TRANSDUCTION OF *SALMONELLA TYPHIMURIUM*-*SALMONELLA MONTEVIDEO* HYBRIDS¹

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IN 1964 DEMEREC and OHTA reported on the use of *Salmonella typhimurium*-*Escherichia coli* hybrids in transductional studies as a technique for obtaining information about the *Salmonella* linkage map. The term "hybrid," as used in the study, referred to a strain of *S. typhimurium* possessing some genic material of *E. coli* origin in its chromosome. When such hybrids were used as donor strains in transduction with *S. typhimurium* auxotrophs as recipients, few wild-type recombinants were produced if the transducing fragment involved contained *E. coli* genes. This reduced amount of transduction observed with "hybrid" fragments has been explained as the result of limited recombination, due to the differences in the "microhomology" (base pair sequence) between the *S. typhimurium* chromosome of the recipient and the *E. coli* genes of the fragment (ZINDER 1960; DEMEREC and OHTA 1964). With the use of appropriate *S. typhimurium* recipient strains, it was possible to determine the extent of the *E. coli* region in the chromosome of a hybrid. This information was also used as evidence for the arrangement of loci in the *Salmonella* chromosome. A study involving the use of interspecific *Salmonella typhimurium*-*Salmonella montevideo* hybrids (TM hybrids) for similar purposes was initiated, and the details and results of this endeavor are presented herein.

MATERIALS AND METHODS

The mutant strains of *S. typhimurium* used in this study were supplied by Drs. M. DEMEREC and K. E. SANDERSON of Brookhaven National Laboratory. The minimal medium of DAVIS, as described by LEDERBERG (1950), was used as the basal medium. Single-enriched (EM) and double-enriched (EEM) media were prepared by adding 1 and 2%, respectively, Difco nutrient broth to minimal medium (v/v).

Selection for hybrid strains: The hybrid strains were obtained by F-mediated conjugation. The female parents (F⁻) included the following *S. typhimurium* auxotrophs: *ilvA8*, *ilvC16* (isoleucine-valine), *metE338*, *metF185*, *metA53* (methionine) and *argA4* (arginine). The male parent (F⁺) of all hybrids, obtained from Dr. K. E. SANDERSON, was *S. montevideo pur-258* (a purine-requiring mutant derived from *S. montevideo* SH672). Aliquots (0.5 ml) of overnight cultures of male and female strains were mixed and kept at room temperature for 5 to 10 minutes;

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0.1 ml aliquots of the mixture were then spread on each of 5 minimal-medium agar plates. Control plates were spread with 0.1 ml aliquots of one parent strain (no mating). The plates were incubated at 37° for 24 to 48 hr and then examined under low magnification of a dissecting microscope for the selection of prototrophic hybrid clones (selected for large size, smoothness and absence of sectoring). The following numbers of hybrids were selected from each *S. typhimurium* parent: *ilvA*=20, *ilvC*=20, *metE*=20, *metF*=10, *argA*=10 and *metA*=12. Each hybrid was picked into a saline drop and streaked on EEM agar plates to select against any parental auxotrophs which may have been picked with the prototrophic recombinant. No revertants were present on the control plates. This isolation procedure was repeated once again, and all plates contained nonsectored, large colonies which were assumed to be stable hybrids. The hybrids were named according to the convention devised by Dr. M. DEMEREC and his coworkers, e.g. TM-*ilvA4* = fourth *S. typhimurium*-*S. montevideo* hybrid isolated by selection at the *ilvA* locus of the *S. typhimurium* parent.

Transduction procedures:—The procedures used for P22-mediated transduction have been described elsewhere (ARMSTRONG and WAGNER 1964). In this study 0.1 ml of the transduction mixture was spread on each of the three EM agar plates which were then incubated at 37° for 28 to 30 hr before the recombinants were counted.

The hybrid strains that were investigated were selected either at the *ilv* region (*ilvA* and *C* loci) and the closely linked *metE* locus (ROTH and HARTMAN 1965) or the *met-arg* region (*metF* and *argA* loci) and the closely linked *metA* locus. Figure 1 depicts not only the region of hybrid selection but also the section of the chromosome, as presented by SANDERSON and DEMEREC (1965), that was analyzed in this study. A mutant of the *cysC* locus was used as the control marker (see below). The following *S. typhimurium* auxotrophs were used as recipients: *cysC1021*, *cysG382*, *cysE911*, *pyrE125*, *aroB34*, *aroC36*, *pdx-4*, *ilvC16*, *ilvB10*, *ile-12*, *ilvA8*, *metE338*, *metF185*, *argH93*, *argA4*, *purD55*, *metA53* and *purA65*.

