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Histidine Regulatory Mutants in *Salmonella typhimurium*

V.† Two New Classes of Histidine Regulatory Mutants

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Two new genetic classes of histidine regulatory mutants have been identified among mutants of *Salmonella typhimurium* resistant to the histidine analog 1,2,4 triazole-3-alanine. They bring to six the number of known histidine regulatory genes. One of the new classes, *hisU*, maps between *ilvC* and *pyrE* genes in conjugation crosses, and the other, *hisW*, is located between (*purF-aroD*) and *metG* loci. Their function in the control of the histidine operon is still unknown.

1. Introduction

Four genetic classes of histidine regulatory mutants have been identified among mutants of *Salmonella typhimurium* selected for resistance to the histidine analog 1,2,4 triazole-3-alanine (Roth, Antón & Hartman, 1966). TRA§ is incorporated into protein and also represses the synthesis of the histidine biosynthetic enzymes (Levin & Hartman, 1963).

Two of the regulatory genes are concerned with the synthesis of histidyl-tRNA. One, *hisS*, which maps between the markers *guaA* and *strB* (Roth *et al.*, 1966), is the structural gene for histidyl-tRNA synthetase (Roth & Ames, 1966), and the other, *hisR*, located between *metE* and the *ilv* cluster (Roth & Hartman, 1965), is involved in the synthesis of functionally active histidine-specific tRNA (Silbert, Fink & Ames, 1966).

Mutants of one of the remaining classes, *hisO*, are characterized as operator constitutive mutants by their location at one end of the histidine operon (Roth *et al.*, 1966), their dominance over the wild-type allele and the fact that they affect only the structural genes located in position *cis* to the *hisO* mutation (Fink & Roth, 1968). The last class is *hisT*, which maps between the *purF* and *aroD* loci, and the function of which is still unknown (Roth *et al.*, 1966).

Each of these four classes is defined on the basis of linkage, in transduction tests with markers of the *Salmonella* chromosome. Several histidine regulatory mutations however, did not show linkage, in transduction tests, with any of those markers and, therefore, were not classified into any of the four groups (Roth *et al.*, 1966).

Those mutations have now been mapped by conjugation crosses. They belong to two new genetic classes of histidine regulatory mutants and have been designated *hisU* and *hisW*.

† Paper IV in this series is Fink, Klopotowski & Ames, 1967.

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§ Abbreviation used: TRA, 1, 2, 4 triazole-3-alanine.

2. Materials and Methods

(a) *Bacterial strains*

The histidine regulatory mutants used are derivatives of *Salmonella typhimurium* LT2 *ara-9*, except *hisU1206* which was isolated in a derivative (*hisH107*) of strain LT7. The methods used for their isolation have been described by Roth *et al.* (1966). Mutant *hisR1827* was induced by diethyl sulfate in *hisH107* and selected for resistance to TRA.

Mutant strains *purF145*, *aroD5*, *metE338*, *ilvA8*, *strB57* and *pyrE123* were kindly provided by Dr M. Demerec. Strains SB109 (*metE338 ilvC401 ara-9* streptomycin resistant (*str*)), SB560 (*purF145 aroD5*), SB751 (*aroD5 hisT1529*) and SB110 (*metE338 hisR1203*) were constructed and donated by Dr J. R. Roth. Strain SR305 (*hisD23 gal-50* HfrA) was received from Dr K. E. Sanderson and is described in Sanderson & Demerec (1965). Strain SB93 (*hisE35 purG302* (formerly *purG2*) *metE319 str*) has been described by Hartman, Rusgis & Stahl (1965).

Strain SB622 (*metE338 hisU1819 str*) was prepared by crossing the donor *hisU1819* (F'*lac*$^+$) with recipient SB109 (*metE338 ilvC401 ara-9 str*). Recombinants with the *ilvC*$^+$ *str* markers were selected on minimal medium containing methionine, streptomycin and 2% dextrose and examined for the unselected markers *metE* and *hisU*. A wrinkled colony (*hisU1819*) which required methionine (*metE338*) was obtained. Strain SB621 (*ilvC401 hisU1819 str*) was isolated by the same procedure among recombinants carrying the markers *metE*$^+$ *str* selected in the same cross as SB622. Strains SB627 (*metE338 hisR1813 str*) and SB628 (*ilvC401 hisU1820 str*) were prepared in a similar way using as donor the F'*lac*$^+$ derivative of the corresponding histidine regulatory mutant. Strain SB565 (*pyrE123 hisU1819 str*) is the quadruple cross-over recombinant obtained in the cross described in Table 8.

