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The Nutritive Value of Colostrum for the Calf

6. The 'K' Antigens of *Bacterium coli**

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Smith & Little (1922) drew attention to the protective properties of colostrum against white scours of calves. Since 1946 members of the staffs of the Department of Pathology of the Royal Veterinary College and of the National Institute for Research in Dairying, University of Reading, have collaborated in experiments planned to assess the relative importance of the nutritional and the immunological properties of colostrum for the calf, and have published their findings in several papers (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). These workers showed that small quantities of colostrum, of its aqueous fraction and of globulin constituents, protected calves against white scours; the indication was that the mechanism of such protection might be of an immunological nature. The work reported in the

* This work formed part of a thesis submitted to the University of Reading for the degree of Ph.D., and the substance of it was read at a meeting of the Pathological Society on 6 January 1950.

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present paper was undertaken to provide a basis for an investigation of the immunological aspects of the protective action of colostrum. The results of that investigation are recorded in a subsequent paper (Briggs, Lovell, Aschaffenburg, Bartlett, Kon, Roy, Thompson & Walker, 1951).

White scours is frequently associated with *Bacterium coli* infection, and several workers believe that certain races of *Bact. coli* are potentially pathogenic for calves. Jensen (1892–3), Smith & Little (1922) and Smith & Orcutt (1925) based their conclusion largely on the classification of strains by biochemical reactions. Lovell (1937) reached a similar conclusion by classification on serological grounds. His precipitin tests with serums and extracts of strains of *Bact. coli* that showed grey-mucoid variation gave unequivocal results, but agglutination tests indicated the presence of two separate antigens apart from a flagellar one; these were associated with the body of the bacterium and the capsule respectively.

The antigens of *Bact. coli* have been studied by Knipschildt (1945), Vahlne (1945) and Kauffmann (1947), and a serological classification of the *coli* group has been established. Apart from the 'O' or somatic antigens there are two main 'K' or 'capsular' antigens, the 'A' antigens and the 'L' (or 'B') antigens. 'A' antigens are thermostable and responsible for 'O'-inagglutinability of suspensions of living bacteria and of those heated at 100° for 1 hr.; they are destroyed by heating to 120° for 2 hr. 'A' strains produce mucoid colonies of capsulated bacteria. 'L' (or 'B') antigens are thermolabile and responsible for 'O'-inagglutinability of suspensions of living bacteria; they are destroyed by heating to 100° for 1 hr. 'L' strains produce grey colonies of non-capsulated bacteria. 'B' antigens closely resemble 'L' antigens but are very rare.

Kauffman (1947) considers that it is possible to differentiate between 'L' and 'A' antigens by the determination of 'O'-inagglutinability and that the rare 'B' antigens can be largely ignored. In the light of these observations a study has been made of the 'K' antigens of *Bact. coli* isolated from calves to determine their significance in the pathogenesis of white scours.

METHODS

Source of strains. The calves that died of white scours in the experiments of Aschaffenburg, Bartlett, Kon, Terry *et al.* (1949) and Aschaffenburg, Bartlett, Kon, Walker *et al.* (1949) constituted the main source of the strains of *Bact. coli* studied in this investigation. Six cases of naturally occurring white scours were also obtained for purposes of comparison. The majority of the strains were recovered from the heart blood and bone marrow of the calves.

Cultivation of strains. Cultures were made initially on blood agar and McConkey agar plates, after which a preliminary identification of the strains was made, based on their biochemical reactions. The strains were preserved in wax-sealed stabs of 0·5 % agar at room temperature in the dark, and later by freeze-drying.

Immune serums. These were prepared in rabbits by a series of injections of 0·25 % formalinized suspensions of mucoid or grey cultures. The strains were from representative experimental calves and the relevant data are given on the next page.

Serum reference	No. of calf and of strain	Type of strain	Type of colony*	Presence of capsules (microscopic examination)†
RR	28	*Bact. coli* intermediate type II	M	+(−)
H	4	*Bact. aerogenes*	M	+(−)
JJ	23	*Bact. coli* type I	M	+(−)
TT	29	*Bact. coli* type I	M	+(−)
LLL	2A	*Bact. coli* type I	G	−

* M = mucoid; G = grey. † (−) = a very few organisms did not show capsules.

Precipitin tests were made by mixing immune serum with alkali extracts of strains as prepared by Smith (1927).

