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Escherichia coli K Bacteriophages

I. Isolation and Introductory Characterization of Five *Escherichia coli* K Bacteriophages

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A set of five *Escherichia coli* K phages has been isolated. These phages are adsorbed to and lyse the capsular forms of the host bacteria, whereas their spontaneous, acapsular mutants are not affected. All host strains are heavily encapsulated test strains for *E. coli* K antigens of the thermostable A type and they readily segregate acapsular mutants. In four of the phage-host systems, all secondary growth obtained was found to be acapsular. When tested for host-range mutants on 38 strains of *E. coli* and *Klebsiella*, less than one mutant per 10^5 plaque-forming units was found. No cross-reacting neutralizing antibodies were obtained when rabbits were immunized with the K phages. The latent periods (between 16 and 30 min) and average burst sizes (between 145 and 580) were determined by one-step growth experiments.

Many K-specific, acidic polysaccharides from the capsules of *Escherichia coli* strains have recently been isolated and their structures were elucidated (7, 8, 20). Further capsular polysaccharides carrying other *E. coli* K specificities are being investigated.

The isolation of these materials is comparatively easy (7, 8, 41) and they constitute morphologically (5, 21), serologically (12, 21), and chemically (20) well-defined parts of the bacterial surface. They are not found in the acapsular (K^-) mutants of the organisms (7, 8, 20).

It has long been known that there are phages capable of lysing such encapsulated strains of *E. coli* (e.g., 27) and that some of them (to which the term K phages will be restricted in this paper) do not affect the noncapsulated mutants of the host bacteria (35, 36, 37).

Thus, it is evident that *E. coli* K phages and the corresponding capsular polysaccharides would be suitable for a study in receptor biochemistry. Especially so, since a hitherto unrecognized type of phage-receptor relationship, reminiscent of the influenza virus system, has recently been demonstrated in a similar case.

Starting from an observation of Koziński and Opara (16), K. Taylor and co-workers (33, 34) showed that Formalinized erythrocytes, coated with Vi-specific polysaccharide, adsorb large amounts (10^{11} plaque-forming units (PFU)/μg of polysaccharide) of Vi-phage II at 0 C and that the phages are reeluted from the modified cells at 37 C without loss of infectivity. Vi-specific polysaccharide, treated at 37 C with Vi-phage II, lost its phage-adsorbing activity (31) together with all its *O*-acetyl and most *N*-acetyl groups (32). Reacetylation restored the activity.

Vi-phage II is adsorbed to and lyses the Vi-form of *Salmonella typhi* only, whereas the W-form, without Vi-specific, acidic polysaccharide, is not affected. Erythrocytes, coated with K42-specific polysaccharide from *E. coli* A295b (antigenic formula: 08:K42(A):H^-), were found to adsorb at least 3×10^9 PFU of K phage 42 per μg at 0 C. At 37 C, essentially all the plaque-forming activity was eluted again (S. Stirm, *in press*).

In this report, the introductory description of five *E. coli* K phages is given. Two of the host organisms develop capsular polysaccharides, the structure of which has been analyzed in detail (7, 8).

MATERIALS AND METHODS

The techniques used were generally those described by M. Adams (1) and F. Kauffmann (12).

Media. Ox heart infusion broth with 1.6% agar added was used for plates and beef broth was used for fluid medium. In both cases, 1% peptone, 0.3% NaCl, and 0.2% $Na_2HPO_4 \cdot 12H_2O$ were added.

Host strains. The host strains were all serological test strains for *E. coli* K antigens of the thermostable A type (12) used at the International Escherichia

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TABLE 1. *Serological test strains for E. coli K antigens*

Strains	Antigens
Bi449/42	O9:K26(A):H$^-$
E69	O9:K30(A):H12
H36	O9:K32(A):H19
A121a	O9:K39(A):H9
A295b	O8:K42(A):H$^-$
A45a[a]	O9:K32(A):H$^-$
A95b[a]	O9:K32(A):H5
A139[a]	O8:K42(A):H4
U14/41[b]	O3:K2(L):H2
G3404/41[b]	O8:K8(L):H4
Bi316/42[b]	O9:K9(L):H12
E19a[b]	O21:K20(L):H$^-$
Bi7575/41[c]	O8:K25(B):H9
H509d[c]	O9:K57(B):H32
Stokew[c]	O111:K58(B):H$^-$
F41[c]	O26:K60(B):H$^-$
4932/53[c]	O127a:K63(B):H$^-$
Guanabara[c]	O112ac:K66(B):H$^-$
Katwijk[c]	O28:K73(B):H$^-$
E56b[d]	O8:K27(A):H$^-$
K14a[d]	O9:K28(A):H$^-$
Bi161/42[d]	O9:K29(A):H$^-$
Su2973/41[d]	O9:K31(A):H$^-$
App289[d]	O9:K33(A):H$^-$
E75[d]	O9:K34(A):H$^-$
A84a[d]	O9:K37(A):H$^-$
A262a[d]	O9:K38(A):H$^-$

[a] Other strains of *E. coli* carrying the same K antigen as one of the host strains.

