Genetic Transfer of *Salmonella* O Antigens to *Escherichia coli* O8

By W. KIEFER, G. SCHMIDT, BARBARA JANN AND K. JANN

Max-Planck-Institut für Immunbiologie, D-78 Freiburg i.Br., Germany

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SUMMARY

His⁺ hybrids from a cross between a *Salmonella typhimurium* donor and an *Escherichia coli* O8 recipient expressed *E. coli* O8 specificity and in addition *Salmonella* O_{4,12}-specificity. This indicated that the recipients had received the *his*-linked donor *rfb* cluster determining the synthesis of *S. typhimurium* O-specific repeat units and that the *rfb* genes of both mating partners are functional in these hybrids. Chemical analyses showed that the hybrids contained an *E. coli* O8 lipopolysaccharide (O antigen) and a *S. typhimurium* specific lipopolysaccharide with only one O-specific repeat unit (SR antigen).

O8-negative mutants selected from the O8-positive hybrids retained the *Salmonella* O-specificity and represent semi-rough (SR) forms, because the *rfc* gene(s) determining the polymerization of repeat units has not been transferred. Attempts to introduce the *S. typhimurium rfc* locus into *E. coli* O8 remained unsuccessful.

Crosses between a *S. typhi* donor and *E. coli* O8 gave rise to smooth (S) and SR His⁺ recombinants exhibiting only *S. typhi* O-specificity. The smooth recombinants are assumed to have obtained the *his*-linked *rfb* cluster and in addition the *rfc* gene(s) of the donor. The exchange of the *rfb* region of such smooth recombinants by that of a *S. typhimurium* donor led to smooth hybrids with O_{4,(5)}, 12-specificity.

The phenotypically smooth recombinants exhibited concomitantly S- and SR-lipopolysaccharides of *S. typhi* and *S. typhimurium* O-specificity, respectively.

INTRODUCTION

The polysaccharide portion of the O-antigenic lipopolysaccharide (LPS) of Enterobacteriaceae consists of two regions, the basal or core oligosaccharide (LPS core) and the O-specific chains composed of repeating oligosaccharide units (Lüderitz *et al.*, 1971).

The composition and structure of O-specific chains vary widely among the different serotypes whereas the LPS core is less variable. Thus all *Salmonella* serotypes are thought to have an identical core structure. On the other hand four different core types (R1 to R4) have been identified thus far in *Escherichia coli* and *Shigella* (Schmidt, Jann & Jann, 1969, 1974; Mayer & Schmidt, 1973).

The genetic background of the LPS synthesis has mainly been studied with *Salmonella typhimurium*. These investigations have shown that most of the genes responsible for core synthesis (*rfa* genes) are clustered in the *cysE-pyrE* region of the chromosome, whereas the genes determining the synthesis of O-specific repeat units form a cluster (*rfb* genes) closely linked to the histidine (*his*) operon. The polymerization of repeat units is under the control of the *rfc* gene(s) which is located outside the main *rfa* and *rfb* clusters, adjacent to the *trp* operon (Stocker & Mäkelä, 1971). A defect in the *rfc* region leads to SR mutants which can attach only single unpolymerized repeat units to the core.

The repeat unit of *S. typhimurium* determined by the *rfb* cluster represents an abequosyl-mannosyl-rhamnosyl-galactose oligosaccharide (Lüderitz *et al.*, 1971), corresponding

Table 1. *Characteristics of bacterial strains*

Strain	Serotype	Auxotrophic markers	Carbohydrate utilization	Response to streptomycin
Donors				
<i>S. typhimurium</i> Hfrk9	O4,5,12:i; 1,2	<i>thr</i>	<i>lac</i> ⁻	s
<i>S. typhi</i> WR4000	O9,12,Vi:d	<i>cys trp</i>	<i>rha</i> ⁻ <i>ara</i> ⁻ <i>xyl</i> ⁻	s
Recipients				
<i>E. coli</i> F210	O8:K ⁻ :H ⁻	<i>met pro his trp</i>	<i>mtl</i> ⁻ <i>ara</i> ⁻	r
<i>E. coli</i> F464	O8:K ⁻ :H ⁻	<i>met pro his</i>	<i>mtl</i> ⁻ <i>ara</i> ⁻	r

thr, threonine; *cys*, cysteine; *trp*, tryptophan; *met*, methionine; *pro*, proline; *his*, histidine; *lac*, lactose; *rha*, rhamnose; *ara*, arabinose; *xyl*, xylose; *mtl*, mannitol; s, sensitive; r, resistant.

serologically to the O antigen 4 and at least to parts of the 12 antigen complex (Mäkelä, 1966; Stocker & Mäkelä, 1971). In addition, the O chain of *S. typhimurium* is modified by O-acetylation of abequose (factor O5) and by the attachment of a glucose branch in α -1,4 linkage to the galactose unit. The glucosylation is serologically reflected in the appearance of the O12₂ factor, a sub-component of the O12 antigen complex. The genetic determinants (*oaf* genes) responsible for these modifications are located outside the *rfb* cluster (Stocker & Mäkelä, 1971; Mäkelä, 1973). The expression of the O1 factor results from a conversion of the glucose-galactose linkage from α -1,4 into α -1,6, depending on the presence of the lyso-genizing phage P22 (Stocker & Mäkelä, 1971).

