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Genetic Transfer of *Shigella flexneri* Antigens to *Escherichia coli* K-12[1]

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The genes controlling synthesis of *Shigella flexneri* group- and type-specific antigens were transferred to *Escherichia coli* K-12 recipients by conjugation with an *S. flexneri* Hfr. After mating *E. coli* with an Hfr strain of *S. flexneri* 2a and selecting for *his*$^+$ recombinants, a high proportion of the *E. coli* hybrids agglutinated in *S. flexneri* grouping serum. None of these hybrids expressed *S. flexneri* type-specific antigen II. When an *E. coli his*$^+$ hybrid possessing the *S. flexneri* group antigen was remated with the same Hfr with selection for *pro*$^+$ hybrids, a high proportion now expressed the type-specific antigen as well as the previously inherited group antigen. If such crosses were performed in reverse order (i.e., *pro*$^+$ followed by *his*$^+$ selection), a different pattern of serological behavior was observed. None of the *pro*$^+$ hybrids showed the type-specific antigen. Subsequent mating for *his*$^+$ resulted in hybrids with both the group- and type-specific antigens. These results show that genes controlling the synthesis of *S. flexneri* group antigen (linked to the *his* locus) and type-specific antigen (linked to the *pro* locus) are widely separated on the chromosome. Expression of the type-specific antigen II depends on the presence of the group antigen.

In recent years, the fertility system of *Escherichia coli* K-12 has been extended to different genera of the family *Enterobacteriaceae*. This has been accomplished by the conjugal transfer of the *E. coli* K-12 sex factor (termed F) or its related F-merogenotes to *Salmonella* (23, 26, 35), *Shigella* (17, 31), *Serratia* (3, 24), and *Proteus* species (5). Generally, such F-infected strains behave as sexual donors of their episomal element in intraspecies, interspecies, and intergeneric matings. *Salmonella* and *Shigella* Hfr derivatives have been isolated either from F-infected strains (19, 35) or by means of selection for a terminal marker linked to F (15, 26, 31). In addition to transferring chromosomal genes in both intraspecies and interspecies crosses (15, 18, 23, 26, 31, 35), some of these Hfr strains have been employed as donors to mediate intergeneric chromosomal hybridizations. Makela (20) transferred the genes controlling the structure and phase variation of flagella antigens from a *Salmonella abony* Hfr strain into a recipient *E. coli* strain while Johnson and Baron (14) hybridized *E. coli* recipients with the Vi antigen of a *S. typhosa* Hfr. In another study, Schneider and Falkow (31) employed *E. coli* recipients in matings with a *Shigella flexneri* Hfr donor and were able to select recombinant hybrids for a number of different nutritional characters. These authors concluded that the order of genes of *S. flexneri* was similar to that of *E. coli* in the *pro* through *his* chromosomal region. Although the use of such Hfr strains in crosses with *E. coli* recipients has not been extensive, the findings indicate that *E. coli* cells can conserve and express at least some of the chromosomal genes received during conjugation.

In light of these observations, it seemed reasonable to expect that *E. coli* hybrids capable of expressing the group- and type-specific antigens of *Shigella* could be constructed. This report describes experiments involving the genetic transfer of these serological determinants from an *S. flexneri* 2a Hfr to an *E. coli* K-12 recipient strain and the genetic and serological characterization of the resulting hybrids.

MATERIALS AND METHODS

Bacterial strains. The characteristics of strains employed in this study are summarized in Table 1. *E. coli* K-12 Hfr P4X-6, the donor strain employed to prepare an Hfr derivative of *S. flexneri* 2a strain M42-43, transfers its chromosomal genes in the order *pro - met - arg - ile* -, with *lac* as the terminal marker linked to the sex factor.

