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*GENETIC RECOMBINATION BETWEEN ESCHERICHIA COLI AND SALMONELLA TYPHIMURIUM**

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Introduction.—The phenomenon of genetic recombination by sexual mating in bacteria, described by Tatum and Lederberg[1] has been investigated intensively by Lederberg *et al.*[2] and many other workers. These classical studies were carried out almost entirely with the K-12 strain of *Escherichia coli*, although comparable results were obtained with a small number of other strains of *E. coli*.[3,4]

In further studies, Cavalli[5] found that the frequency of recombination could be greatly increased by using a highly fertile mutant of the K-12 strain. Additional high frequency of recombination (Hfr) strains of K-12 have been isolated in a number of laboratories.[6,7] The concept of compatibility among certain K-12 derivatives has led to a detailed description of the fertility factor (F), so that it is now understood that mating consists of a unilateral contribution of genetic material from the Hfr or the F^+ strain to the recipient Hfr, F^- or F^+ organism.[8–11]

In an attempt to demonstrate recombination between diverse species of bacteria, Zinder and Lederberg[12] discovered another mode of genetic transfer involving bacteriophage particles as vectors of genetic material. Intensive investigation of this phenomenon, referred to as genetic transduction, was undertaken by Stocker *et al.*,[13] Lederberg and Edwards,[14] and Zinder,[15] using the phage PLT-22. Studies of additional bacterial viruses competent in transduction have been reported by Baron *et al.*,[16] Spilman *et al.*,[17] Sakai and Iseki,[18] Lennox,[19] and Morse *et al.*[20] Many of these results have dealt with the phage-mediated transfer of genetic material between different species of bacteria and even organisms classified in different genera have been altered by a suitable phage.[19]

Genetic recombination between bacteria of different genera, however, has only recently been reported by Luria and Burrous[21] with the demonstration of mating between *E. coli* K-12 and various species of Shigella. The results of these workers have indicated the presence of the same fertility system in Shigella species as is known to exist in the *E. coli* mating strains. Attempts at recombination of *E. coli* with Salmonella species by a number of workers, however, have been uniformly negative.

It is the purpose of this communication to describe experiments which involve the genetic recombination of *E. coli* and *Salmonella typhimurium* at high frequency. Certain of the results which have been obtained more recently suggest a generalized ability of other Salmonella species to mate with Hfr strain of *E. coli* at a much lower frequency. Preliminary data concerning this ability in other Salmonella species have been reported by Baron *et al.*[22] and these results will be detailed in a future communication.

Materials and Methods.—Cultures: A number of previously described derivatives of *E. coli* strain K-12 were employed; W1895 (Hfr), W-6 (F^+), and W1485 were obtained from Dr. P. D. Skaar.

S. typhimurium strain TM-9 was from the culture collection of the Walter Reed Army Institute of Research, as were other Salmonella species employed in some experiments. These strains were originally obtained from Dr. P. R. Edwards.

Isolation of streptomycin resistant mutants (S^r) was accomplished by plating dense suspensions of streptomycin sensitive cells (S^s) on meat extract agar containing 600 micrograms per ml of streptomycin sulfate (Abbott or Pfizer).

Phages: The T series of coliphages was obtained from Dr. G. Bertani and were propagated on *E. coli* B and other host strains; phage PLT-22 was received from Dr. J. Lederberg and propagated on a number of suitable host strains of Salmonella. Phage sensitivity was checked by cross-streaking the phage preparations with the bacterial suspension to be tested on meat extract agar.

Media: Meat extract agar was made to contain: Meat extract 3 g, peptone 10 g, NaCl 5 g, agar 20 g, and distilled water 1,000 ml.

Minimal medium consisted of a separately autoclaved Difco Noble agar base made up double strength (14 g/400 ml water), to which was added 300 ml of physiological saline, 40 ml of a solution of KH_2PO_4 (60 g/l) and K_2HPO_4 (140 g/l), 40 ml of a solution of $(NH_4)_2SO_4$ (20 g/l) and $MgSO_4\ 7H_2O$ (2 g/l), and 10 ml of a 20 per cent solution of the appropriate carbohydrate. The carbohydrates employed (lactose, *l*-arabinose, *l*-rhamnose, etc.) were Pfanstiehl products which were sterilized by filtration.