Genes equivalent in site and function to the *Salmonella rfa* and *rfb* genes could also be demonstrated in *E. coli* (Ørskov & Ørskov, 1962; Schmidt, Jann & Jann, 1970).

Jones, Koeltzow & Stocker (1972) reported experiments involving the transfer of the *Salmonella rfb* cluster into *E. coli* K12. They showed that the *Salmonella rfb* genes are maintained but not expressed in *E. coli* K12 unless the K12 LPS core is modified by the introduction of *Salmonella rfa* genes. Apparently, the K12 LPS core cannot accept *Salmonella* O-specific units. Hämmerling *et al.* (1971) obtained *E. coli* hybrids with *Salmonella* O-specificity from crosses between a *Salmonella abony* donor and an *E. coli* O100 recipient. One reason for the difference in the capacity to express *Salmonella* O factors may be the presence of different LPS core structures in the above-mentioned *E. coli* recipients. Thus the *E. coli* O100 LPS core (R2) resembles that of *Salmonella* (Hämmerling *et al.* 1971), while the *E. coli* K12 core is distinct from both these core types (Schmidt, 1973).

Our interest was focused on *E. coli* hybrids expressing *Salmonella* O-specificity since they may be the basis for the development of an effective avirulent live vaccine against typhoid. The main purpose of our investigations was the introduction of *Salmonella rfb* and *rfc* genes into *E. coli* O8 and the characterization of the resulting hybrids.

METHODS

Bacteria. The following strains were used: *Salmonella typhimurium* Hfrk9 (Sanderson *et al.* 1972) and *S. typhi* WR4000 (Johnson, Falkow & Baron, 1964) as donors, and the *E. coli* O8 strains F210 and F464 as recipients. The characteristics of the bacterial strains used are given in Table 1. The *E. coli* O8 strains are K⁻ derivatives of *E. coli* E56 (O8:K27:H⁻) having a LPS core of the R1 type (Schmidt *et al.* 1969).

Media. For minimal agar, 25 g Difco-Bacto agar and 0.75 g sodium citrate.5H₂O were mixed in 1 l distilled water and sterilized (solution 1). A salt solution sterilized separately, contained (g/l distilled water): NH₄Cl, 20; NH₄NO₃, 4; Na₂SO₄, 8; K₂HPO₄, 12; KH₂PO₄, 4;

MgSO₄, 0.4 (solution 2). Then 900 ml solution 1 and 300 ml solution 2 were mixed together and glucose, separately sterilized, was added to a final concentration of 0.3%. Recombinants from matings between *S. typhimurium* and *E. coli* were selected on minimal agar containing lactose instead of glucose. Selection of recombinants from crosses with the *S. typhi* donor WR4000 was performed on selective minimal agar, which was minimal agar plus 400 µg streptomycin/ml. Amino acids for supplementation of minimal agar were added to a final concentration of 20 µg/ml.

Merck Standard I broth and D1,5-agar (Schlecht & Westphal, 1966) were used as complete media.

Mating experiments. Donor and recipient strains freshly grown in broth were mixed in such a way that approximately 5×10^7 donor cells and 5×10^8 recipient cells/ml were present in the mixture. The mating mixture was incubated for 4 h at 37 °C without agitation and then centrifuged. The sediment was suspended in sterile water and appropriately diluted. Portions (0.1 ml) were plated on selective minimal agar and the agar plates incubated at 37 °C for 48 h. Recombinant colonies were first transferred on to the same medium used for selection and then streaked on to complete agar from which single colonies were isolated.

Bacteriophages. For the characterization of bacterial strains the following bacteriophages were used: P22, P22c2, Ø8, Felix O-1 (FO), C21 and 6SR. Phage P22 (Zinder, 1953; Wilkinson, Gemski & Stocker, 1972) attacks smooth *Salmonella* strains with O antigen 12. P22c2 is a non-lysogenizing clear plaque mutant of P22. The FO phage (Kallings, 1967) lyses smooth (S) strains of most *Salmonella* serotypes and also rough (R) forms having the complete *Salmonella* LPS core (Lindberg, 1967). The C21 phage is active on certain rough mutants of *Salmonella* and *E. coli* (Lindberg, 1973) and on smooth *E. coli* O8 and other serotypes possessing the LPS core of the R1 type (G. Schmidt, unpublished observation). The phage 6SR, known as 'anti-rough' phage (Kay, 1962) lyses *Salmonella* and *E. coli* rough mutants. Phage Ø8 specifically attacks strains with the *E. coli* O8 antigen (Jann *et al.*, 1971).