Strain AB1133, a multiply auxotrophic *E. coli* K-12, was used as a recipient line in conjugation experiments

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TABLE 1. *Bacterial strains*[a]

Working designation	Species	Source	Genetic characteristics											Agglutination in antisera			Mating polarity
			pro	*thr*	*leu*	*arg*	*his*	*nic*	*asp*	*met*	*lac*	*str*	*mot*	*Shigella* Group	*Shigella* Type II	*E. coli* K-12	
P4X-6	*E. coli* K-12	F. Jacob	+	+	+	+	+	+	+	−	+	S	+	−	−	+	Hfr
M43-43	*S. flexneri* 2a	WRAIR	+	+	+	+	+	−	−	+	−	S	−	+	+	−	F−
256	*S. flexneri* 2a[b]	WRAIR	+	+	+	+	+	−	−	+	+	S	−	+	+	−	Hfr
AB 1133	*E. coli* K-12	A. L. Taylor	−	−	−	−	−	+	+	+	−	R	+	−	−	+	F−
AB 1133-H55	Hybrid[c]	WRAIR	−	+	+	+	−	+	+	+	−	R	+	−	−	+	F−
AB 1133-H1	Hybrid	WRAIR	−	−	−	+	−	+	+	+	−	R	+	−	−	+	F−
AB 1133-H3	Hybrid	WRAIR	−	+	+	−	−	+	+	+	−	R	+	−	−	+	F−
AB 1133-H14	Hybrid	WRAIR	+	+	+	−	−	+	+	+	−	R	+	−	−	+	F−
AB 1133-H30	Hybrid	WRAIR	+	−	−	−	−	+	+	+	−	R	+	−	−	−	F−
AB 1133-H23	Hybrid	WRAIR	+	+	+	+	−	+	+	+	−	R	+	−	−	+	F−
AB 1133-H24	Hybrid	WRAIR	+	+	+	+	−	+	+	+	−	R	+	−	−	+	F−
AB 1133-H96	Hybrid	WRAIR	−	+	+	+	+	+	+	+	−	R	−	+	−	−	F−
AB 1133-H6	Hybrid	WRAIR	+	−	−	−	−	+	+	+	−	R	+	−	−	+	F−
AB 1133-H8	Hybrid	WRAIR	+	+	−	−	−	+	+	+	−	R	+	−	−	+	F−

[a] Abbreviations: *pro*, proline; *thr*, threonine; *leu*, leucine; *arg*, arginine; *his*, histidine; *nic*, nicotinic acid; *mot*, motility; *asp*, aspartic acid; *met*, methionine; *lac*, lactose; *str*, streptomycin; S, sensitive; R, resistant; F−, recipient; Hfr, high frequency recombination donor; WRAIR, Walter Reed Army Institute of Research.

[b] Derivation of this Hfr is described in the present paper.

[c] Hybrids of AB 1133 × Hfr 256, prepared as described in this paper.

with the *S. flexneri* 2a Hfr donor. In many of the crosses, hybrid derivatives of AB1133 served as females because it was observed that certain classes of hybrids functioned as better recipients of *Shigella* genes than the original strain (Formal, *unpublished data*).

Media. Brain Heart Infusion (Difco), Penassay Broth (Difco), and meat extract agar (MEA) were used for routine cultivation of organisms. The composition of minimal medium, used for selection and scoring recombinants, has been described (4). When required, amino acids and other growth factors were added to the minimal medium at a final concentration of 25 μg/ml. Streptomycin, employed as a counter-selective agent against donor cells in some of the matings, was incorporated into selective media at a concentration of 500 μg/ml. Fermentation characters were scored either on MacConkey indicator medium, which consisted of MacConkey Agar Base (Difco) supplemented with an appropriate carbohydrate at a concentration of 1%, or by culturing in Phenol Red Broth (Difco) containing appropriate carbohydrate (1%).

Mating procedures. Donor and recipient strains were grown overnight at 37 C in either Penassay Broth or Brain Heart Infusion Broth without agitation. On the next day, 10-ml portions of each culture were washed three times with 0.85% saline and suspended in 1 ml of saline (about 5×10^9 cells per ml). At this time, 0.1 ml of the Hfr cell suspension and 0.1 ml of the recipient cell suspension were plated separately (for controls) and together (for mating) on appropriate selective medium. After incubation for 2 days at 37 C, isolated recombinant clones were picked and purified by streaking on the original selective medium.

Antigenic analysis of recombinants. The presence of *S. flexneri* somatic antigens was scored by slide agglutination tests performed on purified recombinants. The type-specific II antigen was detected by the use of absorbed type-specific II antiserum (Difco). An organism was considered to have only group antigens if it agglutinated in unadsorbed *S. flexneri* 2a antiserum (1:50) and failed to agglutinate in type-specific II serum. In some cases, group factor (3, 4) serum was also employed. Unadsorbed *E. coli* K-12 antiserum was employed to detect the presence of *E. coli* antigens.

