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Genetic Mapping of Vi and Somatic Antigenic Determinants in *Salmonella*

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ABSTRACT

JOHNSON, E. M. (Walter Reed Army Institute of Research, Washington, D.C.), BARBARA KRAUSKOPF, AND L. S. BARON. Genetic mapping of Vi and somatic antigenic determinants in *Salmonella*. J. Bacteriol. **90**:302–308. 1965.—The Vi antigen and somatic antigen 9 were transferred to *Salmonella typhimurium* recipients by mating with *S. typhosa* Hfr TD-7, and the genetic determinants of these antigens were located. A gene responsible for Vi antigen expression, *ViB*, was found to be associated with the *inl-purA-pyrB* linkage group, and the order *ViB-inl-purA-pyrB* was established. The determinant of somatic antigen 9 was found closely linked to the *his* gene, and cotransduction of these determinants was accomplished with phage PLT-22. Moreover, all conjugation and transduction hybrids which received the somatic antigen 9 determinant concurrently lost somatic antigen 4. Similarly, *S. typhosa* hybrids produced by transfer of *his* and the gene for somatic antigen 4 from *S. typhimurium* Hfr B2, or by cotransduction tion of these genes with PLT-22, also lost somatic antigen 9. These results indicated that the genetic determinants of the somatic antigens 9 and 4 are probably allelic. A second Vi antigen determinant, *ViA*, located near *his*, was discovered in matings of *S. typhimurium* Hfr B2 with a Vi-negative *S. typhosa* recipient. Vi-positive *S. typhosa* hybrids were obtained from this cross in which neither parent expressed the Vi antigen, indicating that this Vi determinant of *S. typhosa* is present also in *S. typhimurium*.

The intricate serological pattern exhibited by members of the family Enterobacteriaceae has come, in recent years, within the scope of genetic analysis. Transductional techniques were employed by Lederberg and Edwards (1953) to examine the genetic relationships of the flagellar antigens of a wide variety of *Salmonella* serotypes. These studies showed that the genes determining the various phase 1 flagellar antigens comprised an allelic series at one chromosomal locus, H_1, and those specifying phase 2 antigens constituted a homologous group at a second locus, H_2. Later, Hfr donors were used in genetic matings to map the H_1 and H_2 determinants in *S. abony* (Mäkelä, 1962), and to locate the genes specifying the somatic (O), flagellar (H), and capsular (K) antigens in *Escherichia coli* (Ørskov and Ørskov, 1962). Mäkelä (1964) employed Hfr strains in intergeneric crosses to demonstrate allelism of the H_1 gene of *Salmonella* and the single flagellar antigenic determinant, *H*, of *E. coli*.

The allelic nature of the flagellar antigen genes among *Salmonella* species and between *Escherichia* and *Salmonella*, coupled with other demonstrated chromosomal homologies among these organisms (Zinder, 1960; Falkow, Rownd, and Baron, 1962; Mäkelä, 1963), suggests the existence of further allelic patterns in their somatic and capsular antigens. With the availability of a *S. typhosa* Hfr strain capable of transferring chromosomal material to *S. typhimurium* recipients (Johnson, Falkow, and Baron, 1964*b*), it has been possible to study these two organisms with regard to the genetic relationship of their somatic as well as their flagellar antigens. In addition, an opportunity was afforded to examine the genetic basis of the classical Vi antigen of Felix and Pitt (1934).

MATERIALS AND METHODS

Organisms. The bacterial strains employed are described in Table 1. *S. typhimurium* strains have the somatic antigenic constitution 1, 4, 5, 12, and display flagellar antigens i (phase 1) and 1,2 (phase 2); *S. typhosa* is of the serotype 9, 12, Vi:d. However, *S. typhosa* strains 643WS^r*his*$^-$ and 643WS^{r}HM lack the Vi antigen, and *S. typhimurium* 74R-1S^r is nonflagellated. The orientation of chromosome transfer by the Hfr strains and the chromosomal locations of the genetic markers used are shown in Fig. 1.