Eosin-Methylene Blue agar was made following the usual formula (Difco), but excluding lactose; in addition, the Baltimore Biological Laboratories kindly supplied a preparation of EMB agar which did not contain lactose. Where required, 20 ml of a 20 per cent solution of the appropriate sugar was added to each liter of this medium. Minimal EMB agar was prepared by the addition of the necessary amounts of the dyes to the minimal medium described above. EMB streptomycin agar contained 600 micrograms of streptomycin sulfate per ml.

The Difco products, Penassay broth, Nutrient agar, Bacto-agar, and Phenol red broth were employed for routine cultivation, transfer, and testing of the strains.

Antisera: Sera to *E. coli*, Salmonella species, and recombinants were prepared in rabbits by a series of injections of approximately 10^9 living cells of these organisms, or by injections of suspensions of organisms which were heat-killed at 100 C for two hours. Shigella hybrid F22 (from Shigella x coli) and antiserum to this strain[21] were very kindly supplied by Dr. S. E. Luria.

Results.—A number of species of Salmonella were tested for their ability to mate with the Hfr or the F^+ strain of *E. coli* K-12 in preliminary experiments. The species of Salmonella selected consisted of representative serotypes typical of

the various groups of the Kaufmann-White diagnostic scheme. No attempt was made at this time to undertake a complete screening of the approximately four hundred or more characterized serotypes of the genus Salmonella, or of the huge number of isolates of any one serotype which were available.

The cultures of *E. coli* and Salmonella were grown together in Penassay broth for approximately 24 hours at 37 C without aeration, centrifuged and washed three times with physiological saline, and then plated on minimal agar plates containing lactose (Lac) as the sole carbon source. The *E. coli* Hfr and F^+ strains require methionine (M^-) for growth, while an important criterion for classification of organisms in the genus Salmonella is their inability to ferment or utilize lactose. The minimal lactose (ML) agar medium was employed since it would fail to support the growth of either parental mating strain, but could detect M^+ Lac^+ progeny. For providing a control, the organisms also were grown separately; all the strains tested were stable lactose negatives (Lac^-) and reversions to methionine independence by the *E. coli* strains were observed only rarely in control platings.

In the initial experiment, a smooth culture of *S. typhimurium* strain TM-9, with the antigenic structure IV V XII:i,1,2 mated with the Hfr strain of *E. coli* at low frequency; recombination of this strain could *not* be detected with the *E. coli* F^+ strain. The *E. coli* Hfr would represent the best available strain for demonstration of lactose positive (Lac^+) recombinants since it has been characterized by its high frequency ability for the lactose factor.[5] Failure to observe recombination using the F^+ strain is to be expected when the frequency of mating with the Hfr strain is low. The colonies which appeared on the ML plates in the mating with the *E. coli* Hfr were examined and found to be Lac^+ forms exhibiting the antigenic characteristics of the *S. typhimurium* parent strain.

In further experiments, the plating procedure of mixing washed suspensions of the mating strains directly on ML agar or other selective media as described by Luria and Burrous[21] was used. In agreement with their observations, frequencies of recombination were only slightly higher when parent strains were incubated together for a short time prior to plating.

The frequency of recombination of *S. typhimurium* strain TM-9 with the *E. coli* Hfr strain (expressed as the ratio of recombinants to the number of Hfr parent cells) was approximately 4×10^{-8} using the procedure of mixing the strains directly on ML agar. About 2×10^9 cells of the recipient parent strain were employed usually. Frequencies of this low order have been observed with a number of other species of Salmonella by Baron *et al.*[22]

For purposes of convenience in selection and purification of recombinants, streptomycin-resistant variants of strain TM-9 were obtained as described earlier. Individual streptomycin-resistant clones (S^r) were tested for their ability to cross with the Hfr strain of *E. coli* on minimal lactose and on EMB agar, both containing 600 micrograms of streptomycin per ml. Five S^r clones checked in this experiment were capable of crossing with the Hfr strain at a greatly increased frequency, while one S^r colony obtained from the same plate of streptomycin agar continued to recombine at the low frequency characteristic of the original strain. For further studies, an S^r isolate, referred to as *S. typhimurium* strain TM-9S^r-2, was selected which showed a recombination frequency of 1×10^{-4} when plated on selective media with or without streptomycin. It was also now possible to detect recom-

binants of this TM-9S^r-2 strain by mating it with the F$^+$ strain of *E. coli*. Table 1

TABLE 1

FREQUENCY OF RECOMBINATION OF *Salmonella Typhimurium* STRAINS (SELECTION FOR LAC$^+$ HYBRIDS)

Recipient	*E. coli* Hfr	*E. coli* F$^+$
S. typhimurium 9	4×10^{-8}	0
S. typhimurium 9S^r-2	1×10^{-4}	2.8×10^{-6}
S. typhimurium LT-7	1×10^{-8}	0
S. typhimurium HB	1×10^{-8}	0

depicts the frequencies of recombination of several strains of *S. typhimurium* mated with Hfr and F$^+$ *E. coli*.