The phages were propagated on suitable hosts in broth (Adams, 1959) and stored over chloroform at 4 °C. To determine the susceptibility of bacterial strains towards phages, drops of the lysates containing about 10^8 plaque-forming units/ml were deposited on to bacterial lawns on agar plates. The phage reaction was scored after overnight incubation at 37 °C.

Serological methods. Antisera against *S. typhimurium* (O_{4,5,12}) and *S. typhi* (O_{9,12,12₂}, 12₃ and O_{9,12,1,12₃}) were prepared by immunization with heat-killed saline suspensions of agar cultures. The following *Salmonella* factor sera, kindly supplied by Dr S. Schlecht of this Institute, were used: anti-O₄, anti-O₅, and anti-O₁₂. An anti-O_{9,12₃} serum was obtained by absorption of an anti-O_{9,12,1,12₂,12₃} serum with *S. reading* (O_{4,12,1,12₂}).

The presence of O-antigenic factors was determined by slide agglutination using antisera diluted 1:5 in 0.2% NaCl instead of 0.9%, to avoid spontaneous agglutination of rough and semi-rough (SR) mutants.

Isolation and purification of lipopolysaccharides. The lipopolysaccharides were extracted from whole bacteria using a mixture of 90% phenol and water (1:1, v/v) at 68 °C for 5 min as described by Lüderitz *et al.* (1971) and purified by repeated ultracentrifugation. The final sediment was dissolved in water and freeze-dried. At that stage of purification the lipopolysaccharides were obtained in yields of 1 to 2% based on the dry weight of the cells.

Fractionation of the lipopolysaccharides. The fractionation of lipopolysaccharide by mild acid hydrolysis has been described (Müller-Seitz, Jann & Jann, 1968; Schmidt *et al.*, 1969).

In short, the lipopolysaccharides (100 mg/ml) were hydrolysed at 100 °C for 90 min in 1% (v/v) acetic acid. This splits off lipid A, which was removed by centrifugation. The supernatant fractions containing the polysaccharide moiety of the lipopolysaccharides were freeze-dried. For further purification of the polysaccharides the freeze-dried materials were dissolved in small amounts of pyridine-acetic acid-water (10:4:886, by vol.) applied to columns of Sephadex G50 and eluted with the same solvent mixture. The eluants were monitored with a phenol-sulphuric acid reagent according to Dubois *et al.* (1955). From the peaks the corresponding materials were obtained by freeze-drying. They were then analysed further.

Analytical procedures. Sugars were identified by thin-layer chromatography of the neutralized hydrolysates (0.5 M-H₂SO₄ at 100 °C for 4 h) of lipopolysaccharides on cellulose plates (Merck), with butanol-pyridine-water (6:4:3, by vol.) as solvent. Spots were detected with alkaline silver nitrate.

Quantitative sugar analyses were performed using gas-liquid chromatography of peracetylated alditols following the method of Holme *et al.* (1968). A Varian aerograph series 1520 was used with ECNSS-M (3% 100-120 Gaschrom Q) as a stationary phase. Temperature was 180 °C. Glucosamine was determined with the Elson-Morgan reaction as modified by Strominger, Park & Thompson (1959); abequose and tyvelose were determined by the thiobarbituric acid assay as modified by Waravdekar & Saslaw (1959).

RESULTS

Introduction of the S. typhimurium rfb region into E. coli O8

Because the *rfb* gene cluster of *S. typhimurium* is closely linked to the *his* operon (Stocker & Mäkelä, 1971), His⁺ recombinants were selected from crosses of a *S. typhimurium* donor and *E. coli* F210 (O8) on suitably supplemented minimal agar with lactose as sole carbon source, for counterselection of the *lac*⁻ *Salmonella* donor. As donor, *S. typhimurium* HfrK9 was used, which transfers the *his* locus as a leading marker (Sanderson *et al.*, 1972). The cross is presented in scheme 1 (Fig. 1). The frequency of His⁺ hybrids was approximately 10⁻⁶/donor cell, but most of the His⁺ hybrids were rather unstable and segregated His⁻ clones. After repeated single colony isolations on the selective minimal medium used for primary isolation, ten stable His⁺ hybrids (F961 to F970) were finally obtained, which had retained the *pro met trp ara* markers of the *E. coli* recipient. With one exception they were also mannitol non-fermenters, like *E. coli* F210.

The hybrids could not be agglutinated in 0.3% auramine or in 3.5% (w/v) NaCl and formed homogenous suspensions even after heating at 100 °C for 1 h. Thus the His⁺ hybrids behaved like smooth enterobacteria. In slide tests the hybrids were strongly agglutinated in anti-O8 serum. In addition, they showed a weak reaction in *Salmonella* factor-O4 antiserum.