Media. The minimal agar selective medium was described in a previous communication (Johnson, Falkow, and Baron, 1964*a*). In crosses where the

TABLE 1. *Characteristics of the bacterial strains**

Strain	Source	Loci controlling auxotrophic characters	Carbohydrate utilization				Response to SM
			ara	*inl*	*rha*	*fuc*	
Salmonella typhosa							
Hfr strain							
TD-7	WRAIR	*cys, try*	−	−	−	−	S
Recipient strains							
643S^r	WRAIR	*cys, try*	−	−	−	−	R
643WS^r *his*$^-$	WRAIR	*cys, try, his*	−	−	−	−	R
643WS^r HM	WRAIR	*cys, try, his, metA*	−	−	−	−	R
S. typhimurium							
Hfr strains							
Hfr B2	K. E. Sanderson	*metA*	+	+	+	+	S
Hfr A	K. E. Sanderson	*purC*	+	+	+	+	S
Recipient strains							
74R-1S^r	WRAIR	*proA, argA, ile, his*	+	−	+	+	R
HMXS^r	K. E. Sanderson	*his, metA*	+	+	+	+	R
Pan S^r	K. E. Sanderson	*pan*	+	+	+	+	R
Pur-AS^r	K. E. Sanderson	*purA*	−	+	+	+	R
Pyr-BS^r	K. E. Sanderson	*pyrB*	+	+	+	+	R

* Abbreviations and symbols: *cys*, cystine; *try*, tryptophan; *his*, histidine; *metA*, methionine; *proA*, proline; *argA*, arginine; *ile*, isoleucine; *pan*, pantothenic acid; *purA* and *purC*, purine; *pyrB*, uracil; *ara*, L-arabinose; *inl*, inositol; *rha*, L-rhamnose; *fuc*, L-fucose; SM, streptomycin; S, sensitive; R, resistant; +, utilized; −, not utilized; WRAIR, Walter Reed Army Institute of Research.

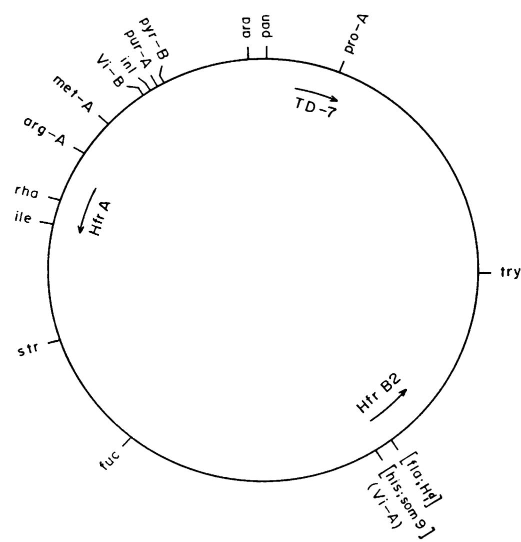

FIG. 1. *Chromosome of Salmonella typhosa showing locations of genetic markers employed or referenced. Arrows indicate transfer orientation of the Hfr strains. Abbreviations are defined in Table 1, except for the following: str, streptomycin resistance; Vi-A and Vi-B, determinants of Vi antigen; som 9, somatic antigen 9; fla, flagella; $H_1{}^d$, flagellar antigen d. Markers enclosed in brackets are cotransducible by phage PLT-22; position of marker enclosed in parentheses is appoximate.*

utilization of a particular carbohydrate was the selected genetic trait, that carbohydrate was added as the sole carbon source at a concentration of 0.4%. Essential amino acids were added at a concentration of 25 μg/ml. Selection of prototrophic recombinants was accomplished by omission of the required amino acid, purine, or pyrimidine, and, in these crosses, glucose at a concentration of 0.4% served as the carbon source. Streptomycin sulfate at a concentration of 625 μg/ml was employed as a counter selective agent.

