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Non-parental-type Recombinants in Crosses between Different Restriction-Modification Systems in SALMONELLA

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Abstract

NON-PARENTAL-TYPE RECOMBINANTS IN CROSSES BETWEEN DIFFERENT RESTRICTION-MODIFICATION SYSTEMS IN *SALMONELLA*

by Mary M. Ball

Many new restriction-modification (R-M) systems which are closely linked to *serB* and allelic to the hsd_{SB} system of *S. typhimurium* occur in different *Salmonella* serotypes. In P1 transduction studies with these systems, recombinants were isolated which fell into one of three classes with reference to their specific restriction phenotype. Recombinants that restricted like the donor, recombinants that restricted like the recipient and recombinants that had lost the ability to restrict. This latter group having lost the recipient SB restriction without gaining the specific donor restriction and here called "zero recombinants," were of special interest and were investigated.

Zero recombinants were selected from among *serB+* recombinants derived from transductions using phage P1, to *E. coli*/*S. typhimurium* LT2 hybrids 4662 (SB+) and 4617 (SA+SB+). Backcrosses of these recombinants to 4662 and 4617 were also done. The modification phenotype of all recombinants was determined.

Three different phenotypes of zero recombinants were isolated in the transduction with *S. eastbourne* as donor: $r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$, $r^-_{SB}m^+_{SB}r^-_{SEA}m^-_{SEA}$, and $r^-_{SB}m^-_{SB}r^-_{SEA}m^+_{SEA}$. Since the SB and SEA R-M systems behaved as true alleles, the simplest explanation of the origin of these recombinant types was as the result of crossovers within the *s* and *r* genes of the two R-M systems. It is possible that the observation that zero recombinants were isolated more frequently when the co-transduction frequency of the complete R-M system was low (less than 5%)

might be related to the fact that the R-M system would be close to the end of the transduced piece of DNA. Recent experiments have indicated that genetic recombination is initiated at the ends of single-stranded pieces of DNA, (Hollman, Wiegand, Hoessli, and Radding, 1975). This could help to explain the high frequency of transduction of the "zero" phenotype.

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NON-PARENTAL--TYPE RECOMBINANTS IN CROSSES BETWEEN DIFFERENT RESTRICTION-MODIFICATION SYSTEMS IN SALMONELLA

by

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Each person whose signature appears below certifies that this thesis, in his or her opinion, is adequate in scope and quality as a thesis for the degree of Master of Science.

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INTRODUCTION

Modification and restriction of DNA was described in 1962, by Arber and Dussoix. They showed that the DNA of bacteriophage lambda was specifically modified so that a particular strain of bacterium could be infected with bacteriophage only when the bacteriophage carried the DNA modification of the host strain. Phages that did not carry the specific modification were restricted. Thus modification and restriction were related processes. The ability of the bacteria to recognize whether the phage DNA was correctly modified or not was referred to as the specificity. Restriction was recognized as the degradation of foreign DNA (DNA without proper modification) by enzymes known as restriction endonucleases. Modification was the process whereby DNA was labeled by a specific methylase which methylated nucleotide bases at specific sites.

Restriction endonucleases are divided into two types based on various properties. Type I enzymes have stringent requirements for ATP, S-adenosyl methionine (SAM), and Mg++, are of a high molecular weight, (250,000 daltons), and produce double-stranded breaks in DNA at sites different from the methylation sites. Type II enzymes are of a smaller molecular weight (100,00 daltons), require only Mg++ as a co-factor, and attack double-stranded DNA at the same sites where methylation occurs, (Boyer, 1974).

All restriction-modification (R-M) systems that are known to produce type I enzymes are coded by systems of genes on the bacterial chromosome. Type II enzymes are coded by systems of genes on plasmids. In Salmonella typhimurium LT2 three different systems of R-M have been

recognized, LT (Colson, 1971), SA (Colson and Van Pel, 1974), and SB (Colson and Van Pel, 1974). Bullas and Colson (1975) found that the LT system was widespread in Salmonella, while the SA system was detected in no other strain. Moreover, many Salmonella possessed a specific R-M system which in genetic crosses behaved as an allele of the SB system in S. typhimurium (Pittman, 1976). Since Colson found that SB was an allele of SB, the allelic system of R-M genes would appear to unite the Escherichia coli and the Salmonella groups of enteric bacteria. Moreover, genetic relatedness between the systems in both genera has been shown to exist by complementation or functional allelism tests (Boyer and Roulland-Dussoix, 1969; Glover 1968, 1970).

Complementation (Boyer and Roulland-Dussoix, 1969; Arber and Linn 1969; Glover 1968, 1970) and transduction recombination studies (Glover and Colson, 1969) between mutants in the R-M systems in E. coli have been done. The isolation of one-step r-m mutants and the derivation of r+m+recombinants in crosses between different R-M mutants, together have suggested that at least three genes are involved in restriction and modification (Meselson, Yuan, and Heywood, 1972); m for modification, r for restriction and s for specificity. Crosses between different mutants of E. coli (Glover, 1970) and of Salmonella (Bullas and Colson, 1975) support the order of the genes as m s r serB.

In our laboratory, in transductions between different E. coli/S. typhimurium LT2 hybrids which contained the S. typhimurium SB system and different Salmonella strains, recombinants of an unexpected type arose at unusually high frequencies. Two types of recombinants would be expected from such crosses; those with the restriction phenotype of the

donor and those with the restriction phenotype of the recipient. With some Salmonella, however, a third recombinant phenotype arose which expressed neither the SB restriction phenotype of the recipient nor the restriction phenotype of the donor. One recombinant of this nature had been isolated earlier by Bullas, Colson, and Van Pel (1976). The much higher frequency with which they were isolated in our crosses suggested that they may have arisen by a different genetic event.

In this thesis, I report on my investigation of these recombinants that not only failed to receive the donor R-M restriction but also lost the recipient SB system.

MATERIALS AND METHODS

A. Bacteria and Bacteriophages

The strains of E. coli, Salmonella typhimurium LT2 and E. coli/ Salmonella LT2 hybrids are listed in Table 1. The different Salmonella serotypes used are shown in Table 2. Bacterial cultures were maintained on nutrient agar slants at room temperature. Daily cultures were inoculated from isolated colonies on nutrient agar plates, which were kept at room temperature, and restreaked from the stock cultures at approximately bi-weekly intervals.