Analysis of transduction data: The results of transduction tests that employed bacteriophage prepared on the hybrid strains and *S. typhimurium* recipients were compared to those involving homologous genes of *S. typhimurium* origin. To obtain a meaningful comparison, it was necessary to account for (1) differences in transduction efficiency among the various recipient strains and (2) differences observed in the titers of the bacteriophage preparations. A control recipient marker, *cysC1021*, was used for this purpose. The *cysC* locus is located a distance from the selected "hybrid" region (Figure 1), and it is presumed that the genetic material of such a distal marker would be of *S. typhimurium* origin in the hybrids. In each test, the total number of recombinants obtained in a cross between wild-type *S. typhimurium* bacteriophage and *cysC1021*, representative of a homologous (*S. typhimurium*-*S. typhimurium*) transduction, served as the "control value." All recipient strains were also tested with wild-type (WT) *S. typhimurium* bacteriophage, and all bacteriophage prepared on the hybrids were tested with *cysC1021*. The

(Based on Sanderson and Demerec, 1965)

FIGURE 1.—Chromosome map of the isoleucine-valine region of *S. typhimurium*.

results of these crosses allowed for the adjustment of transduction counts to the control value. The following example serves as an illustration of the treatment of data. In one test the following values were obtained: WT bacteriophage $\times$ *cysC1021* = 843 (control value); WT bacteriophage $\times$ *pyrE125* = 554; TM-*metE17* (donor) $\times$ *cysC1021* = 615; TM-*metE17* (donor) $\times$ *pyrE125* = 420. To adjust the TM-*metE17* $\times$ *pyrE125* results to that of the control value, 420 is multiplied by the factors 1.52 (transduction correction for *pyrE* locus = 843/554) and 1.37 (TM-*metE17* bacteriophage correction = 843/615) to obtain a corrected value of 876. The ratio of the corrected value to the control value (876/843) is expressed as "percent homology," a term that was arbitrarily adopted to serve as an expression of the transduction efficiency relative to a homologous transduction with *cysC1021*. Values of 70% or higher imply that the particular locus in the hybrid strain is of *S. typhimurium* origin (*pyrE* in TM-*metE17* = 104%; reduced values (2 to 40%) are assumed to be reflections of differences in "microhomology" and are considered to be indicative of genic material of *S. montevideo* origin.

In the three-plate analysis used in this survey of TM hybrids, it is not unusual to obtain values of percent homology of 130 or higher. A number of such analyses was repeated, and these tests produced values much closer to 100% homology. The main testing efforts, however, have been devoted to verifying the low and intermediate values observed. Hence, any high values listed in the tables are accepted as 100% and do not represent the results of a thorough analysis. On the other hand, the low and intermediate values of homology that are presented and discussed have been shown to be reproducible and do reflect the results of comprehensive study.

Abortive transduction procedures: Amino-acid enriched minimal agar plates used contained all 20 of the amino acids, at a concentration of 20 μ g per ml, except arginine, cysteine, isoleucine, methionine and valine. Donor bacteriophage employed in these tests were P22 (LT-2) and TM-*ilvA3*. Transduction mixtures were plated to give approximately 100 stable recombinants per plate. The plates were incubated for 24 to 48 hr, then the abortive colonies were counted (24 $\times$ magnification, dissecting microscope) in a 2 cm ring pattern on each of two petri plates per recipient marker. Stable transductants were counted on every plate examined for abortive colonies. The ratio of the frequency of abortive transduction to the frequency of stable transduction was then computed, as well as the coincidence ratio for each recipient marker.

Somatic antigen agglutination tests: The TM-*ilv* and *metE* hybrids, as well as the parent strains, were tested with somatic antigen Type O antisera, groups B (*S. typhimurium*) and C1 (*S. montevideo*).

RESULTS

All 60 hybrid strains selected at the *ilv* and *metE* loci were found to be stable, i.e. no indication of heterogenotes. Seven months after initial isolation 12 hybrids were taken from nutrient agar slants at random and tested for the presence of auxotrophic segregants. No evidence of segregation was obtained. All 60 hybrids agglutinated with Salmonella antiserum Type O, group B, indicating that they retained the somatic antigen type of the *S. typhimurium* parent. Bacteriophage could be grown efficiently on 48 of the hybrids. The remaining 12 hybrids produced titers of 1×10^{10} or less, which were not high enough for use in the study.

