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Serology, Chemistry, and Genetics of O and K Antigens of *Escherichia coli*

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INTRODUCTION

Several reviews and monographs that cover some of the themes of this review have been published in the last decade (39, 112, 155, 192, 279). An increased interest in *Escherichia coli* in recent years from both human and veterinary medicine has been followed by an interest in the surface structures of these bacteria because of their special role in pathophysiological processes, their usefulness in epidemiological studies, and their importance for the normal immunological status of the host. We therefore agree that the present review covering all of the immunogenic surface structures, with emphasis on the K antigens, is needed. It is based on new methods and insight and should thus hopefully give a coherent description, taking into consideration structure, chemistry, genetics, and serology of the relevant surface antigens.

HISTORY

In this section only few fundamental data will be presented. The first successful attempt to classify *E. coli* by serological methods was carried out by Kauffmann (127), who was able to subdivide *E. coli* into a number of O groups. Using a boiled culture for O-antiserum production and as an antigen in agglutination tests, he was able to establish well-defined *Escherichia* O groups. The first antigenic scheme, comprising 20 O groups, was thus established by Kauffmann (128). Knipschildt (138) added another 5 O antigens, so the scheme contained 25 O groups. Since then, many O antigens have been added, and the antigenic scheme presented here comprises 164 O groups (see below).

During his investigations Kauffmann observed that many freshly isolated strains were not agglutinated when examined in the nonheated state in O antiserum. A similar phenomenon was well known, e.g., from *Salmonella typhi*, which is also nonagglutinable in O serum (O inagglutinable) when equipped with the Vi surface antigen; this inhibition of agglutinability in O serum could be overcome by heat treatment. It was similarly possible to describe a series of additional surface *E. coli* antigens with somewhat different physical and serological characters, which all caused inagglutinability in O serum that could be overcome by heat treatment. For a more detailed discussion of these antigens, the L, A, and B antigens, later collectively called the K antigens, see below. For a detailed first-hand description of the historical development of *E. coli* serology, see Kauffmann (130).

SEROLOGY OF O ANTIGENS

The O antigen is a thermostable surface antigen found in all smooth (S forms) *Enterobacteriaceae*. It is thermostable in the sense that the bacteria keep the immunogenic, agglutinating, and agglutinin-binding capacity after boiling. The O antigen is the O-specific polysaccharide of the cell wall lipopolysaccharide (LPS). Mutants that have lost the O specificity are R forms. As R serology is of little or no value for serotyping, it will not be discussed here.

Techniques for O-Antigen Determination

Sera for O determination are produced by immunization of rabbits with cultures heated at 100°C for 2 h. Broth cultures or agar plate suspensions heated at 100°C for 1 h are used as antigens. With these two ingredients, one will have an agglutination system that, to a large extent, involves only one antigen and its homologous antibody, and therefore the bacterial agglutination method is very simple and sensitive for qualitative O determinations. Some mucoid *E. coli* strains with a special heat-resistant capsular antigen will only be agglutinable in O serum after autoclaving (120°C for 2 h). In the manual on *Enterobacteriaceae* by Edwards and Ewing (39), detailed descriptions of procedures for O-antiserum production and O-antigen determination can be found. Ørskov and Ørskov (191) recently published another detailed account of general O-antigen determination. The test O sera, at present, are collected into pools, each containing cross-reacting O sera. An unknown strain is first examined in the pools and, if positive in one pool, it is examined in the single sera of that pool. Finally, it is titrated in that serum (those sera) that has given a positive reaction. In most laboratories O determination of *E. coli* strains is carried out in tubes or trays (microtiter system), but may of course also be carried out on slides, if only the antigen has been heat treated at 100°C before use. If many strains have to be examined, it may be advantageous to use a more or less automatized agglutination method. Such methods are described in some recent publications (8, 69, 82).

Bacterial agglutination is still the best and most simple technique for O-group determination of *E. coli*, although other techniques are also used. Indirect (passive) hemagglutination, which inadvertently led to the discovery of the enterobacterial common antigen, was used by Kunin and Beard (144) and Kunin et al. (145). Different immunoprecipitation tests may also be employed for O-grouping purposes, but, probably because of the simplicity and reliability of the bacterial agglutination test, gel precipitation has not been used routinely for O-group determination. However, gel precipita-

tion in two dimensions and especially immunoelectrophoresis (219, 241) have been used extensively in many O analyses and comparative examinations (19, 101, 102, 194, 203, 204). Immunoelectrophoresis will also give information on the electric mobility of the O-antigen molecule (194).

Test O Antigens

Table 1 gives an antigenic scheme, listing test strains for all established O, K, and H antigens arranged according to their O antigens, with the origin of the strains and the relevant references included. It should be stressed that this is a restricted antigenic scheme, i.e., that the O, K, and H antigens presented have been found in many other combinations and, furthermore, that antigens listed with the same symbol may be and in many cases are nonidentical. It is also important to remember that new strains are often found which, even though they can be assigned to a certain existing O group, will show cross-reactions not described hitherto.

The first O groups, O1 to O110, were established in the forties by Kauffmann and Knipschildt (see Kauffmann [130, 132]). However, because K and H antigens were not examined in most strains, O26 to O110 were never published in a formal antigenic scheme. Some O groups are cancelled: O31 and O47 because of near identity to other groups and O67, O72, and O94 because they turned out to be *Citrobacter freundii*. Among the first 110 O groups are many that are only found infrequently, the reason being that practically any *E. coli* strain that could not be grouped with available O sera at that time was established as a new O group. At that time—in the beginning of the forties—there was only scanty interest in medical application of *E. coli* typing. Later, when it was found that some well-defined serotypes were associated with outbreaks of infantile diarrhea, this attitude was changed, and serotyping has since been applied to many different problems within medical microbiology. It has been necessary, however, to restrict the number of newly established O antigens in order to keep the number of test antigens within a handy size. Therefore, only strains that have a special importance from a medical, epidemiological, or scientific point of view have been established as new O test antigens. It has also been considered important that any new test antigen, before being officially established, is examined in at least two laboratories equipped with all test antisera and test strains. Among the strains with numbers higher than 110, several will be found that have been isolated from pathological conditions in animals.

Cross-Reaction Between O Antigens of *E. coli*

Many cross-reactions exist between the single O antigens, and a great number of cross-absorbed O sera (factor sera) are necessary for a precise O diagnosis. However, even using these absorbed sera, we are unable to tell whether an unknown strain, assigned to a certain O group, is really O-antigen identical with the test strain of that O group. Only serum production with the unknown strain followed by mutual cross-absorption can give the definite answer. Table 2 shows the stronger cross-reactions between all established O-antigen test strains and the corresponding specific O antisera. These results are based primarily on the results of Ørskov and Ørskov in Copenhagen, but they also include data from the examinations of Ewing (39, 50).

Cross-Reactions Between O Antigens of *E. coli* and *Shigella*, and *Salmonella* and *Klebsiella*

Many O-antigenic cross-reactions have been described between *E. coli* and *Shigella* O antigens. Ewing et al. (46) have gathered data that show that only two *Shigella* serovars are not related to one or more of the *E. coli* antigens O1 to O148. He further shows that a number of sub judice *Shigella* serovars are related to *E. coli* O groups. O relationships between *E. coli* O149 to O163 and *Shigella* have been examined by Rowe et al. (237). These findings stress the well-known close relationship between the two genera. A number of invasive *E. coli* strains described in recent years, which can give rise to dysentery-like disease, have O antigens closely related to *Shigella* O antigens (Table 3).

Kauffmann (130, 132) and Frantzen (57) have described O-antigen cross-reactions between *E. coli* and *Salmonella*, and recently Refai and Rohde (229) have carried out similar investigations with 142 *E. coli* O-antigenic test strains in available *Salmonella* and *Arizona* sera. Table 4 shows the results of Refai and Rohde together with the earlier results in a condensed form.

Cross-reactions between O antigens of *E. coli* and *Klebsiella pneumoniae* have been described by Kauffmann (129) and Ørskov (198) (Table 5). Most of these cross-reactions are mutual, but identity is not found in all cases.

Cross-Reactions Between *E. coli* O Antigens and Antigens Outside the *Enterobacteriaceae* Group

Winkle et al. (307) described O-antigen relationships between *Vibrio cholerae* and *E. coli*, *Salmonella*, and *Citrobacter* strains. Springer (269, 271) has analyzed the cross-reactions between *E. coli* O86 antigen and human blood

TABLE 1. *E. coli antigenic scheme comprising all O-, K-, and H-antigenic test strains February 1977; arranged according to O-antigen numbers*

O	K	H	Culture no.	Isolated from[a]	References	Former K designation
1[b]	1	7	U 5-41	Hu U	128	L
1	51	–	A 183a	Hu A	296	L
2	1	4	U 9-41	Hu U	128	L
2	7 (56)[c]	7	H 17b	Hu F	138	B
2	1	6	A 20a	Hu A	296	L
2	2	1	Su 1242	Hu B	128	L
2	*NE*[d]	8	Ap 320c	Hu A	138	NE
3	2ab	2	U 14-41	Hu U	128	L
3	NE	31	K 15	Ca S	183	NE
3	NE	44	781-55	Hu D	24	NE
4	3	5	U 4-41	Hu U	128	L
4	6	5	Bi 7457-41	Hu U	128	L
4	12[e]	–	Su 65-42	Hu Pus	128	L
4	52	–	A 103	Hu A	296	L
5	4	4	U 1-41	Hu U	128	L
6	2ac	1	Bi 7458-41	Hu U	128	L
6	13	1	Su 4344-41	Hu U	128	L
6	15	16	F 8316-41	Hu F	128	L
6	53	–	PA 236	Hu P	296	L
6	54	10	A 12b	Hu A	39	L
6	13	49	2147-59	Hu F	128	L
7	1	–	Bi 7509-41	Hu U	128	L
7	7	4	Pus 3432-41	Hu P	128	L
8	8	4	G 3404-41	Hu B	128	L
8	25	9	Bi 7575-41	Hu U	128	B
8	27 (A)	–	E 56b	Hu P	138	A
8	40	9	A 51d	Hu A	296	A
8	41	11	A 433a	Hu A	296	A
8	42 (A)	–	A 295b	Hu A	296	A
8	43	11	A 195a	Hu A	296	A
8	44 (A)	–	A 168a	Hu A	296	A
8	45	9	A 169a	Hu A	296	A
8	46	30	A 236a	Hu A	296	A
8	47	2	A 282a	Hu A	296	A
8	48	9	A 290a	Hu A	296	A
8	49	21	A 180a	Hu A	296	A
8	50	–	PA 80c	Hu P	296	A
8	84 (A)[f]	–	H 308b	Hu F	138	–
8	87	19	D 227 = G 7, K88$^-$	Sw D	211	B
8	102 (A)	–	6CB10-1	Hu F	*[g]	–
8	NE	20	H 330b	Hu F	296	NE
8	NE	21	U 11a-44	Hu U	296	NE
8, 60	NE	51	C 218-70	Hu S	146	NE
9	9	12	Bi 316-42	Hu P	128	L
9a	26 (A)	–	Bi 449-42	Hu P	128	A
9ab	28 (A)	–	K 14a	Hu F	138	A
9	29 (A)	–	Bi 161-42	Hu P	128	A
9	30 (A)	12	E 69	Hu P	138	A
9	31 (A)	–	Su 3973-41	Hu U	128	A
9	32 (A)	19	H 36	Hu F	138	A
9	33 (A)	–	Ap 289	Hu A	296	A
9	34 (A)	–	E 75	Hu P	138	A
9	35 (A)	–	A 104a	Hu A	296	A
9	36	19	A 198a	Hu A	296	A
9	37 (A)	–	A 84a	Hu A	296	A
9	38 (A)	–	A 262a	Hu A	296	A

TABLE 1—*Continued*

O	K	H	Culture no.	Isolated from[a]	References	Former K designation
9	39 (A)	9	A 121a	Hu A	296	A
9	55	–	N 24c	Hu NA	296	A
9	57	32	H 509d	Hu F	138	B
9	NE	19	A 18d	Hu A	296	NE
10	5	4	Bi 8337-41	Hu P	128	L
11	10	10	Bi 623-42	Hu P	128	L
11	NE	33	K 181	Ca S	183	NE
11	NE	52	C 2187-69	Hu S	146	NE
12	5	–	Bi 626-42	Hu P	128	L
13	11	11	Su 4321-41	Hu P	128	L
14[h]	7	–	Su 4411-41	Hu U	128	L
15	14	4	F 7902-41	Hu F	128	L
15	NE	17	P 12b	Hu F	128	NE
15	NE	25	N 234	Ca F	183	NE
15	NE	27	K 50	Ca S	183	NE
16	1	–	F 11119-41	Hu F	128	L
16	NE	48	P 4	Sw D	201	NE
17	16	18	K 12a	Hu F	128	L
18ab	(76)	14	F 10018-41	Hu F	128	B
18ac	(77)	7	D-M 3219-54	Hu D	48	B
19ab	NE	7	F 8188-41	Hu F	128	B
20	17	–	P 7a	Hu F	128	L
20	83	26	CDC 134-51	Hu D	287	B
20	84	26	CDC 2292-55	Hu D	287	B
20	101	–	1473	Sw D	*.	–
21	20	–	E 19a	Hu P	138	L
22	13	1	E 14a	Hu P	138	L
23	18	15	E 39a	Hu P	138	L
23	(21)[i]	15	H 38	Hu F	138	L
23	22	15	H 67	Hu F	138	L
24	+	–	E 41a	Hu P	138	–
25	19	12	E 47a	Hu P	138	L
25	23	1	H 54	Hu F	138	L
26	(60)	–	H 311b	Hu F	138	B
26	(60)	–	F 41	Hu D	181	B
26	(60)	46	5306-56	Ch F	22	B
27	–	–	F 9884-41	Hu F	128	–
28	–	–	K la	Hu F	128	–
28	(73)	–	Kattwijk	Hu D	50	B
29	–	10	Su 4338-41	Hu U	128	–
30	–	–	P 2a	Hu F	128	–
32	–	19	P 6a	Hu F	128	–
33	–	–	E 40	Hu P	138	–
34	–	10	H 304	Hu F	138	–
35	–	10	E 77a	Hu P	138	–
36	–	9	H 502a	Hu F	138	–
37	–	10	H 510c	Hu F	138	–
38	–	26	F 11621-41	Hu F	138	–
38	NE	30	N 157	Ca F	183	NE
39	–	–	H 7	Hu F	138	–
40	–	4	H 316	Hu F	138	–
41	–	40	H 710c	Hu F	138	–
42	–	37	P 11a	Hu F	128	–
43	–	2	Bi 7455-41	Hu U	128	–
44	74	18	H 702c	Hu F	138	L
45	1	10	H 61	Hu F	138	L
45	NE	23	K 42	Ca S	183	NE

