Some Observations on Linkage Effects in Genetic Recombination in *Escherichia coli* K-12

BY R. C. CLOWES AND D. ROWLEY

*The Wright-Fleming Institute of Microbiology, St Mary's
Hospital Medical School, London, W. 2*

SUMMARY: Further recombination data have been obtained from matings involving different F^+ auxotrophic mutants of *Escherichia coli* strain K-12 with the same K-12 F^- auxotroph. Variations in the distribution of sugar fermentation markers among the progeny have been explained by the influence of the 'contra-selected' F^+ auxotrophic marker. It is suggested that the data supports the conception of a single linear chromosome in K-12, which fragments randomly during recombination.

Genetic investigation of inhibition of the K-12 strain of *Escherichia coli* by norleucine (Clowes & Rowley, 1954) showed that the segregation of six sugar markers among progeny from the matings of the same ($T^-L^-B_1^-$) F^- strain with two different ($T^+L^+B_1^+$) F^+ strains was comparable. This suggested that the same factors T^+ , L^+ , and B_1^+ were transferred from the F^+ to the F^- parent in each case, accompanied by transfer of some unselected markers carried by the F^+ strain, in proportions depending only upon their proximity to the loci T^+ , L^+ and B_1^+ . This interpretation, based on the one-way transfer of genetic material proposed by Hayes (1952), suggests that the essential and uncomplicated function of the F^+ parent is to contribute that fragment of the bacterial chromosome (or chromosomes) needed by the F^- parent to provide the complete genotype demanded by the selecting medium. However, further matings involving the same F^- strain showed differences in the distributions of these sugar markers among the progeny when compared with similar data from the previous matings. That these differences are due to complications introduced by the relative positions of the selected markers of the F^+ parent with respect to those of the F^- parent and to these sugar markers is shown in the following.

MATERIALS AND METHODS

Strains of Escherichia coli used

(a) **Wild type Strain K-12.** This strain grows on a minimal medium (Davis & Mingioli, 1950) unsupplemented by amino acids or vitamins. It is sensitive to streptomycin and ferments maltose, lactose, D-galactose, D-xylose, D-mannitol and L-arabinose (Mal^+ , Lac^+ , Gal^+ , Xyl^+ , Mtl^+ , $Arab^+$). This strain crosses with F^- strains and is thus F^+ .

(b) **Strain 58-161.** A methionine requiring mutant (M^-) of K-12, having the same fermentation pattern (Mal^+ , Lac^+ , Gal^+ , Xyl^+ , Mtl^+ , $Arab^+$) as the parent strain. Both F^+ and F^- types were used.

(c) **Strain W-677.** A threonine, leucine and thiamine (T^- , L^- , B_1^-) requiring auxotroph of K-12. This strain was derived from strain W-1 (T^- , L^- , B_1^- ,

Mal⁻, Lac⁻) by incorporating the inability to ferment galactose, xylose, mannitol and arabinose in a series of separate steps, the Gal⁻ locus being designated Gal₅⁻ (E. M. Lederberg, personal communication). F⁺ and F⁻ forms of this mutant were used together with the streptomycin-resistant mutant (St^R) of the F⁻ form (W-677/St^R/F⁻). The genetic markers carried by W-677 are allelic with those of strain W-945, which has been the basis of other genetic investigations (Cavalli, Lederberg & Lederberg, 1958), for the loci T, L, B₁, Mal and Lac since both strains are derived from W-1. The St and Xyl loci have been assumed to be allelic and the allelism of the Xyl loci is supported by recombination data (L. L. Cavalli-Sforza, personal communication).

(d) The other auxotrophic strains of K-12 (designated 'J' strains) used in this investigation are listed in Table 1.

