Genetic Determination of the O Antigens of *Salmonella* Groups B (4,5,12) and C₁ (6,7)

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ABSTRACT

MÄKELÄ, P. HELENA (University of Helsinki, Helsinki, Finland). Genetic determination of the O antigens of *Salmonella* groups B (4,5,12) and C₁ (6,7). *J. Bacteriol.* 91:1115-1125. 1966.—The genetic determination of the O antigens of *Salmonella* was studied by Hfr or F' crosses between strains of groups B (antigens 4,5,12) and C₁ (antigens 6,7). The main genetic determinants of the specificities 4 of group B and 7 of group C₁ behaved as alleles of one locus, called *O* or *O*-4/7. This is probably identical with *O*-4/9, responsible for the serological difference between groups B and D, and with the "rough" locus *rouB*. At least parts of the antigens 12 of group B and 6₂ of group C are determined at the same locus. The gene *O*-5 is closely linked to *O*-4/7, both mapping in the approximately 2 minutes distance between *his* and *metG* in the order *his* - *O*-4/7 - *O*-5 - *metG*. In crosses of group B donors with group C₁ recipients, a serologically new type, called semi-rough (SR), appeared in most recombinants that had inherited the *O*-4 allele of the group B donor. These SR forms are serologically intermediate between smooth and rough forms, showing poor stability in saline but possessing the specificities 4 and 12 and (some of them) 5. On the basis of previous biochemical studies, the hypothesis has been put forward that the side chains of their lipopolysaccharide are much shorter than normal group B side chains, probably containing only one repeating unit per side chain. A gene *SR*-4 responsible for the elongation of group B side chains beyond the first repeating unit was mapped between *gal* and *try*, group B and D bacteria being *SR*-4⁺, and group C being *SR*-4⁻.

The specificity of the O antigens of *Salmonella* is determined by the three to five monosaccharides constituting the repeating unit of the long side chains of a lipopolysaccharide (LPS) molecule (22, 25). This O-specific part of the side chain is distal to a short proximal portion or "stub," which, together with a backbone of heptose phosphate-ketodeoxyoctonate, forms a core believed to be common to all *Salmonella* species (Fig. 1). The only hexose components of the core are D-glucose, D-galactose, and N-acetyl-D-glucosamine, whereas a wide variety of hexoses, notably 6-deoxy- and 3,6-dideoxyhexoses, are met with in the O-specific part of the side chains (8). The sugar components not present in the core are conveniently referred to as O-specific.

Rough (R) forms do not possess the O-antigenic specificity of the bacteria from which they were derived; their LPS is correspondingly de-

void of O-specific components and is more or less equivalent to the core (7, 12).

The synthesis of the LPS is under genetic control, part of which is known. Apart from loci governing the synthesis of uridine diphosphoglucose and uridine diphosphogalactose, there are two main loci—far apart from each other—defects at which lead to a rough mutant (28). The locus called *rouA* is probably concerned with the synthesis of the central part of the side chain, and *rouA* mutants have the RI type of LPS, lacking the terminal unit(s) of the side-chain "stubs," and thus the attachment site for the O-specific part. *RouA* mutants can synthesize the O-specific side-chain material, which accumulates as a relatively low-molecular-weight haptenic polysaccharide (1).

Mutations of the other locus, *rouB*, which is closely linked to *his*, the locus for histidine biosynthesis, lead to RII-type LPS, which contains the complete core (1). *RouB* mutants do not

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FIG. 1. Schematic representation of the somatic lipopolysaccharide (LPS) of *Salmonella* (21). The structure of a repeating unit with the specificities 4, 5, 12 is shown in Fig. 3. Symbols: P = phosphate; KDO = 2-keto-3-deoxyoctonic acid; Hept = L-glycero-D-mannoheptose; Glu = D-glucose; Gal = D-galactose; GNAc = N-acetyl-D-glucosamine.

accumulate O-specific polysaccharide, and it therefore seems likely that the *rouB* locus comprises one or more genes concerned with the synthesis and polymerization of the repeating unit of the O-specific part of the LPS side chain. These genes would be expected to comprise genes for the synthesis of nucleotides of the smooth-specific sugars (i.e., mannose, rhamnose, and abequose in the case of *S. typhimurium*) as well as genes for the transferases responsible for their ordered assembly. One *rouB* mutant of *S. typhimurium* was indeed shown to be defective in the synthesis of thymidine diphosphorhamnose, whereas the synthesis of nucleotide sugars was completely normal in several others (19).

More direct evidence of the role of a locus close to *his* in the specificity of LPS was obtained from genetic crosses between *Salmonella* strains possessing different O antigens (5, 14). The specificities 4 (of group B) and 9 (of group D) segregated among recombinants as if determined by alleles of a locus, called O-4/9, close to *his*. The main, perhaps the only, difference between these two antigens is the nature of one of the smooth-specific sugars: one dideoxyhexose, abequose, in 4, and another, tyvelose, in 9 (25). An O locus in *Escherichia coli* has likewise been described as closely linked to *his* (20). In *S. typhimurium*, factor 5 segregates in crosses between 4, 5, 12 and 4, 12 strains as if determined by a locus O-5 located near *his*, between *his* and *purC* (24). Group D (9, 12) bacteria seem to lack this gene. In crosses between 4, 5, 12 and 9, 12 organisms, it segregates in the same manner as in crosses of 4, 5, 12 and 4, 12, but is expressed only together with factor 4 (5, 14).