TABLE 1—*Continued*

O	K	H	Culture no.	Isolated from[a]	References	Former K designation
46	–	16	P 1c	Hu F	128	–
48	–	–	U 8-41	Hu U	128	–
49	+	12	U 12-41	Hu U	128	–
50	–	4	U 18-41	Hu U	128	–
51	–	24	U 19-41	Hu U	128	–
51	NE	24	K 72	Ca S	183	–
52	–	10	U 20-41	Hu U	128	–
52	NE	45	4106-54	Hu D	24	NE
53	–	3	Bi 7327-41	Hu U	128	–
54	–	2	Su 3972-41	Hu U	128	–
55	(59)	–	Su 3912-41	Hu Pus	128	B
55	(59)	6	Aberdeen 1064	Hu D	133	B
56	+	–	Su 3684-41	Hu Mes	128	–
57	–	–	F 8198-41	Hu F	128	–
58	–	27	F 8962-41	Hu F	128	–
59	–	19	F 9095-41	Hu F	128	–
60	–	33	F 10167a-41	Hu F	128	–
61	–	19	F 10167b-41	Hu F	128	–
62	–	30	F 10524-41	Hu F	128	–
63	–	–	F 10598-41	Hu F	128	–
64	–	–	K 6b	Hu F	128	–
65	–	–	K 11a	Hu F	128	–
66	–	25	P 1a	Hu F	128	–
68	–	4	P 7d	Hu F	128	–
69	–	38	P 9b	Hu F	128	–
70	–	42	P 9c	Hu F	24, 128	–
71	–	12	P 10a	Hu F	128	–
73	–	31	P 12a	Hu F	128	–
73	92	34	6181-66	Hu D	39	L
74	–	39	E 3a	Hu P	138	–
75	95	5	E 3b	Hu P	138, 205	–
75	100	5	F 147	Hu D	205	–
76	–	8	E 5d	Hu P	138	–
77	96	–	E 10	Hu P	138, 205	–
78	(80)	–	E 38	Hu P	138	B
79	–	40	E 49	Hu P	138	–
80	–	26	E 71	Hu P	138	–
81	97	–	H 5	Hu F	138, 205	–
82	–	–	H 14	Hu F	138	–
83	–	31	H 17a	Hu F	138	–
83	24	31	H45	Hu F	138	L
84	–	21	H 19	Hu F	138	–
85	–	1	H 23	Hu F	138	–
86	–	25	H 35	Hu F	138	–
86	(61)	–	E 990	Hu D	184	B
86	2 (62)	2	F 1961	Hu D	184	L
86	(64)	36	5017-53	Hu D	47	B
86	(61)	34	BP 12665	Hu D	184	B
86	NE	47	1755-58	Hu D	44	B
87	–	12	H 40	Hu F	138	–
88	–	25	H 53	Hu F	138	–
89	–	16	H 68	Hu F	138	–
90	–	–	H 77	Hu F	138	–
91	–	–	H 307b	Hu F	138	–
92	–	33	H 308a	Hu F	138	–
95	+	33	H 311a	Hu F	138	–
96	–	19	H 319	Hu F	138	–
97	–	–	H 320a	Hu F	138	–
98	–	8	H 501d	Hu F	138	–

TABLE 1—*Continued*

O	K	H	Culture no.	Isolated from[a]	References	Former K designation
99	–	33	H 504c	Hu F	138	–
100	–	2	H 509a	Hu F	138	–
101	–	33	H 510a	Hu F	138	–
101	99 (L)	–	B 41	Ca D	212	–
101	103 (A)	–	8CE275-6	Hu F	*	–
102	–	8	H 511	Hu F	138	–
103	+	8	H 515b	Hu F	138	–
104	–	12	H 519	Hu F	138	–
105	–	8	H 520b	Hu F	138	–
106	–	33	H 521a	Hu F	138	–
107	98	27	H 705	Hu F	138, 205	–
108	–	10	H 708b	Hu F	138	–
109	–	19	H 709c	Hu F	138	–
110	–	39	H 711c	Hu F	138	–
111	(58)	–	Stoke W	Hu D	133	B
112ab	(68)	18	1411-50	Hu D	44	B
112ac	(66)	–	Guanabara (1685)	Hu D	44	B
113	(75)	21	6182-50	Hu F	39	B
114	(90)	32	26w (= K10 = HW36)	Ca S	182, 183	B
115	–	18	27w	Ca S	182	B
116	+	10	28w	Ca S	182	B
117	98	4	30w	Ca S	182	–
118	–	–	31w	Ca S	182	–
119	(69)	27	34w	Ca S	182	B
120	+	6	35w	Ca S	182	–
121	–	10	39w	Ca S	182	–
123	–	16	43w	Ca S	182	–
124	(72)	30	Ew 227	Hu D	42	B
125ab	(70)	19	Canioni	Hu D	289	B
125ac	(70)	6	Ew 2129-54	Hu F	44	B
126	(71)	2	E 611	Hu D	289	B
127a	(63)	–	4932-53	Hu D	47	B
127ab	(65)	4	2160-53	Hu F	39	B
128	(67)	2	Cigleris	Hu D	290	B
129	–	11	Seeliger 178-54	Hu D	257	–
130	–	9	Ew 4866-53	Hu ?	50	–
131	–	26	S 239	Ca S	183	–
132	+	28	N 87	Ca F	183	–
133	–	29	N 282	Ca F	183	–
134	–	35	4370-53	Hu F	50	–
135	–	–	Coli Pecs	Hu D	185	–
136	(78)	–	1111-55	Hu D	185	B
137	(79)	41	RVC 1787	Ca D	24, 185	L
138	(81)	–	CDC 62-57	Sw D	49	B
139	12 (82)	1	CDC 63-57	Sw D	49	B
139	–	56	SN3N/1	Hu F	209	–
140	–	43	CDC 149-51	Hu D	24	–
141	(85)	4	RVC 2907	Sw D	195	B
141	(85) 88ab (L)	4	E 68	Sw D	211	B, L
142	(86)	6	C 771	Hu D	195	B
143	–	–	4608-58	Hu D	39	–
144	–	–	1624-56	Hu D	39	–
145	–	–	E 1385 (3)	Hu D	–	–
146	–	21	CDC 2950-54	Hu D	39	–
147	(89) 88ac(L)	19	G 1253	Sw D	213	B, L
147	(89)	19	D 357 = G 1253 K88$^-$	Sw D	213	B
148	–	28	E 519-66	Hu D	193	–
148a	NE	53	E 480-68	Hu D	207	NE
149	(91)	10	D 616 = CS 1483 K88$^-$	Sw D	214	B
150	93	6	1935	Ch S	60	–

TABLE 1—*Continued*

O	K	H	Culture no.	Isolated from[a]	Reference	Former K designation
151	–	10	880-67	Hu D	193	–
152	–	–	1184-68	Hu D	193	–
153	–	7	14097	Hu D	193	–
154	94	4	E 1541-68	Hu F	206	–
155	–	9	E 1529-68	Hu F	206	–
156	–	47	E 1585-68	Hu F	206	–
157	88ac(L)	19	A 2	Sw D	60	L
158	–	23	E 1020-72	Hu D	207	–
159	–	20	E 2476-72	Hu D	207	–
160	–	34	E 110-69	Hu D	207	–
161	–	54	E 223-69	Hu D	207	–
162	–	10	10B1/1	Hu F	209	–
163	–	19	SN3B/1	Hu F	209	–
164	–	–	145/46	Hu D	237b	–

[a] Abbreviations: Hu, human being; Ca, calf; Sw, swine (piglet); Ch, chicken; U, urine; A, appendix (appendicitis); NA, normal appendix; S, blood (septicemia); P, peritoneum (most often appendicitic peritonitis); B, bile; F, feces from healthy individual; D, diarrhea; Mes, mesenterial lymph node.

[b] Bold-faced numbers = test (reference) antigens.

[c] Bold-faced numbers in parentheses = former reference antigens, now proposed to be deleted. These numbers should not be used for new K antigens.

[d] NE, Presence of K antigen not examined. – in K column = no K antigen detected; + in K column = polysaccharide K antigen not numbered; – in H column = nonmotile. The following polysaccharide K antigens have been found to be closely related or identical (260): **K2ab** ~ **K2ac** ~ **K62**, **K7** = **K56**, **K12** ~ **K82**, **K13** ~ **K23**, **K18** ~ **K22**, **K16** ~ **K37** ~ **K97**, **K53** ~ **K93**, **K54** ~ **K96**. The scheme only contains the serotype formulas of the reference strains used at the World Health Organization Collaborative Center for Reference and Research on Escherichia and thus comprises all hitherto officially established *Escherichia* antigens. It is evident that nothing about prevalence of single antigens can be deduced from the data found in this scheme. The strains have been collected over more than 30 years, and many different considerations have determined the selection.

[e] The famous "K-12" strain used by molecular biologists and biochemists should not be mistaken for the test strain for K antigen K12 (Su65-42).

[f] This strain is the previous test strain of O-antigen O93 = O93:?:H^- (see text) with K antigen related to K84.

[g] *, This review.

[h] O14 contains no S-LPS but R-LPS.

[i] The K21 antigen has now been lost.

[j] Test strain of K24 was previously assigned to O-group 22.

group B antigen. There are also reports on cross-reactions between *E. coli* O antigens and surface antigens of mammalian cells (29, 100, 103, 272).

SEROLOGY OF K ANTIGENS

In 1945, Kauffmann and Vahlne (135) introduced the term K antigen (from the German word for capsule, Kapsel) as a symbol to denote either envelope or capsule antigens, of which three different types had been described, the L, A, and B antigens.

The original description of K antigens was based solely on the bacterial agglutination reaction, and the inagglutinability of nonheated bacteria in O antiserum was considered to be the primary criterion of the presence of a K antigen (127, 128, 132, 138, 296).

Classical L A B Subdivision in the Light of Recent Findings

Since the K antigens (L, A, and B) were first defined, more than 30 years have passed, and it is only timely to consider whether any adjustments or revisions are required. Already Kauffmann and Vahlne (135, 296) pointed out the difficulties that might be encountered in distinguishing between L and B antigens.

On several occasions we have run into such difficulties, either in the laboratory in Copenhagen or when evaluating work carried out in other laboratories. In the next portion, some of these problems will be mentioned.

It was found that the same *E. coli* strain could contain two K antigens, e.g., one labeled B (K87) and the other labeled L (K88) (211). Furthermore, repeated examinations of the same strain would at one time label a strain as L and the next time as B. In the classical "L or B experiment" (Table 6), in which an OK serum was absorbed by homologous boiled culture, the outcome was dependent on variable factors, e.g., the amount of boiled bacteria used for

TABLE 2. *Cross-reactions between E. coli O-antigen test strains*[a]

O serum	O antigens
1	2, 10, 14, 50, 53, 107, 115, 117, 148, 149, 150, 154
2	1, 50, 53, 74, 117
3	13, 23, 53, 115
4	12, 13, 16, 18, 19, 102
5	7, 65, 70, 71, 114
6	57
7	5, 19, 25, 36, 71, 116, 141
8	32, 46, 60
9	
10	
11	125
12	4, 15, 16, 123
13	3, 16, 18, 19, 50, 62, 69, 129, 133, 147
14	24
15	12, 40, 45, 143
16	4, 46, 129, 135
17	18, 44, 62, 73, 77, 106
18	4, 13, 16, 19, 23, 133, 138, 147
19	13, 33, 39, 133, 147
20	
21	22, 32, 83
23	3, 13, 18, 38, 68
24	14, 56
25	4, 7, 13, 18, 19, 26, 36, 68, 102, 133, 138, 147, 158
26	4, 13, 25, 32, 100, 102
27	
28	
29	
30	
32	8, 21, 26, 83
33	
34	4, 85, 140
35	
36	25, 43, 109
37	48
38	23
39	7, 91
40	15
41	
42	
43	36, 118
44	68, 73, 77, 106
45	15, 54, 66
46	8, 16, 134
48	19, 54, 59
49	
50	1, 2, 13, 19, 44, 53, 107, 117, 133, 135
51	
52	
53	1, 2, 3, 50, 149
54	45, 48, 59
55	
56	24
57	6
58	
59	48, 54
60	8
61	108
62	13, 16, 17, 40, 68, 73, 106
63	
64	154
65	5, 70, 71
66	45
68	7, 13, 18, 25, 36, 44, 62, 102
69	13, 51, 150
70	5, 65, 70, 74, 116
71	5, 7, 65, 70
73	13, 17, 44, 56, 62, 68, 77, 106
74	2, 40
75	1, 163
76	22
77	17, 44, 66, 73
78	92, 116, 137
79	41
81	37, 51
82	
83	21, 22, 32, 46
84	
85	34, 140
86	19, 48, 90, 127
87	41, 48, 76, 116
88	141
89	115
90	19, 86, 127
91	39
92	19, 78, 91
95	
96	
97	
98	
99	13, 63
100	26
101	117, 162
102	4, 25, 26, 36
103	
104	
105	
106	17, 44, 62, 73
107	50, 102, 117, 123
108	61
109	
110	
111	
112	144, 149
113	112, 117, 131
114	5, 48
115	1, 3, 152
116	7, 123
117	50, 76, 101, 122
118	121, 123
119	3, 48
120	53, 102, 105, 115, 117
121	101, 116, 123
123	12, 116, 121
124	
125	11, 73
126	
127	86, 90, 128
128	
129	13, 16, 133, 135
130	
131	113, 125, 143
132	
133	13, 129, 135, 147
134	46
135	13, 16, 17, 50, 129, 133
136	
137	78
138	7, 18, 25, 148, 150
139	102
140	34
141	7, 88
142	
143	3, 15, 131
144	112, 149
145	13
146	
147	19, 102, 133
148	1, 138
149	1, 50, 53, 112, 144
150	1, 69, 138
151	
152	3, 115
153	
154	1, 64
155	
156	
157	7
158	25
159	
160	
161	
162	101
163	75

[a] The data in this table have been collected mainly from examinations through many years in the laboratory in Copenhagen. However, some of the data of Edwards and Ewing (39) are included, especially those reactions that have been detected in both laboratories, even if titers may have been low. Several strong reactions found in their laboratory but not in Copenhagen are also listed. Titers are not recorded, as it is our experience that titers of cross-reacting antigens may vary greatly among sera produced at different times. The reactions recorded are generally those that have been found in serum dilutions not less than five twofold titer steps below the homologous titer. The papers by Kampelmacher (122) and Glantz (69), who have made similar studies, should also be consulted by those who are interested in cross-reacting *E. coli* O antigens.

TABLE 3. *Cross-reactions between O antigens of E. coli from dysentery-like disease and Shigella (according to Edwards and Ewing [39])*[a]

E. coli	*Shigella*
O28ac (Kattwijk)	*S. boydii* 13
O112ac (Guanabara)	*S. dysenteriae* 2, identical
O124	*S. dysenteriae* 3, identical
O136	
O143	*S. boydii* 8, identical
O144	*S. dysenteriae* 10
O152	Not examined

[a] Other strong O-antigen cross-reactions between *E. coli* and *Shigella* are: O32/*S. boydii* 14, O53/*S. boydii* 4, O58/*S. dysenteriae* 5, O79/*S. boydii* 5, O87ab/*S. boydii* 2, O105ab/*S. boydii* 11, O112ab/*S. boydii* 15, O129/*S. flexneri* 5, and O55/*S. flexneri* 4b.

TABLE 4. *Cross-reactions between O antigens of E. coli and Salmonella based on the results of Kauffmann (130), Frantzen (57), and Refai and Rohde (229)*

E. coli	*Salmonella*
O1	$O42_1$
O2	O55
O6	$O40_1 40_3$
O15	O59
O21	O38
O23	O51
O44, O62, O68 O70, O73, O99 O106 and O129	O6, 14
O55	$O50_1 50_2 50_4$
O75	O11
O85	O17
O86, O90	O43
O111	O35
O132	O17
O134	O36

TABLE 5. *Cross-reactions between E. coli and K. pneumoniae O antigens*

E. coli	*K. pneumoniae*
O19ab	O1, identical to O19b
O9	O3, identical
O20	O4, strong relationship
O8	O5, identical

absorption. Very often, most, if not all, antibodies against the polysaccharide K antigen were removed by the standard absorption and, if not, repeated absorptions would remove the rest. The antibodies of a so-called pure L serum were therefore either a mixture of residual antibodies against the polysaccharide K antigen and other thermolabile surface antigens or simply antibodies against such thermolabile structures (fimbriae, fimbria-like antigens, H antigens, or other, undefined structures). If no detectable antibodies were left against thermolabile structures or against the acidic polysaccharide K antigen, the strain was labeled B. The above-mentioned facts were not recognized when the L and B definitions were made originally. As the quantitative development of the different surface structures can vary much from time to time and from laboratory to laboratory, dependent on variations in growth conditions, the observed variable outcome of the absorption experiment is easy to understand. Thus, the agglutination experiment, although it can be helpful in the preliminary examination, cannot be relied upon as the sole test for antigenic analysis. Methods that can separate the single antigen-antibody reaction must be introduced. At present, the agar precipitation techniques are the best methods for this purpose.