Strain SB563 (*hisE35 metG319 hisW1824 str*) was obtained by crossing donor *hisW1824* (F'*lac*$^+$) with strain SB93 (*purG302 hisE35 metG319 str*). Recombinants carrying the markers *purG*$^+$ *str* were selected and analyzed by replica plating for unselected markers, including wrinkled colony morphology on nutrient agar containing 2% glucose. A recombinant which required only histidine (*hisE35*) and methionine (*metG319*) and showed a wrinkled colony phenotype (*hisW1824*) was obtained. Strain SB562 (*hisW1824 purF145 aroD5 metG319 str*) is the recombinant of class 7 obtained in the cross SB563 × SB560 (F'*lac*$^+$) reported in Fig. 3.

The mutation to streptomycin resistance of strain SB558 (*purF145 str-125*) was induced by diethyl sulfate in mutant *purF145*, and was selected on medium containing 1·2 mg streptomycin/ml. to secure high-level resistance.

The F'*lac* factor used to make donor strains was a thermosensitive episome originally present in *Escherichia coli* strain E7023 (*pyrF try his str-r su-2*$^+$ and carrying *lac* mutation YA536 both in the chromosome and in F'*lac*) (supplied by Dr J. Beckwith). The F'*lac*$^-$ thermosensitive episome was transferred to an amber suppressor-carrying *Salmonella* strain by selection for *lac*$^+$ colonies, and then non-selectively transferred to *trp-109* which does not carry a suppressor mutation. A reversion to *lac*$^+$ was selected and found to be carried on the episome. This strain, SB394, was obtained from Dr David Berkowitz, who constructed it.

The genetic symbols and nomenclature used are described in Sanderson & Demerec (1965) and Demerec, Adelberg, Clark & Hartman (1966).

(b) *Media*

Media used have been described by Roth *et al.* (1966); those for scoring the wrinkled colony morphology characteristic of de-repressed strains contained 2% dextrose. When needed, streptomycin was used at a concentration of 1·2 mg/ml.

Minimal medium used for the selection of F'*lac*$^+$ strains was a modified A medium (Hartman, 1956) lacking citrate and containing as only carbon source 1% lactose (A-Lac).

(c) *Construction of F'*lac$^+$ *strains*

The F'*lac*$^+$ episome of strain SB394 was transferred to each of the histidine regulatory mutants studied in this work and to several other mutants.

The strain to be made F'*lac*$^+$ was grown overnight in broth, centrifuged and the cells resuspended in liquid minimal medium. A portion was spread on a plate of A-Lac medium supplemented with the amino acids required for the recipient but lacking some required for the F'*lac*$^+$ donor, usually tryptophan. As *Salmonella* is *lac* negative, the recipient cannot grow on this medium unless it acquires the *lac*$^+$ character from the donor.

The F'*lac*$^+$ donor was streaked on the recipient lawn, and the plate was incubated for 2 days at 37°C. Several of the large *lac*$^+$ colonies appearing along the streak were picked up, purified through several re-isolations on the same selective medium and used, then, as donors.

(d) *Transduction and conjugation tests*

Transductions were carried out as described by Roth *et al.* (1966).

Strains to be used in conjugation tests were grown in broth, with aeration, to a density of about 3×10^8 cells/ml. Matings were performed by mixing in a test tube 6 ml. of donor with 4 ml. of recipient. The mixture was immediately filtered on a sterile Millipore filter (HA, 0·45 μ, 25 mm) which was placed then on a fresh nutrient agar plate at 37°C for 2 hr.

Following this incubation, the filter was put in a tube containing 2 ml. of liquid minimal medium without dextrose, and the cells were resuspended by shaking with a vortex mixer. Different amounts of this suspension were plated on the selective media.

When the donor was an Hfr strain, the proportion of donor to recipient in the mixture was changed to 1:1, and the mating suspension was diluted from 10 to 100 times before plating.

The mapping of regulatory loci was done by scoring for the wrinkled colony morphology that characterizes strains having de-repressed synthesis of histidine biosynthetic enzymes when grown on media with a high content (2%) of fermentable carbon source (Roth *et al.*, 1966).

3. Results

(a) *The homeless mutants*

Some TRA-resistant mutants have faulty control of the histidine operon; they synthesize the histidine enzymes, even when growing in repressed conditions, at a level several-fold higher than that of the wild-type strain (Roth *et al.*, 1966). A large number of this type of TRA-resistant mutants was isolated and studied by Roth *et al.* (1966). By transduction tests, most of them were classified as belonging to four genetic classes: *hisO, hisR, hisS* and *hisT*.

A few, however, did not fit into any of the four known genetic classes. As seen in Table 1, *hisU1817, hisU1818, hisU1819, hisU1820, hisW1821, hisU1822, hisU1823, hisW1824* and *hisW1825* mutations did not show cotransduction with any of the markers that distinguish the four classes.

Like constitutive mutants belonging to the four previously known groups, "homeless" mutants have high levels of the histidine enzymes (Table 2); and, also like those mutants, the levels increase even further when they are placed in conditions of histidine deprivation (Table 3).