Table 1. *Derivation and requirements of 'J' strains of Escherichia coli*

Strain:	J4-5*	J5-3*	J5-10*	J6-2*	J13-4
Derivation:	Double mutant by irradiation	From the same proline-requiring mutant		From J4-5	From 58-161
Requirements:†	Tryptophan, Histidine (Tr, H ⁻)	Proline, Methionine (P ⁻ , M ₂ ⁻) Proline, Histidine (P ⁻ , H ⁻)		Proline, Histidine, Tryptophan (P ⁻ , H ⁻ , Tr)	Methionine, Isoleucine or α-aminobutyric acid (M ₁ ⁻ , iL ⁻ or α-aB ⁻)

* Derived from K-12 passing through a Lac⁻ mutation (B. D. Davis—personal communication).

† All growth factors required in minimal concentrations of 20 µg./ml.

All five 'J' strains are able to ferment maltose, D-galactose, D-xylose, D-mannitol and L-arabinose. In addition, J5-3, J5-10 and J13-4 ferment lactose but J4-5 and J6-2 are unable to ferment it. All strains produced recombinants with W-677/F⁻ and were inferred to be F⁺. The presence of prototrophic recombinants from the mating of J5-3 (P⁻M⁻) with 58-161 (M⁻) on MM was taken to indicate that the methionine markers carried by these strains are non-allelic. For convenience, the M⁻ locus of 58-161 will be designated M₁⁻, and that of J5-3, M₂⁻.

Media and recombination techniques were the same as described previously (Clowes & Rowley, 1954).

Biochemical tests. Master plates of purified recombinant colonies, prepared as described previously (Clowes & Rowley, 1954), were used for the testing of fermentation reactions and auxotrophic requirements by the replica plating method of Lederberg & Lederberg (1952), all sugar fermentation tests being carried out on eosin-methylene blue medium.

RESULTS

Table 2 shows the segregation of the six sugar markers Mal₁, Lac₁, Gal₅, Xyl, Mtl and Arab among the recombinants resulting from the matings of W-677/F⁻ (or W-677/St^R/F⁻) with K-12, 58-161/F⁺ and the five 'J' strains on minimal

Table 2. Segregation of sugar fermentation markers in progeny of matings of various F^+ strains with strain W-677/ St^B/F^- or W-677/ F^-

F ⁺ strains are Mal ⁺ Gal ⁺ Xyl ⁺ Mtl ⁺ Arab ⁺ . W-677 is T ⁻ L ⁻ B ₁ ⁻ Mal ⁻ Lac ⁻ Gal ⁻ Xyl ⁻ Mtl ⁻ Arab ⁻ .												
Group	F ⁺ parent	F ⁺ markers	Selecting medium	Colonies pro-trophic (%)	No. colonies examined	Recombinants fermenting (%)						
						Mal	Lac	Gal	Xyl	Mtl	Arab	
A	K-12	St ^s	MM+St		226	4	26	1	13	12	0	
			MM+St+B ₁	13	183*	1	35	0	3	2	3	
	58-161	M ₁ ⁻	MM		240	6	27	4	12	12	5	
			MM+B ₁	11	204*	1	37	1	1	1	2	
B	J4-5	H ⁻ , Tr ⁻ , Lac ⁻	MM		351	23	—	8	27	26	10	
			MM+B ₁	27	142*	5	—	4	5	5	4	
	J5-10	P ⁻ , H ⁻	MM		271	24	3	9	26	31	18	
			MM+B ₁	25	223*	8	3	4	7	8	3	
C	J6-2	P ⁻ , H ⁻ , Tr ⁻ , Lac ⁻	MM		122	20	—	7	20	22	9	
			MM+B ₁	27	73*	3	—	0	2	2	0	
	J5-3	P ⁻ , M ₂ ⁻	MM		410	12	5	6	10	11	8	
			MM+B ₁	16	173*	3	2	2	1	2	2	
D	J13-4	M ₁ ⁻ , iL ⁻ or α-aB ⁻	MM		376	8	33	9	5	4	8	
			MM+B ₁	0	100*	1	56	11	3	3	8	

MM = minimal medium; B_1 = thiamine supplement; St = streptomycin supplement.

