THE IMMUNOLOGICAL SIGNIFICANCE OF COLOSTRUM

II. The Initial Feeding of Serum from Normal Cows and Cows Immunized towards B. coli in Place of Colostrum

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It has been shown that in place of colostrum normal cow serum fed and injected will protect most calves so treated against the various early diseases associated with *B. coli*. It has also been shown that in normal cow serum the specific protective bodies against *B. coli* are much weaker than those in colostrum and below the range of values detected by the intraperitoneal injection of mixtures of cultures and serum into guinea pigs.[1] It may be assumed that colostrum, and cow serum in much less degree, contain antibodies towards other endemic infectious agents which are held in more or less complete subjection in older animals. Hence the feeding of a serum containing a high concentration of *B. coli* antibodies might not protect to the same degree against other diseases developing later and more slowly. However, it was thought best in carrying out the following experiments to try the serum of cows hyperimmunized towards *B. coli*, to keep the serum monovalent, and to administer it only by mouth in full imitation of the natural intake of colostrum. Calves were also fed with normal cow serum. Methods for testing the relative protective power of the sera against *B. coli* on guinea pigs are given in another paper.[1]

The Immunization of Cows.—To provide the immune serum cows were treated in several different ways. *B. coli* strain 1192_a[2] was chosen. This was a highly virulent, hemolytic race, not fermenting saccharose. The cows received both intravenous and subcutaneous injections variously spaced in time according to the condition of the animal. One cow was treated with living cultures, another with

[1] Smith, T., *J. Exp. Med.*, 1930, **51**, 474.

[2] Smith, T., and Bryant, G., *J. Exp. Med.*, 1927, **46**, 133.

cultures heated at 62°C. for 30 minutes, a third received heated cultures of the mutant designated 1192_b, and a fourth bouillon culture filtrates. All intravenous injections were followed by more or less prompt reactions on the part of the respiratory system similar to the disturbances produced in calves by the intravenous injection of filtrates.[3] To avoid too great depression as a result of intravenous injections, the subcutaneous route was frequently used. Large local swellings appeared with or without a febrile reaction, which always followed intravenous administration. There was evident a tendency towards hypersensitiveness when the treatment had progressed 6 or more months. The doses then had to be reduced accordingly.

Table I gives a few data concerning these treated cows. The protective titer of the sera as tested on guinea pigs was about the same for

TABLE I

Immunization of Cows to B. coli

No. of cow	Breed	Strain of *B. coli*	Treatment	Treatment begun	Treatment terminated	Protective titer of serum
						cc.
1109	Guernsey	1127_a	Living culture	Nov., 1924	April, 1925	0.1
A	Holstein	1192_a	" "	" 1925	July, 1927	0.005
B	Jersey	1192_a	Heated "	Sept., 1926	" 1927	0.005
C	Guernsey	1192_b	" "	Dec., 1927	March, 1928	0.02*
D	"	1192_a	Culture filtrate	Nov., 1926	June, 1927	0.5

* Towards mutant.

the cows treated with living and heated cultures respectively. The 48 hour bouillon filtrates failed to produce a potent serum. The serum of the cow treated with heated cultures of the *B. coli* mutant had little or no protective power over the original or (a) form but was quite effective in presence of the mutant.

The Feeding of Newborn Calves with Serum from Normal and Immunized Cows in Place of Colostrum. The method adopted for handling the calves selected for the serum treatment did not differ appreciably from that used in earlier work.

The calf was taken from the dam immediately after birth to the warmed Department units and thoroughly dried by rubbing with towels. Every effort was made to keep the animal protected from undue exposure.

[3] Smith, T., and Little, R. B., *J. Exp. Med.*, 1927, **46**, 123.

The serum was fed from a bottle, provided with a nipple, in three doses, each time mixed with milk. In the first and the second group, the first dose, given as soon as possible and usually within an hour after birth, was about 400 cc. serum. The second dose of 200 cc. was given about 6 to 7 hours later, and the third of 100 cc. from 6 to 10 hours after the second. The total amount of serum, 600 to 700 cc., was thus fed within the first 18 hours of life. In the third group larger amounts were given, also in 3 doses. Thereafter milk from cows well along in lactation was fed 3 times a day, at first from bottles and them from a pail. After 4 weeks some hay and grain were added to the ration.

For agglutination and protection tests blood was withdrawn from a jugular by piercing it with a hollow needle and allowing the blood to run into sterile containers. The tests were made as described in another article.[1]

Urine was obtained whenever possible either when passed voluntarily or by gentle manipulation of the external genitals. The examination was limited to the following operations. The deposits of cloudy fluid thrown down with the centrifuge were examined microscopically. Cloudy samples were filtered until clear. The clear urine was slightly acidified with acetic acid, brought to boiling in a graduated centrifuge tube, and the bulk of any coagulum which had formed measured after 24 hours sedimentation. This quantity divided by the total volume of urine in the graduate is given as per cent protein precipitate. There were also recorded the specific gravity, color, and reaction to litmus. The temperature was taken twice daily. Most of these data have not been reproduced in this paper.