(b) *Identification of* hisU *and* hisW *genes*

The lack of linkage displayed by the homeless mutations could be due either to the existence of new classes which are not co-transducible with the markers tested or to failure of those mutants to transduce or be expressed in the recipient bacteria. Therefore, conjugation tests were carried out to determine their location on the *Salmonella* chromosome.

F'*lac*$^+$ derivatives of each homeless mutant were prepared as described in Materials and Methods, and used as donors in crosses with recipients which carried either

TABLE 1

Transduction crosses with hisU *and* hisW *mutants*

Donor	Recipient											
	hisGDC63		*purF145*		*aroD5*		*metE338*		*ilvA8*		*strB57*	
	Colonies scored	%W†	Colonies scored	%W	Colonies scored	%W	Colonies scored	%W	Colonies scored	%W	Colonies scored	%W
hisU1817	200	< 0·5	1060	< 0·1	—	—	5808	< 0·02	2066	< 0·05	207	< 0·5
hisU1818	70	< 1·4	1030	< 0·1	—	—	3933	< 0·03	1260	< 0·08	159	< 0·6
hisU1819	585	< 0·2	1020	< 0·1	—	—	4278	< 0·02	1874	< 0·05	186	< 0·5
hisU1820	506	< 0·2	1030	< 0·1	—	—	2146	< 0·05	389	< 0·3	347	< 0·3
hisW1821	660	< 0·2	2730	< 0·04	3400	< 0·03	6659	< 0·02	677	< 0·2	1692	< 0·06
hisU1822	1028	< 0·1	1020	< 0·1	—	—	2844	< 0·04	860	< 0·1	186	< 0·5
hisU1823	187	< 0·5	1010	< 0·1	—	—	1640	< 0·06	—	—	139	< 0·7
hisW1824	500	< 0·2	1470	< 0·07	1578	< 0·07	2783	< 0·04	545	< 0·2	1164	< 0·1
hisW1825	380	< 0·3	2102	< 0·05	1505	< 0·07	2941	< 0·03	436	< 0·2	701	< 0·1
hisO1812 (control)	570	100·0	—	—	—	—	385	< 0·3	—	—	—	—
hisR1813 (control)	216	< 0·5	—	—	—	—	595	2·4	160	5·0	—	—
hisT1207‡ (control)	—	—	2225	38·9	1068	19·1	—	—	—	—	—	—
hisS1816 (control)	1080	< 0·1§	240	< 0·4	—	—	800	< 0·1	—	—	437	8·3

† W, percentage of transductants which have wrinkled colony morphology.
‡ Data from Roth *et al.* (1966).
§ Recipient strain was *hisG1303*.

TABLE 2

Repressed enzyme levels in hisU *and* hisW *mutants*

Strain	Histidinol phosphate phosphatase (relative specific activity)
ara-9 (control)	1·0
hisU1917	4·4
hisU1818	2·7
hisU1819	5·3
hisU1820	9·1
hisU1822	4·4
hisU1823	8·0
hisW1821	6·2
hisW1824	5·8
hisW1825	4·7

All strains were grown on minimal medium supplemented with 50 μg L-histidine/ml. As an index of the de-repression of the histidine operon, histidinol phosphate phosphatase activity was measured using the toluenized cell assay of Ames, Hartman & Jacob (1963).

TABLE 3

Enzyme levels of hisU *and* hisW *mutants grown on limiting histidine*

Strain	Histidinol phosphate phosphatase (relative specific activity)	
	Minimal medium	Minimal medium + 2-thiazole alanine
ara-9 (control)	1·0	5·6
hisU1817	2·7	9·7
hisU1819	4·8	12·6
hisU1820	5·3	12·9
hisU1823	4·6	14·0
hisW1821	5·3	11·4
hisW1824	5·6	11·8

Histidine limitation was brought about by the action of DL-2-thiazole alanine, a histidine analog, which acts on the first enzyme of the pathway as a false feedback inhibitor and, therefore, produces a deficit in the amount of histidine available to the cell (Martin, 1963; Sheppard, 1964). The procedure used is that described by Berberich, Venetianer & Goldberger (1966); the 2-thiazole alanine was purchased from the Cyclo Chemical Corp. Phosphatase activity was assayed as described by Ames *et al.* (1963), and is expressed as relative to the repressed level of the control strain *ara-9*.

purF145 (strain SB558) or *metE338 ilvC401* (strain SB109) mutations. Both recipient strains were resistant to streptomycin. Prototrophic recombinants were selected on minimal medium containing streptomycin and 2% glucose, and inspected for the appearance of the wrinkled colony phenotype of the donor.