TABLE 6. *Schematic presentation of the agglutination results on which previously used definitions of K antigens (L, B, and A) are based*

K type	Antigen prepn	O serum	OK serum	
			Absorbed by culture heated at 100°C for 2 h	Unabsorbed
L	Live (or Formalin treated)	−*[a]	+[b]	+
	Boiled (100°C for 1 h)	+	−[c]	+
B	Live	−*	−	+
	Boiled	+	−	+
A	Live	−	−	+
	Boiled	−	−	+

[a] −*, Negative or significantly lower than that of boiled culture.
[b] +, Agglutination.
[c] −, No agglutination.

By use of such serological techniques, three representative *E. coli* K antigens of the L variety, K12, K51, and K52, were reexamined by Ørskov and Ørskov (203, 204). In all three strains the presence of heat-stable, extractable K antigens was demonstrated by passive hemagglutination, double diffusion in gel, and immunoelectrophoresis. In the last test they showed a high electrophoretic mobility toward the anode. The three K antigens are acidic polysaccharides; their sugar compositions or structures have not yet been examined.

Similarly, the K2ac antigen, originally described as an L antigen, was studied by Holmgren et al. (101, 102). The antigen was found to be a polysaccharide of high electrophoretic mobility, and it retained its precipitating as well as its agglutinin-fixing capacity after boiling.

Later, immunoelectrophoretic studies in agar of all *E. coli* K-antigenic test strains have shown anodic precipitation arcs in extracts heated at 100°C due to K antigens in all former

A- and L-antigen-containing strains, except O137:K79(L) (194). From 33 strains, in which B antigens had been described, the presence of a K precipitation arc could only be demonstrated in a few cases. These were the three first-established B antigens, K25, K56, and K57, and in addition K82, K83, K84, and K87. When Knipschildt described the three first B antigens, he was able to produce pure B antisera by absorption with other strains of different K types. However, when the next B antigens were numbered in strains from infantile diarrhea [O111:K58(B) and O55:K59(B)], this was done with great hesitation because there was uncertainty as to the existence of a separate K antigen, since it was not possible to produce a pure B antiserum by absorption (133). The presence of B antigens in these cases and with a few exceptions in all later cases was thus based solely on the inagglutinability of the live culture in an O antiserum.

Thus, although it is highly probable that such strains do not contain an acidic polysaccharide K antigen, it is important to remember that antisera produced with a live culture (OK sera) are preferable, and often necessary, for their primary detection by slide agglutination.

Several substances and structures can cause inagglutinability in O serum, and often it will not be possible to point out which factor(s) is responsible in a certain case. The most important factor that interferes with agglutination in O antiserum is the acidic polysaccharide K antigen. However, many strains are found that are agglutinable in O serum, although they are equipped with an acidic polysaccharide capsule, as shown by immunoelectrophoresis (190) or agar electrophoresis combined with Cetavlon precipitation in the second dimension (187). H antigens (flagella), fimbriae, and perhaps other surface structures can cause inagglutinability in O serum. Furthermore, it is a common experience that this inagglutinability can be abolished by changes in the growth medium or the growth temperature. Another common experience is that strains, originally inagglutinable in O serum, become agglutinable after passages in the usual media. This applies to many of the test strains for the former B antigens found in so-called enteropathogenic strains, e.g., O55:K60(B6) and O111:K58(B4). In these cases it is not possible to detect any serological differences between such laboratory strains that are agglutinable in O serum and freshly isolated strains of the same serotype that are inagglutinable in O serum. It should be emphasized that we do not know which factor(s) causes this inagglutinability of the freshly isolated strains, but we believe that the variable agglutinability in O serum alone does not warrant the description of a special K antigen.

In light of the above considerations, we find it misleading to continue labeling K antigens according to the classical L, A, and B criteria and shall therefore propose to restrict the nomenclature of K antigens to (acidic) polysaccharide K antigens and protein (fimbrial) K antigens. The polysaccharide K antigens may be subdivided into two groups: those found in combination with O8, O9, O20, and O101 and those found in probably all other combinations (see below). However, until more knowledge has been gained, we shall abstain from giving any special name to these subgroups. Several capsulated strains belonging to O8, O9, or O101 are equipped with capsules that make the bacteria inagglutinable in O serum even after boiling, but heating at 120°C for 2 h will make them agglutinable. The K antigens of such strains will, for practical reasons, still be denoted K(A). It should be stressed that these strains can lose this special heat resistance of the capsule by mutation, and thus it is not uncommon in one animal to find strains of the same OK serotype with and without the special heat resistance of the K antigen.

Polysaccharide K Antigens

Techniques for K-antigen determination. The bacterial agglutination technique used for the determination of *E. coli* K antigens is performed in tubes or, preferably, on slides with O and OK antisera produced with heated and nonheated cultures, respectively; inagglutinability in O serum and agglutinability of a live culture in OK antiserum can be taken as an indication of the presence of a K antigen.

For slide agglutination, the culture from a plate is suspended directly in a drop of antiserum, whereas a suspension of the culture in saline-formaldehyde is used for tube agglutination. The tubes are incubated for 2 h at 37°C, left at room temperature for about 20 h, and then read.

The agglutination technique, which is fast and sensitive, gives an idea of the combined agglutinating abilities of the different antigens on the bacterial surface, but often little information concerning the role played by the single antigens. Strains subcultured exclusively on solid media may be both flagellated and fimbriated. OK antisera raised with such bacteria will therefore contain antibodies against these structures, and there are probably additional surface components that might cause erroneous results. Agglutination reactions due to these antigen-antibody systems have often been interpreted as being due to thermolabile K anti-

gens (L antigens). Thus, a description of an *E. coli* K antigen should not be based solely on the agglutination reaction, but also on gel diffusion techniques such as double diffusion in gel (218, 219) or immunoelectrophoresis (241), by which the K antigen is directly recognizable.

Slide agglutination in single or pooled K (OK) antisera may be used as a preliminary test, but in most cases the method of choice is the countercurrent technique (18, 76, 260), by which the result is read after a 1-h electrophoretic run. During this time the homologous antibody migrating towards the cathode and the K antigen migrating towards the anode will meet and give a precipitation line. The serum agar technique of Bradshaw et al. (13) has been adapted for K determination of a limited number of K antigens by Kaijser (121a).

Antigen extracts for the diffusion technique can be made in several ways. Holmgren et al. (102) suspend acetone-dried bacteria in Veronal buffer, pH 8.6, at 37°C for 24 h. The supernatant of the suspension after centrifugation is used as the antigen (VE extract). A freeze-press extract can be prepared as described by Edebo (36). Holmgren et al. (102) consider the VE extract to be the most representative antigenic preparation containing the largest number of demonstrable precipitinogens. Glynn and Howard (71) use both crude (homogenized acetone-dried culture) and purified (by precipitation with Cetavlon) extracts for immunoelectrophoresis.

The extracts used in Ørskov's laboratory for immunoelectrophoresis (194) are produced by suspending a culture from agar plates in buffered saline, pH 7.3, and heating at 60°C for 20 min, followed by centrifugation. The supernatant is called the 60°C extract. Part of this is further heated at 100°C for 1 h and called the 60/100°C extract; this extract is useful if only O and K precipitation lines are wanted. The same kind of extracts can be used for the different electrophoretic methods. For countercurrent techniques the extract has to be diluted (e.g., 1:200), or a simple preparation can be used, i.e., a suspension heated directly at 100°C for 1 h without centrifugation.

For the evaluation of the amount of K antigen, Glynn and Howard (71) use the ability of this antigen to inhibit the agglutination of sheep erythrocytes by rabbit antibody, a method described previously with pneumococcal polysaccharides by Ceppellini and Landy (20). Kaijser (121) finds, however, that the amount of K antigen is more specifically measured by crossed immunoelectrophoresis.

Test K antigens. So far, K antigens numbered K1 to K100 have been established (Table 1). According to our present knowledge, only K1 to K57, K62, K74, K82 to K84, K87, K92 to K98, and K100 represent acidic polysaccharide K antigens. K88 and K99 are proteins. The remaining earlier established K numbers, many of which are so-called B antigens, have not been demonstrated as special K antigens independent of the O antigens.

The patterns obtained with all hitherto established K test strains in immunoelectrophoresis in agar are shown in Fig. 1, which is a revised combination of similar figures presented before (190, 194). Previously, an immunoelectrophoretic group was named 1Ab because antibodies against the weakly immunogenic K1 and K5 are difficult to demonstrate. Strains of IE group 1Aa are now combined with those of 1Ab in a common group 1A. IE group 1Ba only contains K antigen strains having O antigens O8, O9, O20, and O101. The K antigens of this group generally do not move far away from the application basin, as opposed to the K antigens of IE group 1A and, furthermore, the O8, O9, O20, and O101 precipitation arcs are usually weak or even missing in strains giving strong K-antigen precipitation arcs (see reference 190).

Many strains listed in IE groups 1Bb and 2b as K-antigen test strains are disregarded as K strains today because in these strains K antigens of the B type were only defined by the inagglutinability of a nonheated culture in O antisera.

K95 to K98 and K100 are the latest reported K antigens (205). K95 to K98 were found in previously established O test strains by immunoelectrophoresis examination. Some additional K antigens were found in other O test strains by this method, but they will not be numbered, mainly because the reactions are difficult to reproduce and too erratic. These strains are denoted K^+. The K antigen in O45 is denoted K1, as it is closely related or identical to test antigen K1.

Form variation in *E. coli* polysaccharide antigens was described by Ørskov et al. (194). Similar to the form variation observed for *Salmonella* O antigens (132), *E. coli* strains with the K1 capsular polysaccharide can be found in two morphologically identical colony types designated $K1^+$ and $K1^-$, based upon agglutination or immunoprecipitation techniques. The variation phenomenon occurs in various K1 strains with a frequency that ranges from 1:20 to 1:50. In some cases, strains can be found that are stable in the $K1^-$ form. It is possible to produce a specific $K1^+$ antiserum by absorption of an OK $K1^+$ serum by a $K1^-$ stable strain. Although the $K1^-$ form is hardly immunogenic,

injection of the whole bacteria in the $K1^+$ form usually gives low-titered antiserum.

Our recent studies have shown that these antigenic differences can be related to structural differences in the $K1^+$ and $K1^-$ form variants. Both $K1^+$ and $K1^-$-purified polysaccharides are homopolymers of alpha-2,8-linked polysialic acid. The $K1^+$ polysaccharide has approximately 80 to 90% of the sialic acid residues *O*-acetylated; in contrast, the $K1^-$ variant has little or no detectable *O*-acetyl groups (F. Ørskov, I. Ørskov, R. Schneerson, W. Egan, A. Sutton, and J. B. Robbins, manuscript in preparation).

Recently, three new K antigens have been serologically examined in strains received for K-antigen determination. These are *E. coli* 1473 (O20:K101:H^-), *E. coli* 6CB10/1 (O8:K102:-H^-), and *E. coli* 8CE275 (O101:K103:H^-). Strain 1473, which was obtained from H. W. Moon, Ames, Iowa, causes diarrheal disease in newborn, colostrum-deprived piglets. The two other strains were isolated by K. A. Bettelheim and S. M. S. Lennox-King, London, in a maternity ward.

The K84 antigen deserves attention. The test strain of this antigen has the O20 antigen. However, a K antigen strongly related to K84 can also be found in combination with O8 as in strain H308b. That strain was until recently the test strain for O antigen O93, and it was accepted that O93 and O8 were cross-reacting, independent O antigens. With the aid of the O-specific phage Ω8, it was shown (114, 302) that O test strain O93 was in fact an O8 strain with a K antigen. The same result was obtained independently by immunoelectrophoretic studies. Thus, the supposed O antibodies in the O93 "O serum" were actually a mixture of antibodies against a heat-stable K(A) antigen and an O8 antigen, overlooked through many years.

Cross-reactions between *E. coli* K antigens. Many *E. coli* strains will agglutinate on slides in a great number of *E. coli* OK antisera, and many kinds of surface antigens may participate in these reactions. Many fewer cross-reactions are found by a gel precipitation technique, such as double diffusion in gel or countercurrent immunoelectrophoresis. Cross-reactions expressing relationships between K antigens have been found among the following K antigens: K18-K22-K100, K13-K20-K23, K53-K93, K54-K96, K16-K97, K37-K97, K12-K82, K2ab-K2ac-K62, and K7-K56. K2 (ab and ac) is so closely related to K62, K7 to K56, and K12 to K82 that K62, K56, and K82 will probably be deleted as the K antigen in favor of K2, K7, and K12, respectively. The other cross-reactions mentioned need further examination.

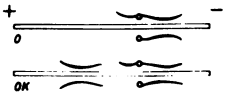
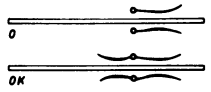
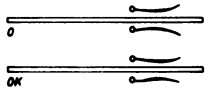
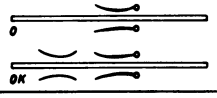
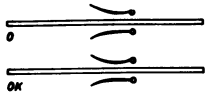
Cross-reactions between *E. coli* O antigens and K antigens. Identity between the unnumbered O antigen in strain 145 and the polysaccharide K antigen K87 found in strain G7 (O8:K87, K88:H19) has been described (211). Furthermore, an antiserum raised with a boiled culture grown at 37°C of strain G7 contains both agglutinins and precipitins against the O8 antigen but not against the antigen having the K87 specificity. However, an O antiserum prepared with a boiled culture of G7 grown at 18°C does contain agglutinins against the K87 antigen. The test strain of K87 was formerly strain 145, but is now a $K88^-$ mutant of G7 called D227 (O8:K87:H19).

Examples of relationships, but not of identity, between O and K antigens are the cross-reaction between the following: K31 and O120 (205), K9 and O104, K44 and O53, and K45 and O74 (unpublished data).

Cross-reactions between *E. coli* K antigens and polysaccharide K antigens outside the *Escherichia* group. Mørch and Knipschildt (170) described cross-reactions between some *E. coli* strains having K(A) antigens and the capsule antigens 9 and 23 of *Streptococcus pneumoniae*. Unfortunately, these strains have not been kept. Cross-reactions between K antigens of *E. coli* and *Klebsiella* have not yet been published. Heidelberger et al. (91) described antigen relations between *E. coli* K30 polysaccharide and pneumococcal polysaccharides Sn II and SV and *E. coli* K42 and Sn XXV, as well as K85 and S II and SV. Grados and Ewing (75) and Kasper et al. (124) demonstrated cross-reactions between *E. coli* K1 and meningococcal group B polysaccharide. Bradshaw et al. (13) used agar plates containing anti-*Haemophilus influenzae* type b serum to discover bacteria producing cross-reacting antigens. Schneerson et al. (252) described an *E. coli* strain with a polysaccharide K antigen closely related to *Haemophilus* type b capsule antigen; the strain was first described as O75:K(F147):H5 and is now test strain O75:K100:H5 for the new K100 antigen (205). Cross-reactions between the capsular antigen of *Neisseria meningitidis* group C and *E. coli* K antigen of strain Bos 12 and other strains were described by Robbins et al. (232). This K antigen was later designated K92; Bos 12 is now O16:K92:H^-. Cross-reactions between *S. pneumoniae* type 3 capsule and *E. coli* K7 antigen were described by Robbins et al. (234).