[b] Test strains for *E. coli* K antigens of the L type.

[c] Test strains for *E. coli* K antigens of the B type.

[d] Test strains for other *E. coli* K antigens of the A type.

Centre, as listed in Table 1. Test strains for *Klebsiella* K antigens were as follows: strain A5054, K1 antigen; B5055, K2; Rh.5046, K3; Oz.D5050, K4; Oz.E5051, K5; Oz.F5052, K6; Aer.4140, K7; 1015, K8; 56, K9; 919, K10; 390, K11; and 1702–49, K21.

Before use, fresh colonies of all strains were checked by slide agglutination with the corresponding O:K sera. In the same way, the *E. coli* strains were also controlled for O-inagglutinability. The host strains were occasionally submitted to a further control: boiled (1 hr) cultures were tested by tube agglutination for O-inagglutinability to exclude A$^\pm$ mutants (12, 13, 38).

Isolation and serological control of K$^-$ mutants. All test strains for *E. coli* K antigens of the A type mentioned readily segregate nonencapsulated (K$^-$) mutants, although with different frequencies. They are best recognized when the plates are kept at room temperature for several days (Fig. 1). K$^-$ growth was isolated from the "wedges" and smooth, transparent colonies were purified. The mutants thus obtained were checked for O-agglutinability and for stability of live and boiled fluid culture. All O-inagglutinable or slightly rough isolations were discarded.

High-titer phage stocks. Phage stocks of 10^{11} to 10^{12} PFU/ml were obtained by washing off confluent plaques from agar plates with 8 to 10 ml of broth per 14-cm diameter plate. The suspensions were centrifuged at low speed, sterilized with chloroform, and stored over chloroform at 4 C. With the exception of K phage 39 suspensions, which lost titer, all stocks were found stable over periods of several months.

All phage titrations were done by the soft agar layer method.

All antibacterial sera were standard sera used for serological typing at the WHO International Escherichia Centre (12, 22).

Rate of adsorption. The velocity of phage adsorption to living bacteria in broth at 37 C was determined, and adsorption was stopped by diluting into 99 volumes of cold (4 C) medium. The diluted samples and the controls were passed through Millipore GS filters (Millipore Corp., New Bedford, Mass.) and titrated. Passage through Millipore filters was found to retain only small and reproducible fractions of the K phages.

Host range. For the determination of host range, a sequence of 10^2, 10^4, 10^6, 10^8, 10^{10} PFU was placed onto a freshly seeded lawn of the bacteria to be tested. A sample of plate culture extract of the host bacteria (obtained in analogy to the phage stocks) was also applied. Effects due to temperate phages or colicines were thus excluded, and the exact titration of the relative efficiency of plating was simplified when host-range mutants appeared.

Antiphage sera. Rabbits were injected with about 10^{11} PFU intravenously and subcutaneously twice weekly for 3 weeks. Bleedings 1 week after the last injection usually yielded sera with K values above 30 min^{-1}, a satisfactory linearity being observed between log (p_0/p) and time, at least for 90 to 99% neutralization. The anti-K-phage sera used are listed in Table 4.

One-step growth experiments. To 0.9 ml of an actively aerated broth culture of the host bacteria, containing about 10^7 to 3×10^7 organisms, approximately 10^7 PFU was added in 0.1 ml. After 3 min, a multiplicity of infection of 0.08 to 0.18 was reached and further adsorption was stopped by diluting 1:10 into sera 3309 (previously diluted 1:100 with broth), 3462 (1:20), 3401 (1:20), 3313 (1:30), or 3132 (1:60) for K phages 26, 30, 32, 39, and 42, respectively, neutralizing at least 99.7 to 99.9% of free phage within 3 to 6 min. All manipulations were carried out at 37 C. Samples from "first and second growth tubes" were plated as usual.

RESULTS

Isolation. The *E. coli* K phages were isolated from Copenhagen sewage and from mouse and human feces. After enrichment in broth cultures of the prospective host bacteria and chloroform sterilization of the suspensions obtained, drops of serial 10-fold dilutions were placed on freshly seeded lawns of a K$^+$ and a K$^-$ variant of the host. After incubation, plaques were picked, which, at a certain dilution, appeared on the lawn of

encapsulated bacteria only. As usual, pure lines of phages were obtained by successive single-plaque isolations.