The phages P1, and λ were the same as used by Bullas and Colson (1975). Phage P1Cm-clr was obtained from Mojica-A (1973). Bacterial lysates of phage λ, and P1 were prepared by extraction from soft layer agar. Lysates of λ, and P1 were sterilized with chloroform. All phage lysates were titered by plating serial dilutions of phage in buffer, into lawns of appropriately sensitive bacterial strains in top laver agar.

B. Notations

The notations for host specificitiy phenotypes and genotypes, and for phage modification follow the recommendations and usage of Arber and Linn (1969), Arber (1974), Colson and Van Pel (1974), Bullas and Colson (1975), and Pittman (1977).

The term hsd (which stands for host specificity for DNA) is the gene designation for R-M system in Salmonella eastbourne, SH the R-M system in S. heidelberg. The restriction phenotype of a particular R-M system was denoted by r the modification phenotype by m. The restriction and modification phenotype of a specific R-M system was

designated by use of the appropriate specificity abbreviation as a subscript to both r and m. The presence or absence of a restriction or modification was indicated by a superscript + or −. Thus, for example, $r^-_{SB}m^+_{SB}$ designates the absence of SB restriction and the presence of SB modification.

The DNA of phages propagated on a specific bacterial strain bears the modification of that strain; phage modification were described by the phage symbol, followed by a period and the appropriate abbreviations for the R-M system separated by commas. Thus, for example, P3.LT, SA, SB represents phage P3 with the LT, SA, and SB modification.

C. Media and Solutions

L broth was used for liquid cultures of bacteria. L broth solidified with 1% agar was used for restriction and modification tests and to propagate phage. 1.5% agar in L broth was used for plating out bacteria. Soft, top-layer agar was used in the spottings tests for determination of the R-M phenotypes of recombinants, and for preparation of phage. MacConkey agar base with added sugar was used for distinguishing between fermentative and non-fermentative bacteria for that sugar. Davis minimal media with appropriately added growth factors was used for selection of the recombinants. Buffer was used for diluting and re-suspending cells and phages.

The composition of the media and solutions per liter was: L Broth (Lennox, 1955), Bacto-tryptone 10g, NaCl 5g, Bacto-yeast extract 5g, dextrose 1g; (pH adjusted to 7.2 by adding 1.5 ml of NaOH.) L Agar (Lennox, 1955) L broth solidified with either 1% or 1.5% Bacto-agar. Top Layer Agar-Bacto-agar 7.5g, .01M $CaCl_2$, .01 M $MgSO_4$

MacConkey Medium-MacConkey agar base 40g, sugar 10g.

Davis Minimal Agar-(DMA, Lederberg, 1950) KH_2PO_4 7g, sodium citrate, 5g, dextrose 1g, Bacto-agar 15g. Growth factors were added only after autoclaving.

Buffer-(Glover, 1962) KH_2PO_4 3g, $NaHPO_4$ 7g, NaCl 4g, $MgSO_4 7.H_2O$ 2g, pH5.5.

D. Use of Phage P1 in Transduction Mapping

Phage P1 is a generalized transducing phage originally isolated from E. coli. P1 was used to transduce serB to the recipient strains and the co-transduced frequency of the new R-M systems was determined. In this way, Bullas and Colson (1975) showed that SP from S.potsdam was closely linked to serB in S. typhimurium but less closely linked to pyrB.

To carry out P1 transductions, either P1 sensitive Salmonella, or P1 lysogens were needed. The method of P1 sensitive serotype selection was with the use of phage FO to select for spontaneous galE mutants (Pittman, 1977). Phage FO characteristically lyses smooth strains of enteric bacteria (Wilkinson, Gemski, and Stocker, 1972). Some of the FO resistant strains (rough) have been found to be mutated at galE and so do not put galactose into the O antigen of the cell wall. Ornellas and Stocker (1974) have shown that some of these mutants are P1 sensitive. S.heidelberg was a P1CM clr lysogen previously derived in our laboratory by Dr. Berney Neufeld.

E. Derivation of Phage P1 for use in transduction

P1 transducing lysates were obtained by induction of P1 lysogens or by soft layer propagation of P1 on P1-sensitive strains. P1 lysogens

were grown in L broth at 30^{o} for three hours, followed by incubation at 42^{o} for ninety minutes with a final incubation at 37^{o} for one hour.

On P1-sensitive Salmonella, high titer lysates of P1 were prepared by plating out P1 at a multiplicity of infection (m.o.i.) between 1 and 10 in soft layer agar on L plates, and incubated overnight at 37^{o}. The phage was extracted from the top layer by centrifugation at 10,000 rpm for 10 minutes to sediment the agar. The supernatant fluid contained the phage and was sterilized with chloroform. Titers between $1x\ 10^{9}$ and $1x\ 10^{10}$ plaque forming units/ ml could be obtained by this method.

F. Transductions

Transductions were performed by adding P1 propagated on the donor strain, to exponential phase broth cultures of the recipient strain to which $CaCl_2$ was added to a final concentration of .01M, at an m.o.i. between 1 and 10. The phage/cell mixture was left standing at room temperature for 30 minutes. The cells were then centrifuged and resuspended in buffer, and plated out on selective medium containing 0.05% sodium citrate to chelate Ca^{++} and inhibit further P1 infection, according to the method of Glover (1962). Transduction recipients were E. coli/ S. typhimurium hybrids 4617 and 4662. Selective medium for 4617 and 4662 was DMA with added thiamine and citrate. The cells were incubated at 37^{o} for three days. Recombinants were purified by streaking out onto selective media.

G. Tests to determine restriction phenotype of recombinants

The method used to determine phenotype of the recombinant was the spotting test described by Colson, Colson, and Van Pel (1970). Tests to

determine restriction phenotypes of recombinants were performed with cultures of purified recombinants. Single colonies were sub-cultured into 0.5 ml of L broth and incubated overnight at 30^{o}. The test phage used for the E. coli/S. typhimurium hybrid recombinants was phage λ . For the test, the purified cultures of recombinants with 2 ml added soft agar were poured onto L agar plates. Test phages with known modifications at three concentrations (10^{7}, 10^{5}, 10^{3} pfu/ml) were dropped onto the lawn of bacteria and allowed to dry in. In most tests four different sets of phages were used, each with a different modification. Bacteria with known R-M systems were used as controls, and spotted with the same phages as the recombinants. Plates were incubated at 37^{o} overnight. Patterns of lysis were observed and compared to the controls to determine the restriction phenotype of the recombinants.