The occurrence of *Salmonella* O4-specificity indicates that the *his*-linked *rfb* region had been introduced from the donor into the respective recipients. The concomitant expression of *E. coli* and *Salmonella* O-specificity suggests that the intact *rfb* genes of both the recipient (O8) and the donor (O4) coexisted in these hybrids. Of the ten hybrids, two (F969, F970) were also slightly agglutinated in *Salmonella* factor-O5 antiserum, indicating the presence of *Salmonella* O5 factor. Therefore these two hybrids must also have inherited the donor *oafA* locus (the genetic determinant of the O5 factor) which is located near to the *rfb* cluster (Stocker & Mäkelä, 1971). Thus two serologically different hybrid types were selected, as presented in Fig. 1.

Fig. 1. Scheme 1. S, smooth; SR, semi-rough.

Table 2. Phage sensitivity, antigen patterns and mannitol utilization of O8-negative *His*⁺ hybrids

Strain designation*	Antigen pattern	Action of phage†						Mannitol utilization‡
		Ω8	P22	P22c2	FO	C21	6SR	
Hybrids								
F972-F979	O4, 12	—	—	—	—	+	—	—
F980	O4, 5, 12	—	—	—	—	+	—	—
F981	O4, 5, 12	—	—	—	+	—	—	+
Parents								
F210	O8	+	—	—	—	+	—	—
HfrK9	O4, 5, 12	—	+	+	+	—	—	+

* See Fig. 3. † +, Lysis; —, no reaction. ‡ +, Fermentation; —, no fermentation.

Selection and properties of hybrids lacking *E. coli* O8 antigen

Hybrids having only Salmonella O-specificities were obtained with the help of the O8-specific phage Ω8. This phage lyses the O8-positive hybrids, and single Ω8-resistant colonies grown within the lysis zone were isolated from each of the hybrids (see Fig. 1). For purification, single colony isolations were repeated several times. Slide agglutination tests revealed that the Ω8-resistant mutants (F972 to F981) derived from the S hybrids (F961 to F970) lacked the *E. coli* O8-specificity but had retained the antigen factors O4 and O4,5, respectively, as indicated in Fig. 1. They were also agglutinated in slide tests with antiserum against *S. typhi* (O₉, 12₁, 12₂, 12₃) but were not reactive in O₁₂₂- or in O₉, 12₃-antiserum. This suggests that the hybrids only express the O₁₂₁-specificity of the Salmonella O₁₂ antigen complex. The O8-positive S hybrids also showed an agglutination reaction in the anti-O₉, 12 sera used here. This seems to be based on an unilateral partial cross-specificity apparently existing between *E. coli* O8 and the Salmonella O₉ factor. Thus cells of *E. coli* O8 are agglutinated in anti-Salmonella O₉ serum but Salmonella bacteria containing O₉ antigen are non-reactive in anti-*E. coli* O8 serum (unpublished observations; M. Heidelberger, personal communication).

Unlike the S hybrids, all Ω8-resistant mutants therefrom were agglutinated in 3.5% NaCl. This is indicative of R as well of SR mutants (Hämmerling *et al.*, 1971) and suggested that

the O8-negative hybrids bearing *Salmonella* O-specificity represent SR-forms which can attach only single O-specific repeat units to the core. This was confirmed when the O8-negative hybrid strains were tested with six S- and R-specific phages (see Table 2). The resistance to the R-specific phage 6SR and to the S-specific phage P22, which needs the presence of polymerized repeat units for adsorption, indicates that the mutants F972 to F981 resemble SR *rfc* mutants of *S. typhimurium* (Wilkinson *et al.*, 1972).

From the sensitivity to phage C21 we conclude that the hybrids F972 to F980 have the R1 LPS core of *E. coli* O8 (Schmidt *et al.*, 1969). The FO-sensitivity of the hybrid F981 indicates the presence of a *Salmonella*-like core (Lindberg, 1967), which means that this hybrid must have received donor *rfa* genes together with the adjacent *mtl*⁺ allele (see Table 2).

The strains F972 (O4,12) and F981 (O4,5,12) were used for the production of antisera in rabbits. In these antisera O4 and O12 antibodies could be demonstrated by agglutination of cells from *S. typhimurium* (O4,5,12), *S. essen* (O4,12) and *S. typhi* (O9,12). In addition, O5 antibodies could be detected in anti-F981 serum adsorbed with *S. essen*.

Thus the immunization experiments confirmed the presence of *Salmonella* O factors in the hybrids.