* Fermentation figures quoted for B_1^- recombinants only

Table 3. Effect of supplementing recombination plates with F⁺ growth factors on segregation of sugar markers among progeny from matings of various F⁺ strains with strain W-677/F⁻ or W-677/St^a/F⁻

F ⁺ parent	Selecting medium	'Contra selected' markers	Ratio no. re-combinants to those on MM	Prototrophic re-combinants (%)	No. of re-combinants examined	Class of re-combinant	Recombinants fermenting (%)					
							Mal	Lac	Gal	Xyl	Mtl	Arab
J 4-5	MM + H	Tr	1.2	100	96	Pr	24	—	7	30	32	14
	MM + Tr	H	1.0	100	97	Pr	28	—	3	33	34	10
J 5-3	MM + P	M ₃	1.3	70	35	P ⁻	14	75	10	28	24	14
	MM + M	P	3.1	35	160	M ⁻	31	3	10	34	39	15
J 5-10	MM + P	H	1.5	66	65	P ⁻	11	60	15	31	32	15
	MM + H	P	0.8	100	121	Pr	27	6	17	30	30	16
J 6-2	MM + P	H, Tr	2.1	70	37	P ⁻	14	—	8	16	16	5
	MM + H	P, Tr	1.1	100	85	Pr	22	—	13	26	23	12
	MM + P + H	Tr	2.0	80	41	P ⁻	17	—	10	20	20	7
	MM + St + P + H + Tr	St	0.8	63	80	Pr	4	—	1	10	17	1
J 13-4	MM + M	iL (or α-aB)	2.5	50	167	M ⁻	11	40	4	5	4	8
	MM + iL	M ₁	1.5	55	61	iL ⁻	17	34	11	23	28	8
	MM + St	St, M ₁ , iL (or α-aB)	0.15	100	49	Pr	6	30	0	8	6	0
	MM + St + M + iL	St	0.2	65	50	Pr	6	21	3	9	6	3
	MM + α-aB	M ₁	0.7	100	50	Pr	12	26	8	2	0	8

Pr denotes prototrophic recombinants.

MM = minimal medium. Supplements: H = histidine; Tr = tryptophan; P = proline; M = methionine; St = streptomycin; iL = isoleucine; α-aB = α-aminobutyric acid.

medium (MM) and minimal medium supplemented with vitamin B₁ (MM + B₁). These matings may be conveniently separated into four groups of similar segregation patterns. It is apparent from these different groups that the genotype of the F⁺ parent has a significant effect on the genotype of the recombinants, under the experimental conditions used. In order to investigate this effect more fully, certain matings were repeated and seeded on minimal media supplemented with the individual growth factors of the F⁺ parent, in cases where this parent has more than one growth requirement. The results of such matings are summarized in Table 3. The effect of the addition of the F⁺ growth factors shows that the supplements methionine, isoleucine and proline affect the recombination rate, the growth requirements of the recombinants and their sugar fermentation pattern. All recombinants arising on MM supplemented with either histidine or tryptophan were capable of growth on un-supplemented MM and their sugar fermentation patterns were similar to that arising on MM alone (Table 2).

Table 4. *Segregation of sugar markers from the mating*
W-677/F⁺ × 58-161/F⁻

Selecting medium	Ratio recombination rate to that on MM	Recombination rate growing on MM (%)	No. colonies tested for sugar fermentation	Progeny not fermenting (%)					
				Mal	Lac	Gal	Xyl	Mtl	Arab
MM	1	100	412	21	4	8	26	25	7
MM + B ₁	1.9	55	130*	23	5	4	40	34	4

* = B₁⁻ recombinants; MM = minimal medium; B₁ = thiamine supplement.

DISCUSSION

One of the difficulties in studying the genetics of *Escherichia coli* K-12 has been to account for the position of the known markers on the basis of one or more straight or branched chromosomes. The present data are discussed on the basis of a multichromosome system and then on the basis of limited transfer of fragments of a single chromosome.

