EXPERIMENTAL KERATOCONJUNCTIVITIS CAUSED BY S. flexneri AND BY ITS GENETIC RECOMBINATIONS

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It has been reported previously that 183 genetic recombinations between E. coli strains HfrH, HfrHd+, HfrC and HfrR and 35 strains of S. flexneri were isolated; data on their biochemical, serological and other properties were also given [2, 3, 4, 8]. In this paper the results of study of their pathogenic properties are presented.

It is known that since the time of isolation of the etiological agent of dysentery [1] there have been many attempts to produce this infection experimentally in laboratory animals (rabbits, guinea pigs, white mice, cats, kittens, dogs, pigeons, etc.). In most cases these attempts were unsuccessful. With the exception of monkeys, no known laboratory animals are susceptible to the infection.

Experiments conducted on cats did not give definite results because the animals succumbed spontaneously to a non-dysenteric enteritis [5]. Some authors [11] considered that it was impossible to produce clinical dysentery in kittens.

Sedan and German in 1924 [13] introduced typhoid organisms onto the conjunctiva of guinea pigs; they produced damage of the cornea and sometimes a generalized infection. During recent years there appeared several papers on the production of a specific keratoconjunctivities in guinea pigs. Thus Sereny [12] used this method with the dysentery organisms of Flexner, Kruze-Sonne and Stutzer-Schmitz. The author in all cases noted the development of a typical keratoconjunctivitis. Gekker [6] and Siroko [9, 10] not only confirmed the results of Sereny but extended their investigations to the Newcastle and Boyd-Novgorodskaya bacteria. Some workers [1] by the use of this method were able to transform atypical strains of dysentery organisms into typical ones. Using formation obtained by the above authors we have attempted to elucidate the interrelationship between original E. coli and S. flexneri and their genetic recombinations. The above-mentioned workers have noted that the pathological process is more easily produced by freshly isolated S. flexneri which is kept for not longer than 6 months under laboratory conditions.

We have attempted to conduct similar experiments with freshly isolated dysenteric bacilli and with their genetic recombinations. By means of five crossings of 15 one-cell cultures Nos. 605, 30, 2155, 751, 713, 597, 737, 734, 768, 777, 832, 181, 87, 91, and 759 of S. flexneri with 4 cultures of E. coli strains HfrH, HfrHd, HfrC and HfrR we have isolated 84 recombinations of S. flexneri.

It is to be noted that experiments with single cell cultures gave results similar to those obtained in our previous experiments.

EXPERIMENTAL METHODS

In this investigation we have used healthy guinea pigs weighing 250-300 grams, obtained from a single source. Infection was made with an 18-h agar culture which was washed off with 0.5 ml of normal saline per culture tube.

Results of Positive Conjunctival Infections of Guinea Pigs with S. flexneri and E. coli and with Their Genetic Recombinations

No. of culture		Results of infection	No. of culture		Results of infection
Genetic recombination	2047	+ (b.s.)	Genetic recombination	2047-Б-3	+ (b.s.)
»	2050	+ (b.s.)	»	2047-Б-6	+ (b.s.)
»	845	+ (b.s.)	»	759-p-1	+
»	2055	+ (b.s.)	»	759-p-2	+
»	75/2	+ (b.s.)	»	759-p-3	+
»	1	+ (b.s.)	»	759-p-4	+
»	2773	+ (b.s.)	»	759-p-5	+
»	605	+	»	759-p-6	+
»	2155	+	»	751-p-1	+
»	751	+	»	751-p-2	+
»	713	+	»	751-p-3	+
»	597	+	»	605-Б-1	+
»	737	+	»	605-Б-2	+
»	734	+	»	605-Б-4	+
»	768	+	»	759-Б-2	+
»	877	+	»	759-Б-3	+
»	832	+	»	759-Б-5	+
»	87	+	»	759-Б-8	+
»	91	+	»	832-Б-1	+
»	759	+	»	832-Б-4	+
Genetic recombination	2047-p-3	+ (b.s.)	»	832-Б-5	+
»	2047-p-4	+ (b.s.)	»	832-Б-6	+
»	845-p-1	+ (b.s.)	»	832-Б-9	+
»	845-p-2	+ (b.s.)	»	832-Б-10	+
»	845-p-3	+ (b.s.)	»	832-Б-11	+
»	75/2-p-1	+ (b.s.)	»	832-Б-12	+
»	75/2-p-21	+ (b.s.)	»	751-Б-6	+
»	2055-Б-3	+ (b.s.)	»	751-Б-8	+
»	2055-Б-3	+ (b.s.)	»	621-Б-1	+ (b.s.)
»	2047-Б-2	+ (b.s.)			

