Genetic Data Concerning *Shigella flexneri* Serotypes 5 and 6

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It is shown that the genetic determinants of type-specific antigens I to V of *Shigella flexneri* controlling similar functions are localized near the lactose-fermentation region on the chromosome; the determinant of antigen VI lies beyond it. The authors support the proposal that *Shigella flexneri* serotype 6 be excluded from *Shigella flexneri* and be considered as a subspecies in correspondence with the Soviet scheme of classification or even as a distinct species as Slopek and Mulczyk suggested. Genetic arguments based on the different behavior of *S. flexneri* strains in recombination with *Escherichia coli* K-12 Hfr are presented which support the division of *S. flexneri* serotype 5 into two subtypes, a and b, in correspondence with a similar division of other serotypes. The advisability of this division is confirmed by receptor analysis demonstrating differences in group-specific factors in strains of serotype 5. The antigenic formula of subtype 5b is V:7 . . . and of 5a, V:4

The genus *Shigella* is generally divided into four subgroups: A, *S. dysenteriae*; B, *S. flexneri*; C, *S. boydii*; and D, *S. sonnei*. Subgroup B consists of six serotypes. Serotype 6 includes type Newcastle, strain boydii 88, type Manchester, and some others.

In the Soviet scheme of classification, serotype 6 is considered to be a subspecies of *S. flexneri* (subspecies *newcastle*); in addition, serotype 5 is divided into two subtypes: 5a and 5b (5).

A number of investigators (2, 12, 18, 22) have suggested excluding serotype 6 (type Newcastle) from *S. flexneri* and considering it as a subspecies or even a species of subgroup B on the basis of multiple differences in characteristics between serotype 6 and the other *S. flexneri* serotypes.

As to serotype 5, N. A. Khomenko (5) demonstrated the independent distribution in nature of strains of this serotype which carried different group-specific factors.

Nevertheless, the classification of *Shigella flexneri* has not changed up to now. In this communication, some genetic arguments are presented concerning the advisability of changing the classification of this organism.

MATERIALS AND METHODS

The strains of *S. flexneri* used in this study were obtained from the Mechnikov Institute of Vaccines and Sera (Moscow). All of the strains were typical in

their biochemical characteristics and in their antigenic structure. As a rule, they required aspartic and nicotinic acids for growth on minimal medium. The donor strains of *Escherichia coli* K-12 Hfr H(*met*⁻) and K-12 Hfr S₁₆₆ (B₁⁻) were obtained from W. Hayes and A. T. Ganesan, respectively. Genetic crosses were performed as previously described (21). The presence of type- and group-specific antigens was determined by agglutination in single-factor sera produced by the Mechnikov Institute (Moscow).

RESULTS AND DISCUSSION

A summary of the published data concerning the salient differences between *S. flexneri* serotype 6 (type Newcastle) and the other serotypes of *S. flexneri* is presented in Table 1.

As is evident from this table, strains of serotype 6 are strikingly different from those of the other serotypes. (i) Biochemical properties are as follows: existence of stable biotypes, higher fermentative activity, gas production by some strains on carbohydrate media, and nutritional requirements (special requirement in pantothenic acid) of serotype 6. (ii) Receptor function is as follows: strains of serotype 6 are less sensitive to colicins and phages than are strains of the other *S. flexneri* serotypes. (iii) Characteristics of the type-specific (main) antigens are: antigen VI is an antigen of type K, whereas antigens I to V are parts of somatic antigens. The immunochemical structure of serotype 6 is distinct from that of the other five serotypes.