A number of subsequent attempts were made to determine whether streptomycin resistance was correlated in some measure with the ability of certain S^r clones to recombine at this higher frequency. A higher rate of recombination could be considered as the frequency typical of a true F$^-$ strain, or could be due to an increase in mutation frequency to the F$^-$ state in certain strains. Although other hypotheses are possible, the assumption was made that strains of *S. typhimurium* are F$^\circ$ (unable to act as recipients), but can mutate at low frequency to the F$^-$ state and that it was these occasional F$^-$ cells in the population which recombined with the Hfr *E. coli*. This assumption bears a similarity to the concept proposed by Jacob and Wollman[6] that only those F$^+$ *E. coli* cells which mutate to or in some manner acquire the Hfr state are capable of mating as donors. The failure to detect recombinants from an *E. coli* F$^+$ mating of strain TM-9 can easily be explained on the basis that it is extremely unlikely that an appropriate cell of *S. typhimurium* (one becoming F$^-$ from the F$^\circ$ state) would encounter an *E. coli* F$^+$ cell which has acquired Hfr ability.

The fortuitous association of F$^-$ strains (or at least strains with an enhanced ability to acquire the F$^-$ condition) with the S^r property in some clones of *S. typhimurium* was indicated when additional S^r clones were examined in further experiments. Approximately fifty individual S^r mutants were studied and these were essentially similar in behavior to the parent *S. typhimurium* strain TM-9 with regard to recombination ability. In addition, S^r mutants of other strains of *S. typhimurium* and of various other Salmonella have been tested without the isolation of other high frequency recipient strains, although slight increases in the ability of certain species to recombine have been noticed.

This approach was investigated in the hope that it might lead to a rational method for the acquisition of the more useful F$^-$ mutants which evidently can occur in many species of Salmonella. Since the Lac$^+$ recombinants (strain TM-9 $\times$ coli Hfr) were uniformly streptomycin sensitive, that does not support any causal association or correlation between resistance to streptomycin and fertility. The limited studies undertaken with the S^r mutants, however, do not rule out the possible existence of linkage involving the streptomycin locus and a determinant controlling recipient ability. This possibility can best be established with a more comprehensive examination by chromosomal mapping methods of suitable material.

Genetic examination of recombinants: A strain of *S. typhimurium* (TM-9S^r-2) was now available with a high enough frequency of recombination to allow for the convenient testing of other genetic changes, namely, the appearance and frequency

of various unselected markers. In order to obtain further factors for linkage analysis, the TM-9S^r-2 strain was treated with ultraviolet light and plated on EMB agar containing various sugars. In this manner, an *l*-arabinose negative (Ara$^-$) and an *l*-rhamnose negative (Rha$^-$) derivative of this culture was isolated, factors which could be studied as both selected and unselected markers. The characteristics of the parent strains employed in these preliminary attempts to establish linkage relationships are listed in Table 2, as are the selected and unselected markers available for testing in the hybrids. Table 3 provides data determined from the frequency of the unselected markers which were transferred from the *E. coli* parent to the Salmonella hybrids (selection for lactose, arabinose, or rhamnose utilization).

TABLE 2

CHARACTERISTICS OF PARENT STRAINS AND SELECTED AND UNSELECTED MARKERS

Salmonella parent	*E. coli* Hfr parent Lac$^+$ Inos$^-$ Rha$^+$ Ara$^+$ Ind$^+$ S^s M$^-$ Dul$^-$ λ^+	
S. typhimurium TM-9S^r-2	Selected	Unselected
Lac$^-$ Inos$^+$ Rha$^+$ Ara$^+$ Dul$^+$ Ind$^-$ S^r λ^r	Lac$^+$	Dul$^-$, Inos$^-$, Ind$^+$, λ^+, S^s
Lac$^-$ Inos$^+$ Rha$^+$ Ara$^-$ Ind$^-$ S^r λ^r	Ara$^+$	Lac$^+$, Inos$^-$, Ind$^+$, λ^+, S$_s$
Lac$^-$ Inos$^+$ Rha$^-$ Ara$^+$ Ind$^-$ S^r λ^r	Rha$^+$	Lac$^+$, Inos$^-$, Ind$^+$, λ^+, S$_s$

Key: Lac = lactose, Ara = arabinose, Dul = dulcitol, Inos = inositol, Ind = indol, M = methionine, Rha = rhamnose, S = streptomycin, and λ = phage lambda.

