn from the jugular vein of the calf shortly after birth and before nursing. The same method of procedure previously outlined for the testing of blood serum was followed, with the exception that fewer gradations of culture broth were employed. A 0.5 cc. portion of the serum-culture mixture was withdrawn at intervals of 6, 12, and 24 hours and plates poured. Plate counts indicating the number of bacteria present in each cubic centimeter of the serum-culture mixture are given in Table 10. Counts made on blood serum of additional calves before nursing are presented in Table 11. Counts are given only for that amount of culture first showing survival and growth of the introduced bacteria.

TABLE 10.—THE GROWTH OF BACILLUS COLI IN THE BLOOD SERUM OF THE CALF BEFORE NURSING

Amount of culture	Bacteria per c.c. after incubation		
	6 hours	12 hours	24 hours
One loopful.....	—	—	—
0.01 c.c.....	50,000	700,000	3,000,000
0.05 c.c.....	200,000	3,000,000	9,000,000
0.1 c.c.....	900,000	12,000,000	30,000,000
0.2 c.c.....	1,500,000	20,000,000	70,000,000

All of the lots of calf serum displayed some inhibitory action. *Bacillus coli* failed to grow in calf serum inoculated with the smaller

gradations of culture. In the other serum-culture mixtures there was survival and growth of the introduced bacteria.

TABLE 11.—THE GROWTH OF *BACILLUS COLI* IN ADDITIONAL LOTS OF CALF SERUM (BEFORE NURSING)

No. of calf	Amount of culture	Bacteria per c.c. after incubation		
		6 hours	12 hours	24 hours
329	0.01 c.c.	60,000	800,000	3,000,000
517	0.01 c.c.	150,000	2,000,000	6,000,000
516	0.05 c.c.	300,000	4,000,000	16,000,000
288	0.01 c.c.	20,000	300,000	1,000,000
127	0.01 c.c.	40,000	700,000	2,000,000

The inhibition of growth in serum inoculated with small amounts of culture indicated that the blood of the calf, even at birth, possessed a weak bacteriolytic action. A more detailed study of calf serum, the effect of serum on the morphology of *Bacillus coli* and the relation of complement to the action, offered confirmation. The addition of a small volume of a saline suspension of *Bacillus coli* to fresh calf serum, followed by incubation at 37° C., resulted in a partial clearing of the suspension observed microscopically. Microscopic examinations of smears, made at intervals, showed a partial agglutination with the formation of small aggregates. Hanging-drop preparations showed clumping but with many free bacteria. As incubation continued there was a progressive alteration in the morphology of many of the bacteria within the clumps, terminated by complete dissolution.

Complement was present in the blood of the calf at birth but the titer was low. Complement titrations were made on the blood serum of a number of calves that had not received colostrum. The same anti-horse hemolytic system, previously mentioned, was used. Diluted serum in no instance caused hemolysis of the cell suspension in the presence of excess amboceptor. The titration figures for the several lots of serum tested are given in Table 12. The serum was undiluted throughout.

TABLE 12.—THE COMPLEMENT TITER OF THE BLOOD SERUM OF CALVES BEFORE NURSING

No. of calf	Amount of serum causing hemolysis				
	.6 c.c.	.5 c.c.	.4 c.c.	.3 c.c.	.2 c.c.
328	++	++	+	—	—
329	++	++	+	—	—
517	++	+	±	—	—
516	++	++	+	±	—
288	++	++	+	—	—
127	++	++	+	±	—

The addition of *Bacillus coli* to the serum of the unsuckled calf resulted in nearly complete removal of the complement. A small volume

of a heavy suspension of *coli* was added to a portion of fresh calf serum, and the mixture incubated at 37° C. for an hour. At the end of the incubation period the tube was centrifuged and the clear serum removed. The complement titer was determined, using the same hemolytic system previously employed. The undiluted serum, in amounts up to 0.4 c.c. gave no hemolysis with a suspension of horse cells in the presence of excess anti-horse hemolysin. The 0.5 c.c. amount gave a trace of hemolysis. There had been nearly complete fixation of complement.

