The Nutritive Value of Colostrum for the Calf

7. Observations on the Nature of the Protective Properties of Colostrum

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The mechanism of the protective action of colostrum in white scours of calves is not understood. Smith & Little (1922) recognized the protective value of the secretion, and Little & Orcutt (1922) demonstrated the passage of agglutinins for *Brucella abortus* from cow to calf in colostrum. Nelson (1924) found a similar transfer of agglutinins for *Bacterium coli* but there was no evidence that these agglutinins were linked with protection. Smith (1930) established that protective antibodies for *Bact. coli* were present in colostrum and that the low content of certain samples was related to the recent entry of a cow into her particular environment; he assumed that such cows had not yet become immunized against the new flora.

The recent work on the antigenic analysis of *Bact. coli* and the evidence that the 'K' antigen-antibody reaction is linked with protection (Briggs, 1951) provided a basis for an immunological study of the protective value of colostrum. This study was made in conjunction with experiments in which newborn calves received various fractions of colostrum as reported in earlier papers of this series (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs & Lovell, 1951*a*, *b*). The essential protective factor against white scours appears to be associated with the lactoglobulin fraction (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949; Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949; Aschaffenburg *et al.* 1951*a*).

The object of this paper is to show that samples of colostrum may contain antibodies against the 'K' antigens of *Bact. coli* and that these antibodies, as in immune serum, are linked with protection.

METHODS

The techniques adopted were similar to those used in the tests with immune serum (see Briggs, 1951), serum being replaced by colostral whey. Samples of colostrum were thawed after removal from storage at $-25°$ and 100 ml. were warmed to blood heat; 3·5 ml. of a 1:10 dilution of a commercial rennet extract were then added. After 10 min. the resulting curd was cut, and the whey was filtered off, bottled and

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stored at −25° until required. Globulin fractions G 2, 4, 5 and 6 and whey fractions WF 2, 4, 5 and 6 were prepared from the same samples of colostrum respectively. Our earlier experiments had shown that a similar degree of protection for the calf was afforded by the globulin-containing whey as by whole colostrum (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949; Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949; Aschaffenburg *et al.* 1951*a*.) In the mouse-protection tests, for each strain tested the quantity of a 20 hr. broth culture that, after intraperitoneal injection, killed about half the mice injected was calculated, and was taken as the LD_{50} dose: this was mostly about 0·12 ml. Mice were inoculated intraperitoneally with the LD_{50} dose of test organisms 2 hr. after injection by the same route of 0·5 ml. of colostral whey diluted 1:5–1:20 according to its titre. The mice were observed for 72 hr. after inoculation, since in preliminary trials no deaths occurred later than this.

RESULTS

Agglutination, agglutinin-absorption and mouse-protection tests. Agglutination and mouse-protection tests showed that whey from colostrum may be examined in the same manner as immune serum (see Briggs, 1951). The 'K' antibody content of colostrum and its protective activity in mice were found to be closely related. Examples with three known strains of *Bact. coli* are given in Table 1. Agglutination tests against suspensions prepared in the three different ways were usually sufficient to determine the presence of a specific 'K' antigen; occasionally, as with serum tests, recourse had to be taken to agglutinin-absorption tests.

Table 1. *Results of agglutination and mouse-protection tests with four samples of colostral whey, and strains of* Bact. coli *recovered from calves (see Briggs, 1951)*

		Colostral whey no.			
Strain no.	Test	1	2	3	4
5(*a*)	'K' ('L') agglutination	–	–	640	–
	'O' agglutination	–	1280	–	–
	Mouse mortality	N.T.	31/70	6/70	40/70
10	'K' ('L') agglutination	–	640	–	–
	'O' agglutination	–	–	320	–
	Mouse mortality	5/10	0/10	4/10	N.T.
25	'K' ('L') agglutination	1280	–	–	–
	'O' agglutination	–	–	40	–
	Mouse mortality	1/10	N.T.	4/10	6/10

In order to differentiate between 'K' ('L') and 'O' agglutination, the suspensions used for agglutination tests were: 1, living, 2, heated at 100° for 1 hr; 3, heated at 120° for 2 hr. The titres in the agglutination tests are expressed as the reciprocals of the dilutions. – = no agglutination at 1:10. Each mouse received one LD_{50} dose of the test organism. The mouse-mortality figures give the mortality as the numerator and the number inoculated as the denominator. N.T. = not tested.