The results of repeated transduction experiments with the TM-*ilv* and *metE* hybrid strains gave reproducible patterns of homology, and an illustration of the types of homology patterns obtained is given in Table 1. Each hybrid listed shows a different pattern of low homology. The results indicate the presence of genetic material of *S. montevideo* origin at the *ilv* loci in TM-*ilvA9*, at the *ilv* and *metE* loci in TM-*ilvA16*, and at the *metE* locus in TM-*metE16*. Hybrid TM-*metE11* shows not only a reduced homology for the *metE* locus but also a unique reduction at the *ilvA* locus. Such an intermediate value of homology, previously un-

TABLE 1

Analysis of various S. typhimurium-S. montevideo hybrids selected at the ilv and metE loci

Hybrid donors	116* <i>pyrE</i>	120 <i>pdx</i>	Recipient markers (<i>typhimurium</i>)							136 <i>purA</i>
			122				128			
			<i>ilvC</i>	<i>ilvB</i>	<i>ile</i>	<i>ilvA</i>	123 <i>metE</i>	<i>metF</i>	<i>argH</i>	
TM- <i>ilvA9</i>	101	95.7	18.0†	6.7	13.6	6.9	96.3	90.4	95.8	90.8
TM- <i>ilvA16</i>	129	153	12.0	14.9	11.9	9.9	32.8	109	106	...
TM- <i>metE16</i>	83.7	142	88.2	88.9	93.6	83.5	21.8	85.6	88.6	...
TM- <i>metE11</i>	120	86.8	98.7	90.9	83.4	58.8‡	16.7	111	98.2	103
TM- <i>metE12</i>	93.0	90.3	9.9	9.0	8.3	6.7	26.6	14.4	13.9	86.3
TM- <i>ilvA6</i>	19.5	88.3	11.6	9.0	4.6	5.1	72.5	74.0	74.7	119

Values expressed as "percent homology" (see METHODS).

* Map position, in minutes (SANDERSON and DEMEREC 1965). Markers included in a single map position are known to be located on the same transducing fragment.

† Italicized values indicate loci of *S. montevideo* origin.‡ This locus was adjacent to a region containing *S. montevideo* genes.

observed at the *ilvA* locus, is interpreted to mean that the *ilvA* locus, although not of *S. montevideo* origin, is adjacent to the "hybrid" region (DEMEREK and OHTA 1964). Three hybrid strains demonstrate this particular pattern of homology. These strains are of interest because they offer information on the orientation of the *ilv* cluster with respect to the *metE* locus. ARMSTRONG and WAGNER (1964) reported that the order of the *ilv* loci with respect to one another is: *ilvA ile ilvB ilvC*; however, the orientation of these loci with respect to the closely linked *metE* was not known. Recently ROTH and SANDERSON (1966) have provided evidence that the *ilvA* locus is closer to the *metE* locus than the *ilvC*, and the following sequence has been proposed: *ilvC ilvB ile ilvA metE*. The data on TM-*metE11* (and two other hybrids that show similar patterns) add to the evidence that the

FIGURE 2.—Diagram of *S. typhimurium*-*S. montevideo* hybrid types selected at the *ilvA*, *ilvC* and *metE* loci.

ilvA locus is indeed adjacent to *metE*. The results obtained with TM-*metE*12 show that *S. montevideo* genes have been integrated from the *ilv* region through the *met-arg* section. This is the longest continuous integration of *S. montevideo* genetic material found in this collection of hybrids and constitutes 4% of the length of the linkage map. Hybrid TM-*ilvA*6 is representative of a hybrid that contains a double integration of *S. montevideo* genes at the *pyrE* and *ilv* regions.

Figure 2 summarizes the type of data found in Table 1 for all 48 hybrids. The three most common types of integration involve the *ilv* or *metE* loci or both regions. These types constitute 69% of the hybrids and have integrated segments of < 1% to 4% of the chromosome map. As mentioned previously, two other hybrids (both selected at the *metE* locus) show patterns similar to TM-*metE*11 of Table 2. Secondary integrations may occur to the right or left of the selected loci, as shown in Figure 2. Twenty percent of the hybrids contain double integrations (particularly the *pyrE* and *ilv* regions). The data on hybrid TM-*ilvA*12 (second hybrid type from bottom in Figure 2) show that only a portion of the *met-arg* region contains *S. montevideo* genes. Transduction tests with this particular hybrid show low homology at the *metF* locus and high homologies at several of the *arg* loci (see Figure 1 for loci on the *met-arg* fragment). Additional data on this region of the chromosome are presented in Table 4.