2. The Reaction of the Calf Blood Serum After Nursing.—A study was made of the growth of *Bacillus coli* in the blood serum of the calf after nursing. Blood was drawn from the calf after a 48-hour period of nursing. Increasing amounts of a broth culture of *coli* were added to equal portions of the serum. At intervals of 6, 12, and 24 hours of incubation a 0.5-c.c. portion of the serum culture mixture was removed from each tube and plated. Plate counts made on the blood serum of additional calves are given in Table 14. Counts are given only for that amount of culture first showing survival and growth of the introduced bacteria.

There was an increase in the bacteriolytic action of the calf serum during the period of nursing. The lytic action more nearly approached that of the blood serum of the cow.

TABLE 13.—THE GROWTH OF *BACILLUS COLI* IN BLOOD SERUM OF THE CALF AFTER NURSING

Amount of culture	Bacteria per c.c. after incubation		
	6 hours	12 hours	24 hours
0.1 c.c.-----	—	—	—
0.2 c.c.-----	—	—	—
0.3 c.c.-----	—	—	—
0.4 c.c.-----	500	—	—
0.5 c.c.-----	3,000	40,000	100,000
0.6 c.c.-----	6,000	150,000	400,000

TABLE 14.—THE GROWTH OF *BACILLUS COLI* IN ADDITIONAL LOTS OF CALF SERUM AFTER NURSING

No. of calf	Amount of culture	Bacteria per c.c. after incubation		
		6 hours	12 hours	24 hours
329	0.4 c.c.	10,000	80,000	400,000
517	0.5 c.c.	2,000	30,000	80,000
516	0.6 c.c.	5,000	60,000	200,000
288	0.3 c.c.	7,000	100,000	500,000
127	0.4 c.c.	—	2,000	100,000

The complement titer of the blood serum of the calf after nursing was determined. The same anti-horse hemolytic system was employed.

Undiluted serum through 0.2 c.c. caused complete hemolysis of a suspension of horse cells in the presence of excess anti-horse hemolysin. The serum diluted 1—2.5 completely hemolyzed the cell suspension in amounts of dilution which varied with different calves. The complement titer of the blood serum of the calves are presented in Table 15. In every case there was a marked increase in the complement titer of the serum during the period of nursing. The serum was diluted 1 - 2.5.

TABLE 15.—THE COMPLEMENT TITER OF THE BLOOD SERUM OF CALVES AFTER NURSING

No. of calf	Amount of serum causing hemolysis				
	.6 c.c.	.5 c.c.	.4 c.c.	.3 c.c.	.2 c.c.
328	++	+	±	—	—
329	++	+	—	—	—
517	++	+	—	—	—
516	++	++	+	±	—
288	+	±	±	—	—
127	++	+	±	—	—

3. **The Reaction of the Blood of the Calf That Has Received Milk in the Place of Colostrum.**—The relative increase in the bacteriolytic action of the blood serum of the calf after nursing indicated the possibility of an antibody transmission to the calf in the colostrum. There is also the possibility that the increase occurred naturally in the calf, that is, uninfluenced by the feeding of colostrum. If the increased bacteriolytic action was due to additional antibodies brought to the calf in the colostrum, then the blood serum of a calf fed ordinary milk in place of colostrum should show no increased lytic action. Milk contains only a trace of globulin and is deficient in antibodies. Even the milk of a highly immune cow is relatively poor in antibody content. It is reasonable to assume that there would be little, if any, normal antibody present in the milk, and that the calf would acquire no additional antibody through the absorption of the lactoglobulin of the milk.

A study was made of the bacteriolytic action of the blood serum of calves fed ordinary milk in place of colostrum. Two calves were removed from their dams, before nursing, and fed cow's milk in place of the colostrum of their dams. Samples of blood were taken soon after birth and again after a 48-hour feeding period. The growth of *Bacillus coli* was determined in the several lots of serum. Plate counts are given in Table 16. Counts are given only for that amount of culture first showing survival and growth of the bacteria. At birth the blood serum of the calves showed a weak bacteriolytic action. After the 48-hour period of feeding there was a considerable increase in the lytic action. The increased bacteriolytic action paralleled that seen with colostrum-fed calves.