The ability of the hybrids to express *Salmonella* O-specificity in consequence of the introduction of *S. typhimurium rfb* genes suggests that *Salmonella* O-specific repeat units can be attached to the R1 LPS core of *E. coli*. The SR character of the O8-resistant mutants derived from the S (O8⁺) hybrids implies that the donor *rfc* locus had not been transferred to the *E. coli* O8 recipient.

Introduction of Salmonella rfb and rfc genes into E. coli O8

To obtain hybrids with the polymerized repeat units of *S. typhimurium* it was necessary to transfer the *rfc* region, in addition to the *rfb* cluster, into *E. coli* recipients. Attempts to transfer the *rfc* gene(s) into O4-positive hybrids by selection of the adjacent *trp* region of appropriate *Salmonella* donors were unsuccessful, but this could be achieved indirectly with the help of *Salmonella typhi*-*E. coli* recombinants.

It was observed that the introduction of the *his* region from a *S. typhi* donor (WR4000) into *E. coli* O8 led to SR and S hybrids having O9,12 antigen factors but lacking *E. coli* O8-specificity (Kiefer, 1974). From this we assumed that, in contrast to the *S. typhimurium*-*E. coli* hybrids, in these recombinants the donor *rfb* cluster has replaced the *rfb* of the *E. coli* recipient. In addition the S recombinants were presumed to have inherited the donor *rfc* region.

The *rfc*-determined polymerase of *S. typhi* is also active on *S. typhimurium* O-repeat units, and vice versa (Mäkelä, 1965). Thus the introduction of *S. typhimurium rfb* genes into such smooth *S. typhi*-*E. coli* hybrids should lead to S forms with the serological specificity of *S. typhimurium*. Therefore the construction of S hybrids with *S. typhimurium* specificity was performed according to scheme 2 (Fig. 2). His⁺ recombinants from a cross of the *S. typhi* donor WR4000 and *E. coli* F464 (O8) were selected on suitably supplemented minimal agar containing streptomycin (400 µg/ml) to counterselect donor cells. One of the smooth His⁺ hybrids (F1008) with O9,12-specificity was chosen for further experiments and was again provided with a defect in histidine synthesis (His⁻) by mutagen treatment. In addition, this smooth His⁻ (O9,12) strain (F1061) does not synthesize methionine and proline (Met⁻Pro⁻) and does not ferment mannitol and arabinose like the original *E. coli* F464.

The hybrid strain F1061 (His⁻) was used as recipient in a subsequent cross with *S. typhimurium* HfrK9, from which 23 His⁺ hybrids were selected on minimal-lactose agar supple-

Fig. 2. Scheme 2. S, smooth; SR, semi-rough; NTG, treatment with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

mented with methionine and proline. The hybrids were purified by repeated single colony isolations. All recombinants had retained the markers *pro met ara mtl* and behaved like S forms. Thus when grown in nutrient broth at 37 °C they formed stable suspensions which remained homogeneously turbid even after heating at 100 °C for 1 h.

The hybrids were further characterized by slide agglutination tests using different antisera and by their sensitivity patterns towards smooth- and rough-specific phages. Twenty-two hybrid strains were agglutinated in anti-O₄ as well as in anti-O_{9,12} serum but not in anti-O_{12₂} and anti-O_{9,12₃} sera. This result shows that these recombinants possess the O_{4,12} factors, evidently in consequence of the introduction of the *his*-linked donor *rfb* genes. The smooth O_{4,12}-specific recombinants were not agglutinated by anti-O_{12₂} and anti-O_{9,12₃} sera, which suggests that, like the SR hybrids, they possess only the O_{12₁} determinant. One of the hybrids was reactive like the recipient F1061 in anti-O_{9,12} and anti-O_{9,46} sera but not in anti-O₄ serum, indicating that no exchange of the *rfb* region had taken place in this case. Of the 22 hybrids with O_{4,12}-specificity, 15 were also agglutinated in an anti-O₅ serum, indicating the presence of the O-factor 5. Thus, these recombinants must have inherited the *oafA* gene in addition to the *rfb* genes of the donor.

Two representative hybrids, one with O_{4,12}-specificity (F1062) and one with O_{4, 5,12}-specificity (F1069) were chosen for further investigations and for production of antisera in rabbits.

Absorption and agglutination tests with these sera confirmed the presence of the above-mentioned antigen factors in these recombinants. The O-antigen factors and the reaction patterns against phages of two representative hybrids are summarized in Table 3. All the

Table 3. *Antigen factors and phage sensitivity of parent strains and His⁺ hybrids from the cross HfrK9 × F1061*

Strain no.	Action of phage*					O factors†			
	P22	P22c2	FO	C21	6SR	4	5	9	12
Hybrids									
F1062	+	+	—	+	—	+	—	—	+
F1069	+	+	—	+	—	+	+	—	+
Parents									
F1061	+	+	—	+	—	—	—	+	+
HfrK9	+	+	+	—	—	+	+	—	+

* +, Lysis; —, no reaction. † +, Present; —, not present.