Results of those crosses (Table 4) demonstrated that seven of the unidentified mutations were linked, by conjugation, to *metE* and *ilvC* markers, even though they were not linked to them in transduction tests (Table 1). Similarly, the other three

TABLE 4

Conjugation crosses with hisU *and* hisW *mutants*

Donor	Recombinant selected			
	metE338$^+$ *ilvC401*$^+$ *str* (SB109 recipient)		*purF145*$^+$ *str* (SB558 recipient)	
	Colonies scored	%W†	Colonies scored	%W
hisU1817 (F′*lac*$^+$)	1830	72·2	—	—
hisU1818 (F′*lac*$^+$)	660	44·3	—	—
hisU1819 (F′*lac*$^+$)	1500	52·5	—	—
hisU1820 (F′*lac*$^+$)	21	62·0	577	<0·2
hisU1822 (F′*lac*$^+$)	62	46·8	—	—
hisU1823 (F′*lac*$^+$)	161	47·9	—	—
hisU1206 (F′*lac*$^+$)	93	58·0	30	<3·3
hisW1821 (F′*lac*$^+$)	103	<1·0	382	>50
hisW1824 (F′*lac*$^+$)	≅ 12000	<0·01	509	57·5
hisW1825 (F′*lac*$^+$)	335	<0·3	721	63·0

Crosses were performed as described in Materials and Methods. Recipient strains SB109 carries mutations *metE338 ilvC401 str* and SB558 is *purF145 str-125*.

† %W, percentage of recombinants which have wrinkled colony morphology.

mutations, which were not cotransducible with the *purF* marker (Table 1), showed, in conjugation crosses, a linkage to this marker of about 60% (Table 4). From each cross, some wrinkled recombinants were purified and their enzyme levels in repressed conditions determined. As shown in Table 5, these wrinkled recombinants had high levels of histidinol phosphate phosphatase characteristic of the constitutive donor strains.

TABLE 5

Enzyme levels in wrinkled recombinants from hisU *and* hisW *conjugation crosses*

Strain	Recombinant Genotype	Recombinant Morphology	Histidinol phosphate phosphatase (relative specific activity)
ara-9 (control)	—	S	1·0
Parental strains:			
hisU1817	—	W	3·5
hisU1819	—	W	5·3
hisU1820	—	W	7·1
hisU1823	—	W	7·2
hisW1821	—	W	7·6
hisW1824	—	W	5·7
Wrinkled recombinants:			
SB109 × *hisU1817* (F′*lac*$^+$)	*metE*$^+$ *ilvC*$^+$ *str*	W	5·3
SB109 × *hisU1819* (F′*lac*$^+$)	*metE*$^+$ *ilvC*$^+$ *str*	W	5·9
SB109 × *hisU1820* (F′*lac*$^+$)	*metE*$^+$ *ilvC*$^+$ *str*	W	9·8
SB109 × *hisU1823* (F′*lac*$^+$)	*metE*$^+$ *ilvC*$^+$ *str*	W	6·0
SB558 × *hisW1821* (F′*lac*$^+$)	*purF*$^+$ *str*	W	6·0
SB558 × *hisW1824* (F′*lac*$^+$)	*purF*$^+$ *str*	W	9·7

Strains were grown on minimal medium containing 50 μg L-histidine/ml. The histidinol phosphate phosphatase assay of Ames *et al.* (1963) was used.
S, smooth; W, wrinkled.

The linkage found in conjugation tests between some homeless mutations and *metE* and *ilvC* loci, and the fact that these loci are linked by transduction to the *hisR* gene, raised the question if those regulatory mutants could be *hisR* mutants unable to express their genotype in transduction. For example, they could be gross chromosomal aberrations that either might not be incorporated into the transducing fragment or the integration of which into the recipient bacteria could be difficult.

To test this possibility several transductional crosses were performed. The recipient was a double mutant, *metE338 hisR1203* (SB110), and six of the mutants in question were the donors. If they were not *hisR* mutants, some of the transducing fragments carrying the *metE*$^+$ marker would carry also the wild-type *hisR* allele and, therefore some *hisR*$^+$ (smooth) colonies would appear among the selected *metE*$^+$ transductants. Constitutive mutants belonging to the other classes, including four *hisR* mutants, were used as donor in control crosses.

Smooth (*hisR*$^+$) colonies appeared among the recombinants from crosses involving every donor used but the four *hisR* strains used as controls (Table 6). It is possible to assert then, that *hisU1817, hisU1818, hisU1819, hisU1820, hisU1822* and *hisU1823* are not *hisR* mutants. The new class of histidine regulatory mutants which they represent has been called *hisU*.

