THE IMMUNOLOGICAL SIGNIFICANCE OF COLOSTRUM

I. THE RELATION BETWEEN COLOSTRUM, SERUM, AND THE MILK OF COWS NORMAL AND IMMUNIZED TOWARDS B. COLI

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The passing on from one generation to another of endemic infectious agents and parasites takes place most frequently at birth or soon after. In some instances the egg is the carrier or intrauterine transmission occurs. The mortality of the young generation is governed largely by the variety, concentration and virulence of the infectious agents and parasites domiciled in the herd or flock. The intrauterine transmission of antibodies towards certain enzootic diseases developed in the female parent and carried in the blood occurs in certain species. In others, such as the bovine species, the temporary protection of the young is accomplished by the storage of antibodies in the udder, quiescent before parturition, and the passage of these through the mucosa of the digestive tract when colostrum is taken during the first, and perhaps the second, day of life. The protection afforded by colostrum is probably limited to certain common, ubiquitous species of bacteria living in the digestive tract and on the mucosa of the respiratory tract. The more formidable invaders, such as tubercle bacilli, may be held off temporarily but no complete immunity is afforded, such as that against pathogenic *B. coli* races. In fact certain pathogenic bacteria tend to parasitize the udder. To prevent the newborn calf's ingesting such infectious agents, efforts have been made to pasteurize colostrum or to replace it with milk, frequently with disastrous results. The importance of colostrum in controlling the early mortality due to *B. coli* has been brought forward in earlier publications.[1] At the same time the possibility of substituting normal cow serum for colostrum

[1] Smith, T., and Little, R. B., *J. Exp. Med.*, 1922, **36**, 181.

was indicated in certain experiments on calves.[2] The use of serum was based on the assumption that it contains at least as much antibody as the colostrum. The experiments to be reported do not wholly substantiate this assumption. In fact the evidence to be presented indicates a much higher concentration in the colostrum than in the serum.

The antibody content towards *B. coli* was singled out, partly because the early diseases of calves are due to members of this group, partly because the antibodies are quantitatively measurable in guinea pigs.

A single strain of *B. coli*, already described,[3] was used in all experiments. This readily mutating strain was employed in the original or (a) form. Several cows were treated subcutaneously and intravenously with living and heated culture suspensions for variable periods of time to furnish a protective serum for control studies.[4] The quantitative estimation of protective antibodies towards *B. coli* was made on guinea pigs weighing 350–400 grams. Exactly 24 hour bouillon cultures were used. The bouillon from the same lot of veal was stored in full bottles in a refrigerator and only this was employed. The minimum fatal dose was determined and $1\frac{1}{4}$ to $1\frac{1}{2}$ times this dose was mixed with the serum or colostrum to be tested and injected after 15 minutes into the peritoneal cavity. Control tests of virulence of the culture were associated with each separate experiment.

The Relative Content of Protective Bodies towards B. coli in Colostrum and in Serum of Untreated Cows.—The protective action of colostrum having been demonstrated indirectly by withholding this fluid in feeding newborn calves, it seemed desirable to use the test on guinea pigs, described above, to determine quantitatively its protective capacity against the species of bacteria chiefly responsible for the early deaths of calves. The results are given in Table I. To simplify the table and still furnish the information at hand, the amounts of colostrum actually tested are tabulated with the sign (+) indicating protection against the fatal dose, and (–) indicating death of the guinea pig. Lower or higher amounts than those given were not tested. Hence the figures do not in every case represent upper and lower limits. The same strain of *B. coli* (1192a)[3] was used unless otherwise indicated.

Attention is called to certain data in this table. (1) The colostrum of normal cows as a rule contains antibodies to a *B. coli* of the scours

[2] Smith, T., and Little, R. B., *J. Exp. Med.*, 1922, **36,** 453.

[3] Smith, T., and Bryant, Gladys, *J. Exp. Med.*, 1927, **46,** 133.

[4] Smith, T., and Little, R. B., *J. Exp. Med.*, 1930, **51,** 483.