The tester phages were placed onto the recombinant seeded agar plate with the use of the Dynadrop MR machine (Dynatech Laboratories Inc., Virginia) calibrated to deliver 0.01 ml drops (Fig. 1). The dispensing manifold was adapted to deliver 12 drops of phage in a 3 by 4 pattern, to fit over the surface of a petri dish. Between uses, the tips of the tubing support needles were immersed in 70% ethyl alcohol in the pressure jar. Before use, the alcohol was run through the system for five minutes, followed by sterile water for three minutes. The test phages suitably diluted, were placed in the reservoir tube rack in the same pattern as they were delivered to the petri dish.

H. Tests to determine modification phenotype of recombinants

To test the modification phenotype of the recombinants, a small

sample of phage propagated on the recombinants in the restriction test was removed and diluted in a 1 ml aliquot of buffer. This phage suspension was further diluted 10^{-2} and 10^{-4}. These phages which bore the modification of the individual transductants were then spotted on various bacterial cells with a known restriction. The phages' modification could then be determined by whether the phage was restricted or not by a certain restriction system. An Oxford micro-doser repetitive pipette, delivering 25 µl/delivery (Oxford laboratories, Inc. California) was used to spot the phage.

RESULTS

A. Detection of serB+recombinants with 4662 and 4617, that had lost the recipient restriction without gaining the donor restriction.

With the use of suitable indicator phages, many Salmonella serotypes have been found to have specific restrictions. Table 3 presents the results of P1 co-transduction experiments with ten Salmonella serotypes each of which had a specific restriction to 4662 or 4617. Eight, S. bareilly, S. gelsenkirchen, S. kaduna, S. eastbourne, S. muenchen, S. enteriditis, and S. thompson co-transduced the new restriction with serB at varying frequencies. With two, S. heidelberg and S. oranienberg no co-transduction for their new restriction with serB was demonstrated. In addition to co-transduction of the new restriction, four, S. bareilly, S. gelsenkirchen, S. eastbourne, and S. enteriditis yielded recombinants that had lost the recipient SB restriction without gaining the donor restriction. No such recombinants were obtained with S. kaduna, S. muenchen, S. blegdam, and S. thompson. However, serB+ recombinants that had lost the recipient SB restriction without gaining the new restriction were also obtained with S. heidelberg and S. oranienberg. With these two strains, however, this result could be interpreted in one of two ways. There was no serB linked R-M system (similar to S. typhi and S. pullorum/gallinarum (Pittman, 1977) or there was an R-M system in the region but it is too far removed from serB to be co-transduced with it. In light of the fact that the majority of the Salmonella examined did have a specific R-M system linked to serB, I assumed the second interpretation to be correct at the beginning of the experiments given here.

To recombinants that had lost the recipient SB restriction without gaining the donor restriction, I applied the term "zero restriction

recombinants." This term will therefore be used throughout the thesis to refer to this type of recombinant. The zero condition will be symbolized by the appropriate specificity designation with a superscript ^{o}. Thus, for example, SEA^{o}, represents a zero strain derived from a cross in which an SEA+ strain was the original donor.

2. Transduction to E. coli/S. typhimurium hybrids 4662, 4617 from S. eastbourne, and S. heidelberg.

Results of P1 transductions from S. eastbourne to 4662 and 4617 are shown in Table 4. Of 280 serB+ recombinants obtained in the cross with 4662, 197 retained the restriction phenotype of the recipient (SB), four of the recombinants (1.4%) co-transduced the SEA phenotype of the donor while 77 (28%) had acquired the zero property in the transduction with 4662.

In the cross with 4617, 138 of the 238 recombinants tested retained the full restriction phenotype of the recipient (SA+SB+), 18 (7%) retained the SA restriction and lost the SB without gaining the SEA restriction, and 12 recombinants gained the SEA phenotype of the donor. Thus a total of 51 serB+ recombinants (21.4%) acquired the zero property in the transduction with 4617.

These results indicated that the zero restriction recombinants could be regularly obtained with S. eastbourne. The frequency with which they were isolated varied in different experiments since the results for Table 4 show an average frequency of 25%, whereas results from Table 1 indicate a frequency of isolation of 6%.

Results of transductions from S. heidelberg to 4662 and 4617 are

also presented in Table 4. In the cross with 4662, 236 of 293 recombinants (80.5%) retained the restriction phenotype of the recipient, 57 (19.5%) expressed no new restriction and therefore expressed the zero restriction property.

In the cross with 4617, 105 of 116 serB+ recombinants (90.5%) retained the full restriction phenotype of the recipient (SA+SB+) while four were SA+ and lacked any other restriction. No recombinants were obtained which expressed a new restriction. Thus a total of 11 serB+ recombinants (9.5%) acquired the zero restriction property in the cross with 4617.

In the crosses involving S. heidelberg, shown in Table 4, the frequency of zero recombinants in the two crosses involving 4662 and 4617, 17% ($\frac{68}{409}$) is comparable to the results of the experiment shown in Table 1 (19%). However, since no serB+ recombinants were obtained which expressed the new S. heidelberg restriction it was still uncertain whether the zero recombinants obtained in the cross with S. heidelberg were of the same type as those obtained with S. eastbourne.

Results with both strains clearly demonstrated, however, that isolation of zero recombinants was independent of the SA R-M system, since both SA+ and SA- recombinants of the zero phenotype were obtained.

3. Modification tests of zero restriction recombinants derived from S. eastbourne x 4662 and 4617 crosses.

Modification tests were carried out using phage grown on selected zero restriction recombinants derived from S. eastbourne to 4662 and 4617 transductions. These results are shown on Tables 5 and 6.