TABLE 6

Recombination between hisR *and* hisU *loci*

Cross	Colonies scored	% Smooth recombinants
SB110 × *hisR1203* (control)	909	< 0·11
SB110 × *hisR1813* (control)	1185	< 0·08
SB110 × *hisR1223* (control)	1226	< 0·08
SB110 × *hisR1827* (control)	10500	< 0·01
SB110 × *hisO1812* (control)	385	0·8
SB110 × *hisT1503* (control)	4021	1·1
SB110 × *hisS1816* (control)	525	1·1
SB110 × *hisW1824* (control)	837	0·5
SB110 × *hisU1817*	1248	0·64
SB110 × *hisU1818*	1235	0·86
SB110 × *hisU1819*	1037	1·6
SB110 × *hisU1820*	449	0·67
SB110 × *hisU1822*	1938	1·4
SB110 × *hisU1823*	1294	0·77

Recipient strain SB110 carries mutations *metE338 hisR1203; metE338*$^+$ transductants were selected in each cross and scored for smooth (*hisR*$^+$) colony phenotype.

A similar test was applied to mutants *hisW1821, hisW1824* and *hisW1825*, to make sure that they were not aberrant *hisT* mutants. This possibility was considered remote since a linkage in transduction of 40% (co-transduction between *hisT* and *purF* loci) should correspond to a linkage, in conjugation, much higher than the 60% detected between *purF* and the three unidentified mutations. In keeping with this interpretation was the observation that the linkage found in conjugation crosses between *purF145* and a *hisT* mutation was 99·4%.

A double mutant, *hisT1529 aroD5* (SB751), was used as recipient, and donors were histidine regulatory mutants from several classes. Recombinants *aroD*$^+$ were selected on minimal medium containing 2% dextrose, and scored for smooth (*hisT*$^+$) colony phenotype. As the co-transduction between *hisT* and *aroD* loci is higher than

TABLE 7

Recombination between hisT *and* hisW *loci*

Cross	Colonies scored	% Smooth recombinants
SB751 × *hisT1529* (control)	1374	0·4†
SB751 × *hisT1503* (control)	767	1·3†
SB751 × *hisT1227* (control)	596	0·8†
SB751 × *hisU1819* (control)	1562	53·1
SB751 × *hisU1820* (control)	1263	45·1
SB751 × *hisW1821*	1230	34·3
SB751 × *hisW1824*	350	25·8
SB751 × *hisW1825*	288	25·4

Recipient strain SB751 carries mutations *aroD5 hisT1529; aroD5*$^+$ transductants were selected in each cross and scored for smooth (*hisT*$^+$) colony phenotype.

† Smooth colonies apparently due to reversion of the recipient *hisT* mutation (see text).

20% (Roth *et al.*, 1966) a high percentage of smooth *aroD*$^+$ recombinants was expected in transductions where the donor did not bear a *hisT* mutation. The results, which appear in Table 7, indicate that the three mutations under study can recombine with a *hisT* mutation at a frequency comparable with that observed when *hisU* donors were used. That is, mutants *hisW1821*, *hisW1824* and *hisW1825* represent a new class of histidine regulatory mutants. This class has been designated *hisW*.

The few smooth colonies which appeared with similar frequency in the control crosses between two *hisT* mutations (Table 7) were probably revertants of the recipient *hisT* mutation, since they also appeared in the first control cross in which the recipient mutation *hisT1529* was used both as recipient and donor.

(c) *Mapping of* hisU *locus*

In order to map more precisely the *hisU* locus, a three-point cross involving *hisU*, *metE* and *pyrE* mutations was performed. The *pyrE* locus is close to the *metE-ilv* region (Sanderson & Demerec, 1965), near which the *hisU* gene appeared also to be located.

The donor strain was a *pyrE123* mutant carrying F'*lac*$^+$; recipients were SB622 (*metE338 hisU1819 str*) and in the control cross involving the *hisR* locus, SB627 (*metE338 hisR1813 str*). In both crosses, recombinants with *metE*$^+$ *str* were selected on minimal medium containing cytosine and streptomycin. About 100 recombinants from each cross were purified on the same selective medium and analyzed by replica plating for the unselected markers *pyrE*, on minimal medium, and *hisU* or *hisR* (depending on the cross) by colony morphology on nutrient agar containing 2% glucose.

TABLE 8

Location of hisU *and* hisR *loci through three-point crosses*

Cross	Unselected markers in *metE*$^+$ *str* recombinants							
	pyrE$^+$W		*pyrE*$^+$S		*pyrE*$^-$S		*pyrE*$^-$W	
	No.	%	No.	%	No.	%	No.	%
SB622 × *pyrE123* (F'*lac*$^+$)	75	64·1	21	18·0	20	17·0	1	0·9
SB627 × *pyrE123* (F'*lac*$^+$)	21	21·7	59	60·8	17	17·5	0	—

Recipient strain SB622 carries mutations *metE338 hisU1819 str*, and strain SB627 carries *metE338 hisR1813 str*. In both crosses, *metE338*$^+$ *str* recombinants were selected. Total number of recombinants scored was 117 in the first cross and 97 in the second.

