BLOOD GROUP ACTIVITY OF GRAM-NEGATIVE BACTERIA*

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The occurrence in bacteria of substances possessing a close serological relationship to A, B, and H(O) erythrocyte agglutinogens was first shown unequivocally by Schiff (1) for one strain of *Shigella shigae*. He grew this bacterium on a chemically fully defined medium free of demonstrable blood group activity. Other Gram-negative bacteria have been reported by Japanese and German workers to be related serologically to human blood groups A, B, and H(O) (cf. references 2, 3), but the preexistence of blood group active substances in the growth medium used by these investigators is almost certain. More recent studies in this laboratory (4) indicated that several different bacteria grown in a chemically fully defined medium exhibited specific blood group A, B, or H(O) activity. A more extensive study of this subject appeared desirable because of the close contact between Gram-negative bacteria and human beings and the possible relation of anti-A and anti-B isohemagglutinin formation to these bacteria. Such an investigation is especially indicated since it has been shown that anti-A and anti-B heterohemagglutinins may be induced by bacteria (5, 6) and that "hyperimmune" blood group agglutinins may be instrumental in the inhibition (5) and even killing (7) of blood group active bacteria.

A correlation between serological specificity and chemistry of blood group active bacteria may further the understanding of the chemical basis for the specificity of human blood group substances.

The present paper reports data obtained during the past 7 years on the blood group activity of 282 Gram-negative bacteria. An attempt is made to relate the blood group activity of some of these bacteria with their content of those monosaccharides which are also building-stones of human A, B, and H(O) blood group mucoids. The data to be presented emphasize the presence

of similar serological and chemical structures in groups as widely separated phylogenetically as bacteria and man.

Materials and Methods

Bacteria.—Gram-negative bacteria were isolated by standard bacteriological procedures from patients' specimens sent for routine analysis to the University Hospital's Bacteriological Laboratory. A random selection was desired, but over the past $1\frac{1}{2}$ years Gram-negative organisms isolated from the blood of patients have been collected predominantly. Such cultures occurred as less than 0.3 per cent of all routine specimens. Furthermore, attempts were made to increase the number of strains tested in those genera from which we had isolated too few organisms to be representative.

Many blood group active strains were sent to Dr. P. R. Edwards and Dr. W. H. Ewing, Communicable Disease Center, Chamblee, Georgia, to check our identification as to species and serotype. In addition, bacterial cultures of known species and serotype were obtained from Dr. Edwards, the American Type Culture Collection in Washington, D. C., Dr. E. Thal, Stockholm, and other laboratories. Strains of the genera *Salmonella, Shigella, Arizona,* and *Pasteurella* were obtained predominantly from outside sources. Most of the organisms studied were Enterobacteriaceae which were classified according to Kauffmann (8), (with the provision that no attempt was made to identify the genus *Cloaca* as a separate entity), but strains of the Pseudomonadaceae, Brucellaceae, and Achromobacteraceae, as well as one strain of *Bacterium anitratum* were also tested.

Preparation of Bacteria.—The smoothness and purity of all cultures were confirmed immediately before use. Test tubes containing 10 ml. of tryptose phosphate broth were inoculated from the fresh agar slants of the organism to be studied. Eight successive subcultures were then made with a loop (diameter 3.5 mm.) into 10 ml. of a fully defined vitamin- and amino acid–free glucose ammonium chloride medium, shown to be devoid of blood group A, B, and H(O) active substances (4). When an occasional strain did not grow in this basal medium, yeast extract (bacto difco) was added in a final concentration of 0.1 per cent. This autolysate did not show any blood group A, B, or H(O) activity when tested at a concentration of 20 mg./ml. or less. Each culture was incubated for 16 to 24 hours at 37°C. before the subsequent transfer. A final subculture was made into two 50 ml. tubes of the same medium. The bacteria were grown approximately 40 hours, then harvested by centrifugation. The sedimented cells were resuspended in distilled water and heated on a boiling water bath for $2\frac{1}{2}$ hours to remove the possible masking effect of heat-labile antigens (8). The final concentration of bacteria was adjusted to a dry weight of 0.5 to 1.5 per cent.

Solutions.—The diluent and the erythrocyte-suspending solution in all tests were 0.85 per cent saline, containing 0.025 M phosphate buffer, pH 7.2.

Erythrocytes.—Human A_1, A_2, B, and H(O) erythrocytes, stored for no more than 2 weeks in one-third volume "anticoagulant acid citrate dextrose" solution (1.47 per cent glucose, 1.32 per cent sodium citrate, 0.44 per cent citric acid) at 1–3°C., were employed. They were obtained from 2 donors for each group. Erythrocytes were added in a 0.5 per cent suspension after 3 saline washings.