Multi-chromosome hypothesis. Watson & Hayes (1953) suggested that the present markers could be arranged in three linkage groups, characterized by the loci St-Mal₁, M₁-B₁ and TL-Lac₁, and Hayes (1953*b*) suggested that recombination data could be interpreted on the basis of at least three discrete chromosomes. In recombination a one-way transfer of usually only one of the chromosomes of the F⁺ parent was postulated. If this transferred chromosome carries one selected marker only, then it follows from the above hypothesis that other loci carried by this chromosome should appear in the progeny to the extent of 50–100 %, depending upon whether there is no linkage (50 %) or complete linkage (100 %) of the unselected marker in question with the selected marker. Other markers on any chromosome not carrying a selected marker should appear in the progeny at a small random rate, depending only upon the frequency with which this chromosome happens to be transferred from the F⁺ parent in addition to that chromosome carrying the selected markers from the F⁺ strain.

In an example quoted by Watson & Hayes, the cross of W-677/F⁺ × 58-161/F⁻ on MM + B₁ where only the chromosome carrying the M₁⁺ locus is being selected, there was a transfer of 61 % B₁ and 21 % Mtl. The loci B₁ and M₁ are postulated to lie on the same chromosome, and Mtl on another, yet there does not appear to be any clear-cut difference between the so-called random transfer of Mtl and the linked transfer of B₁.

In a mating of J5-3 (P⁻M₂⁻B₁⁺T⁺L⁺) and W-677/F⁻ (P⁺M₂⁺B₁⁻T⁻L⁻) on MM + M + B₁ we found that of 82 recombinants examined 53 were M₂⁺B₁⁻, 22 were M₂⁻B₁⁺, 6 were M₂⁺B₁⁺ and 1 was M₂⁻B₁⁻. This shows that the M₂ locus is also linked to B₁ and by the Watson & Hayes hypothesis it is situated on the same chromosome as both M₁ and B₁.

A consideration of some of the present data shows that the proposed random transfer of some markers is considerably affected by the transfer of a supposedly non-homologous chromosome. For example, in the cross J5-3 × W-677/F⁻ on MM (Table 2) the chromosomes carrying the selected markers (B₁⁺, T⁺ and L⁺) would need to be contributed by the F⁺ parent to all viable recombinants. However, since the F⁺ chromosome carrying B₁⁺ also carries the M₂⁻ marker of the F⁺ parent, crossover in this chromosome is necessary to secure a viable recombinant, which must carry the loci M₂⁺ and B₁⁺, i.e. the locus M₂⁻ is 'contra-selected'. On MM + M, the same chromosomes are transferred from the F⁺ parent, but the class of progeny which carry the M₂⁻ locus is now viable. If this class is examined (M₂⁻ colonies, Table 3) the percentage transfer of the loci Mal, Xyl and Mtl is seen to differ significantly from that of the M₂⁺ class of recombinants (see Table 2). In this mating, the transfer of 10-12 % of Mal, Mtl and Xyl on MM (Table 2) would be supposed random on Watson & Hayes's interpretation. However, by transferring an additional piece of another F⁺ chromosome (that containing the M₂⁻ locus) the simultaneous transfer of these sugars is increased to 31-39 %. The inference must be that the transfer of these sugar markers is not random, and furthermore that in some way they are linked with M₂ and thus to B₁.

It is evident on consideration of the recombination data available (Lederberg, 1947; Lederberg, Lederberg, Zinder & Lively, 1951; Cavalli *et al.* 1953; Hayes, 1953*a*; Rothfels, 1952) that the known markers tend to lie in the linkage groups as postulated by Watson & Hayes (1953), but that these form discrete chromosomes is by no means certain as the present data show.

Limited transfer of fragments of a single chromosome. Previous work, as well as the above result, can be interpreted on the basis of a single linear chromosome which fragments at random during recombination, a possibility considered by Watson & Hayes (1953). The three groups of markers must then be considered to occupy small separated segments of this single chromosome. It is necessary to visualize fragmentation of this chromosome at some time during recombination since not all markers are transferred to an extent of 50-100 % even when the F polarity is reversed (e.g. in the case of Gal and Arab in the two crosses of 58-161 and W-677; Tables 2 and 4). The appearance of any unselected marker of the F⁺ parent in the recombinants must depend on the frequency of its incorporation on the same chromosomal fragment as

a selected marker from the F^+ parent, this transfer being proportional to the proximity of the two loci. This implies that any transfer, however small, is due to some degree of linkage.