In the present paper the determination of the smooth-specific part of the side chain is examined by means of crosses between *Salmonella* strains of groups B (4, 5, 12) and C₁ (6, 7). The

absence of any common O factors (6) probably indicates that there are very few structural features common to the respective repeating units. The O-specific sugars in group B are mannose, rhamnose, and abequose; in group C₁, mannose only.

MATERIALS AND METHODS

The bacterial strains came from my collection in Helsinki (SH and SW strains) or from the collection of the Guinness-Lister Research Unit at the Lister Institute (SL strains). Group B (4, 5, 12) *Salmonella* strains used are derivatives of *S. abony* 74 [no. 103 of Edwards and Bruner (2)] or of *S. typhimurium* LT-2 (31). Group C₁ (6, 7) strains are derivatives of *S. montevideo* 2 [no. 129 of Edwards and Bruner (2)].

Mutations were induced in these strains by ultraviolet irradiation or by an alkylating agent, ethyl methane sulfonate or diethyl sulfate (3, 11). The F factor, originally from *E. coli* K-12, was introduced first into *S. abony*, which served as donor of F to other *Salmonella* strains (15). Hfr strains were derived from these F⁺ strains (13); from one *S. abony* Hfr strain, SW 1444, an F' factor, called F-59, was obtained (Mäkelä and Ziegler, *in preparation*). This factor is readily transmissible and converts bacteria acquiring it into donors of the same type as SW 1444. The leading locus and direction of chromosome transfer of the donor strains are shown in Fig. 2, which also shows the locations and abbreviations of genetic markers used.

The motility medium used was nutrient broth (Difco) with 0.5% agar and 8% gelatin (27). The minimal medium was Davis minimal medium as described by Lederberg (10); 1.6% agar, 0.2% glucose, 20 µg/ml of amino acids or adenine (or both), 0.002 µg/ml of thiamine, and 1 mg/ml of streptomycin were added when indicated.

Crosses were made between exponentially growing broth cultures of Hfr or F-59⁺ donor and F⁻ recipient, mixed so as to give about 5 × 10⁷ donor cells and 5 × 10⁸ recipient cells per milliliter. This mating mixture was kept undisturbed at 37°C for 2 hr; then 0.1-ml quantities were plated without washing onto selective minimal medium. Recombinant colonies appearing after 2 days were streaked on nutrient agar, from which discrete colonies were tested for their growth requirements, sugar fermentations, and antigen characters.

H antigens were determined by slide agglutination in rabbit anti-H sera diluted 1:100. Before testing, the strains (or recombinants) were passed through motility medium to enhance their motility.

O antigens were determined by slide agglutination in appropriate sera at predetermined dilutions in 0.2% saline. In many cases the results were checked by tube agglutination of boiled cultures in serial dilutions of the serum, incubated overnight at 45°C. Most sera were prepared by immunization of rabbits with saline suspensions of nutrient agar cultures held at 100°C for 25 hr; single-factor sera were obtained by absorption. The following sera were used: an anti-*S. abortus*equina (SH 117, O antigens 4, 12, 12_s)

FIG. 2. Chromosome map of *Salmonella* showing the location of markers (on the outer circle) and the points of origin and directions of transfer of the donor strains (13, 23, 24). Abbreviations: loci controlling thr = threonine, leu = leucine, pro = proline, try = tryptophan, his = histidine, met = methionine, aro = phenylalanine and tyrosine, pur = purine, ath = adenine and thiamine, iva = isoleucine and valine, arg = arginine biosynthesis; gal = D-galactose, tre = D-trehalose fermentation; str = streptomycin sensitivity (-s = sensitive, -r = resistant); H1 = phase 1, H2 = phase 2 flagellar antigen; rou = rough, SR = semirough character; O-5 = partial antigen 5, O-4/7/9 = partial antigens 4, 7, or 9.

serum used unabsorbed as an anti-4 reagent, as its anti-12 activity was very weak; an anti-*S. typhi* T2AS (SH 116, O antigens 9,12₁,12₂) serum used unabsorbed as anti-12₁,12₂ reagent; an anti-*S. paratyphi* B (SH 108, O antigens 4,5,12) serum used absorbed with SL 681, a *S. typhimurium* LT-2 mutant lacking O antigen 5, as an anti-5 reagent; an anti-*S. montevideo* (SH 541, O antigens 6₁,7) serum used unabsorbed as an anti-6₁,7 reagent; an anti-*S. takoradi* (SH 52, O antigens 6₁,8) serum used unabsorbed as an anti-6₁ reagent; an anti-*S. choleraesuis* var. *kunzendorf* (SH 153, O antigens 6₂,7) serum used absorbed with SH 151, an *S. choleraesuis* var. *kunzendorf* 6₁, 7 strain, as an anti-6₂ reagent; and anti-4 and anti-7 single-factor sera, kindly supplied by the Serological Standards Laboratory, Central Public Health Laboratory, Colindale, London, England.