Antisera.—Human anti-A and anti-B sera, both "natural" and immune, were employed. The latter were prepared by immunization of volunteers with purified blood group substances of hog or horse origin. Eel serum containing potent anti-H(O) agglutinins, after absorption with human A_1B erythrocytes, was used in the agglutination of group O erythrocytes.

A limited amount of human anti-H (Helen Porcano) and rabbit anti-H(O) agglutinins (prepared by injecting human H(O) mucoid conjugated with the protein component of Shiga O antigen) was also used where indicated.

Serological Procedures.—Blood group activity was measured with the hemagglutination inhibition test as previously described (9, 4). This assay was used as a routine, instead of direct bacterial agglutination or absorption of sera, because of its higher sensitivity and greater economy. On occasion, these latter procedures as well as animal immunization have been used as is discussed below. All reagents were employed in 0.1 ml. volume. In brief, the testing procedure was as follows: Twofold serial dilutions of bacterial suspensions were made using a different 0.1 ml. serological pipette for each tube in a titration series. The titrated suspensions were shaken and incubated with 4 to 8 minimum hemagglutinating doses of anti-serum for 2 hours at room temperature (22–26°C.). The appropriate erythrocytes were then added, and the test read microscopically after a further 1 to 2 hours' incubation period at room temperature. Each titration series included controls consisting of a serum standard, diluted to 4 to 8 minimum hemagglutinating doses and then titrated in twofold geometrical dilutions, and a saline suspension of the appropriate erythrocytes. L-Fucose was included as standard in the assays with eel serum. Active materials were tested three or more times.

Bacterial suspensions which did not inhibit at concentrations of 2.5 mg./ml. or less were considered to be inactive. Under the conditions of the present experiments, specificity and reproducibility were found to be wanting at concentrations of more than 5 mg./ml. of inhibitor or less than 4 minimum hemagglutinating doses of serum. Activities are expressed in terms of dilution of the inhibiting material *before* addition of serum and erythrocyte suspension. Final concentrations of inhibitor are obtained by dividing the given values by 3.

Numerous bacteria have also been tested for their ability to inhibit erythrocyte agglutination by anti-D (Rh$_o$), anti-M, and anti-N agglutinins. Agglutinins and red cells used were described previously (10). As anti-N reagent, an extract of *Vicia graminea* seeds grown in this laboratory was used exclusively.

RESULTS

Table I lists bacteria tested for A$_1$, B, and H(O) blood group activity. Almost 50 per cent of the strains examined inhibited anti-human blood group A, B, H(O) agglutinins at concentrations considered to indicate activity. Table I also shows that bacteria with only 1 specificity far outnumber those with 2 or all 3 blood group activities. The activities of a given strain often varied somewhat quantitatively for different batches but not qualitatively.

The most commonly observed blood group specificities were H(O) (against eel serum) and B, either alone or in combination. They predominated in all genera which contained active strains. Thirty-one additional strains of *E. coli* not listed in the tables were tested for A activity exclusively. Only 2 of these strains showed A activity (low).

Tables II and III divide the active bacteria into 2 arbitrary classes, the 10 per cent which were found to be highly active (Table II) and those bacteria (about 38 per cent) of moderate to low activity (Table III). The serotype is given whenever it has been determined. Inactive bacteria of known serotype are listed in Table IV.

As Table II shows, the highest activities were observed among the B specific bacteria. Different serotypes in 6 different genera completely inhibit 4 hemagglutinating doses at concentrations of between 0.5 to 10 µg. of added bacterial suspension; *i.e.*, about 2 to 35 µg./ml. final concentration. Although in general

somewhat less active, 6 different serotypes which inhibited completely at concentrations at or below the 35 μg./ml. final concentration level were found among the H(O) specific bacteria. None of the A active species was active at this low concentration.