Legend: + positive reaction; + (b.s.) positive reaction after bile sensitization.

Two drops of this suspension were introduced onto the mucous membrane of the conjunctiva of guinea pigs and the process was repeated after 3 h.

After an incubation period of 24-48 h the infected guinea pigs showed oedema and lacrymation. Following this there developed a conjunctivitis with a copious mucous and pus exudate. Towards the end of the third and the beginning of the fourth week the guinea pigs recovered. Only the infected eyes became affected, so that no spreading of the infection to the other eye took place.

It was noted that this complex of symptoms was produced only by freshly isolated S. flexneri. There are cases described in the literature, when positive results were obtained with strains kept under laboratory conditions, but infection took place only following sensitization of the conjunctiva with whole ox bile [5, 13, 14].

Sensitization was done in the following manner. Every 8 h for 24 h, two drops of sterile ox bile were introduced onto the conjunctiva of guinea pigs. On the following day two drops of a dense suspension of an 18 h culture of dysentery bacilli and of their genetic recombinations were introduced into the same eye, and the process was repeated after 3 h.

EXPERIMENTAL RESULTS

A total of 322 strains (50 of S. flexneri, 5 of E. coli and 267 of genetic recombinations) were used in the tests for pathogenicity. The accompanying table shows that the symptoms were produced by 12 out of 13 freshly isolated strains of S. flexneri (Nos. 605, 713, 597, 737, 734, 768, 777, 832, 887, 91, 759 and 751). Of 35 strains of dysenteric bacilli kept under laboratory conditions for long periods of time, only 7 (Nos. 2047, 2050, 2055, 845, 72-2, 1 and 2773) produced a reaction, but only after sensitization of conjunctiva with bile. However, strain No. 2155 of S. flexneri which was kept under laboratory conditions for more than 7 months produced symptoms without the bile sensitization.

Among the cultures of genetic recombinations the symptoms were produced by 26 out of 84 cultures obtained after crossing freshly isolated strains of S. flexneri with E. coli (Nos. 759-r-1, 759-r-2, 759-r-3, 759-r-4, 759-r-5, 759-r-6, 751-r-1, 751-r-2, 751-r-3, 605-B-1, 605-B-2, 605-B-4, 759-B-2, 759-B-3, 759-B-5, 759-B-8, 882-B-1, 832-B-4, 832-B-5, 832-B-6, 832-B-9, 832-B-10, 832-B-11, 832-B-12, 751-B-6, 751-B-8).

Of 183 recombinations which were kept under laboratory conditions the reaction which became positive only after sensitization with bile was produced only by strains Nos. 13/2047-r-3, 2047-r-4, 845-r-1, 845-r-2, 845-r-3, 621-r-1, 75/2-r-1, 72/2-r-2, 2055-B-1, 2055-B-3, 2047-B-2, 2047-B-3, and 2047-B-6. Strains of E. coli did not produce any symptoms either before or after sensitization with bile.

Thus, although hybrids of dysentery bacilli differ from the original cultures in their biochemical properties, some of them, especially newly formed ones, are able to retain the pathogenic properties of the original S. flexneri.

SUMMARY

The presence of pathogenic properties was studied in 50 strains of S. flexneri 5 E. coli cultures and 267 sexual recombination by infection of the guinea pig conjunctiva. As noted, 20 strains of S. flexneri and 39 hybrids induced specific keratoconjunctivitis. Although S. flexneri hybrids differed considerably by their biological properties from the initial cultures, they retained their pathogenic properties. E. coli strains possessed no such properties.

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