The immunological implications of these and similar cross-reactions are at present intensively investigated in many laboratories. Robbins et al. (234) have recently reviewed and discussed the relevant literature.

IMMUNOELECTROPHORETIC PATTERNS OF E. COLI O AND K ANTIGEN TEST STRAINS

1A			01 : K1 01 : K51 02 : K1 02 : K56 03 : K2 04 : K3 04 : K6 04 : K12 04 : K52 05 : K4 06 : K2 06 : K13 06 : K15 06 : K53 06 : K54 07 : K1 07 : K7 010 : K5 011 : K10 012 : K5 013 : K11 015 : K14 016 : K1 017 : K16 021 : K20 023 : K18 023 : K22 025 : K19 025 : K23 044 : K74 045 : K1 049 : K+ 073 : K92 075 : K95 075 : K100 077 : K96 086 : K62 0103 : K+ 0107 : K98 0117 : K98 0154 : K94
1B	a		08 : K8 08 : K25 08 : K27 08 : K40 08 : K41 08 : K42 08 : K43 08 : K44 08 : K45 08 : K46 08 : K47 08 : K48 08 : K49 08 : K50 08 : K87 08 : K102 09 : K9 09 : K26 09 : K28 09 : K29 09 : K30 09 : K31 09 : K32 09 : K33 09 : K34 09 : K35 09 : K36 09 : K37 09 : K38 09 : K39 09 : K55 09 : K57 020 : K17 020 : K83 020 : K84 020 : K101 0101 : K103
	b		018 : K76 018 : K77 019 : K− 023 : K21 026 : K60 035 : K− 036 : K− 039 : K− 040 : K− 043 : K− 050 : K− 051 : K− 052 : K− 055 : K59 060 : K− 062 : K− 063 : K− 066 : K− 068 : K− 070 : K− 071 : K− 073 : K− 078 : K80 085 : K− 086 : K− 086 : K61 086 : K64 034 : K− 088 : K− 089 : K− 090 : K− 092 : K− 095 : K− 097 : K− 099 : K− 0101 : K− 0102 : K− 0106 : K− 0109 : K− 0111 : K58 0114 : K90 0118 : K− 0119 : K69 0123 : K− 0125 : K70 0126 : K71 0127 : K63 0127 : K65 0128 : K67 0129 : K− 0132 : K− 0135 : K− 0142 : K86 0145 : K− 0148 : K− 0151 : K− 0153 : K− 0155 : K− 0156 : K− 0157 : K− 0162 : K−
2	a		014 : K7 022 : K13 081 : K97 083 : K24 0116 : K+ 0120 : K+ 0139 : K82 0150 : K93
	b		027 : K− 028ab : K− 028ac : K73 029 : K− 030 : K− 032 : K− 037 : K− 038 : K− 033 : K− 041 : K− 042 : K− 046 : K− 048 : K− 053 : K− 054 : K− 057 : K− 058 : K− 059 : K− 061 : K− 064 : K− 065 : K− 069 : K− 074 : K− 076 : K− 079 : K− 080 : K− 082 : K− 083 : K− 084 : K− 087 : K− 091 : K− 096 : K− 098 : K− 0100 : K− 0104 : K− 0105 : K− 0108 : K− 0110 : K− 0112ab : K68 0112ac : K66 0113 : K75 0115 : K− 0121 : K− 0124 : K72 0130 : K− 0131 : K− 0133 : K− 0134 : K− 0136 : K78 0137 : K79 0138 : K81 0140 : K− 0141 : K85 0143 : K− 0144 : K− 0146 : K− 0147 : K89 0149 : K91 0152 : K− 0158 : K− 0159 : K− 0160 : K− 0161 : K− 0163 : K−

M Antigen

Many *Enterobacteriaceae* strains produce a slime (mucus) antigen that has been termed M antigen (125, 126). Although it forms capsules, it is nonspecific and thus distinct from the K antigens discussed here. It has been suggested by Kauffmann (132) and Henriksen (95, 96) that the M antigen, regardless of the microorganism that elaborates it, in all instances is serologically the same or nearly the same. The M antigen that is also known as capsular antigen (163), colanic acid (73), and slime wall antigen is usually better developed at a growth temperature lower than 37°C. For its formation, the composition of media is very important. It has been shown (1) that solid media with concentrations of solutes giving a high osmotic pressure will cause normally nonmucoid *Enterobacteriaceae* to produce great amounts of mucous substance. The M antigen can be found in many *E. coli* strains.

The M antigen cross-reacts serologically with some type-specific K antigens, such as the K30 and K39 antigens of *E. coli* and the K8, K11, K13, K21, and K35 antigens of *Klebsiella* (97). Antisera against *E. coli* or *Klebsiella* strains of the above K types cannot always be used for demonstration of the M antigen; usually only some of the immunized rabbits will produce these cross-reacting antibodies.

Protein K Antigens (Fimbriae Classified as K Antigens)

One of the *E. coli* K antigens, K88, is known to be a protein (277). According to bacterial agglutination results and classical definitions, this antigen behaves as a typical L antigen (211). In an electron microscope it is seen as long and thin fimbriae covering the whole surface of the bacterial cell (277). The antigen is described as fimbria-like. It can also be demonstrated as a surrounding coat by the immunofluorescence technique (Ørskov and Lind, unpublished data) and after staining with Leifson flagella stain (189). Another similar antigen, K99, is also a protein (105a). Both antigens are found in strains associated with diarrhea in animals, K88 mainly in swine and K99 mainly in calf and lamb strains, and both confer agglutinating ability on blood cells that cannot be inhibited by mannose, K88 on guinea pig erythrocytes (118, 277) and K99 on horse erythrocytes (291). Both are transferable by plasmids and can be found together with chromosomally determined K antigens. No cross-reactions are known to exist between K88 and K99 and other antigens.

Recently a fimbrial antigen has been demonstrated in a strain from diarrhea in humans and claimed to act as a colonization factor (41). Another antigen has been found in some pig strains without the K88 antigen (105a). Furthermore, an antigen found in some calf strains probably belongs to the same class (297a). These have not yet been numbered as K antigens.

Fimbriae Not Classified as K Antigens

Nonflagellar filamentous appendages were first recognized in *E. coli* by Houwink and van Iterson (104). They were later called fimbriae (35) or pili (16). They were demonstrated in many *Enterobacteriaceae* (30, 32, 34, 35) and classified into several types according to morphology and adhesive properties. Some strains undergo a reversible variation between a fimbriate phase and a nonfimbriate phase, which can be influenced by the growth conditions (31). Broth culture is selective for the fimbriate phase, whereas nutrient agar is selective for the nonfimbriate phase.

Type 1 fimbriae, found in most *E. coli*, generally number 100 to 400 per cell in the fimbriate phase. They are characterized by a direct hemagglutination reaction and cause pellicle formation. They consist of protein (16, 180). Some strains are exceptional in that they show hemagglutinating activity but no fimbriae in an electron microscope. *E. coli* strains with fimbriae of type 1 give strong agglutination reactions with erythrocytes from most species, whereas nonfimbriate hemagglutinating strains have a weak activity and a more limited spectrum, and those of different strains agglutinate different erythrocytes. Type 1 fimbriae hemagglutination is inhibited by D-mannose and not affected by temperature. In contrast, nonfimbriate haemagglutinins are not inhibited by mannose and are best demonstrated in the cold at 4°C (31).

FIG. 1. *Immunoelectrophoretic patterns of E. coli O- and K-antigen test strains. In the trough: homologous O or OK antiserum. In the well above the trough: 60°C/20-min extract. In the well below the trough: 60/100°C extract. The right column contains the O:K serotypes of all E. coli O- and K-antigen test strains. Underlined antigens = test antigens. In the test strains of O antigens O24 and O56, anodic lines close to the basin are seen; K antigens have been demonstrated chemically in these strains, but, since we do not know whether the lines in immunoelectrophoresis represent O or K lines, the strains have been omitted from the scheme. The K antigens belonging to immunolectrophoretic groups 1Bb and 2e most likely represent O specificities (LPS) and can thus be left out of the serotype formula (see text and Table 2). (Note: O155:K⁻ should be moved from group 1Bb to 2b.)*

By raising antisera against fimbriate *E. coli* and *Shigella flexneri* type 1 strains and testing these by cross-agglutination and absorptions, Gillies and Duguid (67) obtained results from which they concluded that the fimbriae of *E. coli* contain a major type-specific antigen shared only within groups of related strains, as well as minor coli-flexneri shared antigens. However, no extensive systematic examination of the serology of *E. coli* fimbriae has been undertaken. Probably several morphological as well as antigenic types exist, not only in the *E. coli* group, but also sometimes on a single *E. coli* cell.

In many respects fimbrial antigens could be considered as protein, K antigens; they are thermolabile, superficial, and liable to confer relative inagglutinability in O serum (67).

Other filaments that are necessary for the conjugation process and determined by conjugative plasmids have been described. They are called sex pili (147), as opposed to fimbriae = common pili. Five distinct types of sex pili have been identified and visualized by electron microscopy in *E. coli* K-12. They have characteristic morphologies and serological specificities and are receptors for specific phages (for a review, see reference 23). The first type of sex pilus found was called F because it was determined by the F factor (17, 147). This F pilus is identical to the f^+ antigen (139, 200).

Mention should be made here of previously described surface antigens in *E. coli* such as the α (273) and β (174) antigens. Fimbrial antigens bear resemblance to these, but no thorough comparison has been made. The anodic thermolabile antigen of Seltmann (259) is a common antigen detected by immunoelectrophoresis and found in all *Enterobacteriaceae* strains.

CHEMISTRY OF O ANTIGENS

The O antigens have been studied extensively during the past years, and more general information on them can be found in a number of comprehensive articles and reviews (59, 89, 112, 155–157, 175). They are LPS, the general structural feature of which is given in Fig. 2. The molecule consists of three regions: lipid A, an oligosaccharide termed core, and the O-specific polysaccharide chain shown in Fig. 2 below.

Region 1 (lipid A) is the part of the molecule that by hydrophobic interaction with lipoprotein (14, 15) is buried in the outer membrane of the bacterial cell. It consists of glucosamine phosphate and fatty acids, the most prominent of which is β-hydroxymyristic acid. Its structure has been described recently (158). Lipid A is responsible for the general biological (endotoxic) properties of the LPS (158). It will not be dealt with in this article.

FIG. 2. *Schematic diagram of the general structure of bacterial LPS (from Lüderitz et al. [157]).*

Region 2 (core) is linked to lipid A via a carbohydrate component that is typical for the LPS of gram-negative bacteria, 2-keto-3-deoxymannulosoctonic acid (KDO). Whereas there is only one structure of lipid A, found in all enterobacterial LPS so far studied, five different core oligosaccharides are described. Thus, there is a greater degree of variation in the synthesis of the core oligosaccharide. The core expresses R specificity, which in wild-type S forms is cryptic because of the substitution of the core with region 3.

Region 3 is the O-specific polysaccharide of the LPS of bacterial S forms (S-LPS). It consists of oligosaccharide repeating units. This is a structural principle that is found in all bacterial polysaccharides. Composition and structure of region 3 are the chemical bases of the O specificity of gram-negative bacteria. Thus, with the many distinct O specificities in *E. coli*, many S-LPS with distinct O-specific polysaccharides are found.

From the above considerations it follows that the structural variability becomes increasingly greater in the outermost (probably phylogenetically younger) compartments of the LPS molecule.

Isolation and Purification

Depending on the aim of research, methods were devised to isolate LPS-protein complexes, LPS, or the polysaccharide moiety of LPS. These have been described in several reviews (112, 115–157), and only a few will be mentioned here.

LPS may be extracted from the bacteria with ethylenediaminetetraacetic acid (300) or 45% aqueous phenol at 65°C (303, 304). Due to their high apparent molecular weights (5×10^6 to 50×10^6; see references 236 and 262), they can be purified by repeated ultracentrifugation. More recently, electrodialysis was introduced as a purification procedure (61a).

Chemical Characterization

For chemical characterization, O antigens (S-LPS) are usually hydrolyzed and analyzed as to their sugar constituents by use of chromatographic and electrophoretic methods. This was done with more than 100 *E. coli* test strains, and the results were compiled into a scheme of

chemotypes (197). Due to the common occurrence of glucosamine (from lipid A and often from the core), glucose, galactose, heptose, and KDO (from the core), these sugar constituents were termed basal sugars (for reviews, see references 155 to 157). In many O-specific polysaccharides (region 3) one or more of these basal sugars are missing. Wrong conclusions about the composition of the O-specific polysaccharide may then be drawn from the sugar composition of the whole LPS. This explains why the sugar compositions given in Tables 7 and 9 differ from those published earlier (197).

To study the O-specific polysaccharides, LPS were degraded by mild acid hydrolysis into lipid A (region 1) and carbohydrate moiety (regions 2 and 3). Gel permeation chromatography of the carbohydrate moiety on Sephadex (106, 173, 247) gave elution patterns as shown in Fig. 3. Comparative analyses indicated that the material from peak 1 is the O-specific polysaccharide bound to core oligosaccharide (regions 2 and 3), that from peak 2 is unsubstituted core (region 2), and that from peak 3 is predominantly KDO, which was split off during the acid degradation. The presence of unsubstituted core oligosaccharide in preparations of wild-type S-LPS was an indication of the presence of R-LPS (lacking the O-specific polysaccharide) in S forms of *E. coli*. More recently this was confirmed by polyacrylamide gel electrophoresis of intact LPS in the presence of sodium dodecyl sulfate (106). This method also revealed that S forms often produce LPS molecules with different chain lengths (see also reference 171).

Neutral Polysaccharide Chains

The sugar composition of some neutral O-specific polysaccharide chains is given in Table 7. It is noteworthy that unusual amino sugars and also rhamnose occur quite frequently in *E. coli* (see also references 111 and 112). The O-specific polysaccharides may be composed of up to six different sugar constituents. There are only two O-specific homopolysaccharides hitherto described in *E. coli*, namely, the O8- and O9-specific mannans. O-specific homopolysaccharides (mannans and galactans) are also found in *Klebsiella* (176). The *E. coli* O9 antigen is identical to the *Klebsiella* O3 antigen, and the *E. coli* O8 antigen has a structure that is very similar to that of the *Klebsiella* O5 antigen (see Table 8 and reference 153).

The structures of the neutral O-specific polysaccharides that were previously elucidated are given in Table 8.

Acidic Polysaccharide Chains

For a long time all *E. coli* O antigens were thought to contain only neutral polysaccharide chains, comparable to *Salmonella* O antigens. More recently, LPS were isolated that contained acidic components such as glycerol phosphate (107), hexuronic acids (194; K. Jann, unpublished data), neuraminic acid (109; B. Jann, unpublished data), or 4-*O*-(1'-carboxyethyl)-D-glucose (glucolactilic acid [see reference 28]). Degradation studies and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed that the LPS preparations, obtained in the usual way as the sediment of a preparative ultracentrifugation, contained only little polysaccharide material with acidic components. These preparations consisted largely of LPS with an unsubstituted core (R-LPS) and LPS with only one O-specific repeating unit linked to the core (SR-LPS). An analysis of the supernatant of preparative ultracentrifugation revealed that the major portion of the acidic LPS is not sedimented during ultracentrifugation and can be obtained from the supernatant by fractional precipitation with Cetavlon (see references 105 and 116).