Twenty strains of *E. coli* O_{86} were tested, 1 with unknown K antigen; 16 had the *E. coli* B antigen 7, one B_9, and 2 had no K antigen. One *E. coli* O_{86} without K antigen was isolated from the blood of a patient. Two cultures of *E. coli* O_{86}:B_7 were grown by us from the stools of diarrheic infants and 1 from

TABLE I

Distribution of Blood Group Activity among Bacteria Tested

Genus	Strains tested*	One activity			Several activities				Inactive
		A_1	B	H(O)	A_1BH(O)	A_1B	A_1H(O)	BH(O)	
Escherichia	135	8	18	22	6	3	3	4	71
Salmonella	19	1	2	9	0	0	1	0	6
Arizona	3	0	1	1	0	0	0	1	0
Klebsiella	42	2	6	4	3	1	1	5	20
Citrobacter	24	2	2	2	0	2	2	3	11
Pasteurella	8	0	1	0	0	0	0	2	5
Proteus	20	0	6	2	0	0	0	1	11
Pseudomonas	15	1	1	2	0	0	1	0	10
Serratia	2	0	0	2	0	0	0	0	0
Alcaligenes	8	0	0	0	0	0	1	1	6
Shigella	5	0	0	0	0	0	0	0	5
Herrellea	1	0	1	0	0	0	0	0	0
	282	14	38	44	9	6	9	17	145

* The possibility of having listed the same organism isolated from different patients has been excluded only in those cases in which the serotype has been determined.

the stool of a patient with hemolytic anemia. All strains had similar B and, occasionally, low H(O) activity; however, the single strain with B_9 antigen possessed somewhat higher H(O) activity.

The most highly H(O) active bacterium found was *E. coli* O_{128}. Among 2 strains of *E. coli* O_2, one (13030) was of significant H(O) activity, while the other (O_2:K_1) was inactive (Tables II and IV).

Three different strains of *E. coli* O_{111} showed low B and H(O) activity.

Nine strains of *S. poona*, 5 strains of *S. grumpensis*, and 1 strain each of *S. atlanta* and *S. worthington* were tested (Tables II and III); all strains of these serotypes exhibited quantitatively and qualitatively identical activities. All these serotypes share O antigen 13. There was no difference in activity among those serotypes which contained either O antigen 22 or 23 besides O

TABLE II
Bacteria with High Blood Group Activity

Bacterium	O-antigen (when known)	Minimum amount of boiled bacteria giving complete hemagglutination inhibition*		
		A_1	B	H(O)
		mg./ml.	mg./ml.	mg./ml.
E. COLI				
13030	2	n.a.	n.a.	0.3–2.5
Edwards, L.	7	0.3	1.2	2.5
20 strains	86	n.a.	0.005–0.1	0.6–±2.5
	127	n.a.	n.a.	0.3–1.2
	128	n.a.	n.a.	0.04–0.1
8399		n.a.	0.3–0.6	n.a.
Ivey		0.3	n.a.	n.a.
Hunter		0.3	1.2	0.6
SALMONELLA				
poona (9 strains)	13,22	n.a.	n.a.	0.1
grumpensis (5 strains)	13,23	n.a.	n.a.	0.1
atlanta	13,23	n.a.	n.a.	0.1
berkeley	43	n.a.	0.1	n.a.
ARIZONA				
	9	n.a.	n.a.	0.3–0.6
	21	n.a.	0.02–0.08	n.a.
KLEBSIELLA‡				
	10	0.6	0.3	n.a.
Scott (Ca 484)	63	n.a.	0.1	1.2
4682		2.5	n.a.	0.1–0.2
1598; 1610		0.3–1.2	0.1	1.2
CITROBACTER				
13286		n.a.	n.a.	0.05–0.3
755		0.3	n.a.	2.5
PROTEUS				
mirabilis (Ca 437)		n.a.	0.1–0.2	n.a.
vulgaris (Ca 439)		n.a.	0.05	n.a.
9554		n.a.	0.1–1.2	n.a.
PSEUDOMONAS				
761		n.a.	n.a.	0.3–0.6
741		0.3	n.a.	n.a.
SERRATIA				
marcescens (airborne)		n.a.	n.a.	0.3
HERRELLEA				
Bact. anitratum (3577)		n.a.	0.1	n.a.

n.a., no activity as defined in text.

* Inhibitor concentration before dilution with serum and erythrocyte suspension. For final dilution divide values by 3.

‡ Capsular antigens listed.