The table shows that colostrum containing agglutinins against the 'K' antigen of a strain protects a high proportion of mice against the corresponding strain of *Bact. coli*, that colostrum containing agglutinins against the 'O' antigen affords little protection, and that a sample containing agglutinins against neither type of antigen has no protective activity. It may be concluded, therefore, that the protective power

of a sample of colostrum against a given strain of *Bact. coli* can be estimated by ascertaining its titre of 'K' antibody.

Bact. coli *as a cause of white scours*. There has been some doubt concerning the role of *Bact. coli* in white scours of calves; evidence of its importance additional to that already presented was obtained by the isolation of pure cultures of *Bact. coli* from the jugular blood of two sick calves, one of which died 36 and the other 48 hr. later. By the same technique, no organisms were recovered from the blood of four normal calves or from one suffering from a transient illness. These calves had been used in experiments mentioned on p. 193 of the paper by Aschaffenburg, Bartlett, Kon, Terry *et al.* (1949).

The mechanism of the protective action of colostrum. An epidemic of white scours occurred during experiments in 1949 designed to assess the significance of lecithin in the diet of the newborn calf. Calves given colostrum and expected to live died, as well as those deprived of colostrum. A total of thirteen calves, eleven of which had received 400 ml. colostrum in their first feed, died within a period of 15 days. The opportunity was taken to examine the strains of *Bact. coli* isolated from these calves and to test the relevant 'K' antibody content of the samples of colostrum used. Examination of the strains of *Bact. coli* recovered from the heart blood and bone marrow at post-mortem showed that possibly no more than three or four serological types were responsible for the epidemic. None of the four samples of colostrum which had been bulked for feeding to the calves contained agglutinins against the relevant 'K' antigens, except possibly in two cases (6*c* and 9*c*) in Table 2, but in 6*c*, the calf had been deprived of colostrum. The details are given in Table 2.

The spread of *Bact. coli* from calf to calf has been demonstrated and it is reasonable to assume, therefore, that surviving calves are exposed to infection with strains that kill calves in the same environment. Evidence of a relationship between the protection of calves by colostrum and the presence in the same colostrum of agglutinins against the 'K' antigens of these particular strains, would support the view that the protective action of colostrum is of a specific immunological character.

The results of calf experiments designed to test the protective value of small quantities of colostral proteins have been reported by Aschaffenburg *et al.* (1951*a*): all calves deprived of colostrum died; a few that had received colostrum or one of its fractions also died (see p. 174 of the paper by Aschaffenburg *et al.* (1951*a*)). An attempt was made in the present study to determine whether the presence of 'K' antibody in the colostrum or its fraction used for feeding was correlated with the survival of the majority of the experimental calves.

Accommodation for experimental calves was limited, and relatively few could be subjected to the different treatments at the same time. Since the types of *Bact. coli* vary in experiments in which fresh calves are continuously being introduced, the fractions of colostrum used for feeding were tested against the strains of *Bact. coli* isolated from dead calves that had lived in and been subjected to the same environment at the same time as those that survived. The relevant data are given in Table 3.

The data in Table 3 show that the respective agglutinin titres of globulin fractions G2, 4, 5 and 6 and whey fractions WF2, 4, 5 and 6 were similar. In general, the

Table 2. *The 'K' and 'O' antigens of strains of* Bact. coli *from fatal cases of disease in calves, and the corresponding antibody content of the samples of colostrum fed*