W, wrinkled; S, smooth.

Results of such crosses are presented in Table 8. In the cross involving the *hisU* recipient, recombinants having the alleles *pyrE123 hisU1819* are rare whereas recombinants carrying all other combinations of alleles are frequent. This points to the *pyrE123 hisU1819* as the quadruple cross-over class, and consequently indicates that the *hisU* gene is located between the *pyrE* and *metE* loci (Fig. 1).

The same reasoning applied to the control cross leads to a similar location for the *hisR* gene (Fig. 1), in agreement with the map position obtained by transduction

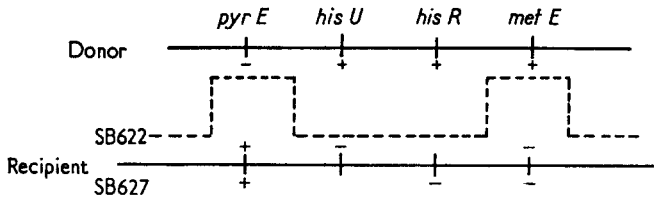

FIG. 1. Three-point crosses involving *hisU* and *hisR* loci.
Selection was made for recombinants carrying *metE*$^+$ marker from the donor and streptomycin resistance (*str*) from the recipient. Unselected markers were *pyrE* and, depending on the cross, *hisU* or *hisR*. The quadruple cross-over class and gene order resulting from the data in Table 8 are shown.

and conjugation tests (Roth & Hartman, 1965; Roth & Sanderson, 1966). Results in Table 8 also show that the linkage between *pyrE* and *hisU* markers (64·1%) is much higher than that between *pyrE* and *hisR* genes (21·7%). Thus, these results are consistent with the map order *pyrE* – *hisU* – *hisR* – *metE* shown in Figure 1.

This order was confirmed by results obtained in crosses involving the HfrA strain SR305, which transfers the *ilv* cluster and *metE* gene very early (Sanderson & Demerec, 1965; Roth & Sanderson, 1966). The purpose of the experiment was to find out whether the *hisU* locus was transferred by this Hfr strain as an early or late marker, and in this way to locate it in relation to the Hfr origin.

The recipient strains were the same two used in the preceding experiment and a third, SB621, carrying mutations *ilvC401 hisU1819 str*. Each recipient was crossed to the Hfr strain and the matings were allowed to proceed for 40 minutes. They were interrupted by shaking in a vortex mixer, and suitable dilutions were plated on selective medium.

TABLE 9

Location of hisU *and* hisR *loci with respect to the origin of transfer in HfrA*

Cross	Recipient	Donor	Recombinant selected	Colonies scored	% Smooth
1	SB627	SR305	*metE338*$^+$ *str*	1550	95·0
2	SB622	SR305	*metE338*$^+$ *str*	569	< 0·2
3	SB621	SR305	*ilvC401*$^+$ *str*	1203	< 0·1

Recipient strain SB627 carries mutations *metE338 hisR1813 str*; strain SB622 carries *metE338 hisU1819 str* and SB621 carries *ilvC401 hisU1819 str*. Donor strain HfrA SR305 is *hisD23 gal-50* and streptomycin sensitive. Its point of origin and order of transfer are shown in Fig. 2. Matings were interrupted 40 min after starting.

Results of these crosses are presented in Table 9. As expected on account of its location between the *metE* and *ilv* loci (Roth & Hartman, 1965), the *hisR* gene behaved like an early marker and most of the *metE*$^+$ *str* recombinants from cross 1 displayed the smooth phenotype of the *hisR*$^+$ donor. On the other hand, none of the *metE*$^+$ *str* or *ilvC*$^+$ *str* recombinants from crosses 2 and 3 received the *hisU* allele from the donor, as demonstrated by the fact that all of them retained the wrinkled colony morphology characteristic of the *hisU* mutation.

This break in the linkage between *hisU* and the *ilvC* or *metE* loci, manifested with an HfrA donor, can be easily explained assuming that the point of origin for chromosomal transfer in HfrA strains lies between *hisU* and the *ilv* gene. In this way, while the *ilvC*, *hisR* and *metE* loci are transferred as early markers, the *hisU* locus is transferred as a terminal one. In summary, the experiments performed with *hisU* mutants locate the *hisU* locus between the *pyrE* gene and the origin of HfrA as shown in Figure 2.

