

EXPERIMENTAL GENETIC RECOMBINATION IN VIVO BETWEEN ESCHERICHIA COLI AND SALMONELLA TYPHIMURIUM

BY H. SCHNEIDER, SAMUEL B. FORMAL, PH.D., AND L. S. BARON, PH.D.

(From the Department of Biology, Catholic University of America, and Division
of Communicable Disease and Immunology, Walter Reed Army Institute
of Research, Washington, D. C.)

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In recent times, a great number of forms showing metabolic and antigenic relationships intermediate between the genera *Escherichia* and *Shigella*, or *Escherichia* and *Salmonella* have been reported and classified in the family Enterobacteriaceae.

Experiments by Luria and Burrous (1) have demonstrated that genetic recombination between fertile strains of *Escherichia coli* and many strains of *Shigella* can occur. Recent work by Baron *et al.* (2) has shown that fertile strains of *E. coli* will recombine also with strains of *Salmonella*. The hybrids produced as a result of crosses between *E. coli* and *Salmonella* retain the antigenic characteristics of the parent *Salmonella* (3), while crosses with *Shigella* may result in antigenic degradation of the *Shigella* (1). In either case, however, hybrids can gain the ability to ferment lactose and acquire other genetically linked characteristics of the male *E. coli* parent as well.

Luria and Burrous (1) have suggested that genetic recombination, easily demonstrated *in vitro* in the laboratory, may be one of the means by which these intermediate forms in the Enterobacteriaceae such as the Arizona and Alkalescens-Dispar groups evolved in nature. The strictly circumscribed environment of the intestine provides a continuous opportunity for intimate contact of cells of various species with each other. When two interfertile strains are present in sufficient numbers in their natural environment, it would appear likely that recombination could take place.

In this report, evidence is presented which shows that genetic recombination can occur in the lumen of the intestine and that hybrids can be detected and enumerated with the use of selective techniques. These results support the premise that genetic recombination may have played a role in the evolution of the family Enterobacteriaceae. The data indicate also that hybrids recovered from *in vivo* matings are similar to those reported from *in vitro* crosses (3, 4).

Materials and Methods

Cultures.—A streptomycin-resistant (*S^r*) mutant of the *E. coli* K-12 Hfr culture, strain W1895 (obtained from Dr. J. Lederberg) was isolated by plating a dense cell suspension on nutrient agar containing 600 micrograms/ml of streptomycin sulfate (Pfizer) and was used

as the male parent. The S^r culture of *Salmonella typhimurium* strain TM-9 which behaves as a high frequency recipient (3) was used as the female (F^-) parent.

Media.—Minimal lactose (ML) agar and Difco EMB agar prepared as described previously (3), each containing 600 micrograms/ml of streptomycin sulfate were used in the selection of hybrid clones. Nutrient agar, and the Difco products, penassay broth, phenol red broth, and tryptone broth were used for the routine cultivation and testing of strains. Carbohydrate solutions were sterilized by filtration.

Antisera.—Antiserum to *E. coli* K-12 and to *S. typhimurium* TM-9 were prepared in rabbits by a series of injections of increasing numbers of living organisms. Agglutination tests for the presence of somatic antigens were done by the usual slide procedure; the presence of pili was confirmed by the hemagglutination of chicken red blood cells (5) and by the use of antiserum prepared with purified pili.

Animals.—Bagg strain mice with an initial weight of 18 to 20 gm were employed.

RESULTS

In vitro recombination experiments with the streptomycin-sensitive strain, *E. coli* W1895, as the male parent and the streptomycin-resistant strain of *S. typhimurium* TM-9 as the female, showed that these two strains recombined at the frequency of 1×10^{-4} on ML agar plates (3). The recombination frequency was the same with the S^r mutant of *E. coli* W1895 and selection on minimal lactose streptomycin (MLS) agar. The relatively high frequency of recombinants obtained in the *in vitro* experiments suggested that this mating system could be suitable for the demonstration of *in vivo* genetic recombination.

A modification of the procedure described by Freter (6) sufficed to render mice susceptible to a chronic non-fatal enteric infection with either of the two parent strains.

Mice were deprived of food and water for 24 hours and then fed by gavage 0.5 ml of distilled water containing 100 mg of streptomycin, 10 mg of erythromycin, and 500 units of mycostatin. The mice were also given drinking water containing 4 mg/ml of streptomycin, 0.1 mg/ml of erythromycin, and 4 units/ml of mycostatin *ad libitum*. Twenty-four hours after antibiotic treatment, the mice were infected orally with 0.5 ml of the appropriate cultures.