Fig. 3. Schematic presentation of the fractionation on Sephadex G50 of degraded polysaccharides obtained from *E. coli* hybrids F962 and F970 (—), and F973 and F981 (---). Eluant, pyridine acetate (10 ml pyridine and 4 ml glacial acetic acid/l, pH 5.4); flow rate, 6 ml/hour. Portions (80 µl) were tested with the phenol-sulphuric acid reagent according to Dubois *et al.* (1955) and extinction was measured at 485 nm.

other hybrids gave the same sensitivity patterns as shown in Table 3. The sensitivity towards phages P22 and P22c2 indicates that the hybrids can synthesize the *Salmonella* O-specific polysaccharide chains which are required for the adsorption of these phages (Wilkinson *et al.*, 1972).

The resistance to phage FO and the sensitivity towards phage C21 indicate the presence of the R1 LPS core of *E. coli* O8 in the hybrids.

Fractionation of the lipopolysaccharides and analyses of the polysaccharide moieties

Degradation and gel permeation chromatography. From different representative hybrid strains lipopolysaccharides were extracted with a phenol-water mixture at 68 °C for 5 min (Lüderitz *et al.*, 1971). All lipopolysaccharides were obtained in comparable yields (1 to 2%) and there were no differences observed during the extraction and work-up procedures.

The lipopolysaccharides were degraded with mild acid and the polysaccharide moieties were obtained by gel permeation chromatography on Sephadex G50. To make the various chromatographic runs directly comparable they were all performed on the same column (2.5 × 65 cm). When polysaccharides from *E. coli* hybrids F962, F970, F973 and F981 (see Fig. 1)

Fig. 4. Schematic presentation of the fractionation on Sephadex G50 of degraded polysaccharides obtained from hybrids F1008, F1061, F1062, F1063 and F1069 (—), and F1009 (---). Details are given in the legend to Fig. 3.

were chromatographed, the elution patterns shown in Fig. 3 were obtained. One peak (I) was always present. With F962 and F970 this was followed by a second peak (II) indicating material of somewhat smaller size which was missing in the elution pattern of F973 and F981. Strains F973 and F981 had been derived from the O8-positive strains F962 and F970 by selection with the *E. coli* O8 phage Ω 8 and resembled SR-forms of *S. typhimurium*. It can be surmised, therefore, that in the patterns of strains F962 and F970, peak II is due to O8-polysaccharide derived from the *E. coli* O8 recipient. Peak III was present in all elution patterns and from its position it was expected to represent unsubstituted core oligosaccharide (Müller-Seitz *et al.*, 1968; Schmidt *et al.*, 1969).

The polysaccharide obtained from recombinants of *S. typhi* and *E. coli* O8 (F1008, F1009 and F1061) as well as those from recombinants obtained in crosses of F1061 with the *S. typhimurium* donor HfrK9 (F1062, F1063 and F1069) were also analysed by gel permeation chromatography on Sephadex G50; their elution patterns are shown in Fig. 4. Peak I, which appeared in all patterns shown in Fig. 3, was missing in all patterns shown in Fig. 4. The significance of this is not yet known. Peaks II and III of Fig. 3 correspond to the first and second peak in Fig. 4, respectively. For better comparison the peaks in Fig. 4 are therefore also designated as peak II and peak III.

Analyses of polysaccharide moieties. The materials from the various peaks of the gel permeation chromatography were analysed as to their sugar components.

The results obtained with the recombinants derived according to scheme 1 (Fig. 1) are shown in Table 4. In the first peaks only glucose was found. The corresponding material (PI) seems to be a glucan. The material from peak II (PII) of F962 and F970 consisted predominantly of mannose, and also contained galactose, glucose and heptose. In PII of F970 a small amount of glucosamine was also found. In F962 the molar ratios of galactose, glucose and heptose in PII were similar to those found in the *E. coli* R1 core (Schmidt *et al.*, 1969), while in F970 (sensitive to phage FO; see Table 2) these molar ratios, together with the glucosamine value, approximated to the values found in the *Salmonella* core (Lüderitz *et al.*, 1971). These analyses indicate that PII is a mannan terminating at the reducing end in a core oligosaccharide. Such a material is obtained after degradation of the LPS from *E. coli* O8 (Reske & Jann, 1972).

Since F973 and F981 are Ω 8 phage-selected strains they do not show a PII fraction. The PIII fractions of all four recombinants contained mainly core components, their molar

Table 4. *Sugar composition of the peak materials obtained by gel permeation chromatography of the degraded polysaccharides from S. typhimurium × E. coli recombinants*

The values are expressed as percentages of the dry weight of each fraction.