No. of calf and of strain	Colostrum	Bacterial suspensions	Agglutination tests: Immune serums RR	H	JJ	TT	LLL	Colostral whey no. 1	2	3	4	Interpretation of results: Antigens of strain	Antibody content of colostrum
3*c*	+	1	—	—	—	160	—	—	—	—	—	'K' and 'O'	None observed
		3	—	—	320	—	320	—	—	—	—		
4*c*	+	1	—	—	—	—	—	—	—	—	—	None observed	'O' alone
		3	—	—	—	—	—	1280	—	160	—		
5*c*	+	1	—	—	—	—	—	—	—	—	—	'O'	None observed
		3	—	—	—	320	—	—	—	—	—		
6*c*	o	1	—	—	—	320	320	1280	—	320	—	Probably 'K' and 'O'	Probably 'K' and 'O'
		3	—	—	—	320	320	1280	—	320	—		
7*c*	+	1	—	—	—	320	—	—	—	—	—	'K' and 'O'	None observed
		3	—	—	320	—	320	—	—	—	—		
8*c*	+	1	—	—	—	—	—	—	—	—	—	None observed	'O' alone
		3	—	—	—	—	—	2560	—	160	—		
9*c*	+	1	—	—	—	320	160	640	—	80	—	Probably 'K' and 'O'	Probably 'K' and 'O'
		3	—	—	—	320	320	1280	—	80	—		
10*c*	+	1	—	—	—	320	—	—	—	—	—	'K' and 'O'	None observed
		3	—	—	320	—	160	—	—	—	—		
11*c*	+	1	—	—	—	—	—	—	—	—	—	'O'	None observed
		3	—	—	—	320	—	—	—	—	—		
12*c*	o	1	—	—	—	—	—	—	—	—	—	'O'	None observed
		3	—	—	—	320	—	—	—	—	—		
13*c*	+	1	—	—	—	—	—	—	—	—	—	None observed	None observed
		3	—	—	—	—	—	—	—	—	—		
14*c*	+	1	—	—	—	320	—	—	—	—	—	'K' and 'O'	None observed
		3	—	—	320	—	160	—	—	—	—		
15*c*	+	1	—	—	—	—	—	—	—	—	—	None observed	'O' alone
		3	—	—	—	—	—	1280	—	80	—		

The suspensions used for agglutination tests were: 1, living; and 3, heated at 120° for 2 hr. Suspensions heated at 100° for 1 hr. were not used and the type of 'K' antigen ('L' or 'A') was therefore not determined. Serums were diluted from 1:10 to 1:320 and colostrum from 1:10 to 1:2560; the titres in the agglutination tests are expressed as the reciprocals of the dilutions: — = no agglutination at 1 in 10. + = calf was fed colostrum samples 1, 2, 3 and 4 bulked. o = calf did not receive colostrum.

fraction of colostrum given to a surviving calf possessed agglutinins against the 'K' antigens of the strains of *Bact. coli* against which it was tested. The titres were relatively low, possibly because the cows providing the colostrum were not the dams of the calves used nor were they necessarily from the same herd.

Table 3. *The agglutination of strains of* Bact. coli *from dead calves by fractions of colostrum given to surviving calves exposed at the same time to similar environmental conditions*

No. of calf and of strain	Disease and age of calf at death	Colostrum	Bacterial suspensions	Agglutination tests: Fractions of colostrum: Globulin: G 2, 4, 5 and 6	Whey: WF 2, 4, 5 and 6	Whey: Col PP 4, 5 and 6	Interpretation of results
23*d*	Typical, 2 days	o	1	40	80	N.T.	'K' ('L') agglutinins
			2	—	—	N.T.	
			3	—	—	N.T.	
24*d*	Atypical, 4 days	o	1	20	40	N.T.	'K' ('L') agglutinins
			2	—	—	N.T.	
			3	—	—	N.T.	
26*d*	Atypical, 7 days	+:G	1	40	80	N.T.	'K' ('L') agglutinins
			2	—	—	N.T.	
			3	—	—	N.T.	
29*d*	Atypical, 5 days	+:WF	1	10	80	N.T.	'K' ('A') agglutinins
			2	20	80	N.T.	
			3	—	—	N.T.	
30*d*	Typical, 2 days	o	1	80	160	N.T.	'K' ('A') agglutinins
			2	80	80	N.T.	
			3	—	—	N.T.	
31*d*	Atypical, 5 days	+:G	1	80	320	N.T.	'K' ('A') agglutinins
			2	80	160	N.T.	
			3	—	—	N.T.	
32*d*	Typical, 6 days	o	1	160	320	N.T.	'K' ('A') agglutinins
			2	160	320	N.T.	
			3	—	—	N.T.	
34*d*	Typical, 21 days	o	1	—	—	160	'K' ('L') agglutinins
			2	—	—	—	
			3	—	—	—	
36*d*	Atypical, 9 days	+:G	1	160	160	320	'K' ('A') agglutinins
			2	80	160	320	
			3	—	—	—	
38*d*	Typical, 9 days	o	1	—	—	80	'K' ('L') agglutinins
			2	—	—	—	
			3	—	—	—	
39*d*	Typical, 12 days	o	1	—	—	160	'K' ('L') agglutinins
			2	—	—	—	
			3	—	—	—	
40*d*	Typical, 14 days	+:WF	1	320	160	320	'K' ('L') agglutinins
			2	—	—	—	
			3	—	—	—	