Of the 34 zero recombinants derived from one transduction to 4662 all lacked modification (Table 5). The R-M phenotype of these recombinants was therefore, $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$. From a second transduction to 4662 (Table 5), 18 zero recombinants were tested for their modification phenotype. Sixteen of these lacked modification, and therefore, had the phenotype $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$. Two were modified for SB and therefore had the phenotype $r^-_{SA}m^-_{SA}r^-_{SB}m^+_{SB}r^-_{SEA}m^-_{SEA}$. From a third transduction (Table 5), 25 zero recombinants were tested. Modification tests determined that 22 lacked any modification, 2 were modified for SB and had the phenotype $r^-_{SA}m^-_{SA}r^-_{SB}m^+_{SB}r^-_{SEA}m^-_{SEA}$. One zero recombinant was modified for SEA only, and had the phenotype $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^+_{SEA}$.

Thirty-one zero recombinants from a S. eastbourne x 4617 cross were tested for modification (Table 6). Eight lacked any modification and thus had the phenotype $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$, while 5 were m+ for the SA but not for SB or SEA, their phenotype was $r^+_{SA}m^+_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$. The remaining 18 zeros were modified for SB and had the phenotype, $r^-_{SA}m^-_{SA}r^-_{SB}m^+_{SB}r^-_{SEA}m^-_{SEA}$.

Twelve zero restriction recombinants from a second S. eastbourne x 4617 cross were tested for their modification phenotype. Four gave SA restriction while eight lacked SA restriction. Three of the SA+ recombinants also modified for SA, and lacked the ability to modify for SB or SEA; they thus had the phenotype $r^+_{SA}m^+_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$. The other restriction recombinant was modified for SA and also was modified for SB but lacked ability to modify SEA, its phenotype was therefore, $r^+_{SA}m^+_{SA}r^-_{SB}M^+_{SB}r^-_{SEA}m^-_{SEA}$. The eight remaining SA- zero recombinants failed to modify for either SB or SEA and thus had the phenotype

$r^-_{SA}{}^{m-}_{SA}{}^{r-}_{SB}{}^{m-}_{SB}{}^{r-}_{SEA}{}^{m-}_{SEA}$.

Thus a majority ($\frac{27}{43}$) of the zero recombinants obtained with the 4617 recipient had not completely lost the SB R-M system, retaining the ability to modify for SB. A smaller percentage of the zero recombinants obtained with the 4662 recipient ($\frac{4}{77}$) had not completely lost the SB R-M system, retaining the ability to modify for SB. One of the zero recombinants had gained the SEA modification without gaining the SEA restriction.

4. Backcross transduction from zero recombinants obtained with S. eastbourne and S. heidelberg donors to 4662 and 4617.

Backcross transductions from zero recombinants obtained with S. eastbourne and S. heidelberg with 4662 and 4617 were done. Table 7 gives the results of seven different $r^-_{SB}{}^{m-}_{SB}{}^{r-}_{SEA}{}^{m-}_{SEA}$ zero combinants backcrosses to 4662. The co-transduction frequency of the zero restriction phenotype in these crosses varied from 1 to 61%. Table 8 shows the results of five $r^-_{SB}{}^{m-}_{SB}{}^{r-}_{SEA}{}^{m-}_{SEA}$ recombinants backcrossed to 4617. All five of these backcrosses yielded SA+ zeros, and two (#2, #4 Table 8) also yielded SA- zeros. The variability in the frequency of zero phenotype in the backcrosses demonstrates that each zero is a unique genetic recombinant.

Five independent $r^-_{SB}{}^{m-}_{SB}{}^{r-}_{SH}{}^{m-}_{SH}$ zero recombinants derived from a S. heidelberg x 4662 cross were transduced to 4662, and 4617 (Table 9 and 10). All of these transductions yielded recombinants with the complete zero R-M phenotype. The co-transduction frequency of the zero phenotype varied from 16%-63%. Both SA+ and SA- recombinants were

obtained.

Four independent $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$ recombinants were backcrossed to 4662, (Table 11). In all four transductions, zero restriction recombinants were obtained. The co-transduction frequency of the zero restriction phenotype varied form 14%-31%. Recombinants #1 and #2 yielded only $r^-_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$ recombinants; two recombinants, #3 and #4, yielded both $r^-_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$ and $r^-_{SB}{}^{m+}{}_{SB}{}^{r+}{}_{SEA}{}^{m-}{}_{SEA}$ recombinants. The co-transduction frequency of the complete donor phenotype $r^-_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$ was 1% in transduction #3 and 6% in transduction #4, whereas the co-transduction frequency of the $r^-_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$ phenotype was 20% in transduction #3 and 30% in transduction #4.

Two $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$ recombinants were backcrossed to 4617 (SA+SB+). Both transductions yielded zero restriction recombinants at a frequency of 19% and 17%. In transduction #1 there were three types of zeros recombinants. One had the phenotype $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$, ten were $r^+_{SA}{}^{m+}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$, and four were $r^+_{SA}{}^{m+}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$. In transduction #2 all 18 zero recombinants were $r^+_{SA}{}^{m+}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$.

The one $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m+}{}_{SEA}$ recombinant obtained from a S. eastbourne x 4662 cross (Table 5) was backcrossed to 4662 and 4617. The $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m+}{}_{SEA}$ donor yielded 38% zero restriction phenotypes and all 14 of the recombinants were not modified for SEA. When the $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m+}{}_{SEA}$ donor was backcrossed to 4617, 14 zero recombinants were obtained (15%). All of these were $r^+_{SA}{}^{m+}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$.

Two zero recombinants of the phenotype $r^+_{SA}{}^{m+}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$ from

crosses of S. eastbourne 4617 (Table 6) were backcrossed to 4662. Both transductions yielded a high percentage of zeros, 71%, and 50% respectively. In the first transduction, 6/84 of the zeros had the $r^{+}_{SA}m^{+}_{SA}r^{-}_{SB}m^{-}_{SB}r^{-}_{SEA}m^{-}_{SEA}$ phenotype of the donor, the remaining 78 were zeros which had not acquired the SA restriction or modification. In the second transduction all zero recombinants had not acquired the SA restriction or modification and had the phenotype, $r^{-}_{SA}m^{-}_{SA}r^{-}_{SB}m^{-}_{SB}r^{-}_{SEA}m^{-}_{SEA}$.