LPS from:		Sugar composition						
Strain	Peak	Mannose	Rhamnose	Abequose	Galactose	Glucose	Heptose	Glucosamine
F962	I	—	—	—	—	79.0	—	—
	II	66.9	—	—	2.0	3.5	4.3	—
	III	9.2	2.4	1.8	13.7	16.6	20.5	—
F970	I	—	—	—	—	95.9	—	—
	II	72.1	—	—	2.4	2.6	4.1	—
	III	6.6	4.3	2.1	16.9	10.2	21.8	7.1
F973*	I	—	—	—	—	80.9	—	—
	III	5.7	5.7	2.1	18.4	18.7	21.4	—
F981*	I	—	—	—	—	95.0	—	—
	III	5.4	4.6	1.8	18.2	11.3	21.9	5.7

* As can be seen in Fig. 3, the elution patterns obtained with the R strains F973 and F981 lack peak II.

Table 5. *Sugar composition of the peak materials obtained by gel permeation chromatography of the degraded polysaccharides from S. typhi × E. coli recombinants (F1008, F1009, F1061) and (S. typhi × E. coli) × S. typhimurium recombinants (F1062, F1063, F1069)*

The values are expressed as percentages of the dry weight of each fraction.

LPS from:		Sugar composition					
Strain	Peak*	Mannose	Rhamnose	DDH†	Galactose	Glucose	Heptose
F1008	II	22.9	19.2	8.7	23.9	5.5	2.3
	III	4.3	5.4	1.7	14.8	13.5	21.8
F1009	II	ND	ND	ND	ND	ND	ND
	III	5.1	5.1	2.4	17.1	16.1	16.4
F1061	II	21.2	18.4	8.1	24.3	8.5	2.9
	III	4.7	5.0	1.7	16.6	16.4	22.0
F1062	II	21.6	18.2	7.3	23.2	5.9	2.8
	III	7.8	5.5	1.8	15.6	14.1	16.9
F1063	II	21.2	18.4	8.1	24.3	5.9	2.9
	III	5.7	5.0	1.7	16.6	16.4	22.0
F1069	II	23.8	20.3	10.3	25.9	2.8	0.9
	III	5.5	5.5	2.4	15.9	14.6	10.5

* Peak designation is such that Figs. 3 and 4 as well as Tables 4 and 5 are directly comparable.

† DDH, 3, 6-dideoxyhexose; tyvelose in F1008, F1009 and F1061; abequose in F1062, F1063 and F1069.

ratios indicating an *E. coli* R1 structure in F962 and F973 and a *Salmonella* core structure in F970 and F981. In addition, they all contain equimolar amounts of mannose and rhamnose, and smaller amounts of abequose. Due to the volatility of their alditol acetates, dideoxyhexoses are partially lost in gas-liquid chromatography. Therefore, it is reasonable to assume that in the PII fractions mannose, rhamnose and abequose were present in equimolar amounts. This is corroborated by the value of galactose, which was higher than expected for the corresponding core ratios by amounts which were about equal to the values of mannose and rhamnose. These results indicate that the PIII fractions are mixtures of unsubstituted core oligosaccharides and core oligosaccharides substituted with one repeating unit (Abe, Gal, Man, Rha) of the *S. typhimurium* polysaccharide; thus, recombinants F962 and F970 are

S-forms with an *E. coli* O8 polysaccharide, and additionally are SR-forms with respect to *S. typhimurium*.

The results obtained with recombinants derived according to scheme 2 (Fig. 2) are shown in Table 5. None of the strains produced a glucan. With F1009, too little PII material was found to be analysed. Analogous to the above reasoning, the values found can be interpreted in the following way: the PIII fraction of F1009, according to its analysis, is a core oligosaccharide of the *E. coli* RI type substituted with one repeating unit of the *S. typhi* polysaccharide. In F1008 and F1061, the PII fraction is the polysaccharide of *S. typhi* (with tyvelose), terminating at the reducing end in the *E. coli* RI core oligosaccharide. The PIII fraction is the core substituted by a *S. typhi* repeating unit. In F1062, F1063 and F1069 the same situation was found, in PII the polysaccharide being that of *S. typhimurium* (with abequose) and in PIII the core being substituted with one repeating unit of the *S. typhimurium* polysaccharide.

All strains analysed also contained unsubstituted core stubs, which explains the asymmetric shape of peak III in Figs. 3 and 4.

DISCUSSION

Genetic crosses between *S. typhimurium* HfrK9 and an *E. coli* O8 recipient generated His⁺ hybrids expressing both Salmonella and *E. coli* O8 specificities. This suggested that the *rfb* gene clusters of both mating partners are functional in these hybrids.

The partial diploid state, apparently stably maintained in the hybrids, may have generated from the insertion of donor genes at novel sites of the recipient chromosome, thus leading to a duplication of the *his-rfb* region (Baron *et al.*, 1968; Mojica-a & Middleton, 1972). Alternatively, the transferred donor DNA may be conserved quite stably in an extra-chromosomal state. Studies are in progress on the mechanism maintaining partial diploidy in these hybrids.