It is possible to partition the TM-*ilv* and *metE* hybrids into three classes according to the locus at which they were selected. The average percent homologies for the *ilv* and *metE* loci for each of these classes are shown in Table 2. The means for the *ilv* loci for the three classes are approximately the same, varying in a range of 7 to 16%. Similar variation is also noted for the *metE* locus. The important percent homologies of this table are those where the recipient marker is identical to the selected locus. Thus, the mean homologies for *ilvA*8, *ilvC*16 and *metE*338 are 8.7%, 16.3% and 22.8%, respectively.

DEMEREK and OHTA (1964) demonstrated that transducing fragments of *S. typhimurium*-*E. coli* hybrids were transferred to auxotrophic recipients by P22 with the same efficiency as transducing fragments of *S. typhimurium* LT-2. This was used as evidence that inefficient transduction of markers by intergeneric hybrids was caused by an interruption of normal recombination and not by an inability of P22 to transfer the integrated *E. coli* genes to the recipient. A similar

TABLE 2

Means and standard errors of the means of reduced percent homologies for ilv and metE markers

Recipient markers	Hybrid class		
	TM- <i>ilvA</i>	TM- <i>ilvC</i>	TM- <i>metE</i>
<i>ilvA</i> 8	8.7 ± 0.98	11.6 ± 1.3	7.0 ± 1.2
<i>ile</i> -12	11.3 ± 1.4	14.9 ± 1.2	8.5*
<i>ilvB</i> 7	11.8 ± 1.6	14.7 ± 1.4	9.6 ± 1.4
<i>ilvC</i> 16	14.5 ± 1.0	16.3 ± 1.6	9.2 ± 1.6
<i>metE</i> 338	29.5 ± 2.2	36.3 ± 2.8	22.8 ± 2.0

* Number of samples was too small to calculate the standard error.

TABLE 3

*Abortive transduction test using LT-2 and hybrid TM-*ilvA3* as donor strains*

Recipient strain	Donor strain	Abortive colonies	Stable colonies	Abortive/stable	Coincidence ratio*
<i>cysG382</i>	A3†	137	179	0.77	0.85
	LT-2	198	217	0.91	
<i>pdx-4</i>	A3	157	148	1.06	0.95
	LT-2	127	113	1.12	
<i>ilvA8</i>	A3	292	6	48.70	36.89
	LT-2	309	234	1.32	
<i>ile-12</i>	A3	199	10	19.90	47.38
	LT-2	83	199	0.42	
<i>ilvB7</i>	A3	182	18	10.10	11.88
	LT-2	216	253	0.85	
<i>ilvC16</i>	A3	250	15	16.70	17.04
	LT-2	181	250	0.98	
<i>metF185</i>	A3	93	143	0.65	0.96
	LT-2	134	198	0.68	
<i>argA4</i>	A3	192	154	1.25	0.95
	LT-2	230	176	1.31	

* [Abortive/Stable (TM-*ilvA3*)]/[Abortive/Stable (LT-2)].† TM-*ilvA3*: hybrid region = *ilv* and *metE* genes of *S. montevideo* origin.

demonstration was attempted with bacteriophage prepared on an *S. typhimurium*-*S. montevideo* hybrid, and the results are listed in Table 3. If a transducing fragment is transferred to an *S. typhimurium* recipient with equal efficiency by both LT-2 and hybrid donor bacteriophage and if stable transduction is not reduced by the low homology of an *S. montevideo* gene, then the ratios of abortive transductants to stable transductants should be the same with both bacteriophage donors. The coincidence of these ratios should be 1.0. If the same conditions prevail but the presence of an *S. montevideo* gene on the fragment carried by the hybrid donor bacteriophage reduces the frequency of stable transduction, then the coincidence ratio will be much greater than 1.0. The coincidence ratios in Table 3 are approximately 1.0 for all markers except the *ilv* genes which have high coincidence ratios. Hybrid TM-*ilvA3* is known to contain *ilv* and *metE* genes of *S. montevideo* origin. Thus, the abortive transduction tests provide substantial evidence that donor fragments of TM hybrids are transferred to *S. typhimurium* recipients with the same efficiency as LT-2 donor fragments.