TABLE 16.—THE GROWTH OF *BACILLUS COLI* IN THE BLOOD SERUM OF NON-COLOSTRUM CALVES AT BIRTH AND AFTER RECEIVING ORDINARY MILK

Number of calf	Time of collection of blood	Amount of culture	Bacteria per c.c. after incubation		
			6 hours	12 hours	24 hours
447	At birth.....	0.01 c.c.	30,000	500,000	2,000,000
447	After receiving milk..	0.5 c.c.	4,000	80,000	400,000
448	At birth.....	0.01 c.c.	50,000	900,000	5,000,000
448	After receiving milk..	0.4 c.c.	8,000	200,000	6,000,000

The complement titer of the blood serum of the two calves was determined, at birth and again at the end of the 48-hour feeding period. As before, an anti-horse hemolytic system was used in the titration. The blood serum of the calves at birth hemolyzed the cell suspension only when used undiluted. The serum, after feeding, gave hemolysis in dilution 1- 2.5. The complement titers are given in Table 17. In both instances the complement titer of the serum was low at birth and increased during the feeding period.

TABLE 17.—THE COMPLEMENT TITER OF THE BLOOD SERUM OF NON-COLOSTRUM CALVES AT BIRTH AND AFTER RECEIVING ORDINARY MILK

No. of calf	Time of collection of blood	Dilution of serum	Amount of serum causing hemolysis				
			.6 c.c.	.5 c.c.	.4 c.c.	.3 c.c.	.2 c.c.
447	Birth.....	Undiluted.....	++	++	+	±	—
447	After receiving milk..	Diluted 1—2.5..	++	+	—	—	—
448	Birth.....	Undiluted.....	++	++	+	—	—
448	After receiving milk..	Diluted 1—2.5..	++	+	±	—	—

The blood serum of the newborn calf displays a weak bacteriolytic action for *Bacillus coli*. After the calf has nursed the lytic action is considerably increased. Since the blood serum of the dam regularly shows a marked bacteriolytic property it was thought that antibodies associated with the reaction might be present in the colostrum. The absorption of such antibodies by the calf could account for the increased bacteriolysis. An experiment with non-colostrum calves showed such an explanation to be utenable. The non-colostrum calf, fed ordinary milk from birth, displayed the same increase in lytic property. It is apparent that some factor other than antibody transmission is responsible for the augmented bacteriolysis.

Complement is deficient in the blood serum of the newborn calf. Shortly after birth, within 48 hours, the complement titer is markedly increased, more closely approaching the titer of blood serum of the cow. The increase occurs not only with calves that have nursed but also with calves that have received a diet of ordinary milk. It does not seem likely that complement is absorbed as such from either the colostrum or the

milk. Ordinary milk is deficient in antibodies generally. Colostrum may contain complement in small amounts, although of colostrum samples from ten different cows on which complement titrations have been made, in only one instance was complement found present. In this case the cow had a mastitis and the colostrum contained a considerable amount of blood. Probably the complement titer of the newborn calf serum is not augmented by any absorption from the colostrum.

Bacteriolysis is dependent on the presence of complement. At birth the bacteriolytic action of the calf serum is weak and there is a deficiency in complement. Shortly after birth the complement titer of the serum is increased and at the same time there is an increased bacteriolysis. It is possible that the augmented lytic action is brought about by the increase in the complement, which occurs as a natural property of the serum. Such an explanation presupposes that there is normally present, in the blood serum of the calf, an anti-coli immune body. The ability of fresh calf serum to fix native complement in the presence of *Bacillus coli* as antigen gives evidence for the presence of such an antibody. The bacteriolytic action of the serum of the newborn calf would indicate that it possesses some natural protective mechanism against *Bacillus coli* invasion.

IV. THE AGGLUTINATING ACTION OF THE BLOOD SERUM OF THE DAIRY COW, AND OF COLOSTRUM, FOR *BACILLUS COLI*

In working with the bacteriolytic action of the blood serum of the dairy cow it was observed that the serum regularly agglutinated a suspension of *Bacillus coli*. With undiluted serum there is sometimes a spontaneous clumping of bacteria, which is independent of the presence of specific agglutinins. With blood serum agglutination occurred in dilutions up to 1-64, which is an indication that native, anti-coli agglutinins were present. Moreover, it was found that the successive addition of small amounts of a coli suspension resulted in a marked decrease in the agglutinating action. There was an absorption of agglutinin by the bacterial antigen. Colostrum was found to have a similar agglutinating action. Anti-coli agglutinins were present, evidently derived from the blood of the dam by a direct passage.