Preparation of antisera. Antisera, for the purpose of absorption experiments and immunodiffusion analysis, were prepared by inoculating individual rabbits with heat-inactivated (1 hr at 100 C) suspensions of the respective parental strains and representative hybrid strains. The animals were inoculated subcutaneously with 2×10^8 bacteria on 3 successive days; one week later, a second course of immunization was given which consisted of three intravenous doses of 5×10^8 cells injected on 3 successive days. The animals were exsanguinated 7 to 10 days after the last inoculation. All sera were stored at -20 C.

Preparation of somatic antigen. O antigens were prepared from representative hybrid and parental strains by the following procedure. Cultures of each, grown for 24 hr at 37 C on MEA supplemented with 0.2% glucose, were harvested and washed twice with saline. After being dried by acetone precipitation, 8 to 10 g of cells was extracted twice with 45% phenol at 68 C for 15 min (34). The aqueous phase was removed after separation by centrifugation. This aqueous phase was then extracted with 20% (v/v) ether to remove any contaminating phenol (29). Residual ether was then removed by passing nitrogen through the aqueous suspension. The extract was dialyzed against distilled water for 24 hr and then centrifuged at $5,000 \times g$ for 15 min to remove any insoluble residue. The crude extract was sedimented at $105,000 \times g$ for 4 hr in an ultracentrifuge. The pellet was dissolved in water and lyophilized. Such partially purified O antigen represented 5 to 10% of the dry weight of cells and was employed for immunodiffusion analysis.

Immunodiffusion analysis. The double diffusion in agar technique of Ouchterlony (30) was used. Glass slides (2 by 3 inches) were coated with 1% Agarose (Fisher Scientific Co., Pittsburgh, Pa.) in 0.01 M sodium phosphate-buffered saline (*p*H 7.2) containing 0.02% sodium azide. Wells (7 mm) on 12-mm centers were cut in the desired patterns. After addition of reagents, the plates were incubated at room temperature for 48 or 72 hr. The lines of precipitation were photographed with an immunodiffusion camera. The slides were washed and dried and were stained with Amidoschwartz (Chroma-Gesellschaft; Roboz Surgical Co., Washington, D.C.). The stained slides were photographed again for comparison with unstained preparations.

RESULTS

Derivation of S. flexneri 2a Hfr 256. *S. flexneri* 2a Hfr 256, the donor strain employed in genetic crosses with *E. coli* recipients, was derived from *S. flexneri* 2a M42-43 by the method of F-linked terminal marker selection (12). Although other methods for recovering shigella donors are possible, Schneider and Falkow (31) showed this approach to be most fruitful for isolating Hfr derivatives. *E. coli* K-12 P4X-6, an Hfr which transfers as its terminal marker the lactose utilization genes (*lac* operon) linked to the F-factor, was mated with *S. flexneri* strain M42-43, and *lac*$^+$ recombinants were selected. Analysis of about 300 such recombinants revealed that a small proportion of them had inherited the sex factor along with the *lac*$^+$ character. One of these, strain 256, was found to be stable for the *lac*$^+$ character and efficiently transferred chromosomal genes in crosses with *E. coli* recipients with a polarity similar to that of P4X-6, i.e., *pro - arg - his - gal - lac* - F. As far as could be detected by genetic analysis for other unselected markers, *S. flexneri* 2a Hfr 256 appeared to have retained only the *lac*$^+$ - F region of the *E. coli* donor genome, and otherwise exhibited the characteristics of a typical *S. flexneri* 2a strain.

Transfer of S. flexneri group antigen to E. coli K-12. The *S. flexneri* 2a Hfr strain 256 was mated with *E. coli* K-12 AB1133, and selections were

TABLE 2. *Analysis of hybrids obtained from crosses between S. flexneri 2a Hfr 256 and E. coli K-12 AB 1133*

Recipient strain	Donor-selected character	No. analyzed	Marker inheritance						Agglutination		
									Shigella		*E. coli* K-12
			arg$^+$	*pro*$^+$	*thr*$^+$	*leu*$^+$	*his*$^+$	*mot*$^{+a}$	Group	Type II	
AB 1133	*his*	63	0	0	0	0	63	20	61	0	20
AB 1133	*pro*	23	5	23	3	1	0	23	0	0	23
AB 1133	*thr*	8	0	0	8	2	0	8	0	0	8
AB 1133	*leu*	7	1	1	7	7	0	7	0	0	7
AB 1133	*arg*	143	143	0	2	0	0	143	0	0	143

[a] Motility character represents a test for the recipient allele, since the *S. flexneri* donor allele is *mot*$^-$.