Agglutination tests. Three suspensions were prepared for differentiating between 'L', 'A' and 'O' agglutination:

(1) Living suspensions prepared from 20 hr. growth on thick, dry, plain agar; this largely suppresses the development of flagellar antigens; at the same time it promotes the development of 'K' antigens (Kauffmann, 1947).

(2) The same suspensions heated at 100° for 1 hr.

(3) Living suspensions heated at 120° for 2 hr.

The serum dilutions used were 1:10–1:5120, and the tubes were incubated at 37° for 18 hr.

Agglutinin-absorption tests. Absorption of serums with thick suspensions of bacteria was made at 4° overnight; the absorbed supernatant fluid was tested against the same bacterial suspensions that were used in the agglutination tests.

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 injected intraperitoneally with 0·5 ml. of a 1:5–1:20 dilution of serum, depending on the agglutinin content of the sample; one LD_{50} of the test strain was similarly given 2 hr. later. The mice were observed for 72 hr. after inoculation, since in preliminary trials no deaths occurred later than this.

RESULTS

Biochemical types of strain. On the basis of biochemical tests most strains were classified as *Bact. coli* type I: a few strains of *Bact. coli* intermediate type II and of *Bact. aerogenes* were also recovered from cases of white scours.

Precipitin tests. Sixty-two strains were submitted to the precipitin test against each of the five samples of immune serum listed above: forty-nine (79 %) reacted with one or other of the serums, as shown below, thirteen strains remaining unclassified.

	Un-classified	Reacting with serum					Total
		RR	H	JJ	TT	LLL	
No. of strains	13	5	4	12	22	6	62

Agglutination tests. Agglutination tests were made with 102 strains of colibacteria and the relationship between the precipitin reaction and the agglutination of a particular

suspension was noted. Some of the strains examined were recovered from the same calf. In general, those labelled (*a*) (e.g. 32(*a*)) were from the blood and bone marrow, and presumably had invasive characteristics. Those labelled (*b*) (e.g. 32(*b*)) were mostly from the intestinal tract or mesenteric lymph nodes. The results for twenty strains are given in Table 1; information concerning haemolytic activity and the type of colony is included.

The interpretation of the agglutination results is based on the reactions obtained with the different suspensions. For example, the agglutination by a particular serum of living suspensions and of those heated to 100° but not of those heated to 120° suggests that the agglutination observed was due to the presence of the thermostable 'A' antigen and its corresponding antibody. The 'L' antigen is thermolabile and its inhibitory effect on 'O' agglutination disappears when suspensions are heated at 100° for 1 hr. The results obtained with strains 3A and 28 are examples of 'A' agglutination and those with strains 17 and 19 of 'L' (or 'B') agglutination.

When one serum agglutinates all three suspensions of a strain to approximately similar titres, agglutinin-absorption tests are necessary in order to distingush between 'K' ('capsular') and 'O' (somatic) agglutination reactions. In some instances with suspensions heated to 120°, agglutinins against the 'O' antigen were in this way completely removed, whereas those against living suspensions and suspensions heated to 100° were left intact: this demonstrated 'A' agglutination. In other instances, absorption with similarly treated suspensions removed agglutinins also against suspensions heated to 100°, thereby indicating that the 'L' antigen and its antibody were involved. Where a suspension heated to 120° proved capable of preventing agglutination by a serum of living and heated suspensions of the strain it was concluded that the test strain did not contain the relevant 'K' antigen, and that the agglutination was in all instances due to the 'O' antibody of the serum.

The relationship between the results obtained by the precipitin technique and the agglutination of the 'K' antigens (either 'L' or 'A') will be discussed later.

Mouse-protection tests. It is impracticable to give here full details of the numerous mouse-protection tests made; they all gave similar results, and typical examples are given in Table 2. The separate total mortality figures for mice receiving serums (*a*) with 'K', (*b*) with ('O'), and (*c*) without 'K' or 'O' antibodies were 3/60, 31/80 and 20/40 respectively. These values were examined by the χ^2 test, incorporating Yates's (1934) correction for continuity; no significant difference at the 5 % level was found between the results of treatments (*b*) and (*c*), but the effects of treatment (*a*) proved highly significantly ($P < 0{\cdot}001$) different from those of either (*b*) or (*c*). These results showed that serums with the relevant 'K' antibody ('L' or 'A' agglutination) conferred a high order of protection, whereas serums lacking 'K' antibody, whether or not containing 'O' antibody ('O' agglutination or no agglutination), conferred no protection. It should be remembered that the test dose of organisms was calculated to kill half the mice inoculated.