TABLE 3

CLASSES AND FREQUENCY OF HYBRID COMBINATIONS OF *S. typhimurium*

Hybrid Classes—Selection for Lac$^+$	No. of Hybrids	Frequency, %
Rac$^+$ Inos$^+$ Dul$^+$ Ind$^-$ λ^r S^r	136	90.6
Lac$^+$ Inos$^+$ Dul$^-$ Ind$^-$ λ^r S^r	8	5.3
Lac$^+$ Inos$^-$ Dul$^+$ Ind$^-$ λ^r S^r	6	4.0
Hybrid Classes—Selection for Ara$^+$		
Ara$^+$ Inos$^+$ Lac$^+$ Ind$^-$ λ^r S^r	91	60.3
Ara$^+$ Inos$^+$ Lac$^-$ Ind$^-$ λ^r S^r	12	7.9
Ara$^+$ Inos$^-$ Lac$^+$ Ind$^-$ λ^r S^r	12	7.9
Ara$^+$ Inos$^-$ Lac$^-$ Ind$^-$ λ^r S^r	36	23.8
Hybrid Classes—Selection for Rha$^+$		
Rha$^+$ Lac$^+$ Inos$^+$ Ind$^+$ λ^r S^r	2	1.0
Rha$^+$ Lac$^+$ Inos$^+$ Ind$^-$ λ^r S^r	4	2.1
Rha$^+$ Lac$^+$ Inos$^-$ Ind$^-$ λ^r S^r	8	4.2
Rha$^+$ Lac$^+$ Inos$^-$ Ind$^+$ λ^r S^r	0	0
Rha$^+$ Lac$^-$ Inos$^-$ Ind$^-$ λ^r S^r	86	44.8
Rha$^+$ Lac$^-$ Inos$^-$ Ind$^+$ λ^r S^r	8	4.2
Rha$^+$ Lac$^-$ Inos$^+$ Ind$^-$ λ^r S^r	78	40.6
Rha$^+$ Lac$^-$ Inos$^+$ Ind$^+$ λ^r S^r	6	3.1

In these experiments, the recombinants which appeared on the minimal agar plates were purified by streaking on EMB streptomycin agar plates and single colonies were selected for further study. The results listed each represent the analysis of between one to two hundred colonies selected and purified in this manner. Carbohydrate fermentation reactions were determined by the inoculation of cell suspensions of the hybrids into tubes of phenol red broth containing 1 per cent of the appropriate carbohydrate.

The results of selective platings demonstrated that the frequency of recombination was highest when selecting for Lac$^+$ recombinants, lower for Ara$^+$ recombinants, and lowest for Rha$^+$ recombinants (Lac$^+$ > Ara$^+$ > Rha$^+$). In addition, from the data in Table 3, it can be concluded that the locus for *l*-arabinose utilization must be linked to the lactose locus, since in the mating with *l*-arabinose as

the selective marker, a large percentage of the recombinants which appeared were also Lac$^+$. It was possible also to demonstrate the presence of recombinants capable of producing indol, but only following the selection of Rha$^+$ recombinants. The introduction of the loss of a fermentative ability, the utilization of inositol was also observed as a consequence of matings with the inositol negative *E. coli* Hfr strain.

The linkage relationships of the biochemical characteristics exhibited by the *S. typhimurium* hybrids as determined in these preliminary experiments seem to be the following: Lac-Ara-Rha-Inos-Ind. Also indicated is the finding that 1 per cent of the hybrids selected for rhamnose utilization were both lactose and indol positive, although unchanged for the utilization of inositol. In contrast, no instance of a lactose and indol positive hybrid has been encountered when lactose was employed as the selective marker.

A number of characters present in the *E. coli* parent strain could not be detected in the recombinants when examined as either selected or unselected markers. For example, a *d*-arabinose positive mutant of the *E. coli* Hfr strain was mated with strain TM-9S^r-2. Selection on minimal *d*-arabinose agar or EMB *d*-arabinose agar failed to yield recombinants, while recombinants selected at the Lac locus remained *d*-arabinose negative. A cystine requiring mutant of TM-9S^r-2 obtained by the penicillin method described by Lederberg[23] likewise did not yield recombinants on minimal glucose agar.