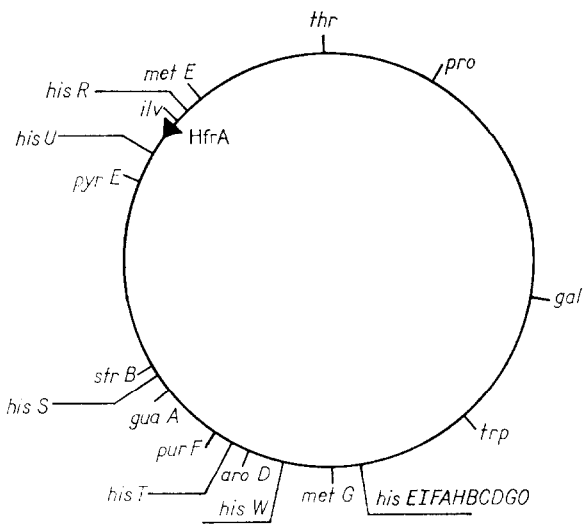

FIG. 2. Genetic map of *Salmonella typhimurium*.
Approximate locations of *his* loci are shown. Markers *thr* (threonine), *pro* (proline), *gal* (galactose) and *trp* (tryptophan) are included for purposes of orientation. The arrow indicates the origin and direction of transfer of HfrA (SR305) strain (Sanderson & Demerec, 1965). The present map is based on that of Sanderson & Demerec (1965), with the additions described by Roth *et al.* (1966) and in this paper.

Several transduction tests using recipients which carried mutations known to be located in the *pyrE-ilv* region (Sanderson & Demerec, 1965) were performed in an attempt to identify a transducing fragment carrying *hisU*. No linkage could be detected between *hisU1819* and *pyrE* (<0·1%), *aroC* (<0·02%), *aroB* (<0·1%), *pdx* (<0·5%) or *cysE* (<0·2%).

To extend the location obtained for *hisU1819* to the other mutations classified as *hisU* on the basis of their linkage to *metE* and *ilvC* markers in conjugation tests (Table 4), several crosses were carried out. The F'*lac*$^+$ derivative of each mutant was used as donor; recipients carried the *hisU1819* mutation and a closely located auxotrophic marker, either *metE338* (SB622) or *pyrE123* (SB565). Strain *hisU1820* (F'*lac*$^+$) was not used as donor because it transfers very poorly the *ilv-pyrE* region (Table 4); recipient strain SB628 (*ilvC401 hisU1820 str*) was used instead, in crosses with the control strains *hisU1819* (F'*lac*$^+$), *hisR1813* (F'*lac*$^+$) and *aroC26* (F'*lac*$^+$). Prototrophic recombinants carrying the recipient *str* marker were selected on minimal medium containing 2% glucose and streptomycin. The appearance of smooth colonies (*hisU*$^+$) among them was scored as evidence of recombination between the donor and recipient regulatory mutations.

TABLE 10

Recombination between hisU *mutations*

Donor	Recipient SB622 Colonies scored	SB622 %S	SB565 Colonies scored	SB565 %S	SB628 Colonies scored	SB628 %S
hisU1817 (F'*lac*$^+$)	599	0·17	1265	< 0·08	—	—
hisU1818 (F'*lac*$^+$)	64	< 1·6	128	0·8	—	—
hisU1822 (F'*lac*$^+$)	200	< 0·5	—	—	—	—
hisU1823 (F'*lac*$^+$)	102	< 1·0	—	—	—	—
Controls						
hisU1819 (F'*lac*$^+$)	186	< 0·5	688	< 0·15	82	< 1·2
hisR1813 (F'*lac*$^+$)	1219	3·3	27	7·4	12	8·3
aroC26 (F'*lac*$^+$)	—	—	309	47·0	100	55·0

Strain SB622 carries the markers *hisU1819 metE338 str*; strain SB565, *hisU1819 pyrE123 str*; and strain SB628, *hisU1820 ilvC401 str*. Recombinants selected carried *metE*$^+$ *str* in the crosses with SB622 recipient; *pyrE*$^+$ *str* with SB565 recipient; and *ilvC*$^+$ *str* with SB628 recipient. Donor strain *aroC26* (F'*lac*$^+$) carries no histidine regulatory mutation. S, smooth.

The recombination observed between *hisU1819* and each of the presumptive *hisU* mutations was very low (Table 10). Thus, the results demonstrate the close proximity of the mutations previously classified as *hisU* and justify their inclusion in one group. On the other hand, the amount of recombination between *hisU1819* and *hisR1813* mutations, though much higher than that detected between *hisU* mutants, was much lower than that obtained when using the wild-type donor *aroC26* (F'*lac*$^+$), corroborating in this way the conclusion reached in the preceding experiments on the vicinity of the *hisU* and *hisR* loci on the chromosome.

(d) *Mapping of* hisW *locus*

To determine the location of the *hisW* locus, a cross with multiply marked strains was carried out. For use as donor, a double mutant *purF145 aroD5* (SB560) was infected with the F'*lac*$^+$ episome of strain SB394. The recipient was strain SB563, which carries mutations *hisE35 metG319 hisW1824* and is streptomycin resistant (*str*).