All 13 test strains for *E. coli* K antigens of the A type (Table 1) were used as prospective hosts, and seven K phages were isolated. They were termed *E. coli* K phage 26, 29, 30, 31, 32, 39, and 42, in agreement with the number of the K antigen that the host strain carries. Five of them (phages 26, 30, 32, 39, and 42) which yielded high-titer stock suspensions without difficulty were selected for further characterization.

Plaque morphology. K phages 26, 30, 31, 32, and 42 yielded plaques without halos, and K phages 29 and 39 yielded plaques with halo, i.e., the latter were surrounded by large zones wherein the bacterial lawn had lost thickness and had become O-agglutinable. In similar cases, such halos have been described (1, 27, 30) as being due to the effect of capsule-degrading enzymes.

Also K phage 26 was first isolated forming plaques with halos. However, a few plaques without zones appeared, and repeated isolations of these mutants finally yielded a population of simple plaques.

Relative efficiency of plating on capsulated and noncapsulated forms of the host strains. A total of 18 acapsular mutants from independent mutations were isolated from each host strain, and the O-agglutination titers were determined by tube agglutination. PFU of the corresponding K phage (10^{10}) were placed onto a lawn of each K^- isolation, and 10^{11} PFU were plated in soft agar layers inoculated with the same K^- strains. No host-range mutants (nor host-induced modifications) were observed (Fig. 1 and 2). The results are summarized in Table 2.

Secondary growth. Young broth cultures of the host strains were infected in the usual way with high multiplicities of the corresponding K phages, incubated until several hours after lysis, and plated in an appropriate dilution to yield single resistant colonies.

With the exception of the system K30 test strain and K phage 30 (*see below*), all colonies thus obtained appeared transparent, like acapsular mutants (Fig. 2). For each K phage, 40 colonies from different lysed cultures were tested by slide agglutination and were found to be stable in saline and O-agglutinable. In each case, 12 clones were purified and their resistance was checked by incubation of 10^{10} PFU on a freshly seeded bacterial lawn. All 12 strains were then tested for stability of live and boiled (1 hr) fluid culture, and the O titers were determined by tube agglutination of Formalinized organisms. With the exception of the K30 system, all resistant strains were found stable and yielded high O titers.

In the K30 system, the majority of the resistant clones were found to be transparent and O-agglutinable. In addition, however, 0 to 30% of opaque, capsulated colonies were obtained from the different lysates plated. Eight such strains were tested and found O-inagglutinable when Formalinized or boiled cultures were used. When checked for resistance as described, mutants of

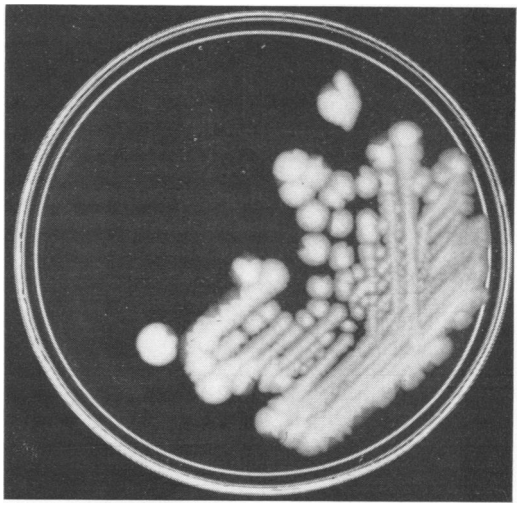

FIG. 1. *Colonies of E. coli A295b (08:K42(A):H$^-$, test strain for K antigen no. 42). Acapsular mutants are seen as darker sectors. The plate was incubated for 18 hr at 37 C and for 2 days at room temperature.*

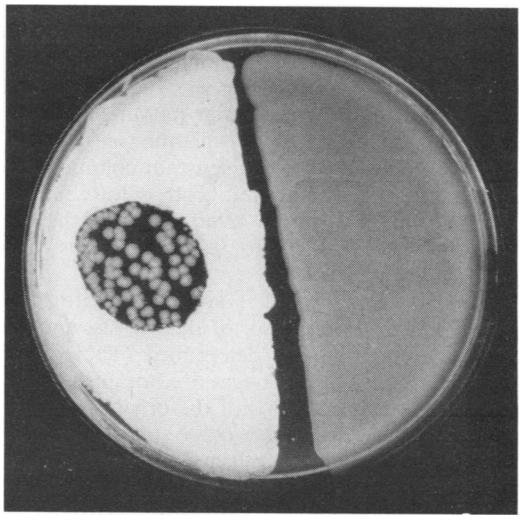

FIG. 2. *Lawns of capsular and acapsular mutant of E. coli A295b (08:K42(A):H$^-$), incubated after application of about 10^{10} PFU of K phage 42. Note acapsular appearance of secondary growth.*