TABLE 1. Summary of differences in characteristics between serotype 6 (Newcastle) and the other serotypes of *Shigella flexneri*

Sero- type	Biochemical characteristics		Receptor functions: sensitivity to		Characteristics of type- specific antigens	
	Enzymatic activity	Synthetic ability	Phages	Colicins	Immunophoretic analysis	Immunodeterminant sugar
I-V	Glucose and mannitol fermented without gas; variations in indole production and in fermentation of rhamnose, maltose, arabinose, and sorbitol (1, 4, 6)	Some prototrophic strains; most require nicotinic and aspartic acids (7, 20)	Wide spectrum (40-69 lysotypes) of sensitivity to phages F ₁ - F ₁₂ (19)	Up to 95% of the strains are sensitive to some of the colicins (up to 91 colicin types) (11, 15)	Component of O antigen; moves to cathode (2)	α -Glucose for type antigens I, II, IV, V; O-acetyl- α -glucose for antigen III (17)
6 (Newcastle)	Seven rather stable biotypes; one mannitol-negative. Carbohydrates actively utilized: 24.1% of the strains produce gas, 100% ferment glycerol, 69% ferment dulcitol, and 21.7% xylose (18)	Auxotrophic, requiring, in addition to the substrates mentioned above, pantothenic acid, cysteine, thiamine, and other (16, 20)	Narrow spectrum; sensitive mainly to phage F ₁₀ (11)	Narrow spectrum; sensitive mainly to colicins E ₂ + I (11); up to 34.1% of the strains are colicin-resistant.	Antigen of K type; moves to anode (2)	Rhamnose residues (17)

Studies of the genetic control of type-specific antigens in recombination between *S. flexneri* of different subtypes and *E. coli* Hfr C showed the loss of some type-specific antigens by most of the hybrids acquiring the ability to utilize lactose (8, 14, 21).

The results of the investigations carried out at our laboratory indicated some regularities in the behavior of type-specific antigens in recombination (21). An analysis of these data showed that, on the *Shigella flexneri* chromosome in the region adjacent to the lactose fermentation (*lac*) locus, there is a locus, which we call Tp (type), responsible for the synthesis of type-specific antigens I, II, IV, and V, but not of III and VI; we observed a high linkage rate (95 to 97%) between the loss of the above-mentioned antigens (I, II, IV, and V) and the acquirement of the *lac* marker, whereas, not a single *lac* hybrid of serotype 3 or 6, produced from mating with *E. coli* Hfr C, had lost antigen III or VI, respectively.

It is important to emphasize that the results reported above paralleled the immunochemical characteristics of the type antigens. The immunodominant sugar of antigens I, II, IV, and V is the same, α -glucose, III-*O*-acetyl- α -glucose, and of antigen VI is rhamnosyl residues (17) (Table 1). Immunochemical studies of hybrids devoid of the antigens showed an absence of α -glucose in their structure in comparison with the parent strains (9).

In our experiments, we observed a complicated behavior of type-specific antigens II and V in combination with group-specific factor 7,8: in strains of serotype 2b, a simultaneous loss of both factors (II and 7,8) occurred, whereas *lac*⁺ hybrids of serotype 5b (V:7,8) kept their antigens (95% *lac*⁺ hybrids of serotype 5a lost V antigen). Hence, the strains of serotype 5(V:3,4) behaved differently, depending on the group-specific factor carried (21).

To prove the same localization of the determinant of antigen V in strains of 5a(V:3,4) and 5b(V:7,8), we carried out a cross between strain 5b and *E. coli* K-12 Hfr H, a strain more effective as a donor than *E. coli* K-12 Hfr C.

The results of an analysis of the antigenic structures of *lac*⁺ hybrids are presented in Table 2.

It is evident from table 2 that most (96) of the *lac*⁺ hybrids of strain 5b in this cross, like the *lac*⁺ hybrids of subtype 5a, had lost type-specific antigen V. By analogy with serotype 2b, many (73) hybrids are devoid of two antigenic factors (V and 7,8) simultaneously.

However, with strain 5b, more complicated changes in antigenic structure were observed after recombination: the loss only of the V antigen (a transition into "x" variant [20 strains] or into strains with formula -:3,4:7,8 [3 strains]) and 1% cultures of "rough" hybrids were observed in previous crosses with *E. coli* K-12 Hfr C as well (21).

In our opinion, the previous observations mentioned above (21) and the data presented in this paper demonstrate differences in genetic behavior of five strains (serotypes 5a and 5b) in spite of the same localization of the determinants of antigen V in both cases. Apparently serotype 5 should be divided, on a genetic basis into two subtypes (5a and 5b).