Technique of genetic crosses. Genetic crosses were performed in the manner previously described by Johnson et al. (1964*a*).

Biochemical and serological analysis of recombinants. Recombinant clones were purified by streaking on minimal medium of the same composition as that on which they were originally isolated. Analysis of unselected markers concerned with the utilization of carbohydrates was accomplished by testing the fermentative ability of the purified recombinants in Purple Broth (Difco) containing the carbohydrate at a concentration of 1%. Unselected amino acid markers were detected by streaking the purified recombinants on minimal glucose selective media. Inheritance of flagella by the nonflagellated 74R-1S^r strain was determined by testing the motility of the purified hybrids on H Antigen Broth medium (Fisher Scientific Co., Pittsburgh, Pa.) solidified by addition of 0.2% Noble Agar (Difco). The presence of Vi and somatic antigens was determined by slide agglutination in single-factor antisera. Determination of

TABLE 2. *Unselected marker inheritance by the progeny of crosses between Salmonella typhimurium Hfr B2 and S. typhimurium recipient strain 74R-1S*^{r}

Selection	Per cent unselected markers*		
	his$^+$	*ile*$^+$	*argA*$^+$
arg$^+$	29	41	All
ile$^+$	36	All	20
his$^+$	All	<1	<1

* Percentages are based on a minimum of 100 tested hybrids of each selection class.

H antigens was carried out by tube agglutination, according to the procedures of Edwards and Ewing (1962).

Preparation of antisera. The procedures described by Edwards and Ewing (1962) were followed in the preparation of somatic 9, 12 antiserum and Vi (against *Citrobacter ballerup*) antiserum by rabbit immunization, and in the absorption of the 9, 12 antiserum to obtain a single factor 9 antiserum. Antiserum prepared against an H-antigen inactivated *Salmonella* 4, 12:b serotype (originally isolated by Joan Taylor in 1951) was absorbed with *S. typhosa* 0901 to obtain pure somatic 4 antiserum. Single factor somatic 5 antiserum and H-antigen antisera (factors d, i, and 1,2) were obtained from Arthur Abrams of the Walter Reed Army Institute of Research.

Transduction technique. Phage PLT-22, grown to a titer of 10^9 particles per milliliter on the bacterial host strain, was used for transduction. The organism to be transduced was suspended in 9 ml of Penassay Broth (Difco; approximately 10^8 cells per milliliter), and 1 ml of the sterile phage lysate was added, making the final phage concentration 10^8 particles per milliliter. After 10 min of incubation at 37 C to allow phage adsorption, the cells were centrifuged, concentrated by suspension in 1 ml of fresh Penassay Broth, and plated on selective medium (0.1 ml per plate).

RESULTS

Transfer of S. typhosa 9, Vi, and d antigens to S. typhimurium. In genetic crosses between the *S. typhosa* Hfr TD-7 and the *S. typhosa* recipient 643WS^{r}HM, where selection was made for the *his*$^+$ marker of the donor, it was observed that 33% of the recombinants also acquired the Vi antigen characteristic of TD-7. On the other hand, all hybrids selected for the *metA*$^+$ marker remained Vi-negative. These results indicated that the genetic determinant of the Vi antigen was located somewhere beyond the *proA* to *metA* (counterclockwise) segment of the TD-7 chromosome, possibly in the vicinity of *his*. However, the scarcity of useful genetic markers in 643WS^{r}HM prevented further exploitation of this system and prompted the use of *S. typhimurium* strains as genetic recipients for TD-7.