Response of hybrids to bacteriophages: The T series of coliphages was tested for lytic ability on *S. typhimurium* strain TM-9S^r-2 with results that indicated host adaptation. When 10^9 particles of T_2 or T_6 (as titered on *E. coli* B) were plated on TM-9S^r-2, not more than 2 to 3 plaques were observed. The phage from these plaques could be propagated on TM-9S^r-2 which were barely visible. The most useful result from the standpoint of genetic study was obtained with a phage originally characterized as a Salmonella phage when isolated at the Army Medical Service Graduate School in 1936. This phage, which will be referred to as phage R, was examined and found to be capable of lysing rough and smooth strains of many species of Shigella, Salmonella, as well as a number of strains of *E. coli*. R phage plated on *E. coli* B, K-12, and *S. typhimurium* produced confluent lysis on these strains. Resistant colonies were observed, although in the case of *E. coli* K-12, these were predominantly mucoid. Further study of resistance to these viruses as genetic markers is planned.

The behavior exhibited by the hybrids toward phage PLT-22 isolated by Zinder and Lederberg[12] was also studied. The parent strain of *S. typhimurium* was sensitive to this phage and showed similar titers to those obtained with *S. typhimurium* LT-2 as indicator strain. It was found that all hybrids obtained following selection for lactose were equally sensitive to PLT-22. When recombinants were selected for *l*-arabinose utilization, approximately 25 per cent of the hybrids obtained were sensitive, an equal number were now resistant to plaque formation by this phage, while the remainder of the hybrids were sensitive, but with low efficiency of plating. Though all of the hybrids tested were identical in antigenic makeup and similar in colonial appearance it is still possible that the differences in sensitivity to PLT-22 may have been due to undetected antigenic changes which may be independent of the mating process. Examination of the parent culture by means

of a dissecting microscope with oblique lighting has revealed some differences in colonial morphology. The significance of these results has not yet been determined.

Although PLT-22 is unable to transmit genetic characteristics directly from *E. coli* to Salmonella, since its host range is limited to Salmonella species possessing the XII antigen and it cannot be grown on *E. coli;* it, nevertheless, could be useful in the transduction of genetic material from *E. coli* × Salmonella hybrids to other susceptible strains of Salmonella. For this reason, lysates of PLT-22 were prepared using a sensitive lactose positive hybrid of *S. typhimurium* TM-9S^r-2. Initial attempts to introduce the lactose marker into other species of Salmonella using preparations containing 10^{10} particles/ml of PLT-22 were unsuccessful, although in these same experiments, this preparation was competent, for example, in the transduction of arabinose utilization to strains of *S. typhosa.* Further attempts with higher-titered phage preparations of PLT-22 (5×10^{11} particles/ml) have resulted in the isolation of Lac$^+$ *S. typhosa* strains at a low frequency; Vi phage lysates of a Lac$^+$ *S. typhosa* strain (also Ara$^+$, Xyl$^+$, Rha$^+$) were prepared in the manner previously described by Baron *et al.*,[16] and used to transduce these factors into a wild type strain of *S. typhosa* (Lac$^-$ Ara$^-$ Xyl$^-$ Rha$^-$). Again transduction to lactose utilization was a rare occurrence, while arabinose positives appeared at high frequency; the frequency of transduction to xylose or rhamnose utilization occurred at a lower rate than that observed for arabinose or nutritional markers using either Vi phage or PLT-22, a result which had been reported earlier.[24, 25] These systems may prove useful in genetic analysis of linkage relationships of *S. typhosa* for confirmatory purposes, since it has been possible to mate *S. typhosa* with *E. coli* at a high frequency.[26]

Transfer of lysogenicity for phage lambda[27] to any of a large number of recombinants of *S. typhimurium* from the lysogenic *E. coli* donor strains has not been detected in these experiments. It should be noted, however, that the strains of Salmonella which were studied were not sensitive to lambda phage nor were they susceptible to lysogenization by this phage under usual conditions.