TABLE III*

Bacteria with Moderate to Low Blood Group Activity

Bacterium	O-antigen (when known)	Minimum amount of boiled bacteria giving complete hemagglutination inhibition		
		A₁	B	H(O)
		mg./ml.	*mg./ml.*	*mg./ml.*
E. COLI				
703; 12415	4	2.5	n.a.	±2.5
	6	n.a.	±2.5	2.5
	7:K₁	n.a.	2.5	n.a.
10716	8	n.a.	2.5	2.5
3 strains	111:B₄	n.a.	1.2–±2.5	±2.5
	125	n.a.	n.a.	1.2
	126	n.a.	n.a.	±2.5
7 strains		0.6–2.5	n.a.	n.a.
16 strains		n.a.	0.6–±2.5	n.a.
17 strains		n.a.	n.a.	0.6–±2.5
1955; 14013; Casey		1.2–2.5	2.5	n.a.
2030; 9069		1.2–2.5	n.a.	1.2–2.5
5346		2.5	2.5	0.6
Tone IB; 14061; K₂₃₅		±2.5	±2.5	±2.5
SALMONELLA				
typhi murium (2 strains‡)	1, 4, (5), 12	n.a.	n.a.	±2.5
worthington	1, 13, 23	n.a.	n.a.	1.2–2.5
typhi (583)	9, 12	±2.5	n.a.	n.a.
typhi (10810)	9, 12	n.a.	1.2–2.5	n.a.
hvittingfoss	16	±2.5	n.a.	2.5
urbana	30	n.a.	n.a.	0.6–1.2
ramat-gat	30	n.a.	n.a.	0.6
sp.	30	n.a.	n.a.	0.6–2.5
ARIZONA				
	25	n.a.	±2.5	±2.5
KLEBSIELLA				
Benton	2	n.a.	2.5	n.a.
	3	n.a.	±2.5	±2.5
	7	n.a.	1.2	n.a.
	8	n.a.	n.a.	1.2
	9	0.6	n.a.	n.a.
9138		2.5	n.a.	n.a.
1924; 1973; Foster (I); Thompson		n.a.	0.6–2.5	n.a.
5192; 9597; Richardson (II)		n.a.	n.a.	1.2–2.5
5276; 6173; A. Johnson (III)		n.a.	0.6–2.5	0.6–2.5
3527		2.5	0.6–1.2	1.2

TABLE III—*Continued*

Bacterium	O-antigen (when known)	Minimum amount of boiled bacteria giving complete hemagglutination inhibition		
		A₁	B	H(O)
		mg./ml.	*mg./ml.*	*mg./ml.*
CITROBACTER				
1922; 8090		2.5	n.a.	n.a.
1971; 9554		n.a.	1.2–2.5	n.a.
2898; 5551		n.a.	n.a.	1.2–±2.5
8006; 8454		±2.5	1.2–2.5	n.a.
5869; 6159		n.a.	1.2–2.5	2.5
4660		2.5	n.a.	2.5
PASTEURELLA *pseudotuberculosis*				
	Group I	n.a.	0.6–1.2	n.a.
	Group II	n.a.	±2.5	n.a.
	Group V	n.a.	1.2–2.5	1.2–2.5
PROTEUS				
X₂	2	n.a.	2.5	n.a.
2892; A. Johnson (IV)		n.a.	1.2	n.a.
2009; 2079		n.a.	n.a.	2.5
14056		n.a.	±2.5	±2.5
PSEUDOMONAS				
8166		n.a.	0.6–2.5	n.a.
4547		n.a.	n.a.	1.2
Paskall		1.2	n.a.	0.6
SERRATIA *marcescens* (9699)		n.a.	n.a.	±2.5
ALCALIGENES				
10969		n.a.	0.6–±2.5	1.2
11982		±2.5	n.a.	0.6

* Same footnotes as Table II.
‡ One strain = var. copenhagen.

antigen 13 (Table II). *S. worthington*, which contains in addition O antigen 1, was less active (Table III). All O antigen 30 containing species tested have similar and exclusive low H(O) activity (Table III).

All 3 *Arizona* serotypes tested were active. *Arizona* O₂₁ possesses high B activity, while *Arizona* O₉ has fairly high H(O) activity, and *Arizona* O₂₅ low H(O) and B activity.

Among the *Klebsiella* tested, roughly as many organisms inhibited only one

agglutinin as several agglutinins. One strain with unknown capsular antigen showed no significantly disproportional action.

In the genus *Citrobacter*, a rather even distribution among the A, B, and H(O) specificities either singly or in combinations of 2 but not of all 3 activities was noted.

TABLE IV

Bacteria of Known Serotype without Blood Group Activity

Bacterium	O-antigen
E. COLI	1:K_1
	2:K_1
	11
	26*
2 strains	55
SALMONELLA	
heidelberg (9787)	4, 5, 12
enteritidis (2 strains)	1, 9, 12
typhi (2 strains); *berta*	9, 12
champaign	39
deversoir	45
KLEBSIELLA‡	
	1
	2
	4
	6
PASTEURELLA	
pseudotuberculosis	Group III
	Group IV
pestis§ (3 strains)	

* One lipopolysaccharide preparation had moderate B activity.