The sugar composition of all acidic O-specific polysaccharides, analyzed in Freiburg until now, is given in Table 9, and the structures of some polysaccharide moieties are presented in Table 10.

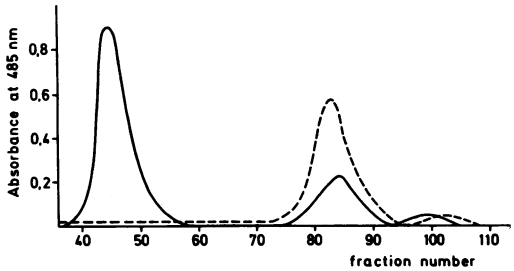


FIG. 3. *Gel permeation chromatography on Sephadex G-50 of degraded polysaccharides from S-LPS (——) and R-LPS (-----).*

CHEMISTRY OF R ANTIGENS

R mutants that arise spontaneously from S forms or that may be produced in the laboratory by treatment with mutagens have LPS lacking an O-specific polysaccharide moiety (region 3 in Fig. 2). These LPS are termed R antigens. They consist of lipid A and the core oligosaccharide, whereby the core may be complete (in *rfb* mutants [245, 247]) or incomplete (in *rfa* mutants [245, 248]). The *E. coli* strains K-12, B, and C are R forms of which the S ancestors are not known. *E. coli* K-12 is an R(*rfb*) mutant, and *E.coli* B is an R(*rfa*) mutant (245).

TABLE 7. *Sugar composition of some neutral O-specific polysaccharides from E. coli*

E. coli O antigen	4-Amino-4,6-dideoxyglucose	4-Amino-4,6-dideoxygalactose	3-Amino-3,6-dideoxyglucose	3-Amino-3,6-dideoxy-D-galactose	2-Amino-2,6-dideoxymannose	2-Amino-2,6-dideoxygalactose	Galactosamine	Glucosamine	Galactose	Glucose	Mannose	Fucose	Rhamnose	6-Deoxytalose	Colitose	Ribose
1								+					+			
2				+				+					+			
3					+			+		+						
4						+							+			
5			+				+		+							+
6							+	+			+					
7	+							+	+		+		+			
8											+					
9											+					
10		+						+	+				+			
19								+	+	+			+			
20									+							+
50								+					+			
55																
71			+				+		+				+			
75								+	+		+		+			
85								+	+							
86							+		+	+		+				
88											+			+		
102								+	+	+			+			
111								+	+	+					+	

For the chemical study of the LPS from R mutants (R-LPS), it is preferable to extract the bacteria with phenol-chloroform-petroleum ether at room temperature (61). Thus, only R-LPS, which may be considered as glycolipids, are extracted, and the purification of R-LPS is easy.

R Antigens with a Complete Core

From early studies it was originally concluded that all gram-negative bacteria had only one and the same core structure in their LPS, a view that holds for all *Salmonella* (see references 122, 156, and 157). Serological studies of Möller (169) in the late 1940s had indicated that two types of R mutants occur in *E. coli* strains. This was verified later by the isolation and analysis of two distinct core types from *E. coli* O8:K27$^-$ and O8:K42$^-$, which were termed coli R1 and coli R2, respectively (247). More core types were found in *E. coli* (244–246), and their compositions are given in Table 11. Their core structures and the *Salmonella* core are the only complete core structures known today. The core types found in *E. coli* also occur in other species, and some examples are included in Table 11. Because the coil core types also occur outside *E. coli*, the specification coil has been omitted from their denomination. Recently, it was shown (249) that *E. coli* O14 is an R strain (lacking O specificity) of the R4 type.

The structures of some core oligosaccharides are presented in Table 12. For comparison, the *Salmonella* core is also shown. All core oligosaccharides seem to have a common basal structure, including the heptose units and the adjoining sequence Glc(Gal) $\xrightarrow{1.3}$ Glc $\xrightarrow{1.3}$ Hep, in which an anomeric exchange of the 4-OH (Glc versus Gal) does not seem to be important for the function of the core region (P. Prehm and K. Jann, manuscript in preparation). The core may be substituted, e.g., on the KDO moiety with galactose in R2 (84) and with rhamnose in some K-12 strains (167) or on the terminal glucose of some K-12 strains with glucosamine or with *N*-acetylmannosaminuronic acid (225, 225a). The significance of these substitutions is not known.

It is important to note that all core oligosac-

TABLE 8. *Structures of some neutral O antigens of E. coli*[a]

O antigen	Repeating unit	Reference
O8	$\xrightarrow{3}$ Man $\xrightarrow[\alpha]{1,2}$ Man $\xrightarrow[\alpha]{1,2}$ Man $\xrightarrow[\alpha]{1}$	230
O9	$\xrightarrow{3}$ Man $\xrightarrow[\alpha]{1,3}$ Man $\xrightarrow[\alpha]{1,2}$ Man $\xrightarrow[\alpha]{1,2}$ Man $\xrightarrow[\alpha]{1,2}$ Man $\xrightarrow[\alpha]{1}$	222
O20	$\xrightarrow{4}$ Galp $\xrightarrow[\alpha]{1,2}$ Ribf $\xrightarrow[\beta]{1}$	298
O75	$\xrightarrow{3}$ GlcNac $\xrightarrow[\alpha]{1,3}$ Gal $\xrightarrow[\alpha]{1,4}$ L-Rha $\xrightarrow{1}$ β ↑ 1,4 Man	40
O86	→ Gal → GalNac → GalNac → ↑ ↑ L-Fuc Glc	270
O111	→ GlcNac $\xrightarrow[\beta]{1,2}$ Glc $\xrightarrow[\alpha]{1,4}$ Gal $\xrightarrow[\alpha]{1}$ α ↑ 1,6 ↑ Col Col	37

[a] Col (colitose), 3,6-dideoxy-L-galactose. If not otherwise indicated, the symbols for sugars used in this and the following tables are those suggested by IUPAC-IUB: Arch. Biochem. Biophys. 115:1-12, 1966.

charides are substituted with phosphate, phosphorylethanolamine, and pyrrophosphorylethanolamine. This together with the carboxyl groups of KDO renders the core region highly charged, may be of importance for the formation of superstructures on the cell wall and the maintenance of the outer membrane, and plays a role in the interaction of bacterial cells with bacteriophages or antibiotics. The full substitution is shown in Fig. 4 with the LPS of *E. coli* B.

R Antigens with an Incomplete Core

E. coli B is an old laboratory strain with an *rfa* (incomplete) core. Treatment of S and R forms with mutagens often leads to R(*rfa*) mutants with incomplete core oligosaccharides. These incomplete structures are a great help in the elucidation of complete core oligosaccharides. In fact, the structures of R1, the K-12 core, and the B core were derived with the aid of such substructures (223, 225; Jann, manuscript in preparation). The structure of the core from *E. coli* B with all of its substituents is shown in Fig. 4.

GENETICS OF O AND R ANTIGENS

For a better understanding of the genetic determination of O antigens (LPS), the chemical structure of these molecules should be known. Information on this is given in the chemical section of this article. The basic structural feature of LPS is shown in Fig. 2.

The genetics of the *Salmonella* LPS have been studied extensively, and much knowledge has been gained about the biosynthesis of the core region and the O-specific polysaccharide by mating and transduction experiments, whereas less is known about *E. coli* in this respect. The *Salmonella* strain particularly studied is *S. typhimurium*. These studies, reviewed by Stocker and Mäkelä (279), have shown that most of the genes determining the biosynthesis of the core region are present in a gene cluster (*rfa*) located in the *cysE pyrE* region on the *Salmonella* chromosome. The genes determining the O-specific repeating units are closely linked to the *his* operon and termed *rfb*. Mutations in either *rfa* or *rfb* result in a rough (R) phenotype. R mutants defective only in the *rfb* locus have a complete core, and R mutants defective in the *rfa* locus have a more or less incomplete core. The *rfc* gene between *trp* and *gal* is involved in the polymerization of the O-specific repeating units in some *Salmonella* O groups; if the *rfc* region is defective, only single repeating units are added to the core, resulting in an SR mutant instead of the normal smooth (S) form. Finally, a gene cluster termed *rfe*, close to *ilv*, appears to be involved in the synthesis of certain types of O antigens (as well as

TABLE 9. *Sugar composition of some acidic O-specific polysaccharides from E. coli*

E. coli O antigen	Pyruvate	Glycerol phosphate	*N*-acetylneuraminic acid	Lactosyl rhamnose	Lactosyl glucose	Glucuronic acid	Galacturonic acid	2-Amino-2,6-dide-oxygalactose	Galactosamine	Glucosamine	Galactose	Glucose	Mannose	Fucose	Rhamnose	6-Deoxytalose	Colitose	Ribose
24			+						+		+	+						
41						+				+	+			+				
54							+			+	+				+			
56			+							+	+	+						
58				+						+			+					
59							+			+			+					
69						+				+	+				+			
80						+			+	+	+			+				
83						+				+	+	+						
87							+		+		+							
96						+				+	+							
104			+						+		+							
105						+				+					+			(+)
112ab	+						+		+	+		+						
112ac						+			+	+		+						
116							+	+	+	+								
120						+			+		+				+			
124					+				+		+	+						
134						+				+	+	+						
139							+			+		(+)						
140						+			+		+	+						
144	+									+	+	+	+					
143						+	+			+	+		(+)					
100		+								+	+				+			
79						+				+	+		+		+			
141						+				+			+		+			
(32)[a]						+		+		+	+	+						

[a] See footnote to Table 10.

of enterobacterial common antigen [160, 161]); *rfe*⁻ *Salmonella* mutants are phenotypically like *rfb*⁻ mutants as no O specific side chain is formed. *rfb*, *rfc*, and *rfe* genes are thus participating in the synthesis of the O-specific side chains.

The genes concerned in LPS biosynthesis of *E. coli*, as far as studied, have been shown to relate to those in *Salmonella*.

In 1962, it was shown by Ørskov and Ørskov (188) that the genes controlling O-antigen specificity in a number of smooth *E. coli* cultures were closely linked to the histidine locus. Strains of O antigens O6, O9, O25, O26, and O100 were studied. Schmidt et al. (247) have similarly found that the genes for O-antigen synthesis in *E. coli* O8:K27 and O8:K42 are near *his*. This O-antigen-determining locus in *E. coli* is probably analogous to the *rfb* locus in *Salmonella*.

By mating some R strains isolated from *E. coli* O8:K27 with S forms of the same strain as donors, it was demonstrated that the chromosomal site of the S → R mutation in these strains was located close to *mtl*, a position similar to the *rfa* locus in *Salmonella* (248). In later experiments Schmidt (245) confirmed the presence of such *rfa* genes linked to *mtl* in the *E. coli* K-12 strain.

One of the several described *rfa* genes in *Salmonella* is termed *rfaL* (279). It determines a component involved in the translocation of newly synthesized O-specific polysaccharide to a complete core. A gene equivalent to this *rfaL* gene was demonstrated in *E. coli* O8:K27 (250).

The presence of a gene close to *ilv* equivalent to *rfe* in *Salmonella* has also been demonstrated in *E. coli* (251). By the transfer of an *rfe* defect from *S. montevideo* to *E. coli* O8, O9, and O100, recombinants were obtained that were blocked in the synthesis of O-specific polysaccharide (Mäkelä and Jann, unpublished data)

TABLE 10. *Structures of some acidic O antigens of E. coli*

O antigen	Repeating unit	References
(O32)[a]	$\xrightarrow{4}$ GlcUA $\xrightarrow[\beta]{1,3}$ FucNAc $\xrightarrow{1,3}$ GlcNAc $\xrightarrow{1,6}$ Gal $\xrightarrow{1}$ β | 1,4 Glc	108, 286
O58	$\xrightarrow{3}$ GlcNAc $\xrightarrow[\alpha]{1,4}$ Man $\xrightarrow[\alpha]{1,4}$ Man $\xrightarrow[\beta]{1}$ α | 1,3 (Man) ; 2(3) | Ac RhaLA	Dmitriev et al., manuscript in preparation
O100	$\xrightarrow{3}$ Gal $\xrightarrow{1,4}$ GlcNAc $\xrightarrow{1,2}$ Rha $\xrightarrow{1,4}$ Rha $\xrightarrow{1}$ P-Glyc	107; Prehm and Jann, manuscript in preparation
O124	$\xrightarrow[\beta]{3}$ GalNAcp $\xrightarrow[\beta]{1,3}$ Galp $\xrightarrow[\alpha]{1,6}$ Galf $\xrightarrow[\beta]{1}$ α | 1,4 (Galp) GlcLA $\xrightarrow[\beta]{1,6}$ Glc	28
O141	$\xrightarrow[4]{2}$ GlcUA $\xrightarrow[1,6]{1,2}$ Man $\xrightarrow{1,3}$ Man $\xrightarrow{1,3}$ GlcNAc $\rightarrow$ Man $\xrightarrow{1,3}$ Man $\xrightarrow{1,3}$ GlcNAc $\xrightarrow{1}$ GlcUA ↓ (Man) ; Rha ↑ (Man) ; Rha ↑ (Man)	113

[a] An O antigen related to O32 was described in the former test strain of antigen K87 (strain designation 145 = (O32):K87:H45) (211): RhaLA (rhamnolactylic acid), 2-*O*-(1'-carboxyethyl)-L-rhamnose; GlcLA (glucolactylic acid), 4-*O*-(1'-carboxyethyl)-D-glucose.

TABLE 11. *Sugar composition and occurrence of various R core types (244-246)*

Core designation	Sugar composition				Also occurring in
	GlcN	Glc	Gal	Hep[a]	
R1		3	2	3	*S. sonnei, S. flexneri*
R2	1	4[b]	2[b]	3	*Arizona*
R3	1	3	<1	3	*Citrobacter, S. flexneri*
R4		3	4	3	*S. flexneri*
K-12		3	1	3	

[a] Hep, L-Glycero-D-mannoheptose.
[b] One residue is linked to KDO.

(and enterobacterial common antigen [160]). The defect (*rfe*) could be restored by mating with an *E. coli* donor strain.

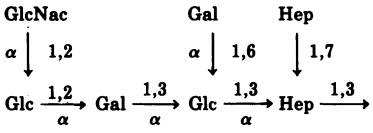
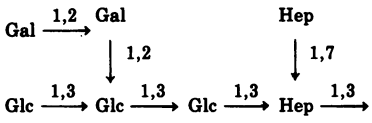
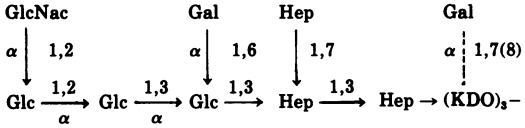
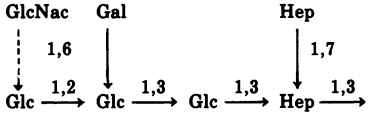
Lack of an O-specific polysaccharide in *E. coli* may be due to a block in its synthesis caused by a mutation located either close to *his* (*rfb*) or *ilv* (*rfe*). It may also be due to the fact that the O-specific polysaccharide is synthesized but not attached to the core and therefore not demonstrable as surface antigen. This may be caused by a mutation in one of the *rfa* genes close to *mtl* determining core structure or in that involved in the translocase system (*rfaL*).