Most of the strains for immunizations were received from F. Kauffmann, International Salmonella Center, State Serum Institute, Copenhagen, Denmark. The *S. choleraesuis* var. *kunzendorf* strains

were received from P. R. Edwards, Communicable Disease Center, Atlanta, Ga. The sera will be described in detail elsewhere (Mäkelä, in preparation).

RESULTS

Crosses with group C₁ (6₁,7) donor to group B (4,5,12) recipients. For a preliminary orientation, the group C₁ donor (Hfr) strain SH 713 was crossed with several group B (*S. typhimurium*) recipients to yield recombinants which had inherited the donor allele of various genes distributed along the length of the *Salmonella* chromosome (Table 1). These crosses were fairly productive, giving approximately 100 recombinants per 5×10^6 donor bacteria per plate, with markers from near the origin (*ade*) to *his*; the number was much less, from 1 to 10 per plate, for more distal markers. When such recombinants were tested for agglutinability in anti-4,12 and anti-6,7 sera, they usually had retained the recipient type of agglutinability (positive with anti-4,12, negative with anti-6,7). Among those recombinants that had inherited the donor *his*⁺ allele, a majority (90%) showed the donor type of agglutinability in anti-6,7 and failure to agglutinate in anti-4,12 serum. Some (14%) of the recombinants with the donor *try*⁺ allele were likewise agglutinated in the anti-6,7 serum. These findings seemed to indicate that the group B and group C₁ O antigen specificities were determined by alleles of one locus, closely linked to the histidine locus.

Upon closer examination, five different agglu-

TABLE 1. O-antigenic composition of recombinants in crosses between a 6,7 Hfr donor (*Salmonella montevideo* SH 713) and various auxotrophic 4,5,12 recipients (*S. typhimurium* LT2 derivatives)

Donor allele* selected	Total tested†	Recombinants		
		4, 5, 12	6, 7	Rough
<i>athA</i> ⁺	47	45‡	1	0
<i>hisE</i> ⁺	315	27‡	283	2
<i>fla</i> ⁺	15	14	1	0
<i>try</i> ⁺	127	111	18	0
<i>gal</i> ⁺	15	15	0	0
<i>pro</i> ⁺	32	32	0	0
<i>metA</i> ⁺	60	60	0	0

* Female marker selected in all cases was *str-r*, which is a very late marker for this donor.

† The recombinants were, in many cases, derived from several crosses with different recipients.

‡ In addition to the 45 and 27 4,5,12 recombinants, there were 1 and 3, respectively, 4,12 recombinants.

mination patterns were detected among the *his*⁺ recombinants (Table 2): (i) 4,5,12 or completely recipient-like, 27 of 315 *his*⁺ recombinants; (ii) 4,12 or like the recipient but lacking factor 5, 3 of 315 *his*⁺; (iii) 6₂,7 or possessing the donor antigen factor 7, but having 6₂ instead of the donor 6₁, 281 of 315 *his*⁺; (iv) 6,7 or completely donorlike, 2 of 315 *his*⁺; (v) rough, 2 of 315.

A tentative interpretation of these results was as follows. The main genetic determinants of the O specificities of groups B and C₁ behave as alleles of one locus, provisionally called O-4/7, closely linked to *his*. Antigen 7 is completely determined at this locus, and it replaces both 4 and 12; to decide whether both of these factors are also determined at the same locus or only suppressed in the O-7 recombinants, the reverse cross is needed. The situation with factor 6 is apparently more complex: the subfactor 6₂ seems to be determined at the same locus, although it is not apparent in the 6₁,7 parent. The 6₂,7 recombinants can be converted to 6₁,7 by a phage isolated from 6₁,7 strains (Mäkelä and Hovi, *in preparation*). Apparently, the few 6₁,7 recombinants (class iv above) obtained have acquired the prophage as well as the O-7 allele of the donor parent, whereas most *his*⁺ recombinants do not carry the prophage of the donor and have O antigens 6₂,7 (class iii). The gene O-5⁺, determining the presence of antigen 5 in group B, is also linked to *his*, but less closely than is O-4/7. If the 6,7 parent lacks this gene (or has a silent allele), the 4,12 recombinants (class ii) result from incorporation of the O-5 region of the 6,7 donor chromosome without incorporation of its O-7 locus. Finally, the rough recombinants (the rare class v) could arise from rough mutants of either donor or recipient, or they might be caused by a crossover, breaking the O locus (18). It should be noted that no recombinant was agglutinated by both group B (4,5 or 12) and group C (6₁,6₂ or 7) sera.