TABLE I

The Antibody Content of Colostrum, Milk, and Serum of Normal Cows towards B. coli

No. of cow	Breed	Source	Entry into herd	Date of test	Dose of: Colostrum (+) = protection	Dose of: Milk	Dose of: Serum	Remarks
				1926				
1	Holstein	Wisconsin	Aug., 1926	Oct.	0.2 (+)	—	—	
2	"	Native	—	Nov. 3	0.2 (+); 0.4 (+)	0.4 (−)	0.2 (−); 0.4 (−)	Milk drawn 7 days later
3	"	"	—	" 3	0.2 (+); 0.4 (+)	—	0.2 (−); 0.4 (−)	Same result with same colostrum refrigerated 7 days
4	Jersey	Oregon	June, 1926	" 15	0.2 (−); 0.4 (−)	—	—	
5	"	"	" 1926	" 19	0.2 (−); 0.4 (+); 0.6 (+)	—	—	
6	Guernsey	Michigan	Oct., 1926	" 19	0.2 (−); 0.4 (+)	—	—	
7	"	Wisconsin	April, 1925	" 24	0.2 (+); 0.4 (+); 0.6 (+)	—	—	
8	Holstein	Native	—	Dec. 3	0.1 (+); 0.075 (−); 0.05 (−)	—		
9	"	"	—	" 7	0.1 (+); 0.075 (−); 0.05 (−)	—	—	
10	"	Michigan	Sept., 1922	" 11	0.1 (+); 0.05 (−)	—	—	
				1927				
11	Jersey	New Jersey	Feb. 2, 1927	Mar. 10	0.2 (+)	—	—	
12	Holstein	Native	—	" 10	0.1 (−); 0.2 (+)	—	—	
13	"	Michigan	Feb. 27, 1927	Apr. 7	0.2 (−); 0.4 (−)	—	—	Colostrum thin, due to loss on account of overdistension of udder and leakage
14	Guernsey	Native	—	" 13	0.2 (−); 0.4 (−); 0.6 (+)	—	—	

type. 0.1 cc. to 0.2 cc. may protect guinea pigs against the surely fatal dose. Tests with quantities less than 0.1 cc., when made, were negative. The protective capacity of certain samples was low. Thus No. 4 failed in 0.4 cc. doses, Nos. 5 and 6 in 0.2 cc. doses. To interpret this deficiency, the dates of entry into the herd are given. These cows came in a short time before calving. The inference, based on perhaps too few cases, is that they were not yet immunized to the new flora. On the other hand, No. 1 came within the range of the native cows and the native No. 14 was low. (2) The milk following the colostrum has lost more or less of its protective power, as shown by No. 2. (3) The

TABLE II

The Relative (Protective) Antibody Content of Serum and of Milk of Two Treated Cows and a Normal Cow

No. of cow*	Treatment	Serum titer	Milk titer	Agglutination titer of serum towards 1192_b (mutant)	$\frac{\text{Milk}}{\text{Serum}}$ (protection)
D	Normal	1.0 (−)	0.8 (−)	—	—
A	Living cultures	0.005 (+)	0.6 (+); 0.4 (−)	1:1,280	$\frac{1}{120}$
B	Heated "	0.005 (+)	0.6 (+); 0.4 (+); 0.2 (+); 0.075(−)	1:2,560	$\frac{1}{40}$

* For data on the treated cows see *J. Exp. Med.*, 1930, **51**, 483.

serum of normal cows drawn at the same time that the colostrum was obtained is distinctly below the colostrum in antibody content. Thus the sera of Nos. 2 and 3 failed to protect in 0.4 cc. doses. The colostra protected in 0.2 cc. doses and probably in less. Earlier, fairly numerous tests of normal cow sera failed to show any protection in doses of 1 and even 2 cc. Hence tests of normal sera were omitted with exceptions noted.

The Relative Antibody Content of Serum and Milk of Immunized Cows.—A number of tests were made to determine the protective titer of the milk of immunized cows. In both cows treated with living and heated cultures, respectively, the milk titer rose in the first to 0.6 cc., and in the second to 0.2 cc. Owing to the fact that the original type of *B. coli* used failed to become agglutinated in the immune sera except in very low dilutions, the mutant was used in agglutination tests. Since the immunized cows were not bred, no colostrum from them was available.

The Effect of Later Feedings of Immune Serum on the Antibody Content of the Blood.—The significance of lactation on the transfer of immune bodies after the two first days of life has not been satisfactorily cleared up in the earlier experiments. The extensive literature on this subject is more or less contradictory, probably due to the use of different species of animals and alien serum. In a former article[5] the absorption of antibodies in the form of *B. abortus* agglutinins from the digestive tract of calves was prompt and abundant when fed in immune cow serum during the early hours of life. One calf fed a serum of high titer when $3\frac{1}{4}$ days old showed no increase of agglutinins thereafter. It was furthermore shown that the feeding of milk of high titer was less favorable to the absorption than either colostrum or serum.