Intraspecies matings involving *S. typhimurium* Hfr and recipient strains do not present quite the same pattern which one observes within the *E. coli* K-12 fertility system. In addition to the reduction of gene-transfer frequencies as compared with K-12, *S. typhimurium* × *S. typhimurium* crosses also display a decreased linkage of unselected genetic characters. When K-12 Hfr donors are crossed with K-12 F$^-$ strains, the inheritance of unselected genetic markers proximal to the selected gene averages about 50% (Jacob and Wollman, 1961). By contrast, analysis of a cross between *S. typhimurium* Hfr B2 and the *S. typhimurium* LT-7 recipient strain 74R-1S^r shows the inheritance of proximal unselected markers to range from 29 to 41% (Table 2).

When *S. typhosa* TD-7 is employed in genetic crosses with *S. typhimurium* 74R-1S^r, no reduction in the frequency of gene transfer by comparison with *S. typhimurium* Hfr strains is observed (Johnson et al., 1964*b*). On the other hand, the linkage of unselected markers is considerably reduced. Linkage analysis of the TD-7 × 74R-1S^r cross is presented in Table 3, and it can be seen that the hybrids inherited most unselected markers in the range of 1 to 13%. Thus, the finding that 75% of the hybrids selected for the *his*$^+$ marker inherited the somatic antigen 9 of TD-7 immediately established the relative chromosomal location of the 9 antigenic determinant. Furthermore, examination of more than 200 74R-1S^r *his*$^+$ hybrids revealed that only those recombinants which failed to receive the 9 antigenic determinant were agglutinated by single factor 4 antiserum. The loss of somatic antigen 4 by all of 153 tested hybrids which had acquired the 9 antigen strongly suggested the allelism of *S. typhimurium* 4 antigen and *S. typhosa* 9 antigen genetic determinants.

The gene determining the formation of flagella

TABLE 3. *Unselected marker inheritance by the progeny of crosses between Salmonella typhosa Hfr TD-7 and S. typhimurium recipient strain 74R-1S*^{r}

Selection	Per cent unselected markers*								
	ara$^-$	*Vi*$^+$	*argA*$^+$	*rha*$^-$	*ile*$^+$	*fuc*$^-$	*his*$^+$	*som 9*	*fla*$^+$
his$^+$	11	3	4	4	1	6	All	75	30
ile$^+$	5	6	13	12	All	8	<1	<1	<1
arg$^+$	2	2	All	10	3	6	<1	<1	<1

* Percentages are based on a minimum of 100 tested hybrids of each selection class. Abbreviation: *som 9* = somatic antigen 9.

TABLE 4. *Unselected marker inheritance by the progeny of crosses between Salmonella typhosa Hfr TD-7 and S. typhimurium LT-2 recipient strains*

Cross	Selection	Per cent unselected markers*							
		ara⁻	*inl⁻*	*Vi⁺*	*metA⁺*	*rha⁻*	*fuc⁻*	*som 9*	*his⁺*
TD-7 × HMXSʳ	*his⁺*	10	5	3	1	3	6	80	All
TD-7 × HMXSʳ	*metA⁺*	1	2	2	All	4	<1	<1	<1
TD-7 × Pur-ASʳ	*purA⁺*	—	42	33	—	NT	NT	NT	—
TD-7 × Pyr-BSʳ	*pyrB⁺*	6	30	18	—	NT	NT	NT	—
TD-7 × PanSʳ	*pan⁺*	31	5	6	—	NT	NT	NT	—

* Percentages are based on a minimum of 100 tested hybrids of each selection class. NT = not tested; *som 9* = somatic antigen 9.

TABLE 5. *Unselected marker inheritance by the progeny of crosses between Salmonella typhimurium Hfr B2 and S. typhosa recipient strain 643WSʳ his⁻*

Selection	Per cent unselected markers*						
	his⁺	4 antigen	*Vi⁺*	*fuc⁺*	*rha⁺*	*inl⁺*	*ara⁺*
ara⁺	6	6	7	8	6	10	All
rha⁺	8	5	6	4	All	<1	2
fuc⁺	7	4	8	All	<1	<1	<1
his⁺	All	73	25	<1	<1	<1	<1

* Percentages are based on a minimum of 100 tested hybrids of each selection class. Per cent inheritance of the 4 antigen is based on 100 hybrids agglutinating in either anti-4 or anti-9 serum, and does not include any O-inagglutinable, Vi antigen-containing hybrids.