Cross A (Table 3) of a 6,7 donor to a 4,5,12 recipient was designed to map more precisely the postulated loci O-4/7 and O-5. The donor was a *S. montevideo* (6,7), a streptomycin-sensitive, Hfr strain injecting the chromosome in the sequence *athA* - *metG* - *his*, with streptomycin as a very late marker. The recipient, SH 162, was a 4,5,12 *S. typhimurium* LT-2 derivative, *hisE*⁻, *metG*⁻, *athA*⁻, streptomycin-resistant. The *metG* locus is known to map very close to *his*, being 2 minutes distant in an interrupted mating experiment (23); it proved very useful, as the O loci appeared to lie within the segment *his* - *metG*. Recombinants with the *metG*⁺ character of the donor and the streptomycin-resistance of the recipient were selected in ex-

periment I; in experiment II the donor alleles of both *metG* and *athA* were selected because of the high reversion frequency of the *metG* mutation. All of the nonselected donor markers tested are distal to (i.e., expected to enter later than) the selected *metG*⁺ and *athA*⁺.

The position of the O loci inferred from cross A (Table 3) is *hisE* - O-4/7 - O-5 - *metG*. With this order, the minimum number of quadruple crossovers is 3 (of 122 recombinants). There is some ambiguity about the location of O-4/7: it could also be on the other side of *hisE*, in which case the minimum number of quadruple crossovers would be increased to 6. Of the 31 recombinants with the donor alleles *metG*⁺ and *athA*⁺ in experiment 2 (where all the *metG*⁺ clones tested are true recombinants, not reversions), probably 27 (87%) have also the donor O-5, 23 (74%) have the donor O-7, and 21 (68%) have the donor *his*⁺. Crossovers between the loci O-4/7 and O-5 are fairly frequent: a total of 13 in the combined experiments 1 and 2. Crossovers between O-4/7 and *hisE* are fewer: a total of seven in experiments 1 and 2. It should also be noted that in experiment 1, when *metG*⁺ of the donor is selected, the earlier locus *athA* is donorlike in only 3 of the 81 recombinants. Such low linkage is typical of *Salmonella* matings (Mäkelä, *unpublished data*; Stocker, *unpublished data*), whereas a linkage of approximately 50% would be expected in a comparable *E. coli* mating situation (4).

Results of selecting *his*⁺ of the donor in the same cross as in Table 3 and in several others between the same *S. montevideo* (group C₁) donor and either *S. typhimurium* or *S. abony* (group B) recipients also support the inferred position and order of the O loci. With this selection, the 4,12 recombinants (class ii in Table 2), genotypically *his*⁺ (donor) O-4 (recipient) O-5 (donor), must arise through quadruple crossovers; their observed rarity is consistent with this view.

Crosses with group B (4/5/12) donors and group C₁ (6,7) recipients. In the reversed, permuted cross, with a 4,5,12 donor to a 6,7 *his*⁻ recipient and selecting the donor *his*⁺ allele, the outcome was complicated. In addition to the serotypes already met with (4,5,12; 4,12; 6₁,7; rough), a new type appeared in large numbers (Table 4). Indeed, the majority of the *his*⁺ recombinants were of this phenotype, which appeared to be novel, and was called semirough (SR).

The SR type was culturally intermediate between the S and R forms, giving smooth-looking colonies but growing with deposit in broth and being unstable in saline suspension. In slide

TABLE 2. Slide agglutination patterns (in 0.2% saline) of parents and *his*⁺ *str-r* recombinants in a cross between a 6₁,7 (*Salmonella montevideo*) Hfr *his*⁺ *str-s* donor, SH 713, and a 4,5,12 (*S. typhimurium*) *his*⁻ *str-r* recipient, SH 162.

Class	C-antigen	No. of recombinants	Anti-4	Anti-5	Anti-12 ₁ , 12 ₂	Anti-7	Anti-6 ₂	Anti-6 ₁	Saline (4%)
Donor Recipient	6 ₁ ,7 4,5,12		— ++	— ++	— ++	++ —	— —	++ —	— —
i	4,5,12	27	++	++	++	—	—	—	—
ii	4,12	3	++	—	++	—	—	—	—
iii	6 ₂ ,7	281	—	—	—	++	++	—	—
iv	6 ₁ ,7	2	—	—	—	++	—	++	—
v	Rough	2	—	—	—	—	—	—	++

TABLE 3. Cross A, between a 6,7 (*Salmonella montevideo* SH 713) Hfr donor and a 4,5,12 (*S. typhimurium* SH 162) recipient*

O serotype	No. of recombinants		Markers tested (in their proposed clockwise map order)								
			<i>str</i>	<i>trc</i>	<i>H1</i>	<i>hisE</i>	O-4/7	O-5	<i>metG</i>	<i>athA</i>	
	Expt I	Expt II								Expt I	Expt II
6,7 4,5,12			s r	+ —	g,m,s i	+ —	6,7 4,12	— 5	+ —	+ —	+ —
4,5,12	40† 3 1	— 4 0	0 0 0	0 0 0	0 0 0	0 0 1	0 0 0	0 0 0	1 1 1	0 1 0	 1 1
4,12	9	4	0	0	0	0	0	1	1	0	1
6,7	3 1 14 8 1	0 1 10 7 3	0 0 0 0 0	0 0 0 0 1	0 1 0 1 1	0 0 1 1 1	1 1 1 1 1	? ? ? ? ?	1 1 1 1 1	0 0 0 0 0	1 1 1 1 1
Rough	1 0	1 1	0 0	0 0	0 0	1 0	? ?	? ?	1 1	0	1 1
Total	81	31	81-31	1-3	10-11	25-21	27-21	?	81-31	3	31

* Recombinants were selected for the alleles printed in italics. Experiment I: *metG*⁺ of donor; experiment II: *metG*⁺ and *ath*⁺ of donor. Symbols: 1 = donor allele; 0 = recipient allele; ? = allele not determined (not expressed in this serotype?). Minimum number of quadruple crossovers: three.