TABLE 2. *Characterization of five E. coli K phage-host systems*

Determination	No. of phage and K(A)-antigen of host				
	26	30	32	39	42
Plaque diameter in mm (18 hr, 37 C)	0.5–1 (no halo)	0.5–1 (no halo)	0.5–1.5 (no halo)	1–2.5 (with halo 2–5)	0.5–2 (no halo)
O-agglutination titer[a] of host cultures, capsular forms, Formalin-killed or heated to 100 C for 1 hr	<20	<20	<20	<20	<20
O-agglutination titer[a] of cultures of acapsular host mutants, Formalin-killed	2,560–≥5,120	2,560–≥5,120	≥5,120	≥5,120	2,560–≥5,120
Relative EOP of phage on capsular/acapsular form of host	$>10^{11}$	$>10^{11}$	$>10^{11}$	$>10^{11}$	$>10^{11}$
O-agglutination titer[a] of phage-resistant secondary colonies of capsular host	2,560–≥5,120[b]	—[c]	2,560–≥5,120[b]	2,560–≥5,120[b]	≥5,120[b]
Rate of adsorption[d] (10^{-10} ml/min) of phage to living cells of host—capsular form	39	37	34.5	40	32
Rate of adsorption[d] (10^{-10} ml/min) of phage to living host—acapsular mutant	<1	<1	<1	<1	<1
Latent period[d] (min)	28–30	17–18	23	17–18	16
Average burst size[d]	580	145	310	340	305

[a] Titer of O sera (O8 or O9) with boiled culture of O test strain: ≥5,120.
[b] All resistant colonies were smooth and yielded stable broth cultures.
[c] Capsular (O9 titer: 20) and acapsular (O9 titer: 2,560–≥5,120) forms were found.
[d] In broth at 37 C.

K phage 30 were detected that lysed a small portion of these organisms. On subculture, these strains readily segregated normally phage-susceptible clones. K phage 30 was found to adsorb to such resistant and capsular mutants of *E. coli* E69 at a rate of 20 × 10^{-10} ml/min (*see* Table 2).

In the 42 system, seven resistant colonies were repeatedly subcultured and broth culture filtrates were tested for K phage 42 on a lawn of the host strain. In all cases, the resistant strains were easily freed of K phage.

Rate of adsorption. The adsorption rate of all K phages to live cultures of their hosts, and K^- mutants thereof, were determined (Table 2). It is evident that the K phages are not adsorbed to the noncapsulated forms of the organism.

Host range. Only three other strains of *E. coli* carrying the same K antigen as one of the host strains were available (Table 1). They were tested with the "homologous" K phages.

K phages 32 and 42 plated with roughly the same efficiency on *E. coli* A45a and A139, respectively, as on the K32 and K42 test strains. K^- mutants of A45a and A139 were found to be resistant to 10^{10} PFU and K phage 42 did not adsorb to a nonencapsulated mutant of A139.

A lysis-from-without phenomenon was produced by K phage 32 on *E. coli* A95b. High-titer phage suspensions, but not cultural filtrate of the K32 test strain, produced confluent lysis on a lawn of A95b. Serial dilutions, however, yielded no plaques, but the lytic activity ceased below 10^9 PFU.

K phages 26, 30, 32, 39, or 42 (10^{10} PFU) yielded no plaques on any of the test strains for *E. coli* K antigens of the L or B type (Table 1), and no effect was observed on the test strains for *Klebsiella* K antigens 1 to 10 (*see* Materials and Methods). K phage 30 was also tested on the *Klebsiella* strains with K antigens 11 and 21, since a serological relationship between these specificities and *E. coli* K 30 has been observed (11; Ida Ørskov, *personal communication*). Even in these cases, however, no host-range mutants were detected.

Neither did 10^{10} PFU of the five K phages yield host-range mutants on most of the 12 test

strains for other *E. coli* K antigens of the A type (Table 1). A few exceptions are listed in Table 3.

Serology. Rabbit antisera against the five K phages were prepared and the velocities of virus neutralization ("K values") were determined (Table 4). Apparently, the neutralizing antiphage antibodies do not cross-react.