For more detailed antigenic characteristics of these subtypes, an analysis of their group-specific antigens was carried out by the method of agglutinin adsorption. In the first experiment, different portions of sera against strains of subtypes 2a and 2b were adsorbed with strains of subtypes 5a and 5b and with control strains of the "y" and "x" variants, respectively. These sera contained complexes of antibodies against the factors 3,4 and 7,8.

The results showed that the control strains "x" and "y" completely adsorbed antibodies against antigens 7,8 and 3,4 respectively, whereas strains 5b and 5a did not adsorb

TABLE 2. Antigenic structure of *lac*⁺ hybrids obtained from mating *Shigella flexneri* 5b 186 (V:7,8) with *Escherichia coli* K-12 Hfr H^a

N hybrid class	No. of hybrids	Antigenic structure	Comments
1	73	-(3,4)	67 Hybrids reacted with serum 3,4; 6 hybrids lost an agglutinability by sera V and 7,8
2	20	-:7,8	
3	3	-:3,4:7,8	
4	3	V:7,8	
5	1	?	Polyagglutinability, tryptaflavine (+) reaction

^a Mroz and Lachowicz (10) described a transition after recombination of a strain of subtype 5b(V:7,8,9) into the "x" variant (-:7,8,9) in 50 of 50 *lac* hybrids studied. The insufficient number of the hybrids obtained and the use of serum with additional factor 9 make a comparison with our results difficult. However, there is a possibility of some deviation in individual behavior of the different strains of subtype 5b in recombination.

TABLE 3. Analysis of group-specific antigens of *Shigella flexneri* 5b and 5a by the method of agglutinin adsorption

Sera against live cultures of <i>S. flexneri</i>	<i>S. flexneri</i> strains and their antigenic formulae (abbreviated) ^a					
	2b-1403 II:7,8	5b-545 V:7	x-1340 -:7,8	2a-1487 II:3,4	5a-249 V:3?4?	y-433 -:3,4
2b Nonadsorbed	25,600	12,800	25,600	128,000	—	—
2b Adsorbed with 5b	12,800	0	6,400	—	—	—
2b Adsorbed with x-var	12,800	0	0	—	—	—
2b Adsorbed with 2b	0	0	0	—	—	—
2a Nonadsorbed	51,200	—	—	102,400	12,800	51,200
2a Adsorbed with 5a	—	—	—	25,600	0	1,600
2a Adsorbed with y-var	—	—	—	12,800	0	0
2a Adsorbed with 2a	—	—	—	0	0	0

^a Symbols: Titers in reciprocal index are shown; 0, absence of agglutination in dilution 1:100 and higher; —, not tested.

TABLE 4. Receptor analysis of group-specific antigens of *Shigella flexneri* 5a in double-adsorbed serum

Serum against live culture of <i>S. flexneri</i> 2a	<i>S. flexneri</i> strains and their antigenic formulae (abbreviated) ^a					
	2a-1487 II:3,4	y-433 -:3,4	1a-1521 I:4	1b-1478 I:4,6	5a-249 V:3?4?	2b-1403 II:7,8
Nonadsorbed	102,400	51,200	51,200	12,800	12,800	51,200
Adsorbed with 2b (complex 3,4)	25,600	6,400	6,400	6,400	1,60	—
Additionally adsorbed with 1a (factor 3)	3,200	800	0	0	0	0

^a Symbols: As in Table 3.

antibodies (Table 3). This indicates that the set of antigenic factors in these strains is incomplete. As to subtype 5b, the results coincided with its known antigenic formula (V:7 . . .).

However, the group-specific antigens of subtype 5a have not yet been studied thoroughly. We therefore performed a double successive adsorption of serum 2a followed by an analysis of the remaining antibodies. At first, the serum was adsorbed with strain 2b (portion I) and then additionally with 1a (portion II). Both portions were studied in tube agglutination reactions with cultures possessing antigens 3,4 (2a and variant "y") or antigenic factor 4 only (1a, 1b) and with strain 5a.