In crosses between *S. typhi* donor WR4000 and *E. coli* O8, smooth His⁺ recombinants were obtained which had lost the *E. coli* O8 antigen and only expressed the *S. typhi* O_{9,12} specificity. In this case the *rfb* region of the donor must have replaced that of the recipient by recombination, thus producing haploid recombinants. Similarly, Johnson & Baron (1969) reported that a high portion of *E. coli* recombinants from crosses between *S. typhi* donor WR4000 and *E. coli* were haploid for several allelic regions transferred from the donor.

Escherichia coli O8 acquires the *rfe* locus in crosses with *S. typhi* but not in crosses with *S. typhimurium*. This, and the fact that *S. typhi* imposes haploidy and *S. typhimurium* diploidy in the *rfb* region of the recombinants, cannot be explained at present.

The smooth and semi-rough His⁺ recombinants exhibiting *S. typhimurium* O-specificity synthesize O-antigen factors 4,(5) and 12. Factor 12 of *S. typhimurium* is complex and divided into sub-factors 12₁ and 12₂ (Kauffmann, 1966). The chemical nature of 12₁ is not known but rhamnose in the polysaccharide chain is supposed to be involved in the 12₁-specificity (Staub *et al.*, 1959). The results of our serological tests suggest the absence of the factor 12₂ in the recombinants. The genes (*oafR,E,F*) responsible for glucosylation of the polysaccharide resulting in 12₂ specificity are located near *purE* and remote from the *his-rfb* region (Rudén & Mäkelä, 1974). This explains the lack of O₁₂ specificity in the hybrids selected here.

The serological findings and bacteriophage patterns obtained were fully corroborated by chemical analyses. Hybrids between *S. typhimurium* and *E. coli* O8 (F962 and F970) exhibited on their surface complete O8 S-lipopolysaccharide, the polysaccharide moiety of which is a

mannan consisting of 20 to 22 trisaccharide units (Reske & Jann, 1972). In addition, they synthesize a *S. typhimurium*-specific SR-lipopolysaccharide which is a core oligosaccharide substituted by one O-specific repeating unit consisting of abequose, mannose, rhamnose and galactose (Lüderitz *et al.*, 1971). Most of the recombinants [of which F962 and F973 (O8⁻) are representative] contain the *E. coli* R1 core, and one hybrid (F970) and its O8-negative derivative (F981) contain the Salmonella core, obviously in consequence of the introduction of donor *rfa* genes. These core types can easily be differentiated by their sugar composition: the *E. coli* R1 core does not contain glucosamine and has a glucose:galactose ratio of 3:2 (Schmidt *et al.*, 1969), whereas the Salmonella core contains glucosamine, glucose and galactose in molar ratios of 1:2:2 (Lüderitz *et al.*, 1971).

Recombinants of *S. typhi* and *E. coli* O8 (F1008, F1009 and F1061) exhibited only *S. typhi*-specific lipopolysaccharides. Degradation and gel permeation chromatography gave rise to (i) fractions containing core oligosaccharide substituted with a polysaccharide consisting of approximately 20 tetrasaccharide units containing tyvelose, mannose, rhamnose and galactose, and (ii) fractions containing core oligosaccharide substituted with only one such oligosaccharide. Thus, recombinants F1008, F1009 and F1061 synthesize S- and SR-lipopolysaccharides of the same specificity, concomitantly. The relative ratios of these surface antigens differed in these strains, strain F1009 producing predominantly SR-lipopolysaccharide and strains F1008 and F1061 producing predominantly S-lipopolysaccharide. The polysaccharides did not contain glucose; this can be explained by the obvious lack of the *oafR*, *E*, *F*-modification (glucosylation) genes in the recombinants.

When in a subsequent cross the (*S. typhi*-specific) *rfb* locus of recombinant F1061 was replaced by the (*S. typhimurium*-specific) *rfb* locus of HfrK9 (see Fig. 2), the ensuing recombinants F1062, F1063 and F1069 produced S- and SR-lipopolysaccharides which contained abequose instead of tyvelose.

Recombinants between *E. coli* O8 (F210) and *S. typhimurium* contained a glucan, and all other recombinants did not. The reason for this is not known. Possibly, the information for the synthesis of this glucan may be determined by the *E. coli* strain, because this strain (F210) is a different isolate of *E. coli* O8 than the one (F464) used in the other mating experiments. We have found previously (Jann and Jann, unpublished observations) that many *E. coli* strains synthesize such a glucan. Of special biochemical interest is the concomitant occurrence of S-, SR- and R-lipopolysaccharides in one bacterial strain. This phenomenon was also observed in *E. coli* wild-type strains which produced acidic lipopolysaccharides (Jann and Jann, unpublished observations).

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