(*fla⁺*) was inherited by 30% of the *his⁺* recombinants. This figure also seemed exceptional when compared with the linkage pattern of other markers in the cross, and indicated the proximity of the *his* and *fla* determinants. Serological analysis of 50 *S. typhimurium* 74R-1Sʳ *his⁺* hybrids which had received the *fla⁺* marker from TD-7 revealed that all were capable of agglutination in 1,2 antiserum. Thus, they expressed the latent phase 2 flagellar antigen specificity determinant (H_2) of 74R-1Sʳ. The lack of involvement of the H_2 determinant in this sample of only 50 hybrids is not surprising in view of its location at some distance from the *his* marker (Mäkelä, 1964), and the low percentages at which the majority of unselected characters were inherited in this cross. On the other hand, the H_1 determinant in *Salmonella* is known to be cotransducible with the *fla* gene (Stocker, Zinder, and Lederberg, 1953). As expected, recombination did occur at the H_1 locus, with 33 of the 50 74R-1Sʳ *his⁺ fla⁺* hybrids receiving the d flagellar antigen of TD-7. The remaining 17 expressed the latent phase 1 antigen, i, of 74R-1Sʳ. The allelism of the d antigen gene of *S. typhosa* with the i antigen determinant of *S. typhimurium* was observed previously by Lederberg and Edwards (1953).

The Vi antigen of TD-7 was acquired by the 74R-1Sʳ recombinants in these crosses at only 2 to 6%, thus precluding the mapping of its determinant with the auxotrophic markers available in this recipient. However, location of this gene was achieved by employing TD-7 in crosses with a number of auxotrophic *S. typhimurium* LT-2 recipients, generously provided for this purpose by Kenneth E. Sanderson. Linkage analyses of some of these crosses are shown in Table 4. Inheritance of unselected genetic characters by *S. typhimurium* HMXSʳ, when selection is made for *his⁺* and *metA⁺* (crosses 1 and 2), parallels the results with *S. typhimurium* 74R-1Sʳ. The 9 antigen is inherited by 80% of the *his⁺* recombinants, and all of the other unselected markers appear in the range of 1 to 10%. In sharp contrast with its 3 and 2% inheritance with *his⁺* and *metA⁺*, respectively, the Vi antigen is acquired by 33% of the *purA⁺* recombinants, of which 42% also inherit the closely linked *inl⁻* marker of TD-7 (cross 3). Selection for the *pyrB⁺* marker, although it is closely linked to *purA*, results in the reduction of Vi inheritance to 18%, and of *inl⁻* to 30% (cross 4). Although these percentages are not all derived from matings within a single strain, the data suggest that the distance between *purA* and Vi is similar to the distance between *pyrB* and *inl*. On this basis, and on Vi antigen inheritance at only 18% with *pyrB*, the best placement of the Vi determinant with respect to the known *inl*, *purA*, and *pyrB* markers would be *ViB-inl-purA-pyrB* (Fig. 1). The gene mapped in this group is designated *ViB*, since, as subsequent investigation was to show, it was not the determinant originally observed in the TD-7 × *S. typhosa* 643WSʳHM cross.

Matings of S. typhimurium Hfr strains with S.