A further study was made of the agglutinating action of the serum and colostrum, and the agglutinin titer of a number of samples determined. A suspension of *Bacillus coli* was made by removing the growth from 12-hour agar slants with physiological saline solution. The suspension was filtered and further diluted with saline solution until its opacity matched that of a standard suspension, which had previously

been found to give the maximum agglutination with blood serum. The serum to be tested was inactivated by heating at a temperature of 56° C. for 30 minutes. The inactivated serum showed no appreciable decrease in the agglutinating action. Equal amounts of the bacterial suspension and the diluted serum were used in the test, with a total volume of one cubic centimeter. The tubes were incubated for five hours in a water bath at 37° C. Readings were made at the end of the first incubation period and again after twelve hours in the cold. The agglutinin titers of the several lots of serum are presented in Table 18. There was a considerable variation in the content of anti-coli agglutinins with different cows.

TABLE 18.—THE ANTI-COLI AGGLUTININ TITER OF NORMAL BLOOD SERUM

No. of cow	Dilution of serum causing agglutination							Control
	1-4	1-8	1-16	1-32	1-64	1-128	1-256	
328	++	++	++	+	—	—	—	—
329	++	++	++	++	±	—	—	—
517	++	++	++	+	±	—	—	—
516	++	++	++	++	++	+	—	—
288	++	++	++	++	+	—	—	—

Legend. ++ = complete agglutination with clearing; + = agglutination without clearing; ± = incomplete agglutination; — = no agglutination.

The agglutinin titer of colostrum, from the same series of cows, was determined. The first colostrum immediately after parturition, was used in each case. The samples were coagulated by the addition of rennet and the whey used in making the tests. The colostrum whey was not inactivated. In other details, the method employed was the same as for blood serum. The anti-coli agglutinin titers of the several lots of colostrum are given in Table 19. In every case the agglutinin content of the colostrum was higher than that of the blood serum of the dam.

TABLE 19.—THE ANTI-COLI AGGLUTININ TITER OF COLOSTRUM

No. of cow	Dilution of serum giving agglutination								Control
	1-4	1-8	1-16	1-32	1-64	1-128	1-256	1-512	
328	++	++	++	++	+	—	—	—	—
329	++	++	++	++	++	+	—	—	—
517	++	++	++	++	++	+	—	—	—
516	++	++	++	++	++	++	+	—	—
228	++	++	++	++	++	+	—	—	—

V. THE AGGLUTINATING ACTION OF NORMAL CALF SERUM FOR BACILLUS COLI

1. The Reaction of the Serum of Normal Calves at Birth and After Nursing.—A comparative study was made of the agglutinating action

of the blood serum of the calf at birth and after suckling. Blood was drawn at birth and after a 48-hour nursing period, and the anti-coli agglutinin titer of the serum determined. The results of the titrations are presented in Table 20. The agglutinin titers of the colostrum and blood serum of the dams of the calves have been given previously. The blood serum of the newborn calves before nursing did not contain anti-coli agglutinins. After a 48-hour nursing period agglutinins appeared in every case. The concentration of agglutinin was regularly less than that of the colostrum or the blood of the dam.

TABLE 20.—THE ANTI-COLI AGGLUTININ TITER OF THE SERUM OF THE CALF AT BIRTH AND AFTER NURSING

No. of calf	Time of collection	Dilution of serum causing agglutination							Control
		1-4	1-8	1-16	1-32	1-64	1-128	1-256	
328	Birth.....	—	—	—	—	—	—	—	—
328	After nursing ..	++	++	+	—	—	—	—	—
329	Birth.....	—	—	—	—	—	—	—	—
329	After nursing ..	++	++	+	±	—	—	—	—
517	Birth.....	—	—	—	—	—	—	—	—
517	After nursing ..	++	++	+	±	—	—	—	—
516	Birth.....	—	—	—	—	—	—	—	—
516	After nursing ..	++	++	++	+	—	—	—	—
288	Birth.....	—	—	—	—	—	—	—	—
288	After nursing ..	++	++	+	—	—	—	—	—

2. **The Reaction of the Serum of Calves Receiving Ordinary Milk in Place of Colostrum.**—The blood serum of the two non-colostrum calves contained no anti-coli agglutinin at birth. The calves were fed milk for a period of 48 hours and the blood retested. The serum was still deficient in agglutinins. There was no absorption of agglutinin from the milk. The results of the titrations are not presented in tabular form as there was no agglutination with any dilution of the serum, either at birth or after receiving milk.