† Many of these are *metG*⁺ reversions of recipient, avoided in experiment II.

agglutination tests with sera diluted to a salt concentration of 0.2%, its reactions seemed fairly specific: there was a strong agglutination in anti-4 and anti-12 sera, and sometimes in anti-5 serum as well. The agglutinates were never the large clumps typical of O agglutination of smooth strains, but were instead an even distribution of small aggregates. In respect to antigen 5, the semiroughs were divided into SR (4,5,12) reacting with anti-5 serum, and SR (4,12), without detectable 5-specificity. This distinction could be made even by slide agglutina-

tion, which made it possible to classify large numbers of recombinants. Absorption and immunization with representative SR (4,12) and SR (4,5,12) recombinants have confirmed the presence in them of the mentioned antigen factors (Mäkelä, *in preparation*).

In cross B (Table 4), the donor was an *S. abony* (4,5,12) Hfr strain injecting clockwise in the sequence *leu*, *pro*, *gal*, *try*, *his*, etc., and the recipient was an *S. montevideo* (6₁,7) *try*⁻, *his*⁻, *gal*⁻ mutant. [This cross was less productive, especially for *his*⁺ recombinants, than were

TABLE 4. Cross B, between a 4,5,12 (*Salmonella abony* SW 1444) Hfr donor and a 6,7 (*S. montevideo* SH 951) recipient*

O serotype	No. of recombinants	Markers tested (in their proposed clockwise map order)							
		gal	"SR"	try	H1	his	O-4/7	O-5	aro
4,5,12 6,7		+	+	+	b g,m,s	+	4,12 6,7	5 —	— +
6,7	3	0	?	0	0	I	0	?	0
	1	0	?	0	1	I	0	?	0
SR (4,12)	13	0	0	0	0	I	1	0	0
	9	0	0	0	1	I	1	0	0
	2	0	0	1	1	I	1	0	0
SR (4,5,12)	2	0	0	0	0	I	1	1	0
4,5,12	1	0	0	0	1	I	1	1	0
	1	0	1	1	1	I	1	1	0
Total his ⁺ aro ⁺	32	0	1?	3	14	32	28	4?	32
6,7	34	0	0	I	0	0	0	?	0
	2	0	0	I	1	0	0	?	0
	1	1	?	I	0	0	0	?	0
SR (4,12)	1	1	0	I	1	1	1	0	0
SR (4,5,12)	1	0	0	I	0	1	1	1	0
	1	0	0	I	1	1	1	1	0
Total try ⁺ aro ⁺	40	2	0?	40	4	3	3	2?	40

* For definitions, see Table 3.

crosses employing the group C₁ donor SH 713 to group B recipients: only 1 to 5 his⁺ recombinant clones were found per plate (5 × 10⁶ donor bacteria).] Of 35 recombinants with the donor his⁺ allele (32 obtained by selecting for his⁺ and 3 found when selecting for try⁺), 31 (88%) had 4 specificity, either SR or smooth, and 6 (17%) had 5 specificity, either 4,5,12 or SR (4,5,12). Only one recombinant was smooth 4,5,12. Of 40 recombinants with the donor try⁺ allele, only those 3 that had the donor his⁺ allele had 4 or 4,5 specificity; the rest had recipient-like O antigens. Thus, the same close linkage of O-4/7 to his was observed as in the reversed crosses between group C₁ donors and group B recipients (Tables 2 and 3).

Genetic composition of the semirough form. An analysis of the LPS of several SR forms—recombinants from cross B (Table 4) selected for the group B his⁺ allele—has shown that it contains the smooth-specific sugars mannose, rhamnose, and abequose of group B, although in much smaller amounts than are present in the smooth group B parent (16). This observa-

tion, together with the serological evidence of the presence of the antigen factors 4 and 12, led to the hypothesis that most genes needed for making group B side chains are located near his, but that an auxiliary gene, SR, not closely linked to his, is needed to enable the normal amount to be made. This gene would be SR⁺ in group B *Salmonella* strains, SR⁻ (or absent) in group C₁; its defect, if O-4⁺ is present, gives semiroughs, either SR (4,5,12) or SR (4,12), depending on the presence or absence of O-5⁺.