On this concept of limited transfer, the experimental evidence may be interpreted as follows. In the cross $J5-3 (P^-M_2^-) \times W-677/F^-$ on MM (Table 2) the loci T^+ , L^+ and B_1^+ are selected from $J5-3$ but the M_2^- locus is contra-selected and the transfer of sugar markers Mal, Xyl and Mtl is small (Mal 12 %, Xyl 10 %, Mtl 11 %). On MM + M however (Table 3), where the M_2^- locus can be carried by viable recombinants, an increased proportion of these latter progeny possess the sugar markers (Mal 31 %, Xyl 84 %, Mtl 39 %), the increased transfer being presumably due to their linkage with M_2 . The similarity in the distribution of these sugar markers (Table 2) among progeny from matings on MM of group 'B', where all F^+ parents carry the M_2^+ allele, and those of $J5-3$ on MM + M supports the explanation that the differences on MM are due to contra-selection of the M_2^- locus. The transfer of the markers Mal, Xyl and Mtl are of a similar order in the crosses both of 58-161/ F^+ and $J5-3$ with $W-677/F^-$ on MM (Table 2), which confirms the proximity of the M_1 and M_2 loci. It seems likely that contra-selection for either M_1^- or M_2^- is the reason for the lower transfer in both these crosses compared with the analogous results within group 'B'.

That strain K-12 also resembles strain 58-161 in this respect could be attributed to the contra-selection of the St^s locus which has also been shown to be linked to Mal, Xyl and Mtl by previous workers (Cavalli *et al.* 1953). Further evidence to support this suggestion can be got from consideration of the cross of $J6-2 \times W-677/St^s/F^-$ (Table 3). On MM the progeny from this mating resembles that of other group 'B' strains, but when contra-selection for the St^s locus is applied, by selecting on MM + St + P + H + Tr , the transfer of markers is reduced, and is similar to that observed in the cross involving K-12 on MM + St .

The differences in Lac^+ transfer between crosses involving either $J5-3$ or $J5-10$ and those in which either 58-161 or K-12 is the F^+ parent may also be explained by the effects of contra-selection, in this case of the P^- locus. In the mating of $J5-10$ with $W-677/F^-$ on MM $\times$ P (Table 3), there is a larger transfer of Lac^+ among the P^- recombinants (60 %) than among the P^+ recombinants (6 %). This suggests P is closely linked with Lac and that when P^- is contra-selected, as in $J5-3$ or $J5-10 \times W-677/F^-$ on MM, a reduced transfer of Lac^+ to the recombinants results.

The matings of $J13-4 (iL^- \text{ or } \alpha\text{-}aB^-, M_1^-)$ are more complex and not entirely explicable. The main distinctions in the progeny from this strain are the very small transfer of the markers Xyl and Mtl on MM which is less than from strains of group A, and the lack of variation in the transfer of these markers on supplementing with M. This would be explained by a close linkage of iL to Xyl and Mtl and the location of this marker between M_1 and the Xyl-Mtl pair. In several recombinations selected on MM + iL , no iL^- progeny could be detected. However, in other recombinations iL^- recombinants were detected but the clones were always much smaller than those of any other class of recombinants.