Table 1. *Results of precipitation and agglutination tests with twenty strains of* Bact. coli *recovered from cases of white scours*

No. of calf and of strain, and type	Type of colony	Serological tests: Haemolysis	Antigenic substance	Serums: RR	H	JJ	TT	LLL	Interpretation of results
17, *Bact. coli* I	G	−	Ext.	−	−	+	−	−	Precipitation
			Susp. 1	−	−	1280	−	−	'L' agglutination
			2	320	−	−	640	−	'O' agglutination
			3	640	−	−	1280	−	'O' agglutination
19, *Bact. coli* I	G	−	Ext.	−	−	−	−	+	Precipitation
			Susp. 1	−	−	−	−	640	'L' agglutination
			2	−	−	−	1280	−	'O' agglutination
			3	−	−	−	1280	−	'O' agglutination
8, *Bact. coli* I	G	−	Ext.	−	+	−	−	−	Precipitation
			Susp. 1	−	1280	−	−	−	'L' agglutination
			2	−	−	−	−	−	—
			3	−	−	−	−	−	—
30, *Bact. coli* I	G	+	Ext.	−	−	−	−	+	Precipitation
			Susp. 1	−	−	−	−	2560	'L' agglutination
			2	−	−	−	640	−	'O' agglutination
			3	160	−	−	1280	−	'O' agglutination
3A, *Bact. coli* I	G	−	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	80	−	−	1280	−	'A' agglutination
			2	80	−	−	640	−	'A' agglutination
			3	2560	−	−	−	−	'O' agglutination
8A, *Bact. coli* I	G	−	Ext.	−	−	+	−	−	Precipitation
			Susp. 1	−	−	1280	−	−	'L' agglutination
			2	−	−	−	1280	−	'O' agglutination
			3	−	−	−	2560	−	'O' agglutination
12, *Bact. coli* I	G	−	Ext.	−	−	+	−	−	Precipitation
			Susp. 1	−	−	2560	−	−	'L' agglutination
			2	−	−	−	−	20	'O' agglutination
			3	−	−	−	−	40	'O' agglutination
22, *Bact. coli* I	G	−	Ext.	−	−	−	−	+	Precipitation
			Susp. 1	−	−	−	−	640	'L' agglutination
			2	−	160	−	2560	−	'O' agglutination
			3	−	80	−	2560	−	'O' agglutination
5A, *Bact. coli* I	G	−	Ext.	−	−	+	−	−	Precipitation
			Susp. 1	−	−	1280	−	−	'L' agglutination
			2	−	−	−	2560	−	'O' agglutination
			3	−	−	−	2560	−	'O' agglutination
28, *Bact. coli* I	M	−	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	2560	−	'A' agglutination
			2	−	−	−	2560	−	'A' agglutination
			3	160	−	−	−	−	'O' agglutination
32(*a*), *Bact. coli* I	M	−	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	2560	80	'A' agglutination
			2	−	−	−	2560	160	'A' agglutination
			3	−	−	−	−	1280	'O' agglutination
32(*b*), *Bact. coli* I	G	+	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	640	−	'A' agglutination
			2	−	−	−	640	−	'A' agglutination
			3	160	−	−	1280	−	'O' agglutination
33(*a*), *Bact. coli* I	G	−	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	1280	80	'A' agglutinatio
			2	−	−	−	1280	80	'A' agglutination
			3	−	−	−	−	2560	'O' agglutination
33(*b*), *Bact. coli* I	M	+	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	640	−	'A' agglutination
			2	−	−	−	1280	−	'A' agglutination
			3	160	−	−	1280	−	'O' agglutination
34(*a*), *Bact. coli* I	G	−	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	1280	−	'A' agglutination
			2	−	−	−	1280	160	'A' agglutination
			3	−	−	−	−	2560	'O' agglutination
34(*b*), *Bact. coli* I	M	+	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	640	−	'A' agglutination
			2	−	−	−	2560	−	'A' agglutination
			3	80	−	−	2560	−	'O' agglutination
35(*a*), *Bact. coli* I	G	−	Ext.	−	−	+	−	−	Precipitation
			Susp. 1	−	−	640	−	−	'L' agglutination
			2	−	−	−	1280	−	'O' agglutination
			3	−	−	−	2560	−	'O' agglutination
35(*b*), *Bact. coli* I	G	+	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	1280	−	'A' agglutination
			2	−	−	−	1280	−	'A' agglutination
			3	80	−	−	1280	−	'O' agglutination
4A, *Bact. coli* intermediate type II	G	+	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	1280	−	'A' agglutination
			2	−	−	−	1280	−	'A' agglutination
			3	−	−	−	−	−	—
14, *Bact. coli* intermediate type I	G	+	Ext.	−	−	−	+	−	Precipitation
			Susp. 1	−	−	−	640	−	'A' agglutination
			2	−	−	−	1280	−	'A' agglutination
			3	−	−	−	−	−	—