DISCUSSION

The results clearly demonstrate that recombinants which had lost the restriction-modification phenotype of the recipient but had not acquired the specific donor R-M phenotype could regularly be obtained in P1 mediated transductions from *Salmonella* to *E. coli*/*S. typhimurium* LT2 hybrid strains 4662 and 4617. These were called "zero restriction recombinants." The derivation of these zero restriction recombinants was independent of the closely located SA R-M system which is nearer to *pyrB*.

Since no *serB+* recombinants with the SH system from *S. heidelberg* were ever obtained, it is impossible to be certain that the zero recombinants with this strain were the result of the SH system either being located in the *serB* region but too far removed from *serB* to be co-transduced with this gene, or being located elsewhere on the chromosome.

With *S. eastbourne* as donor however, SEA+ recombinants were regularly obtained in all crosses at a frequency no greater than 5% whereas zero recombinants were obtained at a frequency of 21-28%. With the other three strains of *Salmonella* which produced zero recombinants with 4662 or 4617, *S. bareilly*, *S. gelsenkirchen*, and *S. enteriditis*, the frequency of co-transduction of the specific R-M system was also low. That a low frequency of co-transduction of the specific R-M system was however, not a necessary pre-requisite to the derivation of zero recombinants with all strains, is illustrated by *S. muenchen* with which no zero recombinants were isolated although the frequency of co-transduction of SM was 0.73%. That zero recombinants could also be isolated from transductions in which the frequency of co-transduction of the specific R-M system was high, was shown by Bullas, Colson, and Van Pel (1976), who, during a systematic

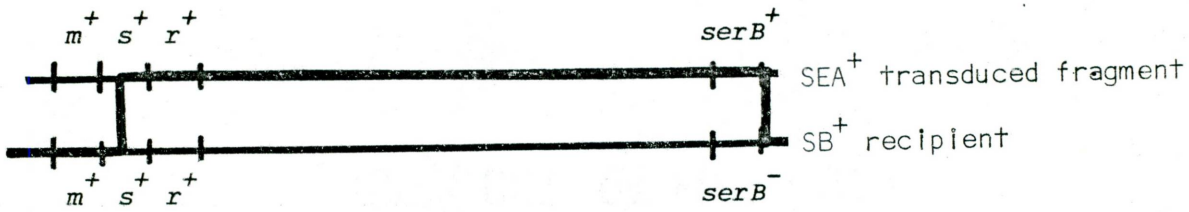
search for such recombinants with S. potsdam as donor, isolated one in 1,443 serB+ recombinants.

These results would indicate that the derivation of zero recombinants is probably a normal genetic event but is more probable in some strains when the specific R-M system is at such a distance from serB that it is co-transduced at only a low frequency. This arrangement would mean that the transducing fragment would contain serB close to one end and the specific R-M system close to the other end.

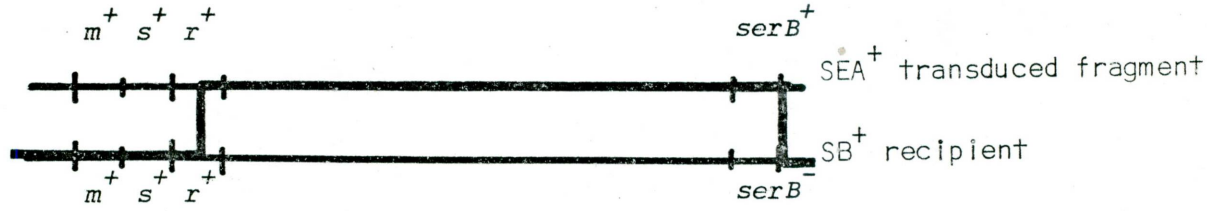
The currently accepted model for the arrangement of genes within R-M systems is the "at least three genes" model of Meselson, Yuan and Heywood (1972). The results of Glover and Colson (1969) with E. coli and Bullas and Colson (1975) support the model that these three genes are arranged in the order, m-s-r-serB. The derivation of the serB+ recombinant in a cross between S. potsdam and 4617 of Bullas, Colson, and Van Pel (1976) which expressed a new specificity SQ, was most simply explained by a cross-over occurring in the s genes of the two allelic R-M systems, SB and SP, to generate a new s gene.

Similarly, the derivation of zero recombinants with S. eastbourne as donor, and probably the other Salmonella donors, may be most simply understood as resulting from cross-overs occurring within the genes of allelic R-M systems. Thus, for example, the explanation of the origin of the r-m- phenotypes, the most frequent of the zero recombinants isolated (at a co-transduction frequency with serB as high as 28% with S. eastbourne as donor) could be the same as that offered by Bullas, Colson and Van Pel--a crossover within the s genes of the two systems but which generates a "nonsense recombinant s gene." The recombinant

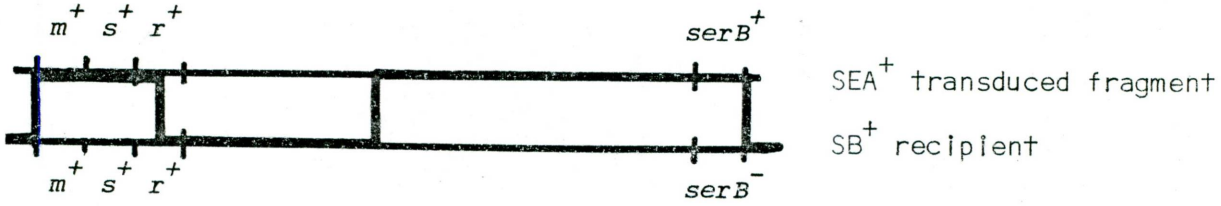
would therefore have the phenotype $r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$. This genetic event is illustrated by the following diagram:



The origin of the $r^-_{SB}m^+_{SB}r^-_{SEA}m^-_{SEA}$ zero recombinants may be similarly explained. Zero recombinants with the phenotype $r^-_{SB}m^+_{SB}r^-_{SEA}m^-_{SEA}$ were isolated at a frequency of 19% (Tables 5 and 6). These could be the result of a cross-over in the r genes of the two systems to generate a recombinant r gene, as shown below:



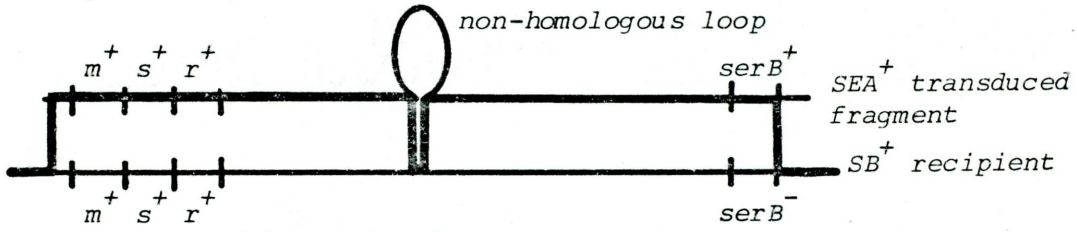
Only one of the 120 zero recombinants isolated had the phenotype, $r^-_{SB}m^-_{SB}r^-_{SEA}m^+_{SEA}$. This very low frequency is in accord with the probable occurance of a triple cross-over event between the R-M systems, as shown below:



Thus we see that cross-overs between either the s or r genes could result in zero recombinants. It would seem that recombinants which were the result of cross-overs between the m genes should also be possible. However, if this were so it would result in a r+m- recombinant which would be a suicidal condition.

The back-cross results verified the allelic nature of the SB and

SEA systems. However, the frequencies of co-transduction with serB of the zero phenotype was significantly higher than was obtained in the forward crosses. This was probably due to a combination of different events. First, there are probably regions of non-homology between serB and the R-M systems in S. typhimurium LT2 and S. eastbourne. These non-homologous regions would not synapse but would be observed as loops. This may be illustrated as below:



Cross-overs on either side of the non-homologous loop would produce a shorter distance between serB and the R-M system in the recombinant. Thus when used in a backcross, the frequency of co-transduction of the R-M system with serB would be higher than in the forward crosses. The high frequency with which zero recombinants were isolated in the forward crosses (28%) may argue against the occurance of these double cross-overs. On the other hand, the presence of such a looped, non-homologous region, may stimulate a cross-over on either side of the loop.

Second, recombinants of the zero phenotype would be derived by both co-transduction of the whole r-m- R-M system of the donor, as well as by the events which produced a zero recombinant, i.e. possibly a cross-over in the s gene, as indicated above. With a zero donor, these recombinants derived by these two events cannot be distinguished, but the overall result would be a higher frequency of zero phenotypes as recombinants.

There are probably other explanations possible for the origin of

zero recombinants. For example, a non-homologous cross-over on either side of the two R-M systems would lead to a deletion in the recombinant of the complete system. However, since SEA and SB behaved as true alleles, (Pittman, 1977), since $r-_{SB}m+_{SB}$ and $r-_{SEA}m+_{SEA}$ phenotypes were both isolated, and no $r-_{SB}m+_{SB}r-_{SEA}m+_{SEA}$ recombinants were isolated, and since a non-homologous cross-over is less likely, certainly at the frequency with which zero recombinants were obtained, such an explanation is less probable than the first one presented.

Recent experiments have indicated that genetic recombination is initiated at the ends of single stranded pieces of DNA (Hollman, Wiegand, Hoessli, and Radding 1975). It is possible that this understanding may explain the occurrence of the high frequency of cross-overs in hsd genes close to the end of the transduced fragment.

Although the P1-transduced Salmonella chromosome fragment is transferred to recipient cells as a double-stranded fragment, it is probable that at the ends of this fragment, short lengths of single stranded DNA, "frayed" ends occur, due to the weakening of hydrogen bonds. The lengths of the single-stranded "frayed" ends could be expected to be slightly different in different fragments. Recombination with single-stranded DNA results in the formation of a triple-stranded intermediate over a length of DNA as long as 90 nucleotides (Hollman, et. al. 1975). This region is the site of specific enzymic attack that opens up the recipient molecule leading to genetic exchange; i. e. the formation of a cross-over. Since there are two, single-stranded ends, it is possible that cross-overs could be initiated by both ends. The stresses produced by the pairing of the single-stranded ends could also lead to breakage of additional

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Table 1. Strains of *E. coli*, *S. typhimurium* and hybrids used

Strain number	Host specificity phenotype	Genotype	Origin
1.			
1228	*Escherichia coli* K-12	*ser B80 leu thi lac* hsd_k	from Bullas and Colson (1975)
2.	*Salmonella typhimurium* LT2		
4529	$r+_{LT}m+_{LT}r+_{SA}m+_{SA}r+_{SB}m+_{SB}$	F'-*gal*$^+$*bio*	from Bullas and Colson (1975)
4532	$r+_{LT}m+_{LT}r+_{SA}m+_{SA}r+_{SB}m+_{SB}$	*met A met B trp B val pyr B124 gal*	from Bullas and Colson (1975)
4536	$r+_{LT}m+_{LT}r+_{SA}m+_{SA}r+_{SB}m+_{SB}$	*met A met B trp B val ser B80 gal*	from Bullas and Colson (1975)
3.	*E. coli*/*S. typhimurium* hybrids		
4617	$r+_{SA}m+_{SA}r+_{SB}m+_{SB}$	(*ser B80 leu*$^+$)* *thi lac*	from Bullas and Colson (1975
4662	$r-_{SA}m-_{SA}r+_{SB}m+_{SB}$	(*ser B80 leu*$^+$) *thi lac*	from Bullas and Colson (1975)
L4029	$r-_{SA}m-_{SA}r-_{SB}m-_{SB}(r+_{SEA}m+_{SEA})$	(*ser B*$^+$ *leu*$^+$) *thi lac hsd* SB	from Bullas and Colson (1975)