In a preliminary experiment, a group of antibiotic-treated mice was fed 0.5 ml of a saline suspension containing approximately 10^{10} cells of *S. typhimurium* and a second group was fed 0.5 ml of an 18 hour penassay broth culture of *E. coli* containing 2×10^9 viable cells. One day later, feces, collected separately from each mouse in both groups, were weighed, emulsified in saline, and diluted 10-fold for plating on EMB streptomycin agar and MLS agar.

The EMB cultures revealed that the mice were now passing pure cultures of the respective parent strains, while the minimal agar plates showed no growth.

Each group of mice was then fed a suspension of the reciprocal parent culture. Twenty-four hours later fecal dilutions were made again in the manner described. Cultures obtained from the group fed first *S. typhimurium* and then the *E. coli* suspension did not show any evidence of *in vivo* recombination on 3 successive days. In each case, pure cultures of the Lac^- *S. typhimurium* were obtained, indicating that the *E. coli* either were not present or were too few in

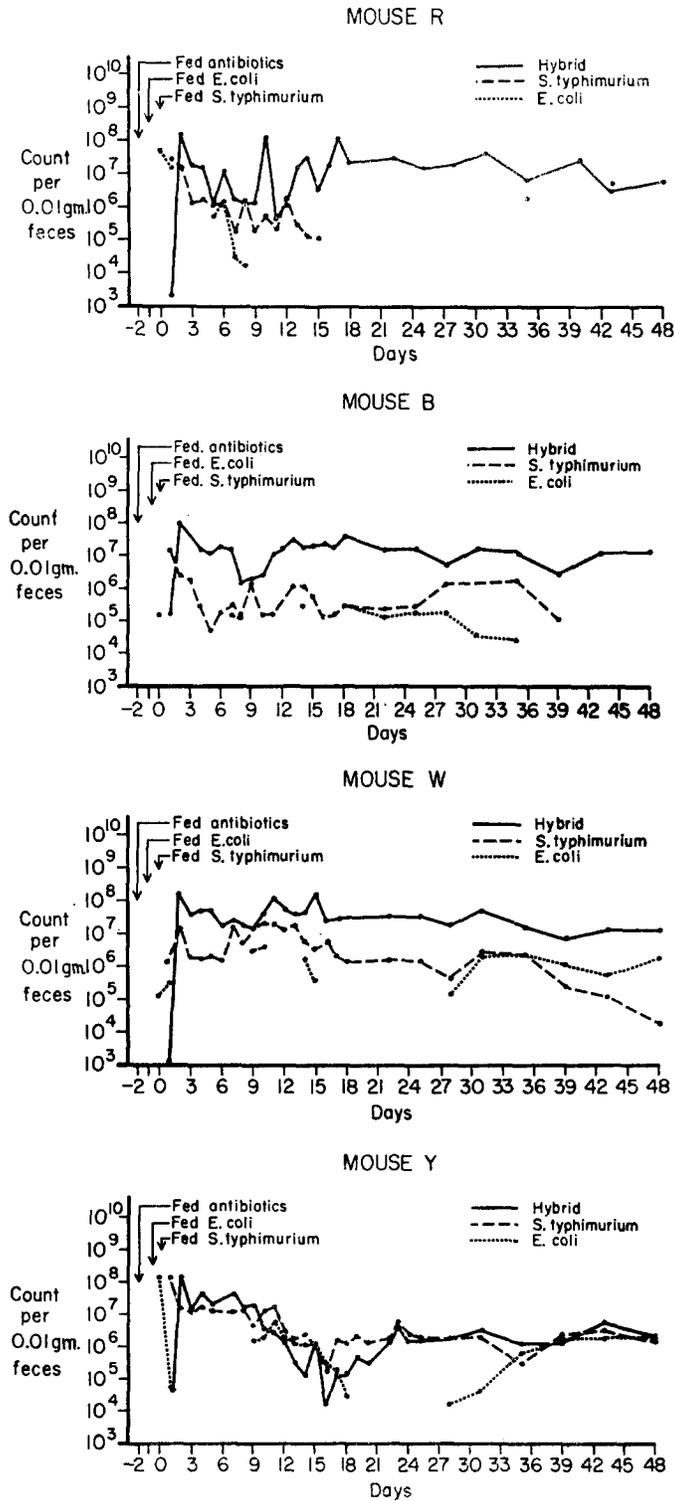
numbers to be detected. These mice were fed a second dose of *E. coli* 4 days after the first dose was administered. Twenty-four hours later small numbers of hybrids were isolated on MLS agar from the feces of these mice.