The experiments were carried out on 3 groups of calves in 3 successive years. The gross results are given in Table II, III, and IV. Each group will be briefly discussed and the results summarized.

First Group (1926–7).—Two calves fed milk from an immune cow both succumbed within a few days after birth. The lesions indicated *B. coli* septicemia associated with unrestricted multiplication of *B. coli* in the small intestine. Nine calves which were fed serum of cows treated with living and dead *B. coli* cultures lived beyond the scours period without manifesting any appreciable disturbance. The serum of the fed calves in nearly all cases contained a demonstrable increase of *B. coli* agglutinins and antibacterial substances. Of three calves receiving serum of an untreated cow, one died on the 9th day. One calf receiving serum of the filtrate-treated cow (D) survived, the other died when 4 days old.

Subsequent happenings in calves kept until at least a month old presented new problems. Two manifested symptoms referable to disturbances of the central nervous system. One of these and three others developed a renal disease differing essentially from that described as the "white-spotted kidney" due to *B. coli*.[4] Two were killed too soon (12 and 21 days respectively) to warrant the inference that

[4] Smith, T., *J. Exp. Med.*, 1925, **41**, 413.

TABLE II

*First Group. Calves Fed Serum From Normal and Immunized Cows (600–700 Cc.)**

No.	Breed and sex	Date of birth	Weight at birth	Fed serum of cow No.	Weight at death	Result
		1926	*lbs.*		*lbs.*	
1369	Guernsey male	May 20	55	(Milk only of A**)	—	Dead in 2 days. *B. coli* septicemia.
1396	" "	Nov. 24	—	Normal serum	—	" " 9 " Hemorrhages in 4th stomach.
1401	Holstein "	Dec. 2	90	" "	92	Killed in 12 days. Slightly fatty liver and renal congestion.
1403	" female	" 7	100	A	127	Killed in 21 days. Normal.
1405	" male	" 8	85	A	149	" " 34 " "
1408	" "	" 21	100	A	—	Sold " 23 " "
		1927				
1411	" "	Jan. 2	70	D***	165	Killed in 77 days. Renal lesions.
1412	" female	" 14	70	Normal serum	—	Used in another experiment when 34 days old.
1416	" "	Feb. 1	70	B****	—	Killed when 97 days old. Renal lesions. Blindness.
1417	Guernsey "	" 4	80	B	—	Killed when 97 days old. Normal.
1418	Holstein "	" 9	70	A	94	Dies when 30 days old in convulsions.
1421	Jersey male	March 1	50	D	—	" in 4 days. Hemorrhages in 4th stomach. Scours.
1423	Holstein "	" 3	85	(Milk only of A)	—	Dies in 5 days. Scours. Peritonitis.
1430	" "	April 1	50	(Control)	214†	Killed when 96 days old. Normal.
1431	" "	" 6	85	B	210	" " 75 " " "
1434	Guernsey female	" 13	40	A	56	Moribund when 44 days old. Hemorrhages in 4th stomach. Renal lesions.
1437	" "	May 5	70	A	130	Killed when 2 months old. Normal.
1449	" male	" 27	90	(Control)	137†	" " 1 month " "
1450	Holstein "	" 27	85	"	138†	" " 6 weeks " "

* The 3 first calves received 600 cc., the rest 700 cc.

** Cow treated with living *B. coli*.

*** Cow treated with *B. coli* filtrates.

**** Cow treated with heated *B. coli*.

† 9 days before slaughter.

TABLE III

Second Group. Calves Reared Normally or Serum-Fed

No.	Breed and sex	Date of birth	Approximate weight at birth	Fed serum of cow	Weight at death	Result
		1927	*lbs.*		*lbs.*	
1461	Holstein male	Sept. 6	119 (at 6 days)	(Control)	171	Killed, 1 mo. 17 days old. Normal.
1466	Jersey "	" 21	40	B	134	" 2 " 8 " " "
1467	Guernsey "	" 21	76	(Control)	184	" 2 " 23 " " "
1469	" "	" 28	80	A	114	Dies when 43 days old in convulsions.
1470	Holstein "	" 28	85	B	220	Killed, 2 mo. 15 days old. Normal.
1473	Guernsey female	Oct. 28	75	(Control)	150	" 2 " 8 " " "
1477	" "	Dec. 12	70	A	142	" 1 " 25 " " "
1478	" male	" 12	60	A	—	" 2 " 1 day " "
1479	Holstein "	" 13	85	(Control)	149	" 1 " 17 days " "
1480	" "	" 28	80	B	181	" 1 " 30 " " "
		1928				
1485	Guernsey "	Jan. 10	65	(Control)	141	" 2 " 2 " " "
1492	Jersey "	Feb. 13	55	A	135	" 1 " 27 " " "
1493	Holstein female	" 17	80	A	171	" 1 " 30 " " "
1494	" male	" 14	85	(Control)	208	" 2 " 9 " " "
1498	" "	March 14	85	(Milk only of B)	91	Dies in 37 hours. *B. coli* septicemia.
1499	Guernsey "	" 15	60	A	92	Killed, 2 mo. old. Scours. *B. coli* cystitis.