In a number of crosses made between various group B Hfr donors and group C recipients, and selecting for the donor his⁺ allele, the proportion of smooth group B-like recombinants, inferred to have both the O-4⁺ and the SR⁺ genes from the group B donor, varied according to the Hfr strain used. None was obtained with donors injecting their chromosome counterclockwise in the sequence *metA* - H2 - *purC* - *his* or H2 - *purC* - *his*; this indicates that the postulated SR locus is not within the segment *metA* - H2 - *purC* - *his*. A few (about 3%) were obtained with donors injecting clockwise in the sequence

thr - *pro* - *try* - *his*; with such donors, up to 30% smooth group B-like recombinants were recovered, when the donor *try*⁺ or *thr*⁺ alleles were selected in addition to *his*⁺. Thus, the inferred *SR* locus seems to map within the segment *thr* - *pro* - *try* - *his*, although no close linkage to any of the markers (*thr*, *ara*, *pro*, *gal*, *try*, *fla*, or *his*) within this segment was detected.

To determine the position of the *SR* gene more exactly, cross C (Table 5) was made between SW 1444, the smooth 4,5,12 Hfr donor strain used in cross B, and a semirough SR (4,12) recipient strain. This semirough strain had been isolated as a *his*⁺ recombinant from another cross, equivalent to cross B (Table 4), between the same 4,5,12 donor and a group C *S. montevideo* recipient; the inferred genetic composition of this SR recipient was *pro*⁻, *gal*⁻, *try*⁻, *H1*-g, *m,s*, *SR*⁻, *O*-4⁺, *O*-5⁻. When, in cross C, the donor *pro*⁺ allele was selected, 6 among a total of 297 recombinants were smooth 4,12, that is,

SR⁺. Five of these six had the donor *gal*⁺ allele, as compared with 30 *gal*⁺ recombinants among the 290 *pro*⁺ which were still semirough, i.e., had not received the donor *SR*⁺ allele. When the donor *try*⁺ allele was selected, 10 of a total of 53 recombinants were smooth 4,12, but none had the donor *gal*⁺ allele. Since the sequence of injection of the donor strain was *pro* - *gal* - *try*, the order *pro* - *gal* - *SR* - *try* was inferred, with *SR* not very close to either *gal* or *try*.

In another cross, the same semirough recipient strain as in cross C was crossed with another 4,5,12 (smooth) donor strain, SW 1403, which injects the chromosome in the opposite direction (with *H2* near the leading end), selection of the donor *try*⁺ allele gave few (6 among a total of 141) smooth *SR*⁺ recombinants. This puts *SR* on the distal side of *try* in this donor (that is, between *try* and *pro*, which agrees with the order inferred above).

Cross C serves also as evidence for the postulated genetic composition *O*-4⁺, *O*-5⁻ of the *SR*

TABLE 5. Cross C, between a 4,5,12 (*Salmonella abony* SW 1444) Hfr donor and semirough SR (4,12) recipient (SH 1006) obtained as a *his*⁺ hybrid from a cross, *S. abony* to *S. montevideo**

O serotype	No. of recombinants	Markers tested (in their proposed clockwise map order)						
		<i>pro</i>	<i>gal</i>	"SR"	<i>try</i>	<i>H1</i>	<i>O</i> -5	<i>aro</i>
4,5,12 SR (4,12)		+	+	+	+	b g,m,s	5	-
		-	-	-	-		-	+
SR (4,12)	260	1	0	0	0	nt	nt	0
	30	1	1	0	0	nt	nt	0
4,12	5	1	1	1	0	nt	nt	0
	1	1	0	1	1	nt	nt	0
Rough	1	1	0	?	0	nt	nt	0
Total <i>pro</i> ⁺ <i>aro</i> ⁺	297	297	35	6?	1			297†
SR (4,12)	53	0	0	0	1	0	0	0
	7	0	0	0	1	1	0	0
	2	0	1	0	1	0	0	0
	1	1	1	0	1	0	0	0
	2	0	1	0	1	1	0	
SR (4,5,12)	3	0	0	0	1	0	1	0
	2	0	0	0	1	1	1	0
	1	0	1	0	1	0	1	0
4,12	4	0	0	1	1	0	0	0
	4	0	0	1	1	1	0	0
	2	1	0	1	1	0	0	0
Total <i>try</i> ⁺ <i>aro</i> ⁺	81	3	6	10	81	15	6	81‡

* Symbols as in Table 3; nt = not tested.

† Minimum number of quadruple crossovers: 1.

‡ Minimum number of quadruple crossovers: 7.

(4,12) type. As soon as the SR^+ gene is introduced (under conditions where the donor O loci are very seldom introduced) into the SR (4,12) recombinant used as recipient, it becomes smooth, with O antigens 4 and 12 but without 5. Several other crosses with SR (4,5,12) or SR (4,12) recipients have confirmed the postulated presence or absence in them of the gene $O-5$. Furthermore, $O-5^+$ could be introduced with his^+ into a 4,12 *S. typhimurium* recipient strain by conjugation from two SR (4,5,12) recombinants converted into genetic donors by introduction of the F' factor F-59. By the same technique, no $O-5^+$ was revealed in SR (4,12) recombinants.