* = genes from *S. typhimurium* LT2

Table 2. Strains of *S. typhimurium* hybrids, and *Salmonella* used

Strain number	Host specificity phenotype	Genotype	Origin
L4030	$r^-_{SA} m^-_{SA} r^-_{SB} m^-_{SB} (r^+_{SEA} m^+_{SEA})$	$(serB+)^{Sea}$ *thi lac* $(leu\ hsd\ SB)^{ST}$	from Bullas and Colson 1973
L4018 L4019	$r^+_{SA} m^+_{SA} r^-_{SB} m^-_{SB} (r^-_{STY} m^-_{STY})$	$(serB+)^{Sty}$ *thi lac* $(leu^+ hsd\ SB)^{Sty}$ P1. *S. typhi* x 4617	"
L4020	$r^-_{SA} m^-_{SA} r^-_{SB} m^-_{SB} (r^+_{SM} m^+_{SM})$	$(serB^+)^{Sm}$ *thi lac* $(leu^+ hsd\ SB)^{St}$ P1. *S. muenchen* x 4662	"
L4023	$r^-_{SA} m^-_{SA} r^-_{SB} m^-_{SB} (r^+_{SK} m^+_{SK})$	$(serB^+)^{Sk}$ *thi lac* $(leu^+ hsd\ SB)^{St}$ P1. *S. kaduna* x 4662	"
L4002	$r^-_{SA} m^-_{SA} r^-_{SB} m^-_{SB} (r^+_{SP} m^+_{SP})$	$(serB^+ hsd\ SA)^{Sp}$ *thi lac* $(leu^+ hsd\ SB)^{St}$ P1. *S. potsdam* x 4617	"

Salmonella serotype

Kauffmann-White group	Name	Antigenic formula	Source
B	*S. heidelberg*	1,4,5,12: r: 1,2	*
D	*S. eastbourne*	1,2,12: e,h: 1,5	*

* N. Atkinson, Dept. of Microbiology, University of Adelaide, Adelaide, Australia

Table 3. P1 co-transduction frequencies with *serB* of R-M systems from different *Salmonella* serotypes in *E. coli*/*S. typhimurium* hybrids 4662 (SA-SB+) and 4617 (SA+SB+)

Salmonella donor	R-M system	Recipient	Total *serB*$^+$ recombinants	Co-transduction frequency of *serB* recombinants with donor specificity	Frequency of *serB*$^+$ recombinants losing recipient specificity but not gaining donor specificity
Kauffmann-White group B:					
S. heidelberg	SH	4662	657	0.15%	19%
		4617	116	0.86%	21%
Kauffmann-White group C_1:					
S. bareilly	SBA	4662	242	5.4%	0.4%
S. gelsenkirchen	SG	4662	33	3.3%	6.6%
S. kaduna	SK	4662	191	30%	0.5%
		4617	32	28%	3.0%
S. oranienburg	SO	4662	233	0.43%	12%
Kauffmann-White group C_2:					
S. muenchen	SM	4617	273	0.73%	0.37%
Kauffmann-White group D:					
S. blegdam	SBL	4662	115	16%	0.9%
S. eastbourne	SEA	4662	145	1.4%	6.2%
		4617	53	1.9%	5.7%
S. enteritidis	SEN	4662	257	1.6%	6.2%
		4617	119	0.84%	3.4%

Table 4. P1 Co-transduction Frequencies with serB+ of R-M Systems in S. eastbourne and S. heidelberg to E. coli/S. typhimurium hybrids 4662 and 4617

Salmonella donor	Recipient	Total serB+	SerB+ recombinants with donor specificity	Co-transduction frequency of serB+ recombinants with donor specificity	SerB+ recombinants with no restriction	Frequency of serB+ recombinants losing recipient specificity but not gaining donor specificity
S. eastbourne	4662	280	4	1.4%	77	28%
S. eastbourne	4617	238	12	5%	51	21.4%
S. heidelberg	4662	293	0	0%	57	19.5%
S. heidelberg	4617	116	0	0%	11	9.5%

Table 5. E. O. P. of phage propagated on "zero" Recombinants from S. eastbourne x 4662 crosses

Suggested phenotype for SerB+ recombinants	Number	1228	SB+ 4662	SA+SB+ 4617	SB+ 4002	SA-SEA+ 4029	SA+SEA+ 4033
$r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	34	1	-2	-2	-2	-4	-4
$r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	16	1	-2	-3	-3	-4	-4
$r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	2	1	1	-2	-3	-2	-2
$r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	22	1	-2	-3	-3		
$r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	2	1	1	-2	-3		-4
$r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m+}{}_{SEA}$	1	1	-2	-2	-3	1	-2

Table 6. E. O. P. of phage propagated on "zero"
Recombinants from S. eastbourne x 4617 crosses

Suggested phenotype for SerB+ recombinants	Number	1228	SB+ 4662	SA+SB+ 4617	SB+ 4002	SA−SEA+ 4029	SA+SEA+ 4033
$r^{-}_{SA}m^{-}_{SA}r^{-}_{SB}m^{-}_{SB}r^{-}_{SEA}m^{-}_{SEA}$	8	1	-2	-2	-4	-4	-4
$r^{-}_{SA}m^{-}_{SA}r^{-}_{SB}m^{+}_{SB}r^{-}_{SEA}m^{-}_{SEA}$	18	1	1	1	-3	-4	-4
$r^{+}_{SA}m^{+}_{SA}r^{-}_{SB}m^{-}_{SB}r^{-}_{SEA}m^{-}_{SEA}$	5	1	1	1	-2	-4	-4
$r^{-}_{SA}m^{-}_{SA}r^{-}_{SB}m^{-}_{SB}r^{-}_{SEA}m^{-}_{SEA}$	8	1	-2	-2	-3	-4	-4
$r^{+}_{SA}m^{+}_{SA}r^{-}_{SB}m^{-}_{SB}r^{-}_{SEA}m^{-}_{SEA}$	3	1	-2	-2	-4	-4	-4
$r^{+}_{SA}m^{+}_{SA}r^{-}_{SB}m^{+}_{SB}r^{-}_{SEA}m^{-}_{SEA}$	1	1	1	1	-4	-4	-4

Table 7. P1 co-transduction frequency with *serB*+ of $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$ R-M systems in recombinants derived from *S. eastbourne* x 4662 crosses, backcrossed to 4662, SA-SB+.

Donor R-M phenotype $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$	Total no. of *serB*+ recombinants	No. of SB- SEA- donor phenotypes	co-transduction frequency of SEA^o
1.	21	8	38%
2.	116	25	22%
3.	110	0	1%
4.	47	4	9%
5.	38	19	50%
6.	69	25	36%
7.	64	39	61%

Table 8. P1 co-transduction with serB+ of $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$ R-M systems in recombinants derived from S. eastbourne x 4662 crosses, backcrossed to 4617, SA+, SB+.