TABLE 3. *Analysis of recombinants obtained from crosses between S. flexneri 2a Hfr 256 and hybrid derivatives of E. coli K-12 AB 1133*

Recipient strain	Donor-selected character	No. analyzed	Marker inheritance					
			his$^+$	*pro*$^+$	*mot*$^{+a}$	Agglutination		
						Shigella		*E. coli* K-12
						Group	Type II	
AB 1133-H55	*his*	134	134	0	68	120	0	68
	pro	39	0	39	39	0	0	39
AB 1133-H1	*his*	61	61	1	23	52	1	19
	pro	33	0	33	33	0	0	33
AB 1133-H3	*his*	24	24	0	6	24	0	24
	pro	32	0	32	32	0	0	32

[a] Motility character represents a test for the recipient allele, since the *S. flexneri* donor allele is *mot*$^-$.

made for hybrids that had inherited *Shigella* genes controlling the biosynthesis of various amino acids. The genetic analysis of such recombinants is summarized in Table 2. Serological examination of various selected classes of hybrids revealed that 61 of 63 recombinants that had inherited the *his*$^+$ marker of the *S. flexneri* donor became agglutinable in unadsorbed *S. flexneri* 2a antiserum. None of these displayed any reaction when tested in absorbed type II antisera, thus indicating that the *S. flexneri* group antigens were responsible for the agglutinin reaction. Twenty of these *his*$^+$ hybrids remained agglutinable in the *E. coli* K-12 antiserum. Unselected marker analysis showed that these 20 recombinants retained their *E. coli* genes for the motility character, so it is likely that much of this serological reactivity is due to the presence of flagellar antigens, for most of those hybrids which no longer agglutinated in K-12 serum were nonmotile.

Recombinants selected for other donor markers in different regions of the chromosome (*pro*, *thr*, *leu*, *arg*) remained unaltered in their serological properties, being agglutinated solely by the K-12 antiserum.

Data from other crosses which indicate a genetic linkage of *S. flexneri* group antigen genes to the *his*$^+$ locus are presented in Table 3. In this instance, we mated the *Shigella* donor with derivatives of *E. coli* AB1133 previously hybridized with some *Shigella* genes, but which remained unaltered in their serological characteristics (*see* Table 1). These *E. coli* hybrids, constructed by mating *S. flexneri* Hfr 256 with *E. coli* AB1133 for hybridization in the *arg* - *thr* - *leu* chromosomal region, were found, when backcrossed with the shigella donor, to act as better recipients of *Shigella* chromosome than the original AB1133 recipient. As previously observed with the AB1133 *E. coli* recipient, a high proportion of *his*$^+$ recombinants from matings of the shigella donor with three different hybrid derivatives of AB1133 also inherited the genetic capability to express the group antigens of *Shigella*. Likewise, most of the hybrids which retained agglutinability in the K-12 antiserum were motile. Furthermore, in the cross

TABLE 4. *Analysis of recombinants obtained from a cross of S. flexneri 2a Hfr 256 with E. coli hybrid AB 1133-H96 his+ group antigen+*

Recipient strain	Donor-selected character	No. analyzed	Marker inheritance				
			pro+	*mot+* [a]	Agglutination		
					Shigella		*E. coli* K-12
					Group	Type II	
AB 1133-H96	*pro*	43	43	0	43	43	0

[a] Motility character represents a test for the recipient allele, since the *S. flexneri* donor allele is *mot*−.

with recipient strain AB1133-H1, one of the group antigen-positive hybrids was found to agglutinate also in type-specific II antiserum.