TABLE 6. *Unselected marker inheritance by the progeny of matings in which Salmonella typhimurium Hfr B2 and S. typhimurium Hfr A are crossed with S. typhosa recipient strain 643S*^{r}

Hfr strain	Selection	Per cent unselected markers*				
		fuc$^+$	*rha*$^+$	*inl*$^+$	*Vi*$^-$	*ara*$^+$
B2	*fuc*$^+$	All	<1	<1	<1	<1
B2	*rha*$^+$	7	All	1	2	<1
B2	*ara*$^+$	6	3	12	13	All
Hfr A	*ara*$^+$	NT	6	16	12	All
Hfr A	*inl*$^+$	NT	2	All	45	13

* Percentages are based on a minimum of 100 tested hybrids of each selection class. NT = not tested.

typhosa recipients. In the earlier crosses of TD-7 with *S. typhosa* 643WS^{r}HM, 33% of the *his*$^+$ recombinants acquired the Vi antigen, and none of the *metA*$^+$ hybrids did so. The inconsistency of these results with the subsequent finding of a Vi determinant linked to *inl* suggested that more than one gene might be involved. Confirmation of the existence of a second *Vi* locus was obtained, in an unusual manner, from matings set up to test the allelism of the 9 and 4 somatic antigenic determinants.

Crosses were performed with the *S. typhimurium* Hfr B2 and the *S. typhosa* recipient 643WS^r *his*$^-$, with single marker selections being made for *his*$^+$, *fuc*$^+$, *rha*$^+$, and *ara*$^+$. Unselected marker analyses of each of these hybrid classes (Table 5) revealed a linkage pattern similar to that observed when TD-7 was employed with *S. typhimurium* recipients. Most unselected markers were inherited at 2 to 10%. On the other hand, 73% of the *his*$^+$ recombinants, as expected, received the somatic antigen 4 of B2. The anticipated loss of the somatic antigen 9 occurred in all hybrids which received the 4 antigenic determinant, thus confirming the probable allelism of the somatic 4 and 9 antigenic determinants. However, the most striking feature of these crosses was the appearance of the Vi antigen among the recombinants. Moreover, the acquisition of the Vi antigen by 25% of the *his*$^+$ recombinants is in significant contrast to its 6 to 8% inheritance among the *ara*$^+$, *rha*$^+$, and *fuc*$^+$ hybrids. This finding, coupled with the 33% inheritance of the Vi antigen among the *his*$^+$ recombinants from the TD-7 × *S. typhosa* 643WS^{r}HM cross, indicated the existence of a second Vi determinant located near *his* and common to both *S. typhosa* and *S. typhimurium*. This determinant was designated *ViA*.

S. typhimurium Hfr strains were also used to confirm the general location of the *ViB* gene. Since this determinant is either altered in, or missing from, *S. typhimurium*, transfer of the *S. typhimurium* allele to a Vi-positive *S. typhosa* should result in loss of Vi antigen by the recipient. Furthermore, this loss should segregate among the hybrids in the same manner as any other unselected genetic marker. The analysis of crosses between B2 and the Vi-containing *S. typhosa* 643S^r is shown in Table 6. Loss of the Vi antigen of 643S^r paralleled the inheritance of the *inl*$^+$ marker, occurring at less than 1% in hybrids selected for *fuc*$^+$, 2% in those selected for *rha*$^+$, and 13% among the *ara*$^+$ recombinants. Moreover, 9 of 13 Vi-negative *ara*$^+$ hybrids had also received the *inl*$^+$ marker. Since the *inl*$^+$ gene proved a difficult selective marker with Hfr B2, selection for this character was made with *S. typhimurium* Hfr A, which transfers *inl*$^+$ as a more proximal marker than does B2. The Hfr A × 643S^r crosses for *ara*$^+$ and *inl*$^+$ are included in Table 6. The inheritance of unselected characters with *ara*$^+$ selection parallels that observed in the B2 cross for this marker, with the Vi antigen being lost by 12% of the hybrids. On the other hand, loss of Vi occurs in 45% of the hybrids selected for *inl*$^+$, thus confirming the linkage of *ViB* with *inl*.