From the results presented in Table 4, it is clear that, in the first portion of the adsorbed serum, all strains were agglutinated in high titers, including strain 5a (1:1600), as this serum contained complex antibodies 3,4. In the second portion (lack of antibodies against factor 4), only the strains possessing antigen 3(2a, y-var) were agglutinated. Strains 1a, 1b, and 5a did not react with it.

This experiment demonstrated that *S. flexneri* 5a lacks antigen 3. Hence, its formula should be designated as V:[4]. . . . Antigen 4 is placed in brackets to show the quantitative antigenic variations in subtype 5a observed in these bacteria.

The division of serotype 5 into two subtypes and the correction of its antigenic formula should be useful in epidemiological analysis of dysentery outbreaks.

Further, we decided to ascertain the sites of the genetic determinants of type-specific antigens III and VI. As mentioned above, neither of the *lac*⁺ hybrids of these serotypes in crosses with *E. coli* K-12 Hfr C lost the corresponding antigen (21).

Antigen III was shown (17) to be an acetylated form of antigen 7,8. This finding, together with our data, indicates that the 14 strains of serotype 3 studied by us are immune to the converting phage 7,8 responsible for the synthesis of this antigen (3, 13), suggesting therefore that phage 7,8 takes part in the synthesis of antigen III as well.

TABLE 5. Characteristics of *lac*⁺ hybrids of *Shigella flexneri* 1575 3a(III:6,7,8) obtained from crosses with *Escherichia coli* K-12 Hfr strains

Donor	Expt	No. of <i>lac</i> ⁺ hybrids with type III antigen	
		Lost	Retained
<i>E. coli</i> K-12 Hfr H	1	28	60
	2	31	49
<i>E. coli</i> K-12 Hfr S 160	1	65	38

TABLE 6. Characteristics of *lac*⁺ hybrids of *Shigella flexneri* serotype 6 (VI:3,4) obtained from crosses with *Escherichia coli* K-12 Hfr strains

Strain (biotype)	Donor <i>E. coli</i> K-12	No. of <i>lac</i> ⁺ hybrids with type VI antigen	
		Lost	Retained
1.1468 (Newcastle)	Hfr C	0	150
2.762 (Manchester)	Hfr C	0	52
3.673 (Boyd-88)	Hfr C	0	22
4.573 (Boyd-88)	Hfr C	0	50
5.281/55 (Boyd-88)	Hfr C	0	100
6.281/55 (Boyd-88)	Hfr H	0	100
7.281/55 (Boyd-88)	Hfr S 160	0	100

Prophage 7,8 was shown to be attached to the site near the Tp locus, i.e., near the *lac* region (13, 14). To determine whether substitution of this region influences the production of antigen III, we crossed strain *S. flexneri* 1575 3a (III:6,7,8) with more active donor strains of *E. coli* K-12 Hfr H and Hfr S₁₆₀. It is evident that, in these crosses, some of the *lac*⁺ hybrids lost the type III antigen (Table 5). Most of the hybrids lost an immunity to the converting phage 7,8 simultaneously. (More detailed data will be presented on this in a subsequent paper.)

In experiments with strains of serotype 6 of different biotypes and with different Hfr strains none of the *lac*⁺ hybrids lost agglutinability with type VI serum (see Table 6).

Hence, in addition to multiple differences between the characteristics of *S. flexneri* serotype 6 and those of the other serotypes (mentioned in Table 1), new genetic data are presented which further differentiate serotype 6 from the others. In spite of the existence of peculiarities in the other *S. flexneri* serotypes, their determinants for type-specific antigens I, II, III, IV, and V are localized near the same region on the chromosome (21; Tables 2 and

5), whereas the determinant of antigen VI is situated beyond this region (Table 6).

These data strongly support the conclusion of many investigators concerning the necessity for excluding serotype 6 from *S. flexneri* and for considering it as subspecies (in correspondence with the Soviet scheme of classification) or, as Slopek and Mulczyk (18) suggested, as a distinct species.

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