If the model involving a genetic difference at an SR locus between 4,5,12 and 6,7 bacteria is correct, it should be possible to introduce the SR^- allele from a 6,7 donor into a 4,5,12 recipient and thus obtain a 4,5,12 semirough (SR^-) recombinant with the same serological characteristics as those obtained by introducing the $O-4$ locus into a 6,7 recipient. This was tried with several 4,5,12 recipient strains. A problem arose from the small numbers of recombinants obtained in the *gal-pro* region in these intergroup crosses with the only available 6,7 donor strain. Among 324 try^+ recombinants, 6 semirough (SR^-) forms were found; they were serologically indistinguishable from the SR recombinants obtained in the reverse situation (cross B). The results of these crosses were also compatible with the location of the SR gene between *try* and *gal*.

DISCUSSION

The main genetic determinants of the specificities of the two widely differing O antigens, 4, (5,) 12 and 6,7 of the group B and group C_1 strains used as parents, behaved as alleles of one O locus, closely linked to *his*. This locus is presumably complex, containing genes for the synthesis of hexose nucleotides as well as genes for the transferases needed for putting together the specific part of the different LPS side chains. It is clear that the group B and group C_1 "alleles" are widely different, as the two antigens differ not only in their antigenic specificities but also in the nature of their smooth-specific sugars. This nonhomology might be the cause of the very few crossovers within the O locus observed. The serotype produced in these cases was rough, consistent with the assumption that these recombinants were unable to synthesize a proper unit and therefore made a LPS equivalent to the core only. No new combinations of group B and group C antigen factors were found.

Genetic determination of the partial antigens.

All the genetic information for the synthesis of antigenic specificity 4 (that is, all such as is not shared by the 6,7 genotype) seems to reside within the O locus. In an accompanying paper (18), this locus has been shown to contain genes for eight enzymes leading to the synthesis of cytidine diphosphoabequose, thymidine diphosphorhamnose, and guanosine diphosphomannose, the known or supposed donors of these sugars to LPS. Two of these sugars, abequose and rhamnose, are not present in the LPS of 6,7 strains, and the enzyme activities for synthesis of their nucleotides are low or absent in 6,7 strains. As rhamnose is a part of the main sequence of the 4,(5,)12 repeating unit (Fig. 3), the repeating unit of group B is certainly very different from that of group C_1 , which lacks rhamnose but unfortunately has not been examined in detail. Consequently, group B and group C_1 strains must also differ in several transferases concerned with the putting together of the respective repeating units; the determinant genes of these transferases must be parts of the $O-4$ and $O-7$ alleles of the O locus.

The presence of at least some component(s) of antigen 12 in the semirough $O-4^+$ recombinants was shown by their agglutination in anti-12₁₂ serum. Moreover, smooth recombinants with 4-specificity always showed 12-specificity also, both when the smooth recombinant was obtained in a cross of a 4,5,12 donor with a 6,7 strain or with a semirough, SR (4,12) or SR (4,5,12), recombinant as recipient. These smooth recombinants were shown to have inherited both the locus $O-4$ and the locus SR^+ from the donor 4,5,12 parent; hence, the genetic determinant(s) of the structures responsible for 12-specificity resides in either $O-4$ or SR^+ loci or in both.

The gene $O-5$, determining antigen 5, that is, O -acetylation of galactose residues in the 4,12 LPS (9), segregated among the his^+ recombinants showing rather loose linkage to *his* and $O-4$. Yet both $O-4$ and $O-5$ map between *his* and

FIG. 3. Schematic representation of the O -specific side chain of the lipopolysaccharide of smooth group B (4, 5, 12) *Salmonella* (17, 25). Abe = abequose; Man = *D*-mannose; Rha = *L*-rhamnose; Glu = *D*-glucose; Gal = *D*-galactose; GNAc = *N*-acetyl-*D*-glucosamine.

metG, a segment which Sanderson and Demerec (23) showed in interrupted mating experiments to be equivalent to only 2 minutes transfer time, i.e., less than 2% of the whole chromosome. As no unrelated genes are known between *O*-4 and *O*-5, it seems possible that they are both part of the same *O* locus, concerned with various aspects of the specific side chain. The 6,7 parent is assumed to lack the *O*-5 locus; some 6,7 recombinants produced in the crosses probably contain the positive allele *O*-5⁺; however, no antigen 5 could be detected in these recombinants. The same situation was observed when the *O*-5⁺ gene was introduced into a 9,12 strain: no antigen 5 could be demonstrated (5, 14).

The gene for antigen 7 behaved as an allele of *O*-4; nearly all recombinants manifested either one or the other of these specificities. The few exceptions to this rule were rough recombinants, at least some of which were apparently due to a crossover within the *O* locus (18).

The *S. montevideo* (6,7) strain used was 6₁,7. The factor 6₁ has been reported to be phage-determined, 6₂,7 bacteria being converted to 6₁,7 by a phage present in, e.g., a 6₁,7 *S. cholerae-suis* var. *kunzendorf* strain but absent in a 6₂,7 variant of the same species (Escobar and Edwards, Bacteriol. Proc., p. 146, 1964). The 6₂,7 recombinants could likewise be converted to 6₁,7 by a phage isolated from 6₁,7 strains (Mäkelä and Hovi, *in preparation*). The recombinants with antigen 7 were 6₁, when the recipient bacterium was 6₁,7, but were 6₂ when the 6₁,7 strain was the parent donating its *O*-7 locus to *S. typhimurium* recipients. Therefore, the 6₂ factor seems to be determined at the *O*-7 locus; the prophage converting this 6₂ antigen to 6₁ is not closely linked to *O*-7.