TABLE IV

Third Group. Calves Reared Normally or Serum-Fed

No.	Breed and sex	Date of birth	Approximate weight at birth	Cow serum fed and amount	Weight at death	Result
		1928	*lbs.*		*lbs.*	
1511	Guernsey male	May 22	—	C, 700 cc.	140	Dies when 1 mo. 28 days old. Renal lesions.
1513	Holstein "	Aug. 16	80	(Control)	158	Killed " 1 " 15 " " Normal.
1514	Guernsey "	Oct. 1	65	C, 700 cc.	133	" " 2 " 2 " " "
1516	" "	" 3	60	C, 700 cc.	112	Sold " 2 " 4 " " Clinically normal.
1518	" "	" 4	55	(Control)	113	" " 2 " 3 " " " "
1522	Holstein "	Nov. 4	85	"	202	Killed " 2 " 1 day " Normal.
1529	" female	Dec. 12	85	Normal serum, 1400 cc.	156	" " 2 " old. Spotted kidney.
		1929				
1531	" male	Jan. 4	110	(Control)	164	" " 2 " 4 days old. Normal.
1532	" female	" 11	75	Normal serum, 700 cc.; D, 700 cc.	147	" " 2 " 8 " " Slight renal lesions.
1533	" male	" 12	95	A, 145 cc.; C, 1260 cc.	172	" " 2 " 3 " " Normal.
1542	" female	Feb. 11	75	Milk only	120	Dies " 1 " 22 " " Large abdominal abscess.
1549	Guernsey male	March 7	75	(Control)	160	Killed " 2 " 16 " " Normal.
1553	Holstein female	" 19	100	Colostrum delayed 18 hr.	190	" " 2 " 1 day " Intestines congested.
1554	" male	" 20	85	Normal serum, 700 cc.	170	" " 2 " 3 days old. Normal.
1556	" "	April 8	85	Colostrum delayed 12 hr.	185	" " 2 " 4 " " "
1562	Jersey female	" 15	45	Normal serum, 700 cc.	93	" " 1 " 27 " " "

they would have remained normal indefinitely. Three of the calves had hemorrhages of the mucosa of the fourth stomach. This condition, to which attention has been called in previous publications, is probably the result of prenatal causes not yet defined. The hemorrhages vary from pale red dots to active petechial, closely set hemorrhages covering the entire mucosa. They may disappear quickly or continue to discharge blood for weeks and are probably the immediate or remote contributory cause of death in such animals. They may have been responsible for the death of Calf 1434. If we exclude this animal, there is one tardy death among 6 fed with serum of Cow A and one calf with renal disease among 3 fed with serum of Cow B.

Three control calves kept with their dams for a few days and then put under the same conditions as the experimental calves remained normal.

Second Group (1927–8).—In planning the experiments of the first group, the possibility that calves which had successfully weathered the early dangerous *B. coli* period might suffer from diseases developing later was not fully realized. Some calves were killed too early. The pathological conditions observed in the first group and in earlier published studies led to various conjectures as to the nature and causes of the conditions. To eliminate some of these a second group was subjected to the same treatment and a few more controls were introduced. These remained with the dam from 4 to 6 days and were then transferred to the Department units. This group was kept under observation long enough to permit the evolution and closer study of the diseases which had appeared in the first group. All serum-fed calves received 700 cc. serum.

From Table III it will be noted that there were 6 controls, 9 serum-fed and 1 milk-fed calf. Those surviving were kept at least 1½ months and most of them 2 months and longer. At the end of this period they were killed and autopsied. All controls remained normal. Of the serum-fed animals, one died in convulsions when 43 days old. One (1499) went through several attacks of scours and later developed a *B. coli* cystitis. The calf receiving milk only from Cow B died of *B. coli* septicemia.