‡ Capsular type listed.

§ One strain, Tjiwidej, showed traces of H(O) and B activities.

One A active *Citrobacter* and 19 *E. coli* were tested against several immune and "natural" anti-A sera and against A_1 and A_2 erythrocytes. Agglutination of A_2 erythrocytes in general was more easily inhibited by these bacterial suspensions than that of A_1 erythrocytes. Not all antisera were inhibited to the same extent. Such variations were not noted in the B- anti-B system.

In the genus *Pasteurella*, strains of both *P. pseudotuberculosis* and *P. pestis* were tested. In spite of all efforts the *P. pseudotuberculosis* strains, groups I-V (11), often became rough during culturing. The results therefore may be an

expression of the properties of a mixture of smooth and rough variants except for groups II and IV which remained smooth. Only groups I and V exhibited significant activity (Table III). Of the 3 different *P. pestis* strains, 2 were inactive in all systems tested, while 1 strain, Tjiwidej, showed traces of H(O) and B activity.

Blood group B activity is the one most often found among the *Proteus* strains tested; it is usually exclusive and sometimes high.

All 3 activities were found among the *Pseudomonas* strains and, except in one instance, the activity was strictly disproportional.

The 2 strains of *Serratia marcescens* tested were exclusively H(O) active. One *Bacterium anitratum* was isolated from a blood culture and showed high B activity only.

A human anti-H serum and a rabbit anti-H(O) serum were both inhibited in their reaction with human O erythrocytes by *S. poona* at the 1 to 2.5 mg./ml. level. *E. coli* O_{128} and *S. typhi murium* 4685 inhibited human anti-H serum to a still lesser extent but not at all the rabbit anti-H(O) serum. *S. atlanta* was of low activity against rabbit anti-H(O) serum. The following bacteria showed borderline or no activity when tested against these 2 sera: *S. grumpensis*, *E. coli* O_{86}, *S. hvittingfoss*, *S. urbana*, and *S. champaign*.

Seventy-six Enterobacteriaceae, both boiled and unboiled, were tested for their ability to inhibit anti-M and anti-N agglutinins, while 64 were investigated for inhibition of anti-D (Rh$_0$) agglutinins. No bacterium gave significant inhibition with the exception of 1 *E. coli* (14061) which inhibited anti-D and anti-N, as well as anti-A, anti-B, and anti-H(O) agglutinins, at approximately the same levels (1.2 to 2.5 mg./ml.). This was probably unspecific inhibition.

DISCUSSION

The frequent occurrence of blood group active antigens in various widely disseminated bacterial species is evident from the data presented here. These data are based on the hemagglutination inhibition assay, which is a valid procedure in the determination of bacterial blood group activity. This has been shown by animal immunization, in which blood group active bacteria evoked potent anti-human blood group agglutinins (5, 6, 12, 13) corresponding to their *in vitro* activities. Bacteria inactive in the hemagglutination inhibition test failed to stimulate anti-human blood group A, B, or H(O) agglutinins. Also, anti-B agglutinins could be absorbed completely and specifically by *E. coli* O_{86} as could anti-A agglutinins by an A active *E. freundii* and anti-H(O) agglutinins by *S. poona* (5, 12–14). This is especially true if the bacteria exhibit disproportional activities, and has been confirmed by a number of investigators (13, 15).

Contamination of test materials with blood group active substances from the medium was excluded. The broth in which all bacteria were first grown,

after being picked from a slant, contained considerable amounts of A and H(O) (but not B, M, N, or D (Rh$_0$)) active material. However, the subsequent 9 daily transfers with a wire loop, from one tube of fully defined medium to the next, dilute out blood group active material. No blood group activity was demonstrated in the first tube with fully defined medium, into which transfer had been made from the broth culture.

False negative results arising from agglutination of erythrocytes by bacteria alone were excluded in the following manner: a cross-section of 33 bacterial suspensions was incubated in different experiments with 4 hemagglutinating doses of anti-H(O) eel serum and a 1:15 dilution of serum from an A$_1$B person. After 90 minutes a 0.5 per cent suspension of an A$_1$, B, and O red cell mixture was added to the human serum and of A$_1$B cells to the eel serum. There was no erythrocyte agglutination by any sample.