The two following cases are further contributions to this subject. Homologous serum was fed to calves $2\frac{1}{4}$ and 18 days old, respectively. In neither animal was an increase in agglutinins demonstrated as a result of the feeding.

Calf 1430. Holstein bull calf, weighing 50–55 lbs., born April 1, 5.30 a.m. Weak and unable to stand at first. Held up to the udder for the first meal when 3 hours old. Left with dam until April 3, 2 a.m. At 10.50 a.m., when about $2\frac{1}{4}$ days old, it was fed with immune serum of Cow B (see Table II) mixed with milk from the same animal. It drank about 350 cc. of serum. At 7.40 p.m. the calf drank about 250 cc. of serum diluted with milk. Animal thereafter fed milk drawn from the udder of the dam.

April 26. Calf has been normal throughout excepting for a short period of soft, whitish fecal discharges a week ago. The agglutinin titer of the calf's serum before and after the feeding of the immune serum is given in Table III. For comparison a sample of the dam's serum is included.

It will be noted that the dam's colostrum had already given the calf's serum a considerable amount of agglutinins towards *B. coli* 1192_b. The feeding of immune serum later failed to raise the titer. The serum titer of the dam was only a little higher than that of the calf.

[5] Smith, T., and Little, R. B., *J. Exp. Med.*, 1923, **37,** 671.

The explanation of this slight difference may be looked for in the high concentration of *B. coli* antibody in the colostrum.

In view of the relation existing between the protein output of the urine and the intake of colostrum during the first days of life[6] the protein content of the urine was taken into consideration. When the calf was 29 hours old and before the serum feeding the protein in the urine was sufficient, when the heat test was applied, to produce a coagulum deposit after 24 hours sedimentation equivalent to 15.3 per cent of the volume of the entire fluid. Fourteen hours after the second feeding of serum, when the calf was 3 days and 4 hours old, the deposit was only 5.3 per cent, and on the following day it was 1.3 per cent. These figures do not present any evidence that any excess of protein had been absorbed from the serum fed.

TABLE III

Titrations of Serum of Calf 1430 against B. coli 1192$_b$ after Ingesting 600 Cc. Serum of Titer 1:2,560

Date of collection of serum	Serum dilutions							
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280
April 3, 10.50 a.m. (just before feeding serum)	C	C	C	++++	+++	+	−	−
April 3, 7.40 p.m. (just before 2nd feeding of serum)	C	C	C	++++	+++	++	±	−
April 4, 9.05 a.m.	++++	++++	++++	+++	++	+	−	−
Titration of Dam's Serum								
April 4, 9.10 a.m.	C	C	C	C	+++	++	−	−

Table IV shows the absence of any protection transmitted by the immune serum to the calf's serum.

Calf 1412. Fed serum and milk of untreated Cow 1229 at start, with only one discharge of fluid feces on 2nd day. When 18 days old, it was fed 1,000 cc. of

[6] Smith, T., and Little, R. B., *J. Exp. Med.*, 1924, **39,** 303.

immune serum of Cow A (see Table II) administered in 3 doses of 500, 300, and 200 cc. respectively, in one day. On the following day blood was withdrawn for tests 24 and 30 hours after the first feeding of serum, and 12 and 18 hours after the third or last feeding. Neither sample showed any appreciable difference in its agglutinin content from the sample drawn 5 days before the feeding. The urine of

TABLE IV

Protective Titer of Serum of Calf 1430 Before and After Ingestion of Immune Serum

Guinea pig No.	Dose of *B. coli* culture	Dose of serum	Result
	cc.	*cc.*	
1	0.04	—	Dead in 9 hours
2	0.044	—	" " 8 "
3	0.044	1.0 (a)*	" " 6 "
4	0.044	0.5 (b)	" " 9 "
5	0.044	1.0 (b)	" " 8 "
6	0.044	0.5 (c)	" " 6 "
7	0.044	1.0 (c)	" " 8 "

* (a) calf serum drawn immediately before feeding; (b) 9 hours after first and just before second feeding; (c) 13 hours after second feeding.