In summary, the *O* locus appears to contain at least most of the information for the synthesis of antigen factors 4 and 7 and of at least parts of factors 12 and 6. Possible functions shared by groups B and C₁, but related with the synthesis of the O-specificities, could still be determined at other loci. So far, it has not been possible to separate the factors 4 and 12 or 6 and 7, and therefore the corresponding alleles should perhaps be called *O*-4,12 and *O*-6,7 instead of the designations *O*-4 and *O*-7 provisionally adopted. Because, however, the factors 12 and 6 contain more than one subfactor, all of which either have not been sufficiently tested (factor 12) or are apparently not determined at the same locus (6₁), it is preferred to retain the shorter forms of the locus symbols.

Semirough recombinants. Among the recombinants of some group B × group C₁ crosses, a serologically novel type appeared as a majority

class among the recombinants selected for the donor group B *his* allele. This type was called semirough (SR) because it shared characteristics of both smooth (S) and rough (R) forms. The LPS of these semirough recombinants has been shown to possess the smooth-specific sugars abequose, rhamnose, and mannose of the O-antigen type of one (4,5,12) parent, whereas the 6,7 parent has mannose only. The quantity of these sugars in the semirough LPS is much less than in the LPS of the smooth 4,5,12 parent form, though the antigenic specificities of the 4,5,12 parent are present. On the basis of this and other facts, the hypothesis has been put forward that the LPS of the semirough recombinants has only one repeating unit in its side chain and that this LPS is thus chemically a transition form between smooth and rough (16). The genetic interpretation tentatively given is verified in all respects by the data now presented: (i) the SR recombinants have the *O*-4 allele instead of *O*-7 at the *O* locus close to *his*; (ii) some of them have the *O*-5⁺ allele of group B, but some do not: SR (4,5,12) and SR (4,12), respectively; (iii) they have a defect at a locus called SR, which maps between *gal* and *try*. The proposed structure of the specific part of the 4,5,12 side chain (Fig. 3) will give a clue as to the function of the SR⁺ gene. The proximal galactose of the 4,5,12 side chain would be attached to the N-acetylglucosamine terminus of the core by a specific transferase. The second and subsequent galactose units, by contrast, would be attached to mannose, and therefore their attachment would presumably be effected by another transferase. A defect of this second galactose transferase would stop the synthesis of the side chain at a stage where the side chain contains only one repeating unit, producing an SR form. If, as now seems more likely, the repeating units are assembled on a lipid acceptor (29, 30) and are then transferred to the LPS, then the SR enzyme would be one transferring second and subsequent repeating units to the mannose of the preceding repeating unit.

Group B (4,5,12) and D (9,12) strains both seem to possess the SR⁺ gene, because an interchange of their *O* loci without interchange of their *gal*-*SR*-*try* region always produces smooth recombinants, either 4,5,12 or 9,12 (14). This is reasonable, too, in view of the close similarity of the repeating units of these groups (25). On the contrary, not only group C₁ (6, 7), as shown in the present paper, but also group C₂ (6, 8) bacteria, lack the SR⁺ gene; the introduction of the gene *O*-4 into them results (Stocker et al., Ann. N.Y. Acad. Sci., *in press*) in semirough types SR (4,5,12) and SR (4,12). This is

the more remarkable, as group C₂ has the same smooth-specific sugars, abequose, rhamnose, and mannose, as group B (8). The structure of its side chain must be very different, however, as these two groups show very little serological cross-reactivity (26).

The group C₁ strains seem to lack any physiological equivalent of the SR gene outside the O locus, because the introduction of the O-7 region alone into group B bacteria suffices to produce smooth 6,7 recombinants. This might reflect a different mode of synthesis of side chains in group C, or a different organization of genetic material. A prediction of this model of the SR form is that mutants of the SR⁺ gene in groups B or D should give the same semirough phenotype. Such mutants were indeed found in *S. typhimurium* strain LT2 as mutants resistant to phage P22 (16), and the mutation mapped at a locus (tentatively called *rouC*) between *gal* and *try*. These mutants resemble the SR recombinants very closely in both cultural and serological properties and in the composition of their LPS.

If the SR form thus is one defined by one repeating unit only per side chain, then this form ought to be possible in almost any serotype. The prerequisite would be that the distal sugar of the repeating unit would not be *N*-acetylglucosamine, so that the second repeating unit could be attached to a different sugar than the first. The kind of repeating unit should therefore be indicated along with the SR form. The SR recombinants described in the present paper are thus SR (4,5,12) and SR (4,12). An SR form with the specificities 9 and 12 has been found as a recombinant from crosses of smooth 9,12 donor bacteria with semirough 4,12 recipients (Stocker et al., *in press*); it should be described as SR (9,12). Mutations responsible for the SR form in other O groups may not always map at the SR locus observed in group B, as in other groups totally different enzymes would be implicated. Thus the SR⁺ gene described in the present paper would better be called SR-4⁺.

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