After our report (4, 5) on the high blood group B activity of $E.\ coli$ O$_{86}$, Iseki, Onuki, and Kashiwagi (13) pointed out the established serological relationship between $E.\ coli$ O$_{86}$, $Arizona$ O$_{21}$, and $Salmonella$ having O antigen 43 (8, 16) and showed by absorption and immunization experiments that $Arizona$ O$_{21}$ and $S.\ milwaukee$ possess blood group B active antigens similar to $E.\ coli$ O$_{86}$. The results of our hemagglutination inhibition experiments with $Arizona$ O$_{21}$ and $S.\ berkeley$ (containing the $Salmonella$ O antigen 43) are in agreement with the findings of the Japanese investigators. The absence of any H(O) activity from $Arizona$ O$_{21}$ and $S.\ berkeley$ points to a difference between the antigens of these bacteria and those of $E.\ coli$ O$_{86}$:B$_7$ and B$_9$, which frequently showed slight H(O) activity.

Although $Arizona$ O$_9$ and $E.\ coli$ O$_{55}$:B$_5$ cross-react with their respective antisera (8), $Arizona$ O$_9$ shows significant blood group activity but $E.\ coli$ O$_{55}$:B$_5$ does not.

Association of H(O) antigen with group antigen 13 of $Salmonella$ appears to be established by earlier observations on 1 strain each of $S.\ poona$ and $S.\ worthington$ (2, 4) and by the present finding of exclusive H(O) activity for all strains of $S.\ poona$, $S.\ grumpensis$, $S.\ atlanta$, and $S.\ worthington$ tested. The first 3 of these species are of equal, rather high activity. They possess, in addition to antigen 13, antigen 22 or 23 but not antigen 1. $S.\ worthington$, on the other hand, possessing O antigens 1, 13, and 23, is of low activity (Tables II and III). O antigen 1 therefore may interfere with the combination of the anti-H(O) agglutinin with its complementary grouping on the bacterial surface.

Bacteria which inhibited the agglutination of O erythrocytes by eel serum were tested with human and rabbit anti-H(O) sera. By this selection other active bacteria may have been omitted. Only some of the bacteria which inhibited eel sera were active against human and rabbit anti-H, and then to a lesser extent than against eel serum.

Chemical investigations, notably those of Morgan and Kabat ($cf.$ references

17–19), have led to the elucidation of portions of the essential chemical structures responsible for the serological specificity of the human blood group mucoids. The identical monosaccharides, namely D-galactose, L-fucose, N-acetyl-D-glucosamine, and N-acetyl-D-galactosamine are present in A, B, and H(O) substances. The evidence obtained indicates that the specificity of these mucopolysaccharides resides in their carbohydrate portion and is associated with oligosaccharide units. The terminal, non-reducing monosaccharides most responsible for serological specificity of each blood group substance appear to be: N-acetyl-D-galactosamine for A substance, D-galactose for B, and L-fucose for H(O) (cf. references 18, 19).

TABLE V

Highly Blood Group Active Bacteria with Known O-Antigen Carbohydrates

Bacterium	O-antigen	Specificity	Sugars shared with human blood group mucoids			
			Fucose	Galactose	Galactosamine	Glucosamine
E. coli	86	B	+	⊕	+	+
	127	H(O)	⊕	+	+	+
	128	H(O)	⊕	+	+	+
S. poona	13, 22	H(O)	⊕	+	+	+
S. grumpensis	13, 23	H(O)	⊕	+	+	+
S. atlanta	13, 23	H(O)	⊕	+	+	+
S. berkeley	43	B	+	⊕	+	+
Arizona	9	H(O)	⊕	+		+
	21	B	+	⊕	+	+

⊕ = Sugar responsible for most of a given activity in human blood group mucoids. For references see text.

Analyses of the polysaccharide antigens of various Gram-negative bacteria by chromatography and on occasion by classical chemical methods (11, 20–32) have given evidence for the presence of carbohydrate building-stones identical with those of the blood group mucoids. To account for closely related serological specificity, blood group active organisms would be expected to possess chemical groups corresponding in nature, structure, and orientation to those present in the human blood group mucoids. This was shown to be probable for the blood group active polysaccharide complex of E. coli O_{86}:B_7 (29).

General agreement was found between the nature of known carbohydrate building-stones (24, 29–32) and the observed blood group activity of the investigated bacteria (Table V). In all cases in which the activity was moderate to high, the H(O) active bacteria contain fucose, A active bacteria N-acetyl-galactosamine, and B active bacteria galactose. It is noteworthy that fucose

was found in all O antigens of the chemically investigated highly active bacteria (Table V), whereas bacteria may have low blood group activity but lack this sugar, e.g. E. coli O_6 and 2 strains of S. typhi (24, 31).