However, the smoothness of an *E. coli* strain does not always indicate the presence of a complete O-specific polysaccharide attached to the LPS core. It may be caused merely by the presence of K antigen. Thus, the antigen established as *E. coli* antigen O14 is not an O antigen, but an R antigen found in a K^+ strain; K^- mutants isolated from O14:K7 are rough. The test O14 strain produces a complete LPS core structure but no O side chain because of a defect in the *rfb* gene cluster (251).

Not all established O antigens are determined by *rfb* genes. Recently it was found that the transfer of the *his* locus from *E. coli* O25:K10 to the O20:K17 strain resulted in the transfer of the O25 antigen, but the recombinants also expressed the O20 specificity. The most likely explanation in this case was not diploidization, but the fact that the genes determining O25 and O20 are not allelic (Ørskov and Ørskov, manuscript in preparation). The *E. coli* O20-specific polysaccharide contains ribose and galactose (Table 8). Similar sugars have been found in the T1 antigen of *Salmonella* (157). Since neither the T1 antigen (279) nor the *E. coli* O20 antigen is determined by the *rfb* locus, a common genetic locus should be looked for.

Genetic crosses involving the transfer of the

TABLE 12. *Structures of some core oligosaccharides*

Core type	Structure[a]	Reference
Salmonella		157
E. coli R1		Jann, manuscript in preparation
E. coli R2		84
E. coli K-12		225

[a] Hep, L-Glycero-D-mannoheptose.

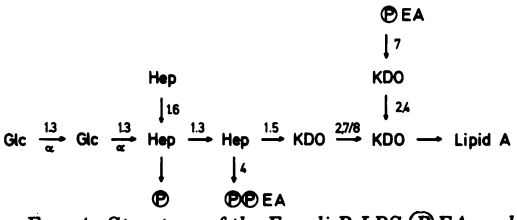


FIG. 4. *Structure of the E. coli B LPS. Ⓟ EA and ⓅⓅ EA are phosphoryl- and pyrophosphorylethanolamine, respectively (from Prehm et al. [223, 224]).*

rfb gene cluster from *Salmonella* to *E. coli* have been reported by various workers (84, 119, 137). The results obtained depend on the strains used. *E. coli* K-12, for example, can receive *S. typhimurium rfb* genes, but will not express them until its LPS core has been modified after the introduction of *Salmonella rfa* genes. When the *rfa* locus of *E. coli* K-12 is introduced into an *E. coli* recipient such as O8:K⁻, the recombinant not only expresses K-12 specificity but is also able to accept O-specific polysaccharides (or oligosaccharides) from other strains such as *S. typhimurium* (225a). *Salmonella* × *E. coli* recombinants may exhibit donor O antigen in a more or less complete state (S or SR), which is assumed to depend in some cases on the simultaneous transfer of the *rfc* locus involved in the polymerization of repeating units. Some experiments with both *E. coli* × *E. coli* and *Salmonella* × *E. coli*, however, result in recombinants that express both donor and recipient O-antigen specificity, suggesting a diploid state in these cases (137). Duplication of gene regions in crosses between *Salmonella* and *E. coli* was described previously (3).

CHEMISTRY OF K ANTIGENS

The K antigens were described as capsular or envelope antigens (130, 131) and can be detected easily by immunoelectrophoresis. They are all polysaccharides, except for two (K88 and K99) that are proteins (105a, 276, 278). Some strains were previously thought to have K antigens (e.g., *E. coli* O111), but no surface antigen other than the O antigen was found in them.

Isolation and Purification of Polysaccharide K Antigens

After extraction of the bacteria with 45% aqueous phenol and isolation of the aqueous phase, acidic capsular polysaccharides are found in the supernatant after subsequent ultracentrifugation (see above, Isolation and Purification). From this, they can be obtained and purified by fractionated precipitation with cetyl trimethyl ammonium bromide (Cetavlon) or cetyl pyridinium chloride (254). This method has been described in detail (105, 166, 303).

K Antigens Occurring in O Groups O8, O9, and O101

A number of K antigens occur exclusively in O groups O8, O9, and O101. K antigens associated with O20 probably belong to this category

also. The K antigens of this group are of two types, one with amino sugars and one without (Table 13). Those devoid of amino sugars have molecular weights ranging from 3×10^5 to 10^6. They are physically heterogeneous and can be rendered homogeneous by treatment with dilute alkali. This reduces their molecular weights to about 10^5 without changes in chemical composition (105, 115, 116, 149). Their similarity to the K antigens of *Klebsiella* is striking (112). Those K antigens that contain amino sugars are homogeneous and have molecular weights ranging from about 1×10^5 to 3×10^5 (Jann, unpublished data).

The K antigens of this group that contain amino sugars were thought to be extracellular, but we have found that they are in fact LPS (101; Jann, manuscript in preparation). Long chains of acidic polysaccharides attached to core lipid A as well as one repeating unit attached to core lipid A (corresponding to SR forms in O-specific LPS) were found. Thus, strains with such K antigens have in fact two cell wall LPS, one of them exhibiting O specificity (O8, O9, or O101) and the other exhibiting K specificity. The amino sugar-free K(A) antigens were also thought to be extracellular (capsular) antigens and not cell wall antigens. However, it was found very recently (B. Jann, P. Prehm, and K. Jann, manuscript in preparation) that in *E. coli* O9:K30 one oligosaccharide repeating unit (GlcUA → Gal → Man) is covalently linked to core lipid A. Thus, in these strains K specificity is, at least in part, also expressed by an LPS. A long-chain polysaccharide bound to core lipid A has not yet been found with these K antigens.

The structures of some K antigens occurring in O groups O8, O9, and O101 are shown in Table 14. By a comparison of Tables 10 and 14, it is striking that one and the same polysaccharide is listed as O specific in Table 10 and as K specific in Table 14. This phenomenon will be discussed below.

K Antigens Occurring in O Groups Other than O8, O9, O20, and O101

These K antigens are all acidic polysaccharides with rather low molecular weights. The compositions of some K antigens of this group are given in Table 15.

They contain sugar constituents that are not frequently found in bacterial polysaccharides, such as *N*-acetylneuraminic acid or *N*-acetylmannosaminuronic acid. The K54 antigen contains stoichiometric amounts of threonine. This constituent has not been found previously in bacterial polysaccharides. In fact, the presence of an amino acid has been reported in only a few cases. Alanine was found in teichoic acid (140), a capsular staphylococcal polysaccharide (85), and in an LPS from *Proteus mirabilis* (72). Lysine was found in an LPS from *P. mirabilis* (77). The role of these amino acids in the LPS and their antigenic specificity are not known in any of the cases mentioned.

The K1 antigen, also known as colominic acid (4), is a poly-*N*-acetylneuraminic acid with the structure shown in Fig. 5. Some of the carboxyl groups form ester bridges with hydroxyl groups of adjacent neuraminic acid residues (168); it is not known, however, whether this is an artifact due to the isolation procedure. There is a form variation in the K1 antigen that is described as

TABLE 13. *Composition of some K antigens occurring in O groups O8, O9, O20, and O101*

K antigen	Acidic sugar	Amino sugar	Neutral sugars					
			Gal	Glc	Man	Fuc	Rha	Rib
K26	GlcUA		+	−	−	−	+	−
K27	GlcUA		+	+	−	+	−	−
K28[a]	GlcUA, GalUA		+	+	+	+	−	−
K29	GlcUA		−	+	+	−	−	−
K30	GlcUA		+	−	+	−	−	−
K31	GlcUA		+	+	−	−	−	−
K32	GlcUA		+	+	−	−	+	−
K34[a]	GlcUA, GalUA		+	+	+	−	−	−
K42	GalUA		+	−	−	+	−	−
K8	GlcUA	GalN, GlcN	+	−	−	−	−	−
K9	NANA	GalN	+	−	−	−	−	−
K17	GlcUA	GlcN	+	−	−	−	−	−
K25	GlcUA	GalN	−	−	−	+	−	−
K57	GalUA	GlcN	+	−	−	−	−	+
K85[b]	GlcUA	GlcN	−	−	+	−	+	−
K87	GlcUA	FucN, GlcN	+	+	−	−	−	−

[a] Data from reference 306.
[b] = O141 (see text).

TABLE 14. *Structures of some K antigens occurring with O groups O8, O9, O20, and O101*

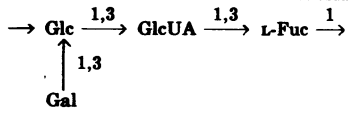
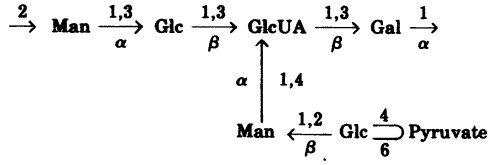
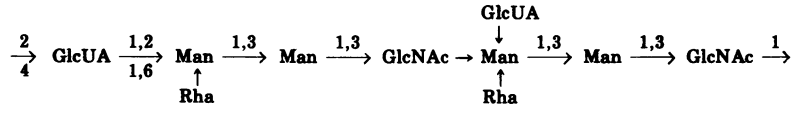
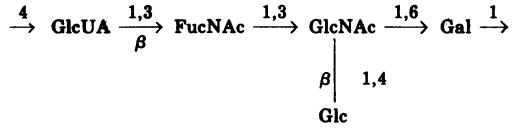
K antigen	Repeating unit	Reference
K27		116
K29		51
K30		105
K31		Jann, unpublished data
K42		115
K85		113
K87		286

TABLE 15. *Composition of some E. coli K antigens in O groups other than O8, O9, O20, and O101*

K antigen	Acidic sugar	Amino sugar	Neutral sugars						References
			Gal	Glc	Man	Fuc	Rha	Threonine	
K1		NANA[a]	–	–	–	–	–	–	4, 168
K7	Mannosaminuronic acid		–	+	–	–	–	–	166
K4	GalUA	GalN	–	+	–	–	–	–	Jann, unpublished data
K54	GlcUA		–	–	–	–	+	+	Jann, unpublished data

[a] *N*-Acetylneuraminic acid.

$K1^+/K1^-$ (194). The $K1^+$ polysaccharide has approximately 80 to 90% of the neuraminic acid residues *O*-acetylated, whereas the $K1^-$ variant has little or no detectable *O*-acetyl groups (see above, Test K antigens).

The K100 antigen of *E. coli*, originally designated Kf147, also belongs to those K antigens in the O groups that are not O8, O9, O20, or O101. It was reported to contain ribitol phosphate (233). Other sugar constituents, e.g., ribose, glucose, mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and *N*-acetylmannosamine, were also found in different preparations of the K100 antigen isolated from various *E. coli* strains (233). The structure of the K100 antigen is not known.

M Antigen

As described above, the M antigen is not a type-specific K antigen, but, since it surrounds the bacterial cell as a thick slime layer, it functions as a capsular antigen. Because of its ubiquitous occurrence and the interesting regulation of its synthesis (123, 163, 164), it was stud-

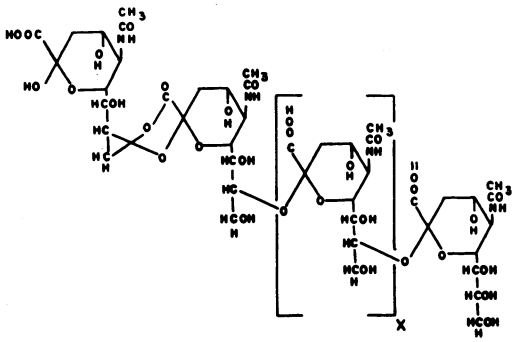


FIG. 5. *Structure of colominic acid (K1 antigen). × = 8 (average) (from McGuire and Binkley [168]).*

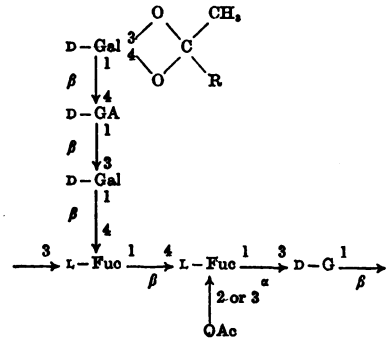


FIG. 6. *Structure of the M antigen. R = CH_3, isopropylidene substitution of galactose; R = H, ethylidene substitution of galactose (from Garegg et al. [63]).*

ied extensively (63, 64, 148, 282). The structure of the M antigen is shown in Fig. 6.

What is commonly termed M antigen actually represents a group of acidic polysaccharide antigens. They all have the same polysaccharide backbone consisting of a hexasaccharide repeating unit. This may be substituted by formaldehyde (methylidene substitution), acetaldehyde (ethylidene substitution), or pyruvate (carboxyethylidene substitution). For all of these substituents, the terminal galactose of the side chain functions as the acceptor (64).

GENETICS OF K ANTIGENS

The main gene loci on the coli chromosome determine two distinct groups of K antigens. One of these is linked to the *his* operon, and the other is linked to *serA*. The linkage map of the *E. coli* chromosome is shown in Fig. 7.

K Antigens Determined near *his*

These K antigens have only been found in O groups O8, O9, and O20, and until now genes have been mapped for the following: K26 (188), K8, K9, K17, and K57 (Ørskov and Ørskov, manuscript in preparation), and K27 (251a). The gene locus controlling the synthesis of these antigens is closely linked to the *rfb* gene cluster that determines the synthesis of the O-specific polysaccharides.

Results obtained recently in crosses involving *his*$^-$ mutants of O8:K8, O8:K25, O9:K9, and O9:K57 as recipients have shown genetic linkage between genes for O and K antigens in these strains. The introduction of donor *his* markers into these strains results in the acquisition of donor O antigen (O1 or O25) simultaneously with a loss not only of recipient O antigens (O8 or O9), but also of recipient K antigens (K8, K9, K25, or K57) (Ørskov and Ørskov, manuscript in preparation).

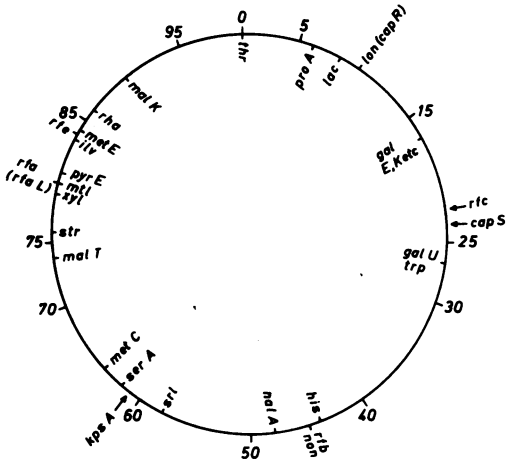


FIG. 7. *Linkage map of E. coli based on the E. coli K-12 map of Bachmann et al. (2). Genes named outside the circle affect polysaccharide structures. Positions not accurately known are indicated by arrows. rf, LPS biosynthesis; rfa, core; rfb, O repeating unit; rfc, polymerization of O repeating units; and rfe, undefined. kps, polysaccharide K-antigen biosynthesis; non, block in capsule formation (M antigen); lon (previously capR), regulation of capsular polysaccharide synthesis (M antigen); capS, regulator gene for capsular polysaccharide synthesis (M antigen); thr, threonine; pro, proline; lac, lactose; gal, galactose; trp, tryptophan; his, histidine; nal, nalidixic acid; srl, sorbitol; ser, serine; met, methionine; mal, maltose; str, streptomycin; xyl, xylose; mtl, mannitol; pyr, pyrimidine; ilv, isoleucine-valine; rha, rhamnose.*

Some of the K antigens of this group require an additional *trp*-linked gene locus for complete expression (251a). This locus may be comparable to the *rfc* locus in *Salmonella* that is necessary for the polymerization of O-specific oligosaccharide repeating units, and its product may thus be a K-specific polymerase.