G = grey; M = mucoid. Ext. = alkali-alcohol extract for precipitin test. Susp. = suspensions for agglutination tests: 1, living; 2, heated, 100° for 1 hr.; 3, heated, 120° for 2 hr. The results of the precipitin tests are expressed as + or −. The titres in the agglutination tests are expressed as the reciprocals of the dilutions; − = no agglutination at 1:10.

Table 2. *Relationship between mouse-protection tests and serological tests in vitro*

Strain no.	Test	Serums RR	H	JJ	TT	LLL
5A	Precipitation	–	–	+	–	–
	'L' agglutination	–	–	1280	–	–
	'O' agglutination	–	–	–	2560	–
	Mouse mortality	N.T.	N.T.	1/20	6/20	10/20
12	Precipitation	–	–	+	–	–
	'L' agglutination	–	–	2560	–	–
	'O' agglutination	–	–	–	–	640
	Mouse mortality	N.T.	N.T.	2/20	12/20	4/20
28	Precipitation	–	–	–	+	–
	'A' agglutination	–	–	–	2560	–
	'O' agglutination	160	–	–	–	–
	Mouse mortality	9/20	N.T.	N.T.	0/20	10/20

The results of the precipitin tests are expressed as + or –. The titres in the agglutination tests are expressed as the reciprocals of the dilutions. Each mouse received one LD_{50} dose of the test organism. The mouse-mortality figures give the mortality in the numerator and the number inoculated as the denominator. – = no agglutination at 1:10; N.T. = not tested.

DISCUSSION

The results show a close relationship between the precipitin reaction and the agglutination of the 'K' antigen of a strain of *Bact. coli*. A similar observation has been recorded by Giles & Sangster (1948) who worked with strains of *Bact. coli* recovered from cases of infantile diarrhoea. Some of their tests were made with strains producing grey colonies (see Lovell, 1937), and the 'K' antigen involved was probably the 'L' type as distinct from the capsular 'A'. The results of protection tests in mice showed that a serum containing the precipitin and the antibody against the 'K' antigen of a given strain of *Bact. coli* protected mice infected with that strain.

Precipitation and agglutination tests permit a classification of colibacteria; they are of value in epidemiological inquiries and indicate the probable spread of strains of *Bact. coli* from calf to calf during an epidemic of white scours. For example, the non-haemolytic *Bact. coli* strains, nos. 32(*a*), 33(*a*) and 34(*a*), were recovered from calves that lived in the same environment and died within a few days of each other: these strains possessed similar antigens and were presumably one and the same strain. Similarly, a series of haemolytic strains, 32(*b*), 33(*b*), 34(*b*) and 35(*b*), was recovered from the same group of calves and these too were serologically identical. The passage of strains from calf to calf in an enclosed community was thus demonstrated. In random cases of white scours occurring in the field, conditions are different; strains recovered from such cases were found to be serologically unrelated. These observations suggest that outbreaks of white scours are associated with the multiplication of special races of *Bact. coli*, which develop their pathogenicity under suitable conditions.

SUMMARY

1. Strains of *Bact. coli* were recovered from calves that had suffered from white scours. Serological examination provided evidence that the 'K' antigens and the pathogenic activities of strains were related.

2. There was a close relationship between the results of 'K' antigen-antibody agglutination tests, precipitin tests using as antigens alkali extracts of strains, and protection tests in mice.

3. Serological classification of coliform organisms demonstrated the spread of potentially pathogenic strains from calf to calf during epidemics of white scours.

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