DISCUSSION

Native anti-coli agglutinins are regularly present in the blood serum of the cow. As with the acquired anti-bodies, there is a direct passage of the native agglutinin to the colostrum. The first colostrum, after parturition, shows an agglutinin titer which with the cows studied is higher than the titer of the blood serum. The serum of the newborn calf does not contain agglutinin. Agglutinins appear shortly after suckling, reaching a concentration which is regularly less than that of the colostrum or the dam's blood. Agglutinins do not appear in the blood serum of calves that have been fed ordinary milk in place of colostrum. The agglutinins, present in the serum of the suckled calf,

apparently are derived from the colostrum by direct absorption. There is no natural increase in the agglutinating action of calf serum as there is in the bacteriolytic action occurring as the result of factors other than the transmission of antibodies in the colostrum. That is to say, there is no immediate increase independent of suckling. Much later, after the passively acquired antibodies have disappeared, there must be a re-appearance of agglutinin due possibly to the assumed immunization by the normal bacteria of the intestinal tract.

Immunity, or the ability of an organism to resist invasion by bacteria, is dependent on many factors. The absence of any one factor may predispose to a susceptibility. Of great importance in determining resistance are the immunity reactions of the blood. One type of immunity reaction appears to be a normal property of the blood of the newborn calf. The bacteriolytic action for *Bacillus coli* which the serum displays suggests a natural means of protection against invasion by that microorganism. Bacteriolysis is only one of the immunity reactions associated with resistance. The antibodies causing agglutination are not present in the serum of the newborn calf. Anti-coli agglutinins appear only after suckling, as the result of absorption from the colostrum. Thus, one of the immunity reactions associated with resistance is lacking entirely at birth. It seems probable that bacteriolysis and agglutination acting in conjunction with one another are responsible for the increased resistance of the colostrum-fed calf. The non-colostrum calf has only the bacteriolytic property of the blood serum as a protective immunity reaction and this may be insufficient to guard against invasion by a particularly active strain of *Bacillus coli*. The calf that has nursed, by reason of agglutinin absorption, has a markedly augmented immunity reaction and a more perfect means of protection.

SUMMARY

1. Fresh, normal serum of the dairy cow displays a marked bacteriolytic action for *Bacillus coli*.
2. The blood serum of the newborn calf, on the other hand, displays but a weak bacteriolytic action for *Bacillus coli*. After nursing, however, the lytic action of the calf serum regularly increases. There is also a marked increase in the concentration of complement in the serum.
3. The blood serum of the non-colostrum calf displays the increased lytic property with age similar to the serum of the colostrum fed calf. The complement titer is likewise increased with age.
4. The increased bacteriolytic action of the calf's serum with age does not, therefore, appear to be directly associated with the transmission of an antibody by way of the colostrum. It appears, rather, that the

increased concentration of the complement with age is the limiting factor in the increase of the bacteriolytic action of the serum.

5. Normal blood serum of the dairy cow regularly contains anti-coli agglutinins. So does colostrum. Indeed, colostrum at parturition usually shows a titer even higher than does the blood.

6. Anti-coli agglutinins are not present in the blood serum of the newborn calf but appear shortly after the calf has nursed. Agglutinins are not present in the blood serum of the calf that has received milk in place of colostrum. Therefore, anti-coli agglutinins must be transmitted from the cow to the calf by the way of colostrum.

7. By reason of the bacteriolytic action of its blood serum the newborn calf appears to possess some means of protection against invasion by *Bacillus coli*. The calf that has nursed is endowed with a more perfect protective mechanism apparently because of the agglutinins which are passively transmitted to its blood stream by the way of colostrum.

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