The distribution of sugar markers among these iL^- recombinants shown in Table 3 shows an increase of transfer of the markers Mal, Mtl and Xyl in proportions similar to that among recombinants where the M_1^- locus is not contra-selected, which would be expected were the iL locus situated between the M_1 locus and the loci Mal, Xyl and Mtl. An attempt was made to investigate the linkage of M_1 and iL by seeding the cross J13-4 $\times$ W-677/St³/F⁻ on MM+St+ iL +M. On this medium all recombinants fell into the two classes St³ $iL^+M_1^+$ and St³ $iL^+M_1^-$ and this lack of iL^- recombinants was confirmed by the fact that recombinants arising on MM+St+ iL were all prototrophic. It was thought that this was due to the closer linkage of St and iL which would prevent their separation. No effects on recombination rate, growth requirements and sugar patterns of the progeny of the cross J13-4 $\times$ W-677/F⁻ were found by supplementing with α -aminobutyric acid. However, the alternative growth requirement of isoleucine or α -aminobutyric acid of this auxotroph makes interpretation of its mating difficult.

It should be emphasized therefore that the division of the F⁺ strains used in this study into the four groups in Table 2 does not imply any fundamental difference in the mechanism of genetic transfer among these strains. The differences in the distribution of the various markers among their recombinants probably arise as a result of contra-selection of F⁺ markers imposed by the selecting medium. If all contra-selection were removed it is likely that the transfer of unselected markers would be comparable in all crosses and would reflect only the various linkages of these markers to T⁺, L⁺ and B₁⁺, the common selecting markers in all crosses.

Construction of a linkage map

On the basis of limited transfer, the data presented and that of other workers may be interpreted to construct a linkage map of the K-12 chromosome as follows.

In all the matings involving W-677/F⁻ on B₁ media (Table 2), the B₁⁺ recombinants form *c.* 20 % of the progeny. We may thus infer that (TL) and B₁ are linked. Of the other quoted markers, selection for TL brings over only a significant amount of Lac, as shown by the crosses of group 'A' on B₁ media, and supports the findings of Watson & Hayes (1953). The introduction of selection for B₁ on MM reduces the transfer of Lac from *c.* 36 to *c.* 26 %. This reduction appears to be significant ($\chi^2=7.3$) and suggests that Lac₁ and B₁ lie on opposite sides of TL.

The large reduction in the transfer of Xyl and Mtl in all crosses on eliminating selection for B₁ (Table 2) implies the linkage of these sugar markers with B₁ and not with TL, although the relative positions of the two sugar markers cannot be fixed unequivocally, and suggests the order

$$(Mtl-Xyl) - B_1 - TL - Lac_1.$$

That M_1 and B₁ are linked has been shown by many other workers (Hayes, 1953*a*; Cavalli *et al.* 1953; Watson & Hayes, 1953) and this is supported by the appearance of *c.* 50 % of the one marker when the other is selected (Tables 3

and 4). The relative positions of M_1 and B_1 is suggested by consideration of the transfer of Xyl and Mtl when either M_1 or B_1 is selected. If we suppose M_1 to be situated to the right of B_1 ; in the cross $W-677/F^+ \times 58-161/F^-$ we would expect on MM where the M_1 locus only is selected that there would be little transfer of Xyl and Mtl but that on B_1 medium a larger transfer would result. If M_1 were situated between B_1 and Mtl+Xyl, however, the reverse effects would be expected, and the transfer on MM should be little affected on supplementing with B_1 . The data (Table 4) show transfers of 26 % Xyl, 25 % Mtl on MM and 40 % Xyl, 34 % Mtl on MM + B_1 . The figures for Mtl do not indicate any significant increase on supplementing with B_1 ($\chi^2=3.85$) but those of Xyl suggest that the variation is not random ($\chi^2=8.5$). These results are difficult to reconcile with either alternative. However, the fact that the transfer of only one of these closely linked markers seems to be increased on selecting for B_1 , and that when M only is transferred there is still a large amount of transfer of both markers throws doubt on the location of B_1 to the right of M. Similarly, in the cross $J5-3 \times W-677/F^-$, the situation of M_2 to the right of B_1 would allow a significant transfer of these sugar markers on MM and the transfer should be little affected on supplementing with M. If M_2 were to the left of B_1 , the transfer on MM should be small and greatly increased on MM + M. The results shown in Tables 2 and 3 suggest that selection for M_2 has a significant effect on the transfer of either Xyl ($\chi^2=36$) or Mtl ($\chi^2=44$). The location of M_2 to the left of B_1 would appear the only satisfactory arrangement. It should be pointed out however that as well as the discrepancy for Xyl transfer in the first mating noted above, the fact that there is a residual transfer of 10 % Xyl and 11 % Mtl on selection for B_1 and contra-selection for M_1 or M_2 would require an unusually high degree of double crossover. The unequivocal location of M_2 to the left of B_1 , and the fact that contra-selection of either of the M_2 or M_1 loci in the presence of selection for B_1 as in the crosses of either J5-3 and 58-161 with $W-677/F^-$ on MM (Table 2) gives similar transfers of Xyl and Mtl suggest that the most probable location of both M_1 and M_2 are to the left of B_1 .