These K antigens determined by a genetic

locus close to *his* were originally described as either L (K8 and K9), B (K25 and K57), or A (K26, K27, K29, K30, K31, and K42).

K Antigens Determined near *serA*

These antigens, of which K1, K4, K10, and K54 have been mapped (199, 208), are probably found in all O groups with the exception of O8, O9, O20, and O101. The gene controlling their synthesis was termed *kpsA*, *k* for K antigen, *ps* for polysaccharide, and *A* because it is the first locus described that is involved in the structure of this type of K antigen.

It was found recently (Schmidt, personal communication) that the synthesis of the K7 antigen is controlled by a gene locus that is not linked to the *his* operon. However, it was not established whether the K7 antigen is controlled by the *kpsA* gene.

From a cross between a $K10^-$ donor and a $K54^-$ recipient, some recombinants were obtained that expressed the latent K antigen of the donor. These results indicated that at least two genes were operating to give a K^+ phenotype, one concerned with the structure (*kpsA*) and the other concerned with the expression of the K antigen (188, 199).

Genetic manipulation allows the construction of *E. coli* strains with both types of K antigens present on one cell. Thus, in crosses between *serA* mutants of *E. coli* O8:K8 or O9:K57 strains as recipients and *E. coli* O25:K10 as the donor, the recombinants expressed both donor and recipient K antigens. Because of the stable presence of two K antigens, the finding was explained as an indication of the fact that the K8 and K57 antigens map at a locus other than K10 (208).

Form variation has been described in the K1 antigen (see above).

M Antigen

Although we do not consider the M antigen as belonging to the true K antigens because it is found with the same specificity in many *Enterobacteriaceae* strains, the regulator genes that control the synthesis of this antigen (163, 164) should be mentioned. Mutations in two regulator genes designated *lon* and *capS* lead to a derepressed synthesis of several enzymes involved in the synthesis of the M antigen and thereby to an overproduction of M-antigen polysaccharide. The structurel genes for some of the enzymes are mapped (152). Two of the enzymes also participate in reactions not necessarily connected with the synthesis of the M-antigen polysaccharide, and thus other structural genes that are more exclusively associated with M-antigen synthesis may be found in an M-antigen polysaccharide operon (165).

Another mutation located near *his*, termed *non-9*, inhibits capsule formation (M antigen) (226).

K Antigens Determined by Plasmids

The fimbria-like K antigens K88 and K99 are proteins (105a, 278). The determinants of both antigens are transferable and located on plasmids (202, 211, 263).

BIOSYNTHESIS OF O AND K ANTIGENS

The principles of the biosynthesis of microbial polysaccharides were established in a number of *Salmonella* strains (172, 175, 215, 217, 235). It was found that oligosaccharide repeating units are first assembled on a carrier lipid in the cytoplasmic membrane. They are then polymerized to the polysaccharide chain that is still attached to the carrier lipid. In the biosynthesis of LPS, the polysaccharide is transferred from the carrier lipid to the core lipid A part of the molecule. The reaction(s) in which free polysaccharides are detached from the carrier lipid is not known. The carrier lipid is a polyprenolphosphate (99, 310), which, after liberation during the polymerization and release of the polysaccharide, is recycled in the synthetic process. The intermediary participation of polyprenolphosphates (bactoprenolphosphate and dolicholphosphate) was also reported for the synthesis of the cell wall glycopeptide (25, 66, 98), capsular polysaccharides of *Klebsiella* (178, 283, 292, 293), and yeast and bacterial mannans (151, 242) as well as polysaccharides of plants (94, 299) and higher organisms (5, 6, 220, 221). It was therefore thought that all polysaccharides would be synthesized by the same mechanism in which membrane-bound lipids function as carrier molecules.

Relatively little is known about the biosynthesis of polysaccharide antigens in *E. coli*. The K30 antigen of *E. coli* O9:K30 was shown to be synthesized via lipid-linked oligosaccharides by a mechanism similar to that described above (D. K. Chandler and K. Jann, manuscript in preparation). It is likely that other K antigens, which are related to the K30 antigen (Tables 13 and 14), are synthesized in the same way.

The in vitro synthesis of the O-specific polysaccharide of *E. coli* O111 was studied by Edstrom and Heath (38). They found that the sugar constituents are incorporated into cell envelope fractions in the sequence galactose-glucose-glucosamine-colitose. No oligosaccharides were detected in the synthesis of the O111 polysaccharide, and no lipid-bound intermediates could be found.

More recently the biosynthesis of the *E. coli*

O9 polysaccharide was studied with membrane vesicles (membrane mixtures and isolated cytoplasmic membrane [see reference 216]). The O9 polysaccharide, which is a mannan, is synthesized from guanosine 5'-diphosphate-mannose without the participation of a lipid intermediate. The synthesis is independent of bivalent metal ions, in contrast to the situation for *Salmonella* and *E. coli* O111, where the incorporation of galactose (the first sugar in the sequence) depended on the presence of magnesium ions. Although extraction with organic solvents failed to show an intermediate of the synthesis, such an intermediate could be detected by polyacrylamide gel electrophoresis in the presence of dodecyl sulfate, as was also shown independently in the synthesis of teichoic acid (55, 56). The synthesizing system can be extracted with 0.01% Triton X-100, which is an advantage for the further study of the system (Winter and Jann, manuscript in preparation). The O9 polysaccharide thus seems to be synthesized in a single chain mechanism that is different from the one described above.

The mechanism of O-polysaccharide biosynthesis found in *E. coli* O9 is also operative in *E. coli* O8 and *Klebsiella* strains O3 and O5 (142; H. C. Flemming and K. Jann, manuscript in preparation). In this context it is interesting to note that in *E. coli* strains O8 and O9 no *rfc* locus (determining the polymerase for lipid-linked oligosaccharides [see reference 279]) was found.

MULTIPLE OCCURRENCE OF POLYSACCHARIDE ANTIGENS

E. coli strain D227, O8:K87 was originally believed to contain a neutral cell wall LPS and, in addition, an acidic capsular polysaccharide (108, 113, 210, 286). It was found later (Jann and Jann, manuscript in preparation) that the K87 antigen was actually an acidic LPS, so the strain contains two LPS in the outer membrane. As described above, the acidic LPS of this strain is still considered to be a K antigen since no K87 antibodies are demonstrable in an O antiserum. The presence of two LPS, one neutral and one acidic, is also found in strains (isolated from calves) serotyped as O8:K85. The K85 and K87 specificities also occur in *E. coli* O141:K85 (strain RVC2907) and (O32):K87 (strain 145), respectively (Jann, unpublished data). This corresponds to the fact that extracts from these strains gave only one anodic line in immunoelectrophoresis (194). The structures of K85 and K87 are shown in Table 14.

One can speculate whether, during evolutionary processes involving host-parasite interactions in terms of invasion and defense, antigenic drifts occur, such as an R strain being converted to *E. coli* O8 which has acquired a neutral O-specific polysaccharide, further to *E. coli* O8:K87 with an additional acidic (lipo)polysaccharide, and finally to (O32):K87 which seems to have lost the neutral (lipo)polysaccharide. A similar sequence can be envisaged with the strains R–O8–O8:K85–O141:K85. In fact, it is possible that all *E. coli* strains with O-specific acidic LPS (Tables 9 and 10) may have arisen in such a fashion, finally arriving at a stage where the properties of a cell wall antigen (lipid A), important for the maintenance of the outer membrane, and those of a capsule (acidic polysaccharide), important for counteracting phagocytosis and adverse influences on the ecosystem, could be combined into one molecule.

PATTERNS OF POLYSACCHARIDE ANTIGENS

The results of the chemical and immunochemical studies are compiled in Table 16. The O antigens that are shown in the first four columns are neutral LPS. Their O-specific polysaccharide chains are mostly heteropolysaccharides; only the O antigens O8 and O9 have O-specific homopolysaccharides. In immunoelectrophoresis in agar, all of these neutral LPS show O lines, which, due to endosmosis, are located toward the cathode. Capsular antigens may occur together with these LPS (as in the first three columns of Table 16). These are either acidic polysaccharides (columns 1 and 2) or acidic LPS (column 3). In immunoelectrophoresis they all give rise to an additional K line, when anti-OK sera are used. K-antigenic acidic polysaccharides of relatively high and relatively low molecular weights can be differentiated in immunoelectrophoresis by their different electrophoretic mobilities. It is, however, not possible to differentiate with this technique acidic polysaccharides of high molecular weight from acidic LPS. Although the acidic LPS have polysaccharide chains of the usual small size (about 10,000), they acquire a very high apparent molecular weight by micelle formation via their lipid A moiety. The O antigens that are shown in columns 5 and 6 of Table 16 are acidic LPS. In some cases (column 6) an additional capsular polysaccharide, probably of low molecular weight, is found. On the whole, the results of chemical and immunoelectrophoretic studies are in good agreement. The designation of the various immunoelectrophoretic groups, as introduced in Fig. 1, are therefore also given in Table 16.

Some antigenic patterns shown in Table 16 are also found in other genera. Thus, *E. coli*

TABLE 16. *Polysaccharide patterns (combinations of polysaccharide antigens) in E. coli based on chemical and immunoelectrophoretic characterizations*

Chemical characterization	In many O groups Neutral LPS Acidic capsular polysaccharide	In O groups O8, O9, O101, (O20) Neutral LPS Acidic capsular polysaccharide	In O groups O8, O9, O101, (O20) Neutral LPS Acidic LPS	In many O groups Neutral LPS	In many O groups Acidic LPS	In many O groups Acidic LPS Acidic capsular polysaccharide
Immunoelectrophoretic characterization	O line: cathodic slow K line: anodic fast	O line: cathodic slow K line: anodic slow		O line: cathodic slow	O line: anodic slow	O line: anodic slow K line: anodic fast
Immunoelectrophoretic groups[a]	1A	1Ba		1Bb	2b	2a

[a] As described in the legend to Fig. 1.

strains with an antigenic pattern (combination of polysaccharide antigens) shown in column 2 closely resemble *Klebsiella*, and those with an antigenic pattern shown in column 5 resemble certain *Shigella* strains (*S. dysenteriae* and *S. boydii*). In fact, identity of surface antigens and serological specificity was found between some *E. coli* and *Shigella* strains (27, 28, 141, 275) as well as between some *E. coli* and *Klebsiella* strains (21, 153, 222, 230).

CHEMICAL BASIS OF ANTIGENIC SPECIFICITY

A number of reviews have been written on the immunochemical aspects of carbohydrate antigens (112, 155–157). Therefore, only information that is essential for the understanding of specificities of *E. coli* polysaccharide antigens and their cross-reactions with other polysaccharides will be given here. Extensive studies in many laboratories revealed that the antigenic specificities of polysaccharides in most cases center around single hexose units and extend along the polysaccharide chain over regions of different lengths. The sugar unit that contributes most to the antigenic specificity (contributes the highest increment of binding energy between the antigenic determinant and the binding site of a homologous antibody) was termed immunodominant sugar (156). In structural formulas this is usually indicated by an arc, according to a suggestion by A. M. Staub (see references 112 and 156).

Immunodominant sugars may be branch substituents of a polysaccharide main chain, such as colitose in the O111 antigen (Fig. 8) and galactose in the K27 antigen (Fig. 8). They may also be part of the polysaccharide chain, as with the K30 and K42 antigens (Fig. 8). In polysaccharide antigens these determinants repeat along the polysaccharide chain. Also, several distinct antigenic determinants may alternate in a polysaccharide.

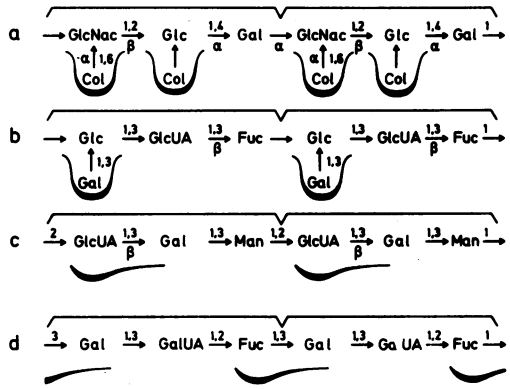


FIG. 8. *Antigenic carbohydrate determinants of the O111 antigen (a), K27 antigen (b), K30 antigen (c), and K42 antigen (d). Immunodominant sugars are indicated by arcs, and repeating oligosaccharide units are indicated by brackets.*

In addition to structural determinants, which are expressed by nature, sequence, and linkage of a few sugar residues, there may exist another type of determinant that could be termed "conformational determinant" (258). It depends on structures of secondary order, i.e., specific spatial arrangements of a polysaccharide such as helix or coil formation. The term conformational determinant was used by Girard and Staub (68) in the immunochemical analysis of the LPS from *Salmonella johannesburg*. The antigenic specificities of the O8 and O9 antigens of *E. coli* seem to be based on a special conformation of the O-specific polysaccharides (α-mannans). In spite of the similar

structures of the O8 and O9 antigens, which contain identical sequences, there is no cross-reaction at all between these two coli antigens. Both (and also the O3 and O5 antigens of *Klebsiella* that cross-react with *E. coli* O9 and O8, respectively) lose their O specificity completely when they are degraded with mild acid, a procedure that liberates the carbohydrate from the (micelle forming) lipid A (230; Jann, unpublished data). It was found (Jann and Jann, unpublished data) that the substitution of the mannans with stearoyl groups (1 to 2 residues per polysaccharide molecule of about 10,000 daltons) restored the serological activity. Since stearoyl groups can associate with each other (micelle formation) or with hydrophobic regions of the polysaccharide (alteration of conformation), this result can be taken as an indication of a secondary structure in the O-specific mannans. In accordance with this, no oligosaccharides with O8 or O9 specificity were found.

Antigenic specificity may also be due, at least in part, to noncarbohydrate substituents such as pyruvic acid in the K29 antigen (Table 14) and the M antigen (Fig. 6) or glycerophosphate in the O100 antigen (Table 10).

CHEMICAL BASIS OF CROSS-REACTIONS

The molecular basis of some cross reactions in which polysaccharide (O and K) antigens of *E. coli* take part has been elucidated (Table 17).

PREVALENCE OF SEROLOGICAL GROUPS AND TYPES OF *E. COLI* IN PATHOLOGICAL CONDITIONS IN HUMANS AND ANIMALS

Little is known about the normal distribution of different serogroups. Best examined are strains from humans and common domestic animals in temperate climates, whereas we know little about the distribution of serogroups and types in warmer geographical regions. Most examinations have been carried out in developed countries.

It is not yet possible to state whether there are significant differences in O-group prevalence between different animal groups. Recently it has been stated that this is not likely between humans and cattle (88). We agree that many frequent O groups are the same, but we would hesitate, on the other hand, to draw this conclusion until further examinations have been carried out. It is astonishing, for example, that O4 and O6 strains hardly occur among frequent cattle strains. When the prevalent O groups from diseased animals and humans are compared, it is evident that most of the strains associated with diarrhea in pigs and humans are different, even though infantile and piglet diarrheas have many other common traits.

E. coli from Humans

In Table 18 the findings from extra-intestinal infections are summarized. Only few of the many reports published are included, but those listed should be representative. Only the most frequent O groups have been included. The percentages for the single O groups differ, but it is probably true that with the use of those O sera that correspond to the O groups listed, it should be possible to determine the O antigen of at least half of all coli strains from urinary and other extra-intestinal infections. It should be noted that these frequent O groups are the same as those found to be prevalent in the normal intestine, but the percentages for feces are lower.

