

GENETIC RECOMBINATIONS IN INTESTINAL BACTERIA.  
REPORT III. A STUDY OF THE GENETIC STRUCTURE  
OF DYSENTERY BACTERIA HYBRIDS (SEROLOGICAL PROPERTIES)

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In the previous reports, we presented evidence of the formation of 183 recombinates, obtained by crossing *E. coli* strains HfrH, HfrR, HfrC, and HfrHλ+, with 35 cultures of *Sh. flexneri*, and provided data on their biochemical properties [1,2].

In this work, we present the results of a serological investigation, performed on the isolated, lactose positive, recombinates of the dysentery bacilli.

#### EXPERIMENTAL METHOD

The serological properties of the recombinates were determined by the widely accepted method, using the reaction of agglutination on glass with Flexner's adsorption serum and monoreceptor sera (a, b, c, d, e, f).

In addition, we prepared (by the immunization of rabbits) agglutinating sera for 5 cultures of *Sh. Flexneri* (3584 titer 1:6400, 2050 titer 1:3200, 1363 titer 1:3200, 2047 titer 1:1600, and 1570 titer 1:800), for 4 strains of *E. coli* (HfrH titer 1:3200, HfrHλ+ titer 1:1600, HfrR titer 1:400, and HfrC titer 1:400), and for 4 strains of the recombinates (2050-r-4 titer 1:1600, 2047-b-5 titer 1:6400, 5008-r-5 titer 1:1600, and 2055-b-1 titer 1:1600). With the latter sera, we set up the agglutination reaction using their serial dilution.

#### EXPERIMENTAL RESULTS

All the recombinates retained the property of being agglutinated by Flexner's antidysentery adsorption serum. After establishing the ability of the recombinates to be agglutinated by Flexner's polyvalent adsorption serum, we also tested their agglutination properties, with monoreceptor sera. It was established that the recombinates 5008-r-1–5008-r-12 stopped being agglutinated by the (original) antiserum type f, and began to enter into reaction with the antitype sera "e" and "c." The recombinates 2047-r-1–2047-r-5, in addition to the original antiserum type "c," began to agglutinate with type "a." The recombinates 2050-r-1–2050-r-28 lost the property of being agglutinated by the original antiserum "b" and began to enter into reaction with the type serums "c" and "a."

One recombinant, 2050-r-3, represented an exception, retaining its ability to be agglutinated by the original antiserum.

An analogous result was noted in the hybrids 2043-r-1, and 2048-r-1–2048-r-8.

Recombinates of Flexner's dysentery bacillus 3584, 628, 2055, and 2047 retained the property of being agglutinated by the original antiserum "c."

Thus, the results of the agglutination reaction with the monoreceptor sera showed, in addition to retention of the ability to be agglutinated by the antisera of the original cultures, the possibility of transferring from one type of dysentery bacilli to another.

No.	Recombinate	Antisera											
		dysentery					against E. coli						
		3584 T 1 : 6400	2050 T 1 : 3200	1363 T 1 : 3200	2047 T 1 : 1600	1570 T 1 : 800	High T 1 : 3200	High T 1 : 1600	H1R T 1 : 400	H1C T 1 : 400	recombinate		
1	5008-r-1—5008-r-12, 2047-r-1—2047-r-5, 2050-r-1—2050-r-28, 5030-r-1—5030-r-10, 845-r-1, 2, 3, 3584-r-1—3584-r-3, 4, 621-r-1, 628-r-2, 2048-r-1, 2, 3, 4, 7, 2046-r-1—2046-r-14, 2044-r-1—2044-r-5, 75/2-r-1, 2, 828-k-1, 970-k-1, 2050-k-3, 4, 13, 16, 18, 23, 2046-m-1, 3584-m-1—3584-m-20, 2050-m-1—2050-m-14, 2055-m-1—2055-m-4, 2055-b-1, 2, 3, 2047-b-1—2047-b-6.	From 1/32 to 1/2 T	From 1/16 to full titer	From 1/32 to full titer	From 1/16 to 1/2 T	From 1/8 to 1/2 T	—	—	—	—			
2	2047-r-1—2047-r-5, 2050-r-1—2050-r-28, 5030-r-1—5030-r-10, 845-r-1, 2, 3, 3584-r-1—3584-r-34, 621-r-1, 628-r-2, 2048-r-1—2048-r-4, 7, 2046-r-1—2046-r-14, 2044-r-2, 3, 4, 5, 75/2-r-1, 2, 828-k-1, 970-k-1, 2049-m-1, 2047-m-6, 8, 3584-m-1—3584-m-20, 2050-m-1, 2050-m-14, 2055-m-1, 2, 3, 4, 2055-b-1, 2, 3, 2047-b-1—2047-b-6										From 1/32 to full titer	From 1/8 to full titer	From 1/32 to full titer
3	5008-r-1—5008-r-12, 5030-r-1—5030-r-10, 845-r-1, 2, 3, 3584-r-1—3584-r-34, 2055-b-1, 2, 3												From 1/32 to full titer