Transfer of S. flexneri type antigen II to E. coli K-12. Unselected marker analysis of the one *E. coli* hybrid which agglutinated in both the group and type II antisera (Table 3) revealed that this recombinant had also inherited the *pro* chromosomal region of *Shigella*. This observation suggested that a genetic locus controlling type II antigenic specificity of *S. flexneri* 2a might be located in this segment of the chromosome. To test for this possibility, we remated the *S. flexneri* Hfr donor with an *E. coli* hybrid capable of producing *Shigella* group antigens and selected for *pro+* recombinants. The results of such a cross (Table 4) indicated a very high linkage of the genetic determinants for type II antigenic specificity to the *pro* locus. All 43 *pro+* hybrids were agglutinated in type-specific II antiserum as well as in the *Shigella* grouping antisera.

These results at first seemed paradoxical because previous experiments (*see* Tables 2 and 3) showed that *E. coli* recombinants selected for the *pro* region of the chromosome remained unchanged in their serological characteristics. The primary difference, however, between these earlier experiments and the present one is that, in the present cross, the recipient bacteria were capable of producing group antigens of *S. flexneri*. In our earlier experiments, the recipient cells did not have this genetic capability. It occurred to us, however, that many such *pro+* hybrids from these earlier crosses may have inherited the genes for type II antigenic specificity but, due to the lack of a genetic ability to synthesize *Shigella* group antigens, were unable to express their type II specific determinant. To test this possibility, a series of *pro+* hybrids from previous crosses (which had been found to be unchanged in their serotype) were remated with the *Shigella* Hfr donor, and selections for *his+* recombinations were made to insure the inheritance of genes controlling *Shigella* group antigens. If our suspicion that these strains had inherited the genes for type II antigen was correct, then one would expect that the introduction of *Shigella* group antigen genes to such cells would result in hybrids capable of expressing both group- and type-specific II antigens. This indeed was found to be the case, as is illustrated by the data in Table 5. With recipients AB1133-H14, AB1133-H30, and AB 1133-H6, all of the *his+* recombinants capable of expressing *Shigella* group antigens now also agglutinated in type-specific II antisera. With recipients AB1133-H8, AB1133-H23, and AB 1133-H24, a slightly different result was observed. Although most of the group antigen-positive hybrids also expressed type II specificity, we recovered hybrids which produced only *Shigella* group antigen. Since some intergeneric hybrids are partial diploids which segregate their hybrid region (1), we suspect that those hybrids lacking type II specificity may have segregated the *pro* region of the chromosome.

Serological characterization of representative hybrids. For the purpose of further characterizing the serological properties of *E. coli* hybrids which had inherited the *Shigella* group- and type-specific II antigens, representative *his+* (group antigen+) hybrids and *his+ pro+* (group and type II+) were chosen for absorption tests and immunodiffusion analysis. Included also in these analyses was a "Y" variant strain of *S. flexneri*, which produces only group antigen and is presumably mutated in the type-specific II antigen locus.

The results of absorption tests, performed by the procedures of Edwards and Ewing (2) on antisera prepared against *S. flexneri* 2a M42-43, are presented in Table 6. The data show that an *E. coli* hybrid capable of expressing both *Shigella* group and type II antigens (*his+ pro+*) behaves as a typical *S. flexneri* 2a in its ability to absorb from antisera the antibodies directed against group- and type-specific determinants. Furthermore, a *his+* hybrid which only can synthesize *Shigella* group antigen was found to behave as

TABLE 5. *Analysis of recombinants obtained from crosses of S. flexneri 2a Hfr 256 with E. coli K-12 AB 1133 hybrids that are pro+*

Recipient strain	Donor-selected character	No. analyzed	Marker inheritance				
					Agglutination		
			his+	*mot*+[a]	*Shigella*		*E. coli* K-12
					Group	Type II	
AB 1133-H14	*his*	40	40	21	40	40	30
AB 1134-H14	*arg*	40	0	40	0	0	43
AB 1133-H30	*his*	32	32	32	32	32	16
AB 1133-H6	*his*	32	32	9	32	32	9
AB 1133-H8	*his*	32	32	7	32	25	9
AB 1133-H23	*his*	62	62	53	48	41	48
AB 1133-H24	*his*	22	22	11	22	21	14

[a] Motility character represents a test for the recipient allele, since the *S. flexneri* donor allele is *mot*−.