The disease from which a calf died is referred to as: Typical = typical white scours with *Bact. coli* septicaemia. Atypical = colibacillosis with peritonitis and pleurisy. o = calf did not receive colostrum: control. +:G = calf received colostral fraction—globulins. +:WF = calf received colostral fraction—whey WF 2, 4, 5, 6. The suspensions used for agglutination tests were: 1, living; 2, heated at 100° for 1 hr.; 3, heated at 120° for 2 hr. The titres in the agglutination tests are expressed as the reciprocals of the dilutions. — = no agglutination at 1:10. N.T. = not tested.

Of the twelve strains of *Bact. coli* used in the tests, seven were from calves that had not received colostrum or one of its fractions; five were from calves that had received such a fraction. Six of the seven calves that had been deprived of colostrum died from typical white scours with a *Bact. coli* septicaemia; four of the five calves receiving a colostral fraction died from a colibacillosis in which there was a marked fibrinous reaction on the pleura and peritoneum. This latter type of colibacillosis might indicate some increased resistance on the part of the individual calf. The evidence is not conclusive, but there appears to be some relationship between the agglutinins against 'K' antigens in the fraction of colostrum and a possible increase in resistance in calves that received it.

DISCUSSION

The value of colostrum to the newborn calf and the possible mechanism of its protective action have been considered in earlier papers (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949; Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949; Aschaffenburg *et al.* 1951*a*). Briggs (1951) showed that most of the strains of *Bact. coli* isolated from calves with white scours possess 'K' antigens, and that these antigens are linked with pathogenicity. Whatever other causes may be involved in this disease, *Bact. coli* is usually the killing factor.

Since 'K' antibody in colostrum appears to be the factor that protects mice against experimental infection, it should be possible to determine the efficacy of a sample of colostrum against a particular strain of *Bact. coli* by agglutination tests. It was shown by this method that colostrum given to calves that died in an epidemic of white scours was deficient in agglutinins against the 'K' antigens of the strains responsible for the deaths of the calves.

In a further experiment, most of the calves receiving colostrum that contained agglutinins against relevant 'K' antigens survived exposure to *Bact. coli* which killed control calves not given colostrum in the same environment. In these experiments the evidence is not complete, but it is suggestive of the immunological nature of the protective action of colostrum. Of the twelve calves supplying the strains of organism, five received a fraction of colostrum containing the relevant agglutinins; these calves were expected to live. Four of them died from an unusual type of colibacillosis, in which there was a marked pleurisy and peritonitis; it is suggested that these showed a resistance greater than that of the control calves.

The evidence is strongly in favour of the immunological, and therefore specific, nature of the protective action of colostrum. Our earlier observation that a very small quantity of colostral protein has a marked protective effect for calves (Aschaffenburg *et al.* 1951*a*) also supports this view. Work on the immunological aspects of the problem is continuing. It would be unwise to assume, on the evidence so far available, that agglutinins against the 'K' antigens of the strains of *Bact. coli* concerned represent the only factor of importance in the protection of calves by colostrum.

SUMMARY

1. Whey samples prepared from colostrum were examined for agglutinins against the 'K' and 'O' antigens of *Bact. coli*, and those samples containing agglutinins against the 'K' antigens protected mice against the corresponding strains of *Bact. coli*.

2. Samples of colostrum given to calves that died in an epidemic of colibacillosis did not contain agglutinins against the 'K' antigens of the strains of *Bact. coli* isolated from the dead calves.

3. In another experiment, protein fractions of colostrum given to fifteen calves that survived contained, in most cases, demonstrable agglutinins against the 'K' antigens of strains of *Bact. coli* recovered from contemporary calves that had died in the same environment.

4. The evidence at the moment is incomplete, but it supports the contention that the protective mechanism of colostrum is largely an immunological one and that it is associated, in colibacillosis, with the possession of antibodies to the 'K' antigens of the infecting strains of *Bact. coli*.

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