One-step growth experiments. Latent period and average burst size of the K phages were determined in one-step growth experiments. The data are listed in Table 2 and the curves are given in Fig. 3.

TABLE 3. *Host-range mutants of K phages on other E. coli test strains for K antigens of the A type*

No. of K phage/no. of K antigen of host	No. of K antigen of new, heterologous host	Relative efficiency of plating K phage[a]
32	26(−)[b]	10^{10}
	39(−)	1.5×10^{9}
	42(−)	10^{7}
39	26(−)	5×10^{5}
	32(−)	3×10^{9}
	42(+)	10^{10}
42	29(+)	3×10^{6}

[a] On host/new host.

[b] Mutant plaques with (+) or without (−) halo.

DISCUSSION

Our knowledge of the chemical basis for the specificity of phage adsorption and penetration is

TABLE 4. *Neutralizing velocities (K values in min^{-1}) of anti-K-phage rabbit sera*

K value with phage	Serum against phage (no. of rabbit)				
	26 (3309)	30 (3462)	32 (3401)	39 (3313)	42 (3132)
26	214	<0.5	<0.5	<0.5	<0.5
30	<0.5	83.5	<0.5	<0.5	<0.5
32	<0.5	<0.5	42	<0.5	<0.5
39	<0.5	<0.5	<0.5	77	1.4
42	<0.5	<0.5	<0.5	<0.5	54

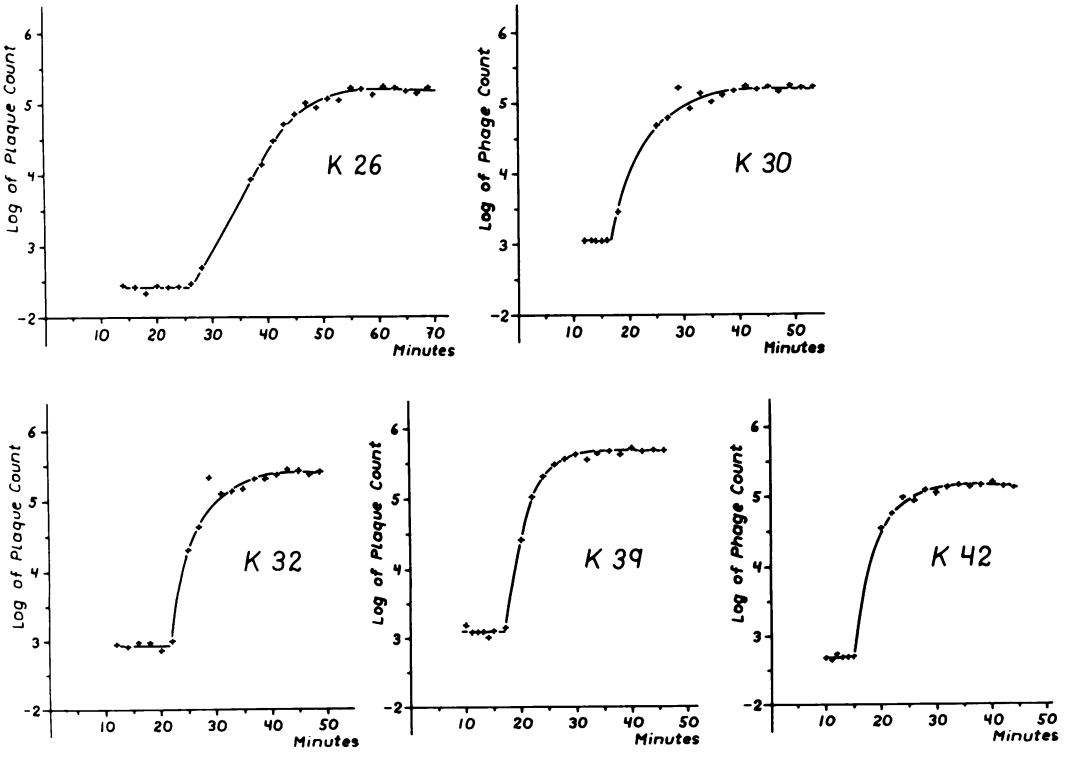

FIG. 3. *Single-step growth curves of E. coli K phages in terms of PFU per ml of first growth tube. Beef broth, 37 C. The hosts are E. coli test strains for different K antigens of the A type.*

still rather vague, in spite of the extensive research on this aspect of the bacteriophage phenomenon (24). Even in phage-host systems as thoroughly investigated as the T-even phages and *E. coli* B, we cannot tell in exact chemical terms just how specific these viruses are, i.e., what receptive structures they have to meet on the bacterial surface for that sequence of events to occur, which begins with the attachment of the tail fibers and ends with the ejection of nucleic acid from the phage head (15, 29, 42).