Tables 3 and 4 show that when M_1 is selected or when B_1 is selected with no contra-selection for M_2 , a small transfer of both Gal and Arab is effected. This suggests that these fermentation loci which have been shown to be closely linked with the locus for resistance to norleucine inhibition, NI (Clowes & Rowley, 1954) are linked to M_1 and M_2 rather than B_1 or TL and implies the arrangement: (NI-Gal₅-Arab)-(Xyl-Mtl)-(M₁-M₂)-B₁-TL-Lac₁.

The Mal₁ locus is seen to be linked to M_1 from Table 4. Its percentage transfer and the results of Cavalli *et al.* (1953) suggest it is linked to B_1 less than Xyl. However, in matings where B_1 is transferred, the application of contra-selection for M_2 would be expected to reduce to the greatest amount those markers nearest to M_2 . The results, shown in Table 3 on contra-selecting for M_2 in the cross of J5-3, give a reduction in transfer of Mal 31 to 12 %, Xyl 34 to 10 % and Mtl 39 to 11 % and do not seem to suggest any difference in the degree of linkage of either Mal₁, Xyl or Mtl with M_2 . The relative position of Mal₁ with respect to Xyl

and Mtl cannot therefore be postulated exactly. The linkage St-Mal₁ has been shown by many workers. The decrease in transfer of St in an analogous manner to that of Mal, Xyl and Mtl when M₁ is contra-selected has been noted in a previous paper (Clowes & Rowley, 1954) where in the cross J5-10/St^B × W-677/F⁻ on MM, 27 % of the recombinants were St^B as compared with a transfer of 10 % in the parallel 58-161/St^B/F⁺ cross. The linkage between St and Mal and Xyl, quoted by Cavalli *et al.* (1953) would suggest the inclusion of these markers in the order:

(Nl-Gal₅-Arab)-(St-Mal₁)-(Xyl-Mtl)-(M₁-M₂)-B₁-TL-Lac₁.

In the cross of K-12 × W-677/St^B/F⁻ on MM + St, the similar degree of transfer of Mal, Xyl and Mtl would be explicable by the linkage of St to these markers to the same extent as M₁, together with contra-selection of the St^s locus. This is substantiated in the cross of J6-2 (Table 3) where contra-selection for the St^s locus causes a reduction in transfer of these sugar markers to values similar to the K-12 cross.

The removal of contra-selection for proline in the crosses of J5-10 (Table 3) allows a greater transfer of Lac, from which linkage of these two loci is inferred; this would explain the small degree of transfer of Lac in crosses involving P⁻F⁺ strains on MM. The data are compatible with the location of P between TL and Lac₁, since imposing contra-selection for P on the selection for TL reduces the transfer of Lac from *c.* 24 to 3 %.

The results of the crosses involving J13-4 can be interpreted if the locus iL is between the M₁-M₂ and the Xyl-Mtl markers. However, in view of the alternative growth requirement of this auxotroph (M-iL or α-aB), this result cannot be more than tentative.

From the above considerations, it seems likely that the investigated markers lie in the order:

(Nl-Gal₅-Arab)-(St-Mal₁)-(Xyl-Mtl)-iL-(M₁-M₂)-B₁-TL-P-Lac₁.

The brackets imply uncertainty as to the relative positions of the markers enclosed.

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