Transduction of somatic antigen 9 and 4 determinants by PLT-22. The 73 to 80% linkage of the somatic 4 and 9 antigenic determinants with the *his* gene in conjugation experiments strongly indicated the feasibility of their cotransduction with this marker. Therefore, phage PLT-22, grown on an *S. typhimurium* 74R-1S^r hybrid which had received the *his*$^+$, *fla*$^+$, 9 antigen, and d antigen determinants from TD-7, was used to transduce the *his*$^+$ marker of this hybrid to 74R-1S^r. Transduction occurred at a frequency of about 10^{-6} per phage particle. Analysis of the *his*$^+$ transductants showed that 24% received the 9 antigen determinant and concurrently lost the somatic 4 antigen. None was observed to receive the *fla*$^+$ gene.

Cotransduction of the *his*$^+$ and somatic antigen 4 determinants was accomplished with a PLT-22 lysate of *S. typhimurium* Pur-AS^r, and use of *S. typhosa* 643WS^r *his*$^-$ as the recipient. Transduction of the *his*$^+$ marker took place at a frequency of about 10^{-7}, with 21% of the transductants also inheriting the *S. typhimurium* somatic antigen 4 determinant. As had been the case with the somatic antigen 9 transductants, all of the 643WS^r *his*$^+$, 4 antigen transductants lost the 9 antigen, again pointing up the probable allelism of the genetic determinants of somatic antigens 9 and 4.

The *S. typhosa* 643WS^r *his*$^+$ transductants which received somatic antigen 4 were tested in single factor somatic 5 antiserum with negative results. The finding that the determinant of somatic antigen 5 is at least outside the range of cotransduction with *his*$^+$ is in accordance with the assumption of Naide et al. (1965) that these determinants are not closely linked. On the other hand, the *S. typhimurium* 74R-1S^r *his*$^+$ transductants which received the somatic antigen 9 determinant (and lost the somatic 4 determinant) also failed to agglutinate in antisomatic 5 serum. This finding, coupled with the fact that the 5 antigen is not found without the 4 antigen among *Salmonella* serotypes, suggests that the determinant of somatic antigen 5 can express itself only in an organism in which somatic antigen 4 is also manifested.

DISCUSSION

In view of the allelism of H-antigen genes among *Salmonella* serotypes (Lederberg and Edwards, 1953), it is not surprising that the present study has revealed further relationships between antigens of *S. typhosa* and *S. typhimurium*. The data presented in this account have demonstrated the existence of two genes involved in the expression of the Vi antigen in *S. typhosa*, and have shown that one of these is present also in *S. typhimurium*. In addition, the data have established the chromosomal location of the *S. typhosa* somatic antigen 9 determinant, and have indicated its probable allelism with the somatic antigen 4 determinant of *S. typhimurium*. Of course, the possibility exists that examination of larger numbers of hybrids might reveal some possessing both (or neither of) these somatic antigenic determinants. However, analysis in this study of over 400 *his*$^+$ conjugation hybrids and 200 *his*$^+$ transduction hybrids indicated only a simple allelic replacement of the markers for the somatic 4 and 9 antigens.

In the crosses between *S. typhosa* TD-7 and *S. typhimurium* 74R-1S^r, it was apparent that unselected markers were inherited in significant numbers with the selected gene only when the determinants in question were closely linked. This finding was true also when TD-7 was mated with the *S. typhimurium* LT-2 auxotrophs, and it provided the basis for mapping of the *ViB* determinant. Thus, the examination in this system of nine selected markers at closely spaced intervals along the *pan* to *argA* chromosome segment (four of these are listed in Table 4) showed unselected inheritance of the Vi antigen exceeding 6% in only two instances—*purA* (33%) and *pyrB* (18%).