One reason for this is probably that phage inactivation was used by most workers as a test for the isolation and evaluation of "phage receptors" (e.g., 3, 4, 10, 18, 19, 40). Rather large pieces of the bacterial surface are likely to be necessary for this effect to occur, since inactivation implies that most (or all) of the steps from the first contact to NA-ejection have been triggered, which may (15, 29, 42) and in most cases does (14) involve rather distant parts (in molecular terms) of both bacterial surface and phage tail.

Another reason seems to be that, in most systems hitherto studied, all receptive structures resided in bacterial surface layers of rather complicated chemistry. Notably, the outer cell wall layers involved in at least the first stages of T-phage adsorption to and penetration into *E. coli* B or other *Enterobacteriaceae* (3, 4) consist of complex carbohydrate, protein, and lipid-containing structures (2, 10, 20, 26, 28, 39). The elucidation of their stereochemical arrangement and even of their primary buildup from monosaccharide, amino acid, etc., subunits is far from being complete.

The Vi-receptor activity discovered by Koziński (16) and by Taylor et al. (34) offers an advantage in both respects: only a small organelle of Vi-phage II is likely to be involved in the enzymatic deacetylation reaction (17), and the bacterial surface layer encompassing the corresponding receptive structures probably consists only of α (1 $\rightarrow$ 4)-linked *N*-acetyl D-galactosamine uronic acid which is partially *O*-acetylated and possibly cross-linked via ester bonds (9, 20). This system appears better suited for an attempt to trace infection specificity back to chemical stipulations, e.g., by estimating the adsorption (at 0 C) or deacetylation (at 37 C) velocities with chemically altered Vi-antigen, its subunits, or structurally related polysaccharides.

The same is true for K phages in general, as long as the capsule material is of comparatively simple chemical composition, and if tests can be developed that measure partial receptive activities involving phage parts of molecular sizes only.

E. coli test strains for K antigens of the A type were selected as prospective host organisms for K phages, since they readily segregate noncapsulated, smooth, transparent, and O-agglutinable mutants (Fig. 1) which differ from the capsulated, opaque, and O-inagglutinable parent strains in that they do not carry capsular polysaccharides (20). Similar to Vi-antigen, these polysaccharides are often *O*-acetylated and contain in their repeating units uronic acids which may be occasionally engaged in ester bridges (7). In contrast to the Vi-substance, however, they are easily hydrolyzed to yield oligosaccharide subunits of different sizes (20). Also, as stated above, preliminary studies revealed a Vi-type receptor activity in the case of K42-specific polysaccharide and K phage 42.

Some serologically distinguishable forms with intermediary capsulation have been recorded in *E. coli* strains carrying K antigens of the A type: O-inagglutinable translucent mutant colonies were called "ns" (not swelling) or "$A^{\pm}$" forms by Kauffmann (13) and Vahlne (38) and found to become more or less O-agglutinable after boiling. A further serological subdivision of these strains appears possible (G. Schmidt, *personal communication*) since boiling may or may not destroy their agglutinability in a K serum, i.e., an antiserum against the strongly capsulated parent form absorbed with a pure K^- variant. In addition, G. Toft (36, 37) found translucent mutant clones, which, though O-agglutinable, could still deplete a serum against the capsular parent form from the K antibodies.

Therefore, opaque growth and O-inagglutinability of Formalinized and boiled cultures were taken as criteria of encapsulation; translucence, smooth growth, O-agglutinability, and stability of live and boiled cultures were selective criteria for acapsular mutants. With the exception of the variants found by Toft (36, 37; *see above*), all known forms of intermediate capsulation and rough mutants (12, 21) were thus excluded, and the data presented demonstrate a correlation between capsulation of the host bacteria and adsorption of and lysis by the K phages.

The fact that spontaneous K^- mutants of the host organisms are K-phage resistant is considered a strong indication that the acapsular bacteria obtained from K-phage lysates of K^+ cultures are just these spontaneous mutants and that conversion phenomena are not involved. In the case of the K42 system, which has been examined in more detail, this view is strenthened by the finding that such colonies are easily freed of K phage on subculture.

Five K(A)-specific acidic polysaccharides from capsular strains of *E. coli* have hitherto been isolated and analyzed (20). Wherever tested, these polysaccharides were not found in the

corresponding K^- mutants, selecting capsular and acapsular variants by the same criteria as above. It is thus concluded that the K-specific polysaccharide capsules of the five host strains encompass chemical structures necessary for the adsorption and penetration of the corresponding K phages.