TABLE 6. *Agglutinin reactions of S. flexneri 2a and Y and E. coli hybrids with unabsorbed and absorbed antisera*

Strain	Group antigen	Type II antigen	*S. flexneri* 2a M42-42 antiserum				
			Unabsorbed	Absorbed with M42-43	Absorbed with *his*+-*pro*+ hybrid	Absorbed with Y strain	Adsorbed with *his*+ hybrid
S. flexneri 2a M42-43	+	+	2,560	<80	<80	640	2,560
S. flexneri Y mutant	+	−	2,560	160	160	<80	<80
S. flexneri × *E. coli his*+ hybrid	+	−	2,560	80	80	80	<80
S. flexneri × *E. coli his*+ *pro*+ hybrid	+	+	2,560	<80	<80	640	1,240

the Y mutant derivative of *S. flexneri* in these absorption tests.

The results of immunodiffusion analysis using antisera against *S. flexneri* 2a, *S. flexneri* Y variant, various *E. coli* hybrids, and partially purified somatic antigens yielded similar conclusions (Fig. 1). *S. flexneri* 2a and *E. coli his*+ *pro*+ hybrid somatic antigens formed lines of identity when either *S. flexneri* 2a or *E. coli his*+ *pro*+ antiserum was employed. *E. coli his*+ hybrid antigen precipitated with these sera but did not yield a line of identity with the major antigen of *S. flexneri* 2a M42-43. Antisera against *S. flexneri* "Y" variant and the *E. coli his*+ hybrid precipitated a line of identity when reacted with *E. coli his*+ hybrid antigen. Both these sera, however, failed to precipitate the major antigenic moiety of *S. flexneri* 2a M42-43.

DISCUSSION

By means of intergeneric crosses, in which an *S. flexneri* 2a Hfr donor strain was mated with an *E. coli* K-12 recipient line, it has been possible to construct *E. coli* hybrids capable of producing both the group- and type-specific antigens of *S. flexneri* 2a. A genetic analysis of such hybrids revealed the existence of two distinct, widely separated chromosomal loci, which control the synthesis and expression of *S. flexneri* 2a smooth somatic antigens. Closely linked to the histidine biosynthesis operon, there is a locus (presumably a cluster of genes) which determines the presence of at least some *Shigella* group antigens. This conclusion is based on our observations that a high proportion of *E. coli* recipients, hybridized with the *his* segment of the *S. flexneri* chromosome, were agglutinated by unadsorbed *S. flexneri* 2a antiserum but not by type-specific II antiserum. This conclusion is further supported by the results of absorption tests and immunodiffusion analyses of representative *his*+ recombinants. Such hybrids were found to behave serologically as typical Y mutants of *S. flexneri* in which only group antigenic determinants are present. The identification of a somatic antigen locus linked to the *his* locus in *Shigella* parallels similar observations reported for the genetic control of smooth somatic antigens of salmonella species. The genes