No.	recombinate	Antisera									
		dysentery					against <i>E. coli</i>				
		3584 T 1 : 6400	2050 T 1 : 3200	1363 T 1 : 3200	2047 T 1 : 1600	1570 T 1 : 800	HfrH T 1 : 3200	HfrH $\lambda$ +	HfrR T 1 : 400	HfrC T 1 : 400	recombinate
4	Original cultures of Sh. Flexneri: 3584	T		.			—	—	—	—	1/8 T
5	2050		T				—	—	—	—	1/8 T
6	1363			T			—	—	—	—	1/4 T
7	2047				T		—	—	—	—	1/8 T
8	1570					T	—	—	—	—	1/4 T
9	<i>E. coli</i> HfrH	—	—	—	—	—	T				—
10	HfrH $\lambda$ +	—	—	—	—	—		T			—
11	HfrH	—	—	—	—	—			T		—
12	HfrC	—	—	—	—	—				T	—

Note. 1. 5008-r-1-5008-r-12 represent the first through the twelfth recombinates. 2. 845-r-1, 2, 3 represent the first, second, and third recombinates. 3. T-titer.

In order to establish more graphically the serological relationship between the recombinates and the original cultures, we subsequently set up the reaction of agglutination with prepared sera.

The table shows that the recombinates 5008-r-1-5008-r-12, 2047-r-1-2047-r-5, 2050-r-1-2050-r-28, 5030-r-1-5030-r-10, 845-r-1, 2, 3, 3584-r-1-3584-r-34, 621-r-1, 628-r-2, 2048-r-1, 2, 3, 4, 7, 2046-r-1-2046-r-14, 2044-r-1-2044-r-5, 75/2-r-1, 2, 828-k-1, 970-k-1, 2050-k-3, 4, 13, 16, 18, 23, 2046-m-1, 3584-m-1-3584-m-20, 2050-m-1-2050-m-14, 2055-m-1, 2, 3, 4, 2055-b-1, 2, 3, and 2047-b-1-2047-b-6 are agglutinated by the anti-dysentery sera 3584, 2050, 1363, 2047, 1570, from 1:32 to full titer. Thus, despite the fact that these cultures differ significantly from the original cultures in their biochemical properties, in high titers they are agglutinated by the antidysentery sera.

At the same time, we tested the serological relationship between the recombinates themselves, using the reaction of agglutination with the corresponding (homologous) antisera. The obtained results permit dividing the recombinates into two groups.

The first group includes the cultures 2047-r-1-2047-r-5, 2050-r-1-2050-r-28, 5030-r-1-5030-r-10, 845-r-1, 2, 3, 3584-r-1-3584-r-34, 621-r-1, 628-r-2, 2048-r-1, 4, 7, 2046-r-1-2046-r-14, 2044-r-2, 3, 4, 5, 75/2-r-1, 1, 2, 828-k-1, 970-k-1, 2049-m-1, 2047-m-6, 3584-m-1-3584-m-20, 2050-m-1-2050-m-14, 2055-m-1, 2, 3, 4, 2055-b-1, 2, 3, 2047-b-1-2047-b-6. The enumerated cultures enter into the reaction of agglutination with antiserum against the recombinant 2050-r-4 from 1:8 to full titer, with antiserum against the recombinant 2047-b-5 from 1:32 to full titer, and with antiserum against the recombinant 2055-b-1, also from 1:32 to full titer.

The recombinates studied, as well as the original strains of the dysentery pathogen, are serologically interrelated, indicating that they belong to one serological group.

We further tested the serological connection between the original dysentery, intestinal bacilli and their recombinates. In this case, we noted that the strains of Flexner's dysentery bacillus 3584, 2050, 2047, 1363, and 1570 enter into the reaction of agglutination with the antisera of recombinates 2050-r-4, 2047-b-5, 5008-r-5, and 2055-b-1, in titer dilutions from 1:8 to 1:2.

It should be emphasized that not a single strain of *Sh. Flexneri* entered into reaction with the antiserum of *E. coli*, and vice versa.

We noted a serological connection in the reverse direction, i.e., between *Sh. Flexneri* and their recombinates.

Thus, although the hybrids of the dysentery bacteria differ markedly in their biochemical properties from the dysentery bacteria, they nonetheless retain the antigenic characteristics of the original strains. Our data coincide with the results of Luria and Burrous [3].

#### SUMMARY

An inquiry was made into the serological properties of lactose positive hybrids of the Flexner bacilli, obtained by crossing *Sh. Flexneri* with *E. coli*. Although these recombinates differed considerably from the maternal *Sh. Flexneri* cells in their chemical properties, they still retained the serological character of the original dysentery strains.

#### LITERATURE CITED

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