Third Group (1928–9).—In this group were 16 calves. The treatment was the same as in the other groups, excepting that the amount and source of the serum fed differed from those of the earlier groups and from animal to animal. Five calves were introduced as controls. Three were fed with serum of Cow C (mutant *B. coli* treatment), and 3 received normal cow serum. One received normal serum and serum of Cow D (*B. coli* filtrate serum) in equal amounts; another chiefly C serum plus 1/10 A serum. In two the first feedings were with milk and colostrum feeding was delayed 12 hours and 18 hours respectively. One was fed milk only. This animal lived nearly 2 months and died within 15 minutes in convulsions. The autopsy showed the presence of a large abdominal abscess, originating in the track of the urachus. All 5 controls remained normal. Of the serum-fed, one died with renal disease. A second, although with normal

clinical history and autopsy, had slightly heavier kidneys and intranuclear bodies in the uriniferous tubules to be described more fully in another paper.[5]

DISCUSSION

The outcome of the three experiments shows that feeding an adequate amount of serum (600 cc. or more), either from normal cows or from those immunized with one type of *B. coli*, in place of colostrum prevented the early fatalities attributable to *B. coli*, but did not protect all calves from renal lesions or fatal convulsive attacks during the 2nd month of life. That the serum is somehow negatively responsible for these delayed morbid conditions is indicated by the significant fact that all 11 control calves of the second and the third group remained normal whereas of the 17 serum-fed animals of these same groups 2 died. The greater success in preventing disease in these groups as compared with the first group cannot be adequately interpreted in default of knowledge concerning the causes of the convulsive symptoms and renal lesions. The rarity and unexpected appearance of these conditions have interfered thus far with a more thorough study of them.

The age of the serum, normal and immune, varied considerably. Some of it was nearly 2 years old when fed. The guinea pig tests indicated very little antibody decline when the serum was kept refrigerated in nearly full containers. All of the serum was passed through Berkefeld filters and stored without the addition of antiseptics.

The value of the monovalent *B. coli* serum as compared with that of normal cows could have been determined only by a large series of tests on account of the genetically variable animal material, the fluctuating conditions of seasons and of the dairy cow during pregnancy. However, the complete history of the calves, of which the details have been omitted, indicated a greater freedom from early diarrheas among those fed immune than among those receiving normal serum. The experiments bring out the fact that there is nothing qualitatively different in colostrum from normal serum but that the former is quantitatively more effective so far as the content of antibodies is involved. It should be borne in mind that the normally fed calf receives an amount

[5] Smith, T., *J. Exp. Med.*, 1930, **51**, 519.

of colostrum much larger than the serum fed. It would require 2 to 3 liters of normal serum to match the antibody content of the usual amount of colostrum ingested. The inference is thus permitted that there is a large factor of safety in the normal feeding of the newborn calf. That colostrum contains favoring substances not present in serum, as has been claimed at times, is not denied, but the experiments indicate that a sufficient quantity of serum is in 9 out of 10 cases capable of replacing colostrum without injury to the calf. The experiments also indicate that the chief and perhaps the only function of colostrum is to protect the young animal against an early invasion of miscellaneous semi-pathogenic microorganisms by way of the digestive and the umbilical tract.

The pathological conditions appearing in the 2nd month in the serum-fed calves are probably not new diseases but such as are occasionally encountered in calves fed naturally. In the practical application of serum feeding to eliminate contact with the dam immediately after birth, it is suggested that the method might be distinctly reenforced by two or three subcutaneous injections of small quantities of serum during the first 24 hours of life.

The advantages of immune serum do not appear to outweigh the cost of production and it is suggested that the normal serum be used, preferably in larger quantities, such as 1200 to 1500 cc. If possible, the source of the serum should be the same herd or at least herds in the same section of the country, until further experiments should demonstrate no important differences between, let us say, serum from the eastern states and that from the middle or western states. The interchange of cattle is, however, of more importance than locality in the dissemination of infectious agents that tend to become endemic.

The feeding of milk alone, even from highly immunized cows, is again shown powerless to prevent rapid or delayed death.

Though the outcome of these experiments appears on the surface of little practical value, it may be safely predicted that the encroachment of the slowly developing, insidious infectious agents—such as tuberculosis, contagious pleuro-pneumonia, paratuberculosis, and diseases due perhaps to other still undefined agents—will be more dangerous in the dairy herds of the future as they continue to grow in size. In the thoroughbred Guernsey and Jersey cattle the spread of paratubercu-

losis may eventually compel the use of some procedure which separates the calf from its dam at birth.

CONCLUSION

Under certain safeguards, such as isolation, calves from a large dairy herd have been raised by feeding normal and immune cow serum in place of colostrum. The losses were about one out of ten in the later experiments. This outcome may probably be improved by the subcutaneous injection of serum during the first day. This loss may be no greater than that under ordinary conditions, since sporadic deaths among calves are not infrequent. However, no satisfactory statistics are available for comparison with results as given above.