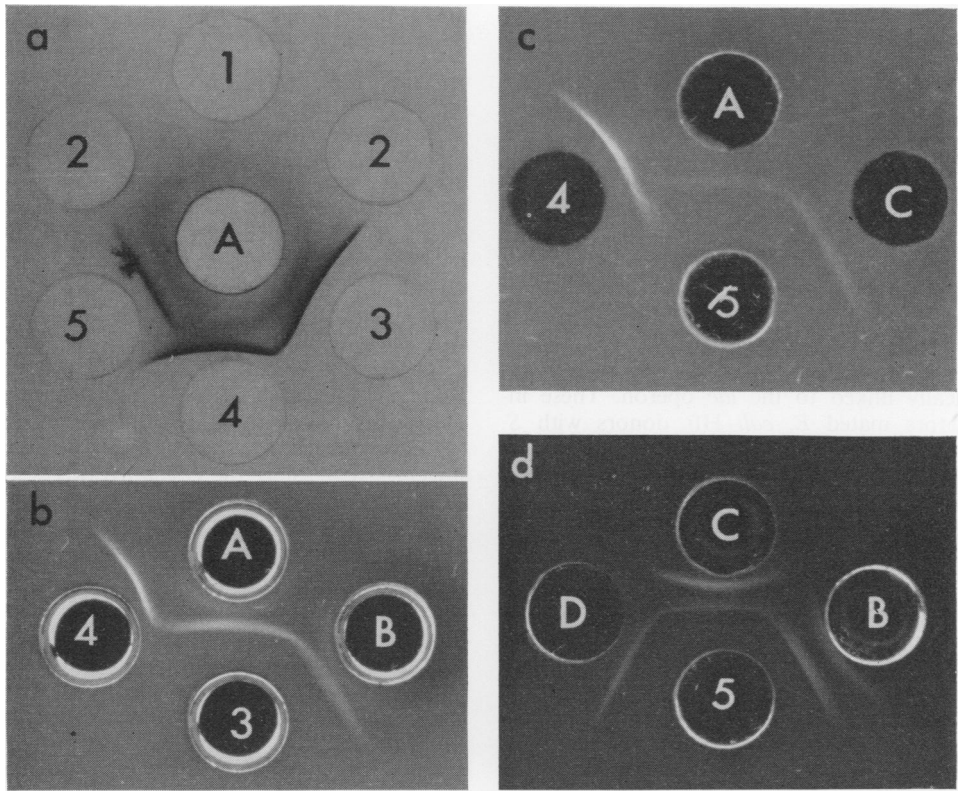

FIG. 1. *Immunodiffusion analysis of E. coli hybrid antigens demonstrating the presence of group factor or type II specific antigens, or both.* (a) *There is a reaction of identity between the S. flexneri 2a and the his+ pro+ hybrid antigens but not the his+ hybrid antigen. Antigens of E. coli recipient strains were not precipitated by the S. flexneri 2a antiserum.* (b) *The reaction of the his+ pro+ hybrid antigen and its antibody is identical to the S. flexneri 2a antigen (II-antigen) and its antiserum.* (c) *The his+ hybrid O-antigen (group factor +) is precipitated by unabsorbed S. flexneri 2a and his+ hybrid antibody, but this reaction is not identical to the reaction between the Shigella O-antigen and its antibody.* (d) *The his+ hybrid antigen is precipitated by the two E. coli hybrid antisera and an S. flexneri Y antiserum demonstrating the presence of group factor antibodies in sera prepared against both classes of E. coli hybrids. The line of identity nearest the antiserum wells has not been identified. Key: (antisera) A, S. flexneri 2a; B, E. coli his+ pro+ hybrid; C, E. coli his+ hybrid; D, S. flexneri Y; (antigens) 1, E. coli K-12; 2, E. coli K-12 arg+; 3, E. coli his+ pro+ hybrid; 4, S. flexneri 2a; 5, E. coli his+ hybrid.*

controlling the synthesis of the O repeating unit in the distal portion of the side chain in cell wall lipopolysaccharide of *Salmonella typhimurium* have been mapped near the *his* locus (33). In addition, smooth somatic antigens of salmonella such as factors 4, 6, 7, 9, and 2, which are specific reflections of the serological reactivity of the O repeating unit, also have been positioned in this segment of the salmonella chromosome (13, 16, 21, 22). Likewise, similar genetic studies in *E. coli* have placed O antigen genes near the *his* operon (28).

As in the case of salmonellae and *E. coli*, the O antigenic specificity of *Shigella flexneri* is determined by the chemical and structural properties of the O repeating units which compose the side chains of smooth lipopolysaccharide (32). *S. flexneri* Y mutants, which serologically contain only group antigenic determinants, have been shown to produce O-specific side chains consisting of a *N*-acetylglucosamine-rhamnose-rhamnose repeat unit (32). It is likely that the *his*-linked group antigen locus of *S. flexneri* controls the synthesis of such repeat units. By means of correlating antigenic factors with the molecular structure of *S. flexneri* Y mutant (group factors 3,4) and *S. flexneri* serotype 2a (group factors 3,4), Simmons (32) has tentatively concluded that the 3,4 group antigens are related to the rhamnosyl-(1 → 4)-rhamnose portion of the repeat unit. In the present study, we have made no attempt to distinguish serologically the group factors which map near the *his* locus. *S. flexneri* 2a produces group factor 1, which is common to all *S. flexneri* strains

regardless of serotype, and group factors 3,4 which are shared only by certain serotypes of *S. flexneri*. We are attempting to determine at the present time whether *E. coli his*$^+$ hybrids, prepared by intergeneric mating procedures or by transduction with phage P1 (Formal, *unpublished data*), express all of these group antigens.