A completely different pattern was noticed in the group of mice fed first *E. coli* and then, *S. typhimurium* 1 day later. The *E. coli* were found to be well established in the intestine as evidenced by the fact that 10^5 to 10^7 cells/0.01 gm of feces were recovered prior to the feeding of the *Salmonella* cells. Twenty-four hours after the *Salmonella* were fed, 10^3 to 10^4 hybrids were recovered. The parent *S. typhimurium* was present in large numbers while the numbers of *E. coli* were depressed. Subsequent cultures from mice in this group every 3 to 4 days indicated that the hybrids were continuing to multiply and had become a part of the intestinal flora. While the rapid establishment of the parent *S. typhimurium* was accompanied by an immediate and marked depression in the numbers of *E. coli* recovered, the number of *E. coli* did increase afterwards.

A detailed study was then undertaken to determine the fate of parent strains and hybrids in the mouse intestine over a period of time. The same infection procedure was followed as described previously. The *E. coli* were fed first, then the *S. typhimurium*; a control group was fed the same number of heat-killed cells of *S. typhimurium*. The following day fecal collections and dilution counts were begun and continued daily for 18 days, and then every 3 to 4 days for an additional 30 days. Lac⁺ *S. typhimurium* isolated on EMB plates were differentiated from *E. coli* by slide agglutination tests. In the control group, the numbers of *E. coli* recovered from each mouse ranged from 10^6 to 10^7 cells/0.01 gm of feces and remained at this level throughout the course of the experiment.

The results obtained from the daily cultures of mice in the experimental group are presented in Fig. 1. 10^3 to 10^5 hybrids were recovered per 0.01 gm of feces from each of the experimental mice within 24 hours after being fed the living *S. typhimurium*. The number of hybrids increased to 10^7 – 10^8 cells/0.01 gm of feces within 48 hours, and remained at approximately 10^7 cells/0.01 gm of feces throughout the course of the experiment. The *E. coli* count showed a marked depression within 48 hours after the *S. typhimurium* feeding and this strain was not present in numbers sufficient enough to be detected by the methods used for at least 5 days. In all but one of the mice, the parent *S. typhimurium* strain was detected on almost every occasion in which the mice were checked; in mouse R, the parent *S. typhimurium* cells were not observed after the 15th experimental day. The numerical relationship between the two parents and between the parents and hybrids varied considerably from mouse to mouse, and with the exception of the continuing presence of hybrids in large numbers, no distinct pattern emerged in this study.

On the 43rd day of the experiment, a number of Lac⁺ hybrid colonies were isolated and checked serologically and biochemically to determine if genetic changes other than the gain in ability to ferment lactose had occurred. The



TEXT-FIG. 1. Population changes of parent Hfr *E. coli* and F⁻ *S. typhimurium* and hybrids after *in vivo* genetic recombination within the intestinal tract of experimentally infected mice.

hybrids were also tested for the presence of the non-flagellar appendages, pili, by their ability to agglutinate chicken red blood cells as described by Brinton and Baron (5). The characteristics of the two parent strains and results of this analysis are presented in Table I. The greatest number of different hybrid classes were found in mouse R, and the fewest in mouse Y.

In addition, classes of hybrids were observed which previously were not detected in matings of the two parent strains performed *in vitro* on ML agar (3). The majority of these hybrid classes were recovered from mice where the numbers of *S. typhimurium* were not detectable or were at least 1 log lower than the total hybrid population. This suggests the possibility that further mating took place between the more available first class of hybrids and *E. coli*, with the

TABLE I
Frequency of Hybrid Classes Recovered from Mice First Fed Hfr E. coli Followed by Feeding F⁻ S. typhimurium

Characteristics of parents: * *E. coli*: Lac⁺ Inos⁻ Ind⁺ Pili⁺; *S. typhimurium*: Lac⁻ Inos⁺ Ind⁻ Pili⁻

				Mouse designation				
Hybrid class				R	W	B	Y	Total
Lac ⁺	Inos ⁺	Ind ⁻	Pili ⁻	36	43	48	47	172
Lac ⁺	Inos ⁻	Ind ⁻	Pili ⁻	7	3	1	1	12
Lac ⁺	Inos ⁻	Ind ⁺	Pili ⁻	1	1	1	0	3
Lac ⁺	Inos ⁻	Ind ⁺	Pili ⁺	6	3	0	0	9

* Key: Lac = Lactose; Inos = Inositol; Ind = Indol.

production of the other classes of hybrids. Although these classes of hybrids were not observed as a consequence of matings between the same two parent strains *in vitro*, it is possible that initial matings with extensive exchange of genetic material could have taken place *in vivo*.