Serology of the hybrids: At present, all of the hybrids isolated at the various selective loci have not been subjected to extensive antigenic analyses for possible changes in somatic or flagellar antigens. Minor cross reactions have been noticed with some hybrid strains of *S. typhimurium* when tested with anti-K-12 serum; all the Lac$^+$ hybrids of *S. typhimurium* which were examined, however, retained their *known* somatic and flagellar components. These hybrids were studied as completely as possible, since Luria and Burrous[21] have reported that a majority of Lac$^+$ hybrids obtained from crosses of *E. coli* and *Shigella flexneri* strain 2aI show a markedly altered serological behavior. In addition, the Lac$^+$ Salmonella hybrids were tested with antisera to Shigella hybrid F22, with this strain as a control, to determine whether such a change had occurred in the *S. typhimurium* hybrids. None of the Lac$^+$ *S. typhimurium* hybrids tested showed any cross-reactivity with anti-F22 serum.

Polarity of the recombinants: A number of attempts have been made to determine the sexual polarity of the *S. typhimurium* hybrids. Lactose selected recombinants obtained from a mating with an F$^+$ *E. coli* strain did not mate with either F$^-$ *E. coli*, or with other Salmonella.

Discussion.—The experiments which are reported here have shown that at least

some species of Salmonella are able to mate with *E. coli*. In addition, the results may provide a further tool for the study of the genetic behavior of *S. typhimurium*, a problem which has been studied primarily by means of transductional recombination as reviewed by Hartman.[28]

The chance isolation, prior to selective recombination, of a high frequency recipient strain supports the possibility that many species of Salmonella may possess cells exhibiting the attributes of F^- or recipient organisms. It is also likely that the rare hybrids which can be obtained from crosses of other strains of Salmonella with *E. coli*, would now act as high frequency recipient organisms on remating with *E. coli*. There are a number of interpretations for the lower frequency of recombination which is evident even in the case of the TM-9S^r-2 strain of *S. typhimurium* when compared with results which have been obtained in *E. coli* Hfr × *E. coli* F^- crosses. Thus, it is conceivable that the TM-9S^r-2 strain represents a form capable of becoming F^- at a higher frequency than strain TM-9 from which it was derived. It may, therefore, not be the typical or ultimate F^- strain of Salmonella which might occur. Another interpretation bears on the possibility of a relatively infrequent integration of the genetic material transferred from the *E. coli* parent to the Salmonella recipient. It would be premature, however, to make any statements concerning the degree of genetic homology between *E. coli* and *S. typhimurium* even though results have indicated that certain of the characteristics of the *E. coli* parent could not be detected in the hybrids. The extent of genetic homology between these organisms may be approached more significantly through the use of different Hfr strains of *E. coli* as has been described by Wollman *et al.*[29]

There may be a number of problems which can be examined effectively by further application of this means of bacterial hybridization. Thus, the problem of the virulence for mice of *S. typhimurium*, investigated by many workers throughout the years, may now be amenable to genetic analysis. Along these lines, preliminary experiments have been initiated to determine if any hybrids of *S. typhimurium* have lost virulence as a consequence of recombination with the mouse avirulent parent strain of *E. coli*. The correlation of antigenic or other types of changes associated with loss of virulence could prove useful for the possible determination of a genetic basis for virulence.

Although preliminary experiments using Salmonella as donor organisms (either F^+ or Hfr) have not been successful, it would still seem feasible for such strains to be isolated within the existing compatibility framework. It should be possible under certain circumstances, with appropriate mating strains and proper selective procedures to transfer Hfr ability to Salmonella species. As a result, it would not be unreasonable to expect successful crosses of Salmonella × Escherichia, Salmonella × Salmonella, or Salmonella × Shigella. The hybrids of such crosses would surely offer material of an exceptionally interesting nature for studies in bacterial virulence.

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*ROLE OF EPISTASIS AND OVERDOMINANCE IN STABILITY OF EQUILIBRIA WITH SELECTION**

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The condition which leads to a stable equilibrium under constant selective values for a single locus with two alleles is simply heterozygote superiority. This condition is also necessary and sufficient for many loci as long as they recombine freely and do not exhibit epistasis. The purpose of this study is to investigate the conditions of stable equilibria when genes do exhibit epistasis. Two alleles per locus, constant genotypic values, free recombination, and random mating are assumed throughout.

Genotypic Variance as Functions of Derivatives of the Mean.—The conditions for stable equilibria must be phrased in terms of derivatives of the mean of a population. These derivatives are essentially gene effects (mono-, di-, tri-genic, and so on) which lead to the same partitions of genotypic variance as those of Cockerham[1] and Kempthorne.[4] This method of obtaining the partitions will be briefly illustrated to establish notation and to tie the conditions for stable equilibria as closely as possible to the genotypic variances. It is worth noting also that the procedure is expeditious