Similarly, only a few polysaccharide K antigens (K1, -2, -3, -5, -12, and -13) are found with high frequency in urinary tract infections and other extra-intestinal infections (121b, 159, 296).

Possibly, invasiveness is paralleled by a further selection of strains already frequent in the intestine.

Today most authors agree that the same O groups are frequent in the normal healthy intestines and extra-intestinal infections. However, in the special discussion about the involvement of *E. coli* in urinary tract infections, two seemingly different views have been expressed. One is named the "special pathogenicity theory," stating that some serovars are more frequent in the urinary tract than in the intestinal tract due to the special pathogenicity of such strains. The other, called the "prevalence theory," says that the serovars found in urinary infections represent the most prevalent types in the gut (81). Unfortunately, comparable data that could make possible a definite choice between the two theories are missing.

Some authors have indicated that differences in O-group distribution exist, e.g., between Europe and the United States (71) and even within different areas in London (80). Others have suggested that prevalence rates of certain O groups could vary with time in the same area (159). Even though such differences do occur, they can probably be explained by the use of different techniques and sera. The overall picture of a similar *E. coli* group prevalence in Western developed countries in temperate climates (301) is not disturbed by these discrepancies.

The results of serotyping carried out on different types of diarheal diseases in humans are summarized in Table 19. Part a lists the most

TABLE 17. *Cross-reactions of some E. coli polysaccharide antigens*

E. coli antigen	Cross-reacting antigen	Probable common antigenic determinant
O8[a]	*Klebsiella* O5[b]	$\xrightarrow[\alpha]{2}$ Man and/or $\xrightarrow[\alpha]{3}$ Man and/or conformational
O9[a]	*Klebsiella* O3[c]	$\xrightarrow[\alpha]{2}$ Man and/or $\xrightarrow[\alpha]{3}$ Man and/or conformational
O19ab	*Klebsiella* O1[d]	$\xrightarrow{3}$ Gal $\xrightarrow[\alpha]{}$
O20[a]	*Klebsiella* O4[e]	$\xrightarrow[\alpha]{2}$ Rib and/or $\xrightarrow[\beta]{4}$ Gal
O58[f]	*S. dysenteriae* 3	Terminal 3-*O*-(1'-carboxyethyl)-L-Rha[f]
O124[g]	*S. dysenteriae* 5	Terminal 4-*O*-(1'-carboxyethyl)-D-Glc[h]
K1[i]	*N. meningitidis* b	$\xrightarrow[\alpha]{8}$ NANA and/or conformational
K4	Pneumococcus XII	GlcUA possibly ManNacUA
K7[j]	Pneumococcus III	$\xrightarrow{4}$ Glc possibly ManNacUA
	Pneumococcus VIII	$\xrightarrow{4}$ Glc
	Pneumococcus XIX	Glc
	Pneumococcus XXII	$\xrightarrow{4}$ Glc
K8	Pneumococcus XXII	Glc and/or Gal
K26	Pneumococcus II	$\longrightarrow$ GlcUA (or terminal in K26) and/or $\xrightarrow{3}$ Rha
	Pneumococcus VI	$\xrightarrow{3}$ Rha
K30[k]	Pneumococcus II	$\longrightarrow$ GlcUA (or terminal in K30)
K31	Pneumococcus XXIII	$\xrightarrow{2}$ Rha (or terminal in K31)
K42[k]	Pneumococcus XXV	$\xrightarrow{3}$ GalUA and/or $\xrightarrow{3}$ Gal
K54	Pneumococcus III	$\xrightarrow{3}$ GlcUA
	Pneumococcus VI	$\longrightarrow$ Gal (2 position in VI or terminal in K54)
K85[k]	Pneumococcus II	Terminal GlcUA
	Pneumococcus V	$\xrightarrow{2}$ GlcUA (in V)
	Pneumococcus X	$\xrightarrow{3}$ GlcNac
K87	Pneumococcus VIII	Gal $\xrightarrow{1,4}$ GlcUA

[a] Structures are given in Table 8.
[b] Structures are given in reference 153.
[c] Structure is given in reference 21.
[d] Structure is given in reference 9.
[e] Structure is given in reference 10.
[f] Dmitriev et al., manuscript in preparation.
[g] Structure is given in Table 10.
[h] Structure is given in references 27, 28, and 141.
[i] Structure is given in Fig. 5.
[j] The K7 antigen contains glucose and *N*-acetylmannosaminuronic acid, the pneumococcal polysaccharides III and VIII contain glucose and glucuronic acid. The charge of the acidic sugar may also play a role in cross-reactions.
[k] The data for K30, K42, and K85 polysaccharides are from reference 91, and the results for the other K antigens are unpublished results of Heidelberger and Jann. For the composition or structures of the pneumococcal polysaccharides, see reference 90.

common enteropathogenic *E. coli* serovars. These types can be associated with infantile diarrhea. They were all originally isolated from severe outbreaks of infantile diarrhea in institutions and are also today most frequently found in such places. Only O groups have been listed, i.e., O26 and O55, since complete serotyping of such strains has been carried out to a very limited extent. However, it was found (45, 185) that some O:H combinations (O55:H6, O111:H2, O86:H34) are much more frequently associated with clear-cut outbreaks than others. The LPS of these strains are neutral, and they have no acidic polysaccharide K antigens (190), which seems to be in accordance with their noninvasive character. These serovars can also be found in institutionalized infants with no diarrhea, and the cause of pathogenicity in these strains cannot yet be explained. Fimbria-like antigens such as those found in piglet and calf strains (211, 212, 263) have not yet been described; neither has a definite proof for a general production of enterotoxin in such strains been given. Recently there has been a tendency to reject the importance of these special serovars completely because few such types examined have been enterotoxigenic. It should be remembered, however, that *E. coli* serotyping has been of great value for the elucidation of many serious outbreaks and epidemics of infantile diarrhea. On the other hand, it is of course true that serotyping of these so-called enteropathogenic types probably is of limited value for the examination of sporadic cases of infantile diarrhea.

TABLE 18. *E. coli O groups from extra-intestinal infections in humans*

Infection	O groups[a]	References
Urinary[b]	O1, O2, O4, O6, O7, O8, O9, O11, O22, O25, O62, O75	7, 43, 62, 81, 159, 177, 227, 294, 296
Septicemia	O1, O2, O4, O6, O7, O8, O9, O11, O18, O22, O25, O75	43, 74, 191
Other	O1, O2, O4, O6, O8, O9, O11, O21, O62	43, 79, 295, 296
Neonatal meningitis[c]	O1, O6, O7, O16, O18, O83	231, 241
Feces (healthy adults and children)	O1, O2, O4, O6, O7, O8, O18, O25, O45, O75, O81	128, 227, 255, 295, 296, 301, 305

[a] Since the prevalence rates have been compiled from many different investigations, the O groups are listed numerically.

[b] A limited number of polysaccharide K antigens are found to dominate among strains from urinary tract infections: K1, -2, -3, -5, -12, and -13. The same K antigens are also frequently found among stool isolates from healthy persons (121b, 159, 296).

[c] The prevalent O groups listed here are characterized by having the same K antigen, K1, when found in this disease.

In part b of Table 19, O groups of the dominant *E. coli* flora of sporadic cases of diarrhea in infants (not colonized by the so-called enteropathogenic serovars) are listed. It is apparent that the common fecal O groups are found (186), a result that might indicate that a possible etiological role of these strains is not linked to the O group.

Part c of Table 19 lists some O:H serovars frequently found among enterotoxigenic strains isolated from diarrheal diseases in adults and children, mostly in warm climates. Some of these O:H types have been isolated from the so-called traveler's diarrhea (196). Recently it has been shown that enterotoxigenic strains may also be the cause of food- and water-borne dis-

TABLE 19. *E. coli O groups and O:H types from intestinal infections in humans*

Infection	O groups and O:H serotypes	References
Infantile diarrhea		
(a) From outbreaks, mostly institutions in developed countries	O20, O26, O44, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, O158, O159	39, 45, 79a, 134, 185, 237a, 238, 256, 288[a]
(b) Sporadic cases excluding enteropathogenic types	O1, O2, O4, O6, O8, O15, O21, O51, O75, O85	78, 186
Diarrhea in adults and children		
(c) Enterotoxigenic strains, mostly sporadic cases	O6:H16, O8:H9, O15:H11, O25:H42, O78:H11, O78:H12, O128:H7, O20:H$^-$	196, 238, 205a, manuscript in preparation
(d) From food-borne disease	O6, O8, O15, O78, O124, O149	162a, 239
(e) Dysentery-like disease	O28ac, O112, O124, O136, O143, O144	179, 240

[a] References given are mostly to reviews in which further references can be found.

eases (239). A more detailed typing, also including K-antigen and biotype determinations, shows that special serofermentative O:K:H types are frequently associated with enterotoxigenicity (205a).

Serogroups associated with dysentery-like diseases are listed in part e of Table 19. In geographical regions where *S. dysenteriae* is a common type of diarrhea, these *E. coli* serovars are also found to be associated with dysentery. By the usual laboratory procedures, they will be labeled *E. coli*, but most of them have close O-antigen relationships to different *Shigella* serogroups (39). They have the same invasiveness as *Shigella* strains, are positive in the Sereny test (261), and cause primarily a disease of epithelial layers of the colon. The last column of Table 19 records a number of references to papers where O-group distribution in normal healthy persons has been examined. It is seen that some O groups can be called the common O groups, among which those listed in parts a and e of Table 19 are not found. The so-called enteropathogenic serotypes from infantile diarrhea and the *Shigella*-like serovars are thus rare in healthy intestines. The O groups from enterotoxin-determined diarrhea are not rare, but there are indications that some of these strains represent well-defined combinations of special O, H, and K antigens and have special fermentation patterns that are not common in healthy intestines (196, 205a).

E. coli from Cattle, Pig, and Poultry

When Jensen in 1893 (117) described the etiology of diarrhea and septicemia in calves, it was the first attempt to associate *E. coli* bacteria with a special disease syndrome.

Table 20 summarizes the knowledge about the association between *E. coli* O groups and certain diseases in cattle. As in the previous similar tables in this paper, results from different investigations have been combined because a general prevalence of some frequent O groups is apparent. The differences in prevalence found among various reports may be real but can, most simply, be explained by differences in the selection of strains and in the typing techniques. It is apparent from Table 20 that few examinations of the *E. coli* O groups in healthy calves have been carried out. If the O groups from sick and healthy calves are compared, only few significant differences in O-group prevalence can be found, the only important one being that Wramby (309) never found O78 strains in healthy animals. Fey (54) confirmed this rare occurrence of O78 strains in healthy animals.

TABLE 20. *E. coli O groups from cattle*

Infection	O groups	References
Diarrhea (white scours) and septicemia in calves	O1, O2, O8, O9, O15, O20, O26, O55, O78, O87, O101, O114, O115, O117, O137	12, 22, 53, 65, 70, 120, 154, 162, 228, 253, 264, 266, 297, 309
Healthy calf feces	O1, O2, O4, O6, O8, O9, O11, O13, O15, O18, O20, O22, O23, O36, O101, O107, O116, O117, O123, O153	70a, 104a, 104b, 309
Mastitis	O2, O8, O9, O21, O81, O86	52

Table 21 shows the *E. coli* O groups that are frequently found in young pigs. For an extensive review, see Sojka (266).

The strains from diarrhea, especially in newborn piglets, very often carry the fimbria-like antigen K88. Most of the O groups listed in Table 21 are usually found as well-defined serofermentative types, i.e., O138:H14 (earlier O138:K81:H14), with a typical fermentative pattern independent of their geographical place of isolation.

It should be mentioned that O2:K1 in most reports is the dominant O:K group from septicemia, and next in frequency are O78 and O1:K1 (Table 22). For an extensive review, see Sojka (266).

SUMMARY AND CONCLUSION

A thorough and precise knowledge about the surface structures of *E. coli* is evidently important. The *E. coli* group (species) consists of a high number of more or less closely related serovars or serofermentative types. These can be differentiated in many ways and by many techniques, but until now the most practical and useful procedure has been based on different antigenic surface structures. The all-important foundation for this work was laid down by Kauffmann 30 years ago, and since then sero-

TABLE 21. *Prevalent E. coli O groups from pig diseases*

Infection	O groups	References
Diarrhea (mostly enterotoxigenic strains from piglets)	O8:K87, O45, O101, O138, O139, O141, O147, O149, O157	83, 136, 143, 228, 265, 268, 284, 308
Edema disease	O138, O139, O141	136, 143, 268

TABLE 22. *E. coli O groups from poultry*

Infection	O groups	References
Coli septicemia (septic pericarditis, air sacculitis, etc.)	O1, O2, O8, O71, O73, O78, O88	86, 87, 92, 93, 267, 285
Hjärre's disease, coli granuloma disease, mucoid strains	O8, O9, O16	309

typing has been carried out mainly based on the O antigens (O-specific polysaccharide of LPS) and the H antigens (protein of flagella). Although the K antigens have been known to exist during this same period, they have played a minor role in general serotyping.

The introduction of some new techniques, mainly based on immunoprecipitation in gels, and the application of chemical and genetic methods have given us a more coherent picture of the capsular or microcapsular (acidic) polysaccharide K antigens. This development has made a more reliable serotyping of these antigens possible. Only few strains have been examined for their polysaccharide K antigens, and only future research will show how many more serologically different capsular antigens exist; the present number is about 70.

So far, the protein surface structures of *E. coli* have played a small role in serotyping procedures, with the exception of the two important K antigens, K88 and K99. Recent interest in many laboratories in fimbrial and pili structures, because of their possible role as adhesive organs, may increase the number of such protein K antigens considerably.

The importance of *Escherichia* strains, both as normal inhabitants of the intestinal tract and as intestinal and extra-intestinal pathogens, has been recognized for many years. However, only recently have we begun to understand the different mechanisms underlying the diverse aspects of *Escherichia* pathogenicity. The pathogenicity of *E. coli* is a very complex phenomenon, in which the chemical nature of the bacterial cell surface plays a prominent part. The negative surface charge due to acidic capsular or cell wall polysaccharides may be important for the penetration of bacteria into the tissues. The presence of fimbriae on bacteria enables them to attach to the mucoid epithelial cell layers, such as intestinal linings. Special immunological techniques and chemical analyses have opened the door to an understanding of the role played by bacterial surface antigens in pathogenicity.

An interesting phenomenon is the prevalence of certain *E. coli* strains in humans or animals and their correlation with pathogenicity. An idea evolving from comparative studies is that of specific recognition of and interaction between bacteria and mammalian cells. It is conceivable that histocompatibility antigens or surface antigens expressed on cells of certain tissues play a role in the attachment of bacteria as the first step in an infection. This may be due to (i) antigens common to the bacterial and mammalian cell surface causing impaired recognition of the bacteria or to (ii) the presence on the mammalian cell of antigenic structures acting as receptors for complementary bacteria surface antigens.

In the interaction of host and parasite, defense mechanisms developed by the host were counteracted by the bacteria, with the production of protective surface structures. As new surface antigens evolved, the production of previous ones, which may have proved to be insufficient, is probably discontinued. Thus, such an evolutionary adaptation to a changing ecosystem may have produced antigenic drifts, and this can be considered as one of the reasons for the enormous diversity in the patterns of *E. coli* antigens. Their study by chemical and genetic approaches, as well as the investigation of the biosynthetic pathways leading to the polysaccharide antigens in question, is certainly exciting. Furthermore, it can easily be appreciated that such studies are essential for our understanding not only of the pathogenicity of *E. coli* but also of bacterial pathogenicity at large.

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