The presence of all 4 sugars of human blood group mucoids has been reported (24, 29–32) for the O antigens of those bacteria listed in Tables V and VI, with the exception of Arizona O_9, which lacks galactosamine. It should be stressed that glucosamine is a constituent of the lipid part of the lipopolysaccharide and not necessarily a component of the polysaccharide as well (11, 22). No active bacterium contained less than 2 of the sugars found in human blood group substances. In all instances in which the specific sugar was not detected in the O antigen, activity observed was weak.

TABLE VI

Bacteria with Low or No Blood Group Activity Possessing All Sugars of Blood Group Mucoids

Bacterium	O-antigen
E. coli	11
	125
S. worthington	1, 13, 23
S. kvittingfoss	16
S. urbana	30
S. ramat-gat	30
S. champaign	39
Arizona	25

For references see text.

The frequently noted B specificity of Proteus strains is in agreement with the presence of galactose in a Proteus OX_{19} polysaccharide. Among the other sugars identified (27), only N-acetylglucosamine is a component of human blood group mucoids.

The absence of activity in the 5 strains of Sh. flexneri tested is understandable, as the only sugar present in both the O antigen of this bacterium and blood group mucoids is glucosamine (21).

The lipopolysaccharides of P. pestis Tjiwidej and of 2 other P. pestis strains have been isolated and characterized by Davies (28). He found only glucose, glucosamine, and an unidentified aldoheptose as component carbohydrates. We observed a trace of H(O) and B activity for the whole Tjiwidej organism and no blood group activity for the 2 other strains.

While so far significant activity in bacteria has not been observed when the monosaccharide responsible for the major part of a given activity in human blood group mucoids was stated to be missing in the O antigen, the converse is not necessarily the case. In addition to those listed in Table VI, S. deversoir, E. coli O_{55}, and E. coli O_{128} have been reported (30, 31) to contain at least 3 sugars of the human blood group mucoids; nevertheless, these bacteria

were either inactive or possessed low activity only. Also, otherwise highly active fucose-containing strains such as *S. berkeley, Arizona* O_{21}, and *E. coli* $O_{86}:B_7$ show no or only borderline H(O) activity.

Assuming a similar proportion of blood group constituent monosaccharides in blood group active bacteria and in those without activity, one would expect that in the latter group the monosaccharides in question are either linked in sequence and orientation in such a way as to render them inaccessible to the antibody or that they are present as the enantiomorphous isomer of the active form. It is generally accepted that only one member of a pair of enantiomorphs is significantly active in a given immunological system (33). It may be predicted therefore that all highly H(O) active bacteria (in the eel serum system) contain L-fucopyranoside in α-glycosidic linkage, all highly B active bacteria a terminal α-D-galactosyl structure, while an α-N-acetyl-D-galactosaminoyl structure most likely is responsible for the activity of A specific bacteria. Other sugars structurally related to the parent compound may also play a role in low blood group activity, as has been shown for compounds closely related structurally to L-fucose (34, 35). Thus, the low H(O) activity of *E. coli* O_{111} may be explained by its content of properly integrated 3-deoxy-L-fucose, although this sugar is inactive as a monosaccharide (34). On the other hand, *E. coli* O_{55} which also contains 3-deoxy-L-fucose (31) possesses no H(O) activity.

Attempts to correlate the blood group activities of bacteria with the chemistry of their O antigens have to take into account that drastic procedures have been employed (22, 24) in the isolation of almost all the O antigens analyzed for carbohydrate components. It may be relevant that more highly B active material was obtained from *E. coli* $O_{86}:B_7$ (29) by a mild extraction method (50 per cent aqueous pyridine, at 37°C. (21)) than with hot 45 per cent aqueous phenol.

Few of the more recent chemical studies of Gram-negative bacteria have considered the K antigens which presumably are carbohydrate but more labile than the O antigens (8, 36). Most of our bacterial suspensions contained K antigens, probably in solution. Unremoved K antigens may mask blood group active O antigens as seems likely for the colominic acid-containing bacterium *E. coli* $O_7:K_1$ which inhibits blood group B agglutinins only after heating on a boiling water bath.

On the other hand, K antigens may contribute to the blood group activities observed and may help explain the borderline H(O) activity of the 2 strains of *S. typhi murium*. These bacteria belong to *Salmonella* group B in whose O antigens no fucose or galactosamine has been found. Another *Salmonella* B, *S. heidelberg*, did not possess demonstrable H(O) activity. Similarly, of 6 strains of *Salmonella* group D_1, which reportedly contain no galactosamine, 1 *S. typhi* showed low A activity, while the remaining 5 *Salmonella* D_1 did not possess A specificity.