Within the host-range tested, the K phages were found specific for strains with the same capsular antigens up to 10^5 PFU. At higher test concentrations, host-range mutants were found for some related strains of *E. coli* (Table 3), i.e., for strains also carrying K antigens of the A type.

A noticeable feature of the single-step growth curves (Table 2; Fig. 3) is the comparatively large burst sizes, especially of K phage 26.

When the 13 prospective host strains were tried, seven K phages were obtained in the first attempt. K phages lysing encapsulated strains of *E. coli* with other K antigens (also of the L type) have been isolated (36). For the genus *Klebsiella*, bacterial viruses have been described that do not lyse acapsular variants of the host organisms and others that yield acapsular secondary growth (6, 23, 25); and the Vi phages may be called K phages (as defined above). It thus appears that K phages are rather common in *Enterobacteriaceae*.

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LITERATURE CITED

1. Adams, M. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Bayer, M. E., and T. F. Anderson. 1965. The surface structure of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **54**:1592–1599.
3. Beumer, J., M. P. Beumer-Jochmans, J. Dirkx, and D. Dekegel. 1965. Etat actuel des connaissances concernant la nature et la localisation des récepteurs des bactériophages dans la paroi cellulaire des *Shigella* et des *Escherichia*. Bull. Acad. Roy. Méd. Belg. **5**:749–790.
4. Beumer, J., M. P. Beumer-Jochmans, J. Dirkx, and D. Dekegel. 1966. Localisation des récepteurs de phages chez des souches de *Shigella* et d'*Escherichia*. Ann. Inst. Pasteur **110**:727–736.
5. Duguid, J. P. 1964. Functional anatomy of *Escherichia coli* with special reference to enteropathogenic *E. coli*. Rev. Latinoam. Microbiol. Suppl. 13–14: 1–16.
6. Humphries, J. C. 1948. Enzymatic activity of bacteriophage-culture lysates. I. A capsule lysin active against Klebsiella pneumoniae type A. J. Bacteriol. **56**:683–693.
7. Hungerer, D., K. Jann, B. Jann, F. Ørskov, and I. Ørskov. 1967. Immunochemistry of K antigens of *Escherichia coli*. 4. The K antigen of *E. coli* 09:K30:H12. European J. Biochem. **2**: 115–126.
8. Jann, K., B. Jann, F. Ørskov, I. Ørskov and O. Westphal. 1965. Immunchemische Untersuchungen an K-Antigenen von *Escherichia coli*. II. Das K-Antigen von *E. coli* 08:K42(A):H^-. Biochem. Z. **342**:1–22.
9. Jarvis, F. G., M. T. Mesenko, D. G. Martin, and T. D. Perrine. 1967. Physicochemical properties of the Vi antigen before and after mild alkaline hydrolysis. J. Bacteriol. **94**:1406–1410.
10. Jesaitis, M. A., and W. F. Goebel. 1955. Lysis of T4 phage by the specific lipocarbohydrate of phase II *Shigella sonnei*. J. Exptl. Med. **102**: 733–752.
11. Kauffmann, F. 1949. On the serology of the *Klebsiella* group. Acta Pathol. Microbiol. Scand. **26**:381–396.
12. Kauffmann, F. 1966. The bacteriology of *Enterobacteriaceae*. Munksgaard, Copenhagen.
13. Kauffmann, F., and G. Vahlne. 1945. Über die Bedeutung des serologischen Formenwechsels für die Bakteriophagen-Wirkung in der *Coli*-Gruppe. Acta Pathol. Microbiol. Scand. **22**: 119–137.
14. Bradley, D. E. 1965. The morphology and physiology of bacteriophages as revealed by the electron microscope. J. Roy. Microscop. Soc. **84**: 257–316.
15. Kellenberger, E., A. Bolle, E. Boy de la Tour, R. H. Epstein, N. C. Franklin, N. K. Jerne, A. Reale-Scafati, J. Sechaud, I. Bendet, D. Goldstein, and M. A. Lauffer. 1965. Function and properties related to the tail fibers of bacteriophage T4. Virology **26**:419–440.
16. Kozinski, A. W., and Z. Opara. 1954. Experiments with substrates of bacterial viruses. Reversibility of reaction and destruction of the substrate. Bull. Acad. Polon. Sci. Classe II **2**:39–43.
17. Kwiatkowski, B. 1966. The structure of Vi-phage II. Acta Microbiol. Polon. **15**:23–26.
18. Levine, P., and A. W. Frisch. 1933. Specific inhibition of bacteriophage by bacterial extracts. Proc. Soc. Exptl. Biol. Med. **30**:993–996.
19. Lindberg, A. A. 1967. Studies of a receptor for Felix O-1 phage in *Salmonella minnesota*. J. Gen. Microbiol. **48**:225–233.
20. Lüderitz, O., K. Jann, and R. Wheat. 1968. Somatic and capsular antigens of gram-negative bacteria, p. 105–228. *In* M. Florkin and E. H. Stotz (ed.), Comprehensive biochemistry, vol. 26A. Elsevier Publishing Co., Amsterdam.
21. Møller, O. 1948. Bacterial variation in *Escherichia coli*. Gleerupska Universitet Bokhandeln, Lund.
22. Ørskov, I., F. Ørskov, W. J. Sojka, and J. M. Leach. 1961. Simultaneous occurrence of *E. coli* B and L antigens in strains from diseased swine. Acta Pathol. Microbiol. Scand. **53**: 404–422.
23. Park, B. H. 1956. An enzyme produced by a