The positioning of the *ViB* gene to the left of the *inl-purA-pyrB* linkage group (Fig. 1) best fits the data available from the *S. typhosa* TD-7 × *S. typhimurium* LT-2 crosses. It would have been preferable to have located this determinant by linkage data derived from matings with a single recipient strain, but such a strain was not available. Thus, the accuracy of *ViB* placement is dependent upon the assumption that the *S. typhimurium* auxotrophs all behave similarly with regard to the integration of *S. typhosa* genes. However, in all crosses performed with these strains in this study, as well as in other experiments not reported here, we have found no evidence to indicate that this assumption is unwarranted.

The placement of the *fla* locus to the right of *his* on the genetic map in Fig. 1 is made in accordance with the marker order *his-fla-try* presented by Subbaiah and Stocker (1964). Its distance from *his* is based on the 30% linkage which it exhibited with this marker in the *S. typhosa* TD-7 × *S. typhimurium* 74R-1S^r mating. Location of the d antigen determinant is in accordance with the reported cotransducibility of H_1 and *fla* determinants (Stocker et al., 1953). It is further substantiated by the finding in this study that 33 of 50 74R-1S^r *fla*$^+$ hybrids from the TD-7 cross were also recombinant for the d antigen. At present, only a tentative position near *his* can be assigned to the *ViA* determinant.

The indication that somatic 9 (group D) and somatic 4 (group B) antigen determinants are alleles at a locus linked to *his* suggests that other *Salmonella* group somatic antigen genes may also be alleles at this site. Thus, the situation would be comparable to the already demonstrated allelism of *Salmonella* flagellar antigenic determinants. Some indication that this may, in fact, be the case is found in the recent report of Naide et al (1965). In crosses between an Hfr *S. abony* (group B; O-antigen 4, 5, 12) and *S. montevideo* (group C; O-antigen 6, 7), these workers observed some *his*$^+$ hybrids which acquired the group B specificity of the donor while losing their native group C specificity. However, in this mating, the majority of *his*$^+$ recombinants were found to be different serologically from either parent and were termed semi-rough (SR). Naide et al. (1965) interpret the SR strains as lacking one or more enzymes necessary for the elongation of the O-antigenic side chain of the cell-wall lipopolysaccharide. They postulate that SR types appear among the *S. montevideo* hybrids because the elongating enzyme(s) of this species cannot extend a 4-antigen-specific (group B) side chain. Since the determinant(s) of the elongation enzyme(s), unlike those responsible for O-specific side chain synthesis,

apparently are not linked to *his*, most hybrids which receive the 4 antigen determinant (and presumably lose the 6 antigen determinant) would be unable to elongate the 4-specific side chain and would be SR. Only the minority hybrid class receiving also the unlinked elongation enzyme determinant(s) of the donor could express its O-4 specificity. It would appear, then, that *S. typhosa* and *S. typhimurium*, unlike *S. montevideo*, possess enzymes capable of elongating O-antigenic side chains other than their own, since, in the present study, no hybrids of the SR type were observed.

At the present time, it is not possible to do more than speculate as to the function of the *ViA* and *ViB* determinants. Clark, McLaughlin, and Webster (1958) suggested that the Vi antigen is a polymer consisting principally of *N*-acetyl aminohexuronic acid units. However, its exact structure is not known, nor has its biosynthetic pathway been elucidated. It is possible, of course, that both genes identified in this study specify enzymes involved in Vi biosynthesis. Alternatively, one of them may have a regulatory rather than a structural function, with the situation being similar to the control of *Salmonella* flagellar phase variation (Lederberg and Iino, 1956). In any case, further studies will be necessary to establish the nature of these genes, as well as the extent to which *ViA* may be shared by other enteric bacteria. The report of Ørskov and Ørskov (1962) that K antigen determinants of the A and B types appear closely linked to *his* in *E. coli* raises the question as to whether a relationship might exist between the *E. coli* K and *Salmonella ViA* determinants. With the hope that the difficulties involved in hybridization of *S. typhosa* with *E. coli* will not prove insurmountable, we are currently endeavoring to answer this question.

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