In addition to the *his*-linked group-antigen locus, genetic analyses of hybrids revealed another locus, closely linked to the proline biosynthesis marker, which controls the type-specific II antigen of *S. flexneri* 2a. Previous studies by Luria and Burrous (17) indicated that the locus controlling type antigen specificity of *S. flexneri* was genetically linked to the *lac* operon. These investigators mated *E. coli* Hfr donors with *S. flexneri* recipients and found that a high proportion of *lac*$^+$ hybrids had lost their type-specific antigen and now behaved serologically as Y strains which only express group antigens. We suspect that the type-specific antigen gene(s) identified by these investigators near the *lac* operon represents the same locus we have shown to be closely linked to the *pro* locus, because the *lac* operon and the *pro* gene cluster are in close proximity on the chromosomal map. Our findings, in addition, indicate that the expression of type-specific antigen II (operationally detected by agglutination in type-specific II antiserum) is dependent on the presence of group antigen. *E. coli* hybrids capable of producing group antigens were found to express the type-specific II antigen after remating with the *Shigella* Hfr donor for the inheritance of the *pro* chromosomal region. Such *his*$^+$ *pro*$^+$ *E. coli* hybrids, analyzed by absorption tests and immunodiffusion studies, behaved serologically as *S. flexneri* 2a. In contrast, if *E. coli* recipients were directly hybridized for the *pro* region and then tested by slide agglutination tests we could not detect the presence of any *Shigella* antigens. Subsequent remating of such serologically unaltered *pro*$^+$ hybrids for the group antigen *his*-linked locus resulted in hybrids capable of producing both type II and group antigens. We thus conclude that the *E. coli pro*$^+$ hybrids had inherited the gene(s) for type II antigen, but were unable to express it without the presence of group antigens. Our genetic findings are in accordance with the structure of *S. flexneri* 2a lipopolysaccharide proposed by Simmons (32), who has shown that α-glucosyl secondary side chains, involved in type II antigen serological specificity, are attached to the primary side chains (group antigens) of the lipopolysaccharide. The type-specific locus in the *pro* - *lac* chromosomal segment may represent a gene cluster controlling the biosynthesis of specific uridine diphosphate-glucose transferases which are essential for the incorporation of secondary side chains into elongating primary side chains (32). Hence, hybrids with a type II antigen-positive, group antigen-negative genotype would not be expected to express their type-specific antigen due to a lack of the capability to synthesize group antigen primary side chains.

The development of *E. coli* hybrids with the serological properties of *S. flexneri* enables us to extend previous studies on the use of intergeneric hybrids as oral dysentery vaccines (6–8). Previous studies of this nature have shown that *S. flexneri* and *S. sonnei* hybrids which have incorporated into their genome the *rha* - *xyl* chromosomal segment of an *E. coli* Hfr were of reduced virulence (5). When employed as a live vaccine against dysentery, the hybrids rendered monkeys resistant to experimental oral challenge with virulent shigellae (6, 8). Since the *E. coli* hybrids described in this report obviate any difficulties with virulence, which is of prime concern in developing live vaccines, it is of interest to determine whether they could prove functional as a protective dysentery vaccine.

In addition to serving in this capacity, such hybrids may prove useful in studies concerned with the immunochemistry and biosynthesis of the O-specific lipopolysaccharide of *S. flexneri*. Hybrids able to synthesize *S. flexneri* group antigens provide an opportunity to isolate and investigate, as a separate entity, the primary side chains of *S. flexneri* somatic antigens with the view of correlating serological determinants with chemical composition and structure. Furthermore, although *pro*$^+$ *his*$^-$ hybrids appear phenotypically negative for type antigen II by slide agglutination tests, it is possible that they synthesize haptens related to factor II which could be isolated and studied immunochemically.

In recent years, it has been reported that the O serological specificity of *S. flexneri* is subject to phage conversion. Matsui (25) converted *S. flexneri* type-specific antigens I and II to type-specific antigen IV by means of a phage recovered from a *S. flexneri* 4c strain. Similarly, phages have been employed to change type-specific antigens of other *S. flexneri* serotypes (9–11, 27). At the present time the genetic and biochemical aspects of phage conversion in *S. flexneri* have not been extensively investigated. The use of *E. coli* hybrids in such studies may prove interesting.

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