phage-host cell system. I. The properties of a *Klebsiella* phage. Virology **2**:711–718.
24. Raettig, H. 1958, 1967. Bakteriophagie 1917–1956 (1957–1965). Gustav Fischer Verlag, Stuttgart, Germany.
25. Rakieten, M. L., A. H. Eggerth, and T. L. Rakieten. 1940. Studies with bacteriophages active against mucoid strains of bacteria. J. Bacteriol. **40**:529–545.
26. Salton, M. R. J. 1964. The bacterial cell wall. Elsevier Publishing Co. Amsterdam.
27. Sertic, V. 1929. Untersuchungen über einen Lysinzonen bildenden Bakteriophagen. 1. Mitteilung. Der Aufbau der Bakteriophagenkolonien. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **110**:125–139.
28. Shands, J. W., J. A. Graham, and K. Nath. 1967. The morphologic structure of isolated bacterial lipopolysaccharide. J. Mol. Biol. **25**:15–21.
29. Simon, L. D., and T. F. Anderson. 1967. The infection of *Escherichia coli* by T2 and T4 bacteriophages as seen in the electron microscope. I. Attachment and penetration. II. Structure and function of the baseplate. Virology **32**:279–297 and 298–305.
30. Sutherland, I. W. 1967. Phage-induced fucosidases hydrolysing the exopolysaccharide of *Klebsiella aerogenes* type 54 [A3(S1)]. Biochem. J. **104**: 278–285.
31. Taylor, K. 1965. A study on the loss of the receptor activity of Vi-polysaccharide during incubation with Vi-phage. Acta Biochim. Polon. **12**:157–166.
32. Taylor, K. 1966. Physical and chemical changes of Vi-polysaccharide due to Vi-phage II action. Acta Biochim. Polon. **13**:97–106.
33. Taylor, K., and B. Kwiatkowski. 1963. Adsorption of Vi-phage II on the Vi-receptor coated erythrocyte membranes, examined in the electron microscope. Acta Microbiol. Polon. **12**: 107–112.
34. Taylor, K., and A. Taylor. 1963. Estimation of Vi-receptor activity. Acta Microbiol. Polon. **12**:97–106.
35. Toft, G. 1946. Demonstration of bacteriophages with effect on capsular strains of *B. coli*. Acta Pathol. Microbiol. Scand. **23**:277–283.
36. Toft, G. 1947. Studies on the specificity of some *colon* bacteriophages, with a special view to the capsulo-phages (K phages). Acta Pathol. Microbiol. Scand. **24**:260–271.
37. Toft, G. 1949. Undersøgelser over Colibacteriophagers Specificitet. Munksgaard, Copenhagen.
38. Vahlne, G. 1945. Serological typing of the *colon* bacteria. Gleerupska Univeristet Bokhandeln, Lund.
39. Weidel, W. 1964. Ein neuer Typ von Makromolekülen. Angew. Chemie **76**:801–807.
40. Weidel, W., G. Koch, and K. Bobosch. 1954. Über die Rezeptor-Substanz für den Phagen T5. I. Extraktion und Reindarstellung aus *E. coli* B. Physikalische, chemische und funktionelle Charakterisierung. Z. Naturforsch. **9b**:573–579.
41. Westphal, O., and K. Jann. 1965. Bacterial lipopolysaccharides, p. 85–91. *In* R. L. Whistler and M. L. Wolfrom (ed.), Methods in carbohydrate chemistry, vol. 5. Academic Press, Inc., New York.
42. Wildy, P., and T. F. Anderson. 1964. Clumping of susceptible bacteria by bacteriophage tail fibers. J. Gen. Microbiol. **34**:273–283.