The loci of the *met-arg* and *pur-metA* transducing fragments are closely linked in the Salmonella linkage map (SANDERSON and DEMEREC 1965). The collection of TM hybrids, selected in this region of the chromosome, were isolated in an attempt to acquire information on the placement and orientation of the loci known to be located in this section of the chromosome. A total of 32 hybrids were selected at the *metF*, *argA* and *metA* loci; only two of the hybrids failed to produce bacteriophage preparations. The results of the analysis of the 30 TM hybrids are presented in Table 4. With the recipient markers used in this study, 11 patterns of homology are observed. A majority of the hybrids show integration

TABLE 4

Analysis of S. typhimurium-S. montevideo hybrids selected at the metF, argA and metA loci

Hybrid donors	Recipient markers (<i>typhimurium</i>)						Frequency	
	123* <i>metE</i>	128		129		136 <i>purA</i>		
		<i>metF</i>	<i>argA</i>	<i>purD</i>	<i>metA</i>			
TM- <i>metF4</i>	136	51†	105	...	129	...	1	
TM- <i>argA10</i>	139	133	44	103	127	...	1	
TM- <i>argA4</i>	118	28	22	87	118	...	4	
TM- <i>argA2</i>	120	106	27	22	33	133	3	
TM- <i>argA9</i>	85	16	18	10	23	131	9	
TM- <i>metF3</i>	96	21	26	47	117	...	2	
TM- <i>metA12</i>	...	101	112	24	17	112	4	
TM- <i>metA16</i>	...	80	87	70	44	23	1	
				128				
	120 <i>pdx</i>	122 <i>ilvA</i>	123 <i>metE</i>	<i>metF</i>	<i>argA</i>	129 <i>metA</i>	136 <i>purA</i>	
TM- <i>argA8</i>	130	10	24	25	19	24	87	3
TM- <i>metA18</i>	...	79	30	20	20	26	21	1
TM- <i>metA3</i>	...	99	29	82	28	20	149	1

Values expressed as "percent homology".

* Map position, in minutes (SANDERSON and DEMEREC 1965). Markers included in a single map position are known to be located on the same transducing fragment.

† Italicized values indicate loci containing genetic material of *S. montevideo* origin.

of *S. montevideo* genetic material at the loci contained in one or both of the transducing fragments. The pattern seen in TM-*metF4* (an intermediate value for the selected locus) is apparently the result of limited incorporation of *S. montevideo* genetic material because the *S. typhimurium* parent, *metF185*, is a multisite mutant and wild-type revertants have never been observed. The pattern of homology for TM-*argA10* is probably due to a similarly limited incorporation. Three hybrids, represented by TM-*argA2* in Table 4, show "partial hybridity" of the *met-arg* fragment, i.e. high homology for the *metF* locus and low homology for the *argA* locus. These data support the orientation for the *met-arg* fragment suggested by the pattern of homology of TM-*ilvA12*, i.e. *metE metF argA metA*. A more comprehensive study on the orientation and order of the seven loci located on the *met-arg* transducing fragment will appear as a separate report.

The following orientation for the *pur-metA* fragment is suggested by the patterns of homology obtained with two of the hybrids (represented by TM-*metF3* in Table 4): *argA purD metA*. However, analysis of more hybrids is required before a definite orientation can be assigned to this particular fragment.

The last three TM hybrids listed in Table 4 include patterns of larger integrations and a pattern of double integration. It should be stressed that in these studies the patterns of homology obtained are limited by the variety of recipient markers tested. On the basis of previous results, it is unlikely that TM-*metA18* and TM-*metA16* (Table 4) contain only *S. montevideo* genetic material in the region between the *metA* and *purA* loci (7 minutes of the linkage map). Additional tests with appropriate markers will be required before a more precise estimate of the *S. montevideo* incorporation in these two hybrids can be made.

The mean "low homology" values for the three selected loci, as calculated from the results of tests in which the recipient marker was identical to the selected locus of the TM hybrids, are: *metF* = 24.1, *argA* = 26.5 and *metA* = 24.1.

DISCUSSION

This study was undertaken to explore the feasibility of utilizing *S. typhimurium*-*S. montevideo* hybrids to obtain mapping information about the *Salmonella* chromosome. Hybrids were selected at the closely linked *ilv* and *metE* loci and at the *met-arg* and *pur-metA* regions of the *S. typhimurium* parent and were used in transduction to examine these particular sections of the *Salmonella* linkage map.