TABLE V

Calf 1416

Agglutinin Titer of Serum before and after Newborn Calf Had Been Fed Serum of Cow B (Heated Cultures) and Normal Milk

Blood drawn	Serum dilutions								
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560
Feb. 1, 5.45 a.m.	++	+	+	±	–	–	–	–	–
" 2, 3.10 p.m.	+++	+++	++++	++++	++	+	–	–	–
Serum of Cow B	C	C	C	C	C	C	C	++++	++++

the two following days was as before the feeding. It showed only a very faint clouding after the heat test. Tests on guinea pigs did not indicate any increase in resistance referable to the serum.

Contrasting with these cases is that of another calf (No. 1416, Table V) fed immune serum in place of colostrum during the 1st day of life.

The agglutinins towards *B. coli* (*b*) were tested in the calf's serum before and after the feeding of immune serum. Serum drawn before the first meal produced a slight deposit of clumps in 1:10 dilution. Twenty-one hours after the first meal the clumping was nearly complete at 1:80. There was a trace at 1:320. The immune serum itself showed nearly complete clumping at 1:2,560. Blood drawn 26 days later had declined in agglutinin content from 1:160 to 1:20.

The protective power of the same samples of serum was distinctly increased by the feeding. The sample drawn before the first meal failed to save the life of the guinea pigs in 1 cc. doses, whereas the sample following the feeding protected in 0.5 cc. but not in 0.25 cc. doses. The serum drawn 26 days later did not protect in a 0.75 cc. dose.

TABLE VI

Calf 1416

Protective Power of Serum before and after Calf Had Been Fed Serum of Cow B

Dose of *B. coli*	Dose of serum	Result
cc.	*cc.*	
0.036	—	Dead in 6 hours
0.04	—	" " 8–9 "
0.04	1.0 (a)*	" " 8–9 "
0.04	0.5 (b)	Lives (40 gram loss in 3 days)
0.04	1.0 "	" (30 " " " 3 ")
0.04	1.0 (a)	Dead in 6 hours
0.04	0.25 (b)	" " 8–9 "
0.04	0.5 "	Lives (no loss in 2 days)

* (a) = serum before feeding; (b) after feeding.

In another calf (1431)[7] fed serum on the 1st day the agglutinin titer rose from /20 to /160. Eleven days later the titer had receded considerably. One cc. of the serum drawn before the first meal failed to save the life of a guinea pig. Serum drawn 23 hours later protected in 1 cc. but not in 0.5 cc. doses. In still another calf (1417) treated in the same way the agglutinin titer rose from /20 to /80 and the protective dose from above 1 cc. to 0.5 cc. On the other hand, in a third calf (1437) neither agglutinin nor protective antibody increase was detected. The calf, however, continued normal until killed when 1⅔ months old.

Another method of determining the end period of effective colostrum feeding is to postpone it. Here the danger of early invasion by in-

[7] These figures refer to calves in the second paper of this series, *J. Exp. Med.*, 1930, **51**, 486.

fectious agents must be taken into consideration. In two calves reported upon elsewhere[8] the feeding of colostrum delayed 12 and 18 hours, respectively, was not followed by abnormal conditions during the 2 months' life of the calves.

SUMMARY

The protective antibody content of normal cow serum is below that of colostrum of the same animal. The method used does not permit the titration of the actual amount of the antibody in serum. Quantities up to 2 cc. have no protective effect. The same limitations apply to the titration of milk owing to the introduction of large quantitites of foreign protein into the peritoneal cavity of the guinea pig. When cows were immunized and a serum of high titer obtained, the antibodies in the milk of such cows rose to within the range of the method of testing. The relation of the protective capacity of serum to that of milk was approximately $\frac{1}{120}$ and $\frac{1}{40}$ in the two animals. These figures do not differ much from those obtained by early investigators titrating the antitoxic content of serum and milk of animals undergoing immunization with diphtheria toxin. In the two experiments on calves, $2\frac{1}{4}$ and 18 days old respectively, fed a highly protective serum, no increase in agglutinins or protective antibodies could be demonstrated. The postponement of colostrum to the 12th and 18th hour, respectively did not prevent normal growth.

[8] Smith, T., and Little, R. B., *J. Exp. Med.*, 1930, **51**, 488.