The trace of blood group activity found in 1 of 3 strains of *P. pestis* cannot

be explained on the basis of the reported chemistry (28) of the O antigen of this strain.

In the *Klebsiella*, capsular and somatic antigens may both contribute to the hemagglutination inhibitions observed, since the capsules are removed from the bacterial surfaces by heating on a boiling water bath but are not destroyed (26). Fucose, glucose, uronic acid, and in some instances galactose have been shown (25, 26) to be present in capsular polysaccharide preparations of the *Klebsiella*. The presence of galactose has been attributed to contamination with somatic material (26). There appears to be no detailed knowledge of the chemical nature of this genus' somatic antigens.

The most active whole B specific bacteria, *E. coli* O_{86}, *Arizona* O_{21}, and *S. berkeley* approximate, in the hemagglutination inhibition test, the activity of crude human blood group B substance preparations obtained from pseudomucinous ovarian cysts and meconium (4, 9).[1] The most highly H(O) active bacterium, *E. coli* O_{128}, has an activity similar to H(O) mucoids of human origin. No A specific bacterium was this active. It is remarkable, even if an anamnestic response is considered, that anti-human A agglutinins obtained in rabbits with a moderately A active *E. freundii* were somewhat more potent than anti-B agglutinins obtained at the same time with *E. coli* O_{86}:B_7 under identical conditions (5, 14).

No marked disproportional activity was noted for 1 *Klebsiella* and a few *E. coli* strains (Tables II and III). In these instances, unspecific inhibition such as that given by some strongly acidic polymers must be excluded before assuming that these bacteria possess substances with similar A, B, and H(O) specificity.

The lack of significant disproportional inhibition of the anti-M, anti-N, and anti-D (Rh_o) agglutinins by the bacteria tested is noteworthy and compatible with the rare occurrence of these agglutinins without deliberate stimulation. Four inactive bacteria, obtained from Dr. W. F. Goebel and Dr. G. T. Barry, are known to contain colominic acid (*cf.* reference 36), a polymer of sialic acid which is a component of M and N specific substances (10, 37, 38).[2]

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[1] In the quantitative assay highly purified B active material from *E. coli* O_{86}:B_7 precipitated in the equivalence zone of an homologous human blood group B mucoid–hyperimmune anti-B serum system approximately one-half as much antibody as did the B mucoid. Furthermore, agar-gel diffusion studies showed a fusion line between human B mucoid and *E. coli* O_{86} polysaccharide complex (Springer, G. F., and Readler, B., data to be published).

[2] This lack of specific inhibition of anti-D (Rh_o), anti-M, and anti-N is in agreement with our observations (unpublished) that N-acetyl- and N-glycolylneuraminic acids, colominic acid, crystallized brain gangliosides, and numerous derivatives of sialic acid did not inhibit anti-M, anti-N, or anti-D (Rh_o) agglutinins under the conditions of our test. Our observations on anti-D (Rh_o) agglutinins are in contrast to a recent report (Dodd, M. C., Bigley, N. J., and Geyer, V. B., Specific inhibition of Rh_o (D) antibody by sialic acids, *Science*, 1960, **132**, 1398).

Of the few Gram-positive bacteria which have been tested, none has shown A, B, or H(O) activity (4, 7).

Our studies of blood group activity in bacteria show that A, B, and H(O) activity is not confined to any particular genus of Gram-negative bacteria. It is noteworthy therefore that the antigens of blood group active *Salmonella* also occur in other bacteria. Saphra and Silberberg (39) reported on 13 coliform strains which possessed the O antigens of the *S. worthington–S. poona* group in various combinations. Similarly Wheeler, Stuart, Rustigian, and Borman (40) found *Klebsiella* and *Escherichia* strains, as well as other coliform organisms, to possess various *Salmonella* O antigens.

SUMMARY

Distribution of blood group A, B, and H(O) activities among 282 aerobic Gram-negative bacteria, many isolated from the blood of patients, has been studied. Almost half of these bacteria were found to be blood group active. About 10 per cent of the organisms exhibited high, disproportional activities, which in some instances approached those of crude human blood group mucoids. No significant, specific D (Rh$_o$), M, or N activity was found in approximately 70 members of the Enterobacteriaceae.

An attempt was made to correlate the observed activity of a given organism of known O somatic antigen with its monosaccharide components. The presence of those sugars which account for the specificity of human blood group mucoids was noted.

The bearing of these findings on the origin of human anti-A and anti-B isoantibodies has been mentioned.

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