It should be pointed out that the term "homology" is usually a general reference to similarities and differences between the genetic material of related organisms. As referred to in this study, "homology" is measured by a recombination parameter and, thus, could be more aptly termed "recombinational homology."

One of the most pronounced characteristics of the summarized hybrid data (Figure 2 and Table 4) is that limited amounts of continuously integrated *S. montevideo* genetic material were found in most of the hybrids. The longest continuous incorporated *S. montevideo* fragment represented approximately 4 to 5% of the *Salmonella* chromosome (the data on TM-*metA18* in Table 4 are not included because of the lack of a more thorough analysis). In a study of *Salmonella*-*Escherichia* hybrids, FALKOW, ROWND and BARON (1962) discovered that segments of about 12% of the *E. coli* chromosome can be integrated into *Salmonella*. Also, several of the *Salmonella*-*Escherichia* hybrids isolated by DEMEREC and OHTA (1964) contained continuously integrated *E. coli* material that amounted to 9% of the *Salmonella* chromosome. It would be interesting to know if this apparent difference in recombination patterns (greater integration with intergeneric matings) is due to limited locations of identical homology where the chromosomes of organisms of two different genera can recombine with one another.

The data on the orientation of the *ilv* cluster with respect to the *metE* locus (as represented by TM-*metE11* in Table 1) are, at best, only suggestive. The low homology of the *metE* marker and the reproducible intermediate value at the *ilvA* locus imply that such hybrids contain *S. montevideo* genetic material that extends far enough into the *ilv* fragment to reduce transduction at the *ilvA* locus but not at the other *ilv* loci. The data, nevertheless, do support the following orientation, as proposed by ROTH and SANDERSON (1966): *ilvC ilvB ile ilvA metE*.

The analysis of the hybrid strains selected at the *metF*, *argA* and *metA* loci permitted a study of a section of the *Salmonella* chromosome that contains loci that are known to be located on two transducing fragments (*met-arg* and *pur-metA*). The results obtained with three hybrids (represented by TM-*argA2* in Table 4), as well as with TM-*ilvA12* (second pattern from bottom in Figure 2) provide the following orientation for the *met-arg* fragment: *metE metF argA*

pur-metA. A more detailed study on the order of the loci known to be located on the *met-arg* fragment will appear as a separate report. Two of the hybrid strains (represented by TM-*metF3* in Table 4) show patterns of hybridity that provide preliminary evidence that the orientation of the *pur-metA* fragment is: *argA purD metA*. However, more hybrids will need to be studied before any definite assignment can be made. The orientation data obtained on both of the transducing fragments, nevertheless, do serve to promote the use of hybrids to procure mapping data of this nature.

The results of the studies on the *ilv* region (containing a known cluster of four loci involved in the biosynthesis of isoleucine and valine) show that among the 44 hybrids containing *S. montevideo* genetic material at the *ilv* loci, none displayed a pattern of "partial hybridity" for these loci, i.e. some genes of *S. typhimurium* origin and others of *S. montevideo* origin. Available data on the four hybrids that show "partial hybridity" of the *met-arg* region produce the same pattern, i.e. each of the two clusters of loci (two *met* and four *arg*) contains only one type of genetic material. Thus, it appears that the probability of obtaining both *S. typhimurium* and *S. montevideo* genic material within a cluster of related loci is low. The results, particularly with the *ilv* fragment, may be an indication of the close linkage of clustered genes.

The data obtained with the TM hybrids are analogous to those reported on Salmonella-Escherichia hybrids (DEMEREK and OHTA 1964). It has been found that the clustered *ilv* genes (Table 2) and different alleles of the *ilvA* locus (unpublished data) display similar values of low homology. However, different regions of the chromosome, e.g. *ilv* and *metE*, do not necessarily show similar values for low homology, and these differences may represent variations of structural relatedness between the *S. typhimurium* and *S. montevideo* genes from one region of the Salmonella chromosome to another (DEMEREK and NEW 1965). The patterns of hybridity obtained in all work has served to confirm the linkage map published by SANDERSON and DEMEREK (1965), and the data support the contention that TM hybrids, like Salmonella-Escherichia hybrids, are useful in mapping studies.

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SUMMARY

A collection of *S. typhimurium*-*S. montevideo* hybrids were analyzed by transduction to obtain mapping information on the *ilv* and *met-arg* regions of the Salmonella linkage map. The results support the proposed orientation of the *ilv* loci, *ilvC ilvB ile ilvA metE*, and provide evidence that the orientation of the *met-arg* region is *metE metF argA purD-metA*.

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