Unfortunately the lumen of the gut is not a satisfactory place to determine the frequency of recombination of these various classes of hybrids. As was shown earlier, the hybrids appeared shortly after the *S. typhimurium* was introduced and increased rapidly indicating hybrid multiplication and making the determination of frequencies difficult.

DISCUSSION

The extensive studies of *in vitro* genetic recombination between *E. coli* and *Shigella* by Luria and Burrous (1) and between *E. coli* and *Salmonella* by Baron *et al.* (2-4) have demonstrated conclusively the interfertility existing between these organisms. Although the occurrence of organisms bearing characteristics

of two established species may be the result of similar genetic mechanisms, the role of this phenomenon in nature has not been established.

The results reported here have demonstrated that under appropriate conditions, recombination between two interfertile strains of bacteria can occur within the intestine. In the preliminary experiment, it was found that *S. typhimurium* could easily be superimposed upon an enteric *E. coli* infection in antibiotic-treated mice and this system was chosen for the final experiments. On the other hand, *E. coli* superimposed upon a pre-existing *S. typhimurium* infection, proved to be more difficult, since it required additional feedings of the *E. coli* cells. The mouse is a natural host for *S. typhimurium*, and is often afflicted with chronic, non-fatal infections. For this reason *S. typhimurium* may more easily become established in the presence of large numbers of other organisms such as *E. coli*.

The recombinants which were isolated showed linkage results equivalent to those reported previously, but hybrids were recovered also which had acquired a more extensive transfer of genetic material than usually observed as a result of *in vitro* crosses. The hybrids multiplied rapidly and were recovered in numbers larger than either of the two parent strains, possibly owing to some selective advantage.

The system of a high frequency donor strain of *E. coli* and a very competent recipient strain of *S. typhimurium* was chosen as that which would most probably yield positive results and lend itself to the detection of hybrids. The presence of large numbers of these interfertile strains within the intestine would provide numerous opportunities for contact and recombination between donor and recipient cells. It is difficult, however, to assess the importance of a necessity for the presence of large numbers of cells of interfertile strains for genetic transfer to occur in nature. The episomic mechanism described by Jacob *et al.* (7) which is both contagious and promiscuous in the diversity of strains affected would not require large populations of classically competent mating cells. The very high frequency (VHf) donor strains described by Taylor and Adelberg (8), if present in nature, would also reduce the necessity for large populations.

Recent results by Baron *et al.* (9, 10) have shown that a lactose-positive strain of *S. typhosa* isolated from a case of typhoid fever was able to transfer the ability to utilize lactose at a very high frequency to many species of *Escherichia*, *Salmonella*, *Shigella*, and *Serratia marcescens*. These strains were then also able to transfer the lactose marker via an episome to other organisms. Mitsuhashi *et al.* (11, 12) have described what appears to be an episomic mechanism for the transfer of antibiotic resistance in a strain of *Shigella* isolated from a patient with dysentery. It may be that such mechanisms of limited transfer of genetic material, occurring at high frequency and indifferent to the established mating system, may be of more importance and significance in nature than the classical sexual recombination. It is possible also that other genetic mechanisms of which we are as yet unaware may play a role as well.

In any case, it is felt that the experiments reported here add further to the theory that recombination between organisms plays a role in the natural evolution of the enteric group of microorganisms. Matings occurring at low frequency and therefore difficult to detect in laboratory experiments or by diagnostic isolation procedures could have taken place in nature in view of the multitude of opportunities over long periods of time.

SUMMARY

Antibiotic-pretreated mice were fed orally an Hfr culture of streptomycin-resistant *E. coli* and 1 day later, a streptomycin-resistant F⁻ *S. typhimurium* culture. Hybrids were recovered in relatively small numbers from the feces of these mice within 24 hours demonstrating that genetic recombination can occur within the intestinal tract of a mammalian host under experimental conditions. These hybrids multiplied rapidly and persisted throughout the course of the experiment. In addition, hybrids were recovered which had not been observed in single matings performed *in vitro*.

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