Donor R-M Phenotype $r^-_{SA}m^-_{SA}r^-_{S}\ m^-_{SB}r^-_{SEA}m^-_{SEA}$	Total no. of SerB+ recombinants	No. of SEA^o	R-M phenotype of these recombinants	co-transduction frequency of SEA^o
1.	97	31	$r^+_{SA}m^+_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$	31%
2.	53	10	9: $r^+_{SA}m^+_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$	19%
			1: $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$	
3.	134	3	$r^+_{SA}m^+_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$	2.2%
4.	60	10	8: $r^+_{SA}m^+_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$	17%
			2: $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SN}r^-_{SEA}m^-_{SEA}$	
5.	135	92	$r^+_{SA}m^+_{SA}r^-_{SB}m^-_{SB}r^-_{SEA}m^-_{SEA}$	68%

Table 9. P1 co-transduction frequency with serB+ of $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SH}m^-_{SH}$ R-M systems in recombinants derived from S. heidelberg x 4662 crosses, backcrossed to 4662.

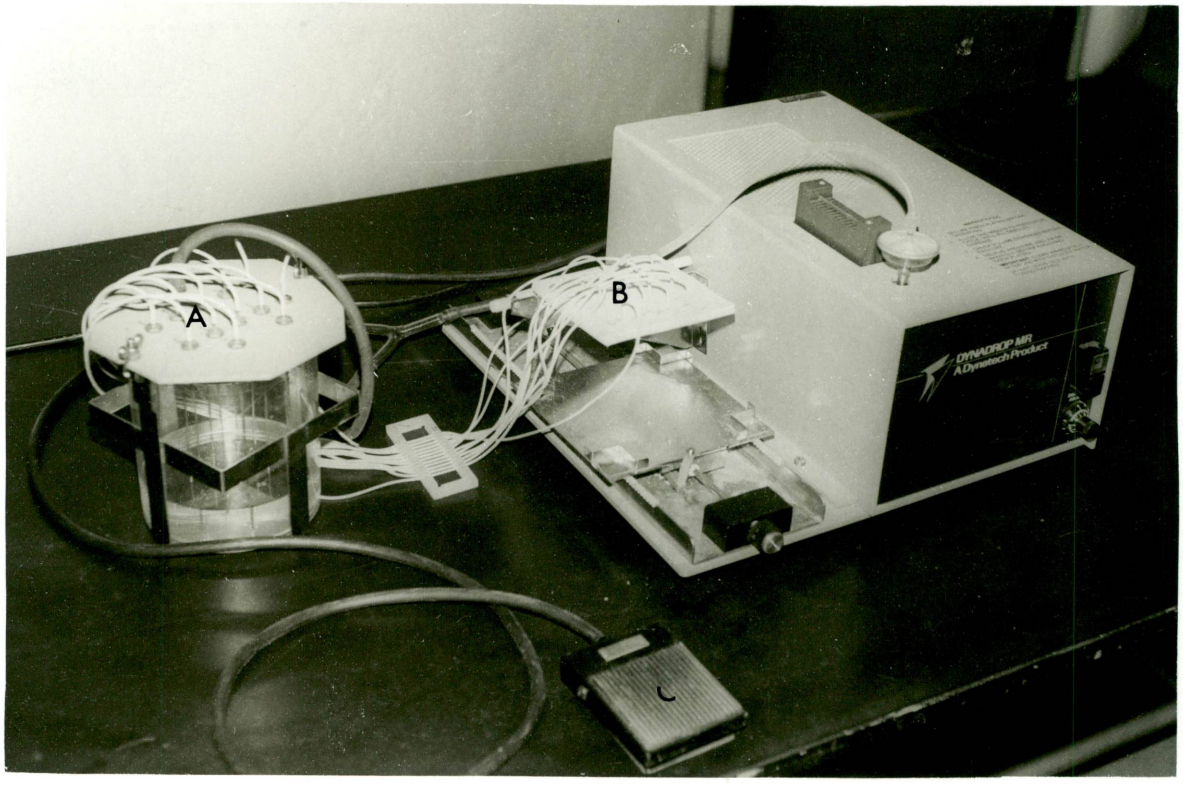
Donor R-M phenotype $r^-_{SA}m^-_{SA}r^-_{SB}m^-_{SB}r^-_{SH}m^-_{SH}$	Total no. of serB+ recombinants	No. of SB- SH- donor phenotypes	co-transduction frequency of SH^o
1.	181	86	48%
2.	46	29	63%
3.	129	27	21%
4.	30	7	23%

Table 10. P1 co-transduction frequency with serB+ of $r-_{SA}m-_{SA}r-_{SB}m-_{SB}r-_{SH}m-_{SH}$ R-M systems in recombinants derived from S. heidelberg x 4662 crosses, backcrossed to 4617, SA+ SB+.

Zero donor R-M phenotype $r-_{SA}m-_{SA}r-_{SB}m-_{SB}$	Total no. of SerB+ recombinants	No. of SH^{o}	R-M phenotypes of these recombinants		Co-transduction frequency of SH^{o}
1.	34	13	12	$r+_{SA}m+_{SA}r-_{SB}m-_{SB}$	38%
			1	$r-_{SA}m-_{SA}r-_{SB}m-_{SB}$	
2.	31	5	4	$r+_{SA}m+_{SA}r-_{SB}m-_{SB}$	16%
			1	$r-_{SA}m-_{SA}r-_{SB}m-_{SB}$	

Table 11. P1 co-transduction frequency with serB+ of $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$ R-M systems to recombinants derived from S. eastbourne x 4662 crosses, backcrossed to 4662, SB+.

Zero donor R-M phenotype $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	Total no. of serB+ recombinants	No. of SEA^o	R-M phenotype of these recombinants	co-transduction frequency
1.	147	31	$r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	21%
2.	21	3	$r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	14%
3.	53	14	3: $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	26%
			11: $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	
4.	91	28	1: $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m+}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	31%
			27: $r^-_{SA}{}^{m-}{}_{SA}{}^{r-}{}_{SB}{}^{m-}{}_{SB}{}^{r-}{}_{SEA}{}^{m-}{}_{SEA}$	



A = Pressure reservoir jar; contains tubes of the phage dilutions

B = The modified manifold to deliver a pattern of twelve drops to Petri dish

C = Foot pedal

Figure 1. Modified Dynadrop MR machine used for the restriction test.