The cross was performed as described in Materials and Methods. Recombinants with the *hisE35*$^+$ and *str* markers were selected on minimal medium containing methionine, adenine, thiamine, phenylalanine, tyrosine and streptomycin. The *hisE*$^+$ *str* recombinants were purified by streaking on the same selective medium, and then analyzed by replica plating for unselected markers. On account of their close linkage (8·1% cotransduction, Roth *et al.*, 1966) markers *purF145* and *aroD5* were considered as only one marker for the analysis of the cross. The few recombinants which had suffered a cross-over between those loci were not included in Table 11, because, as the orientation of the two loci in the *Salmonella* genetic map is not known, they were not significant to the purpose of the cross.

The results of the cross are shown in Table 11, along with three representations of gene order (models 1 to 3) (Fig. 3); a location of *hisW* to the "right" of *hisE* had been ruled out by earlier experiments, and the order of (*purF aroD*) – *metG* – *hisE* had been determined earlier. According to model 1, the four classes of recombinants

TABLE 11

Genetic analysis of hisE35 str *recombinants from cross SB563 × SB560 (F'*lac$^+$)

Class	Unselected markers			Frequency		No. of cross-overs required according to model		
	purF145-aroD5	*hisW1824*	*metG319*	%	(No. colonies)	1	2	3
1	+	−	−	36·0	(69)	2	2	2
2	+	−	+	24·0	(46)	2	2	4
3	−	+	+	16·1	(31)	2	2	2
4	+	+	+	13·5	(26)	2	4	2
5	+	+	−	5·2	(10)	4	4	2
6	−	+	−	1·0	(2)	4	4	4
7	−	−	−	0·5	(1)	4	4	4
8	−	−	+	—	(0)	4	2	4

Donor strain SB560 (F'*lac*$^+$) is *purF145 aroD5* and streptomycin sensitive. Recipient strain SB563 carries mutations *hisE35 hisW1824 metG319 str*. Because of their close linkage, the *purF* and *aroD* loci are used as one marker. Frequencies are based on a total number of recombinants scored of 192. Seven recombinants (three different classes) are not shown in the Table because they showed recombination between the *purF* and *aroD* markers (see text).

which were most frequently found would require two cross-overs each. On the other hand, model 2 predicts a double cross-over class (class 8) which did not appear at all. Model 2 also predicts that class 4, a rather frequent class, would require four cross-overs. Similarly, according to model 3, a quadruple cross-over class (class 2) would be much more frequent than one requiring only two (class 5).

Therefore, assuming that recombinant classes produced by two cross-overs are more frequent than those which require four cross-overs, the order favored by the results is that of model 1, that is, the *hisW* locus is located between the (*purF-aroD*) and *metG* markers (Fig. 2).

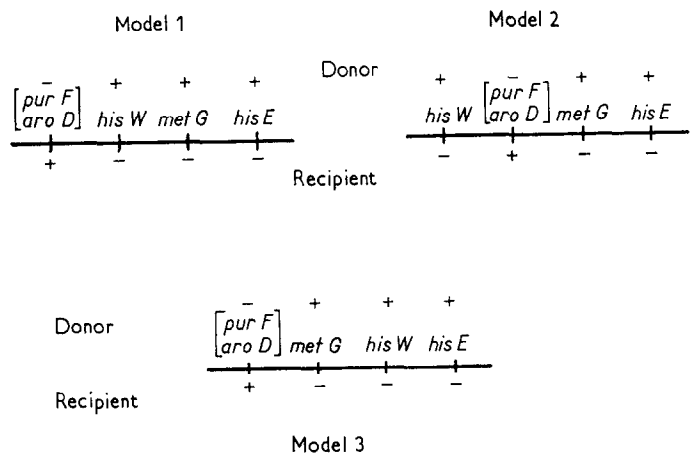

FIG. 3. Possible locations of *hisW* locus.

The symbols above the line correspond to the genotype of the donor in the cross reported in Table 11, and those below the line to the recipient. Description of the cross is given in the text and in the legend to Table 11.

The same conclusion concerning the location of *hisW1821* and *hisW1825* mutations was drawn from crosses designed to test the occurrence of recombination between *hisW1824* and those presumptive *hisW* mutations. The recipient was strain SB562, carrying markers *hisW1824 purF145 aroD5 metG319 str*, and the donors were the F'*lac*$^+$ derivatives of the mutants in question. A wild-type strain (SB394) and *hisW1824* (F'*lac*$^+$) were used as donors in control crosses. Recombinants with the markers *purF*$^+$ *aroD*$^+$ *str* were selected and scored for development of the smooth (*hisW*$^+$) colony phenotype. The low recombination observed (Table 12) indicates that the three regulatory mutations are situated very close together and supports the classification of *hisW1821* and *hisW1825* as *hisW* mutants.

TABLE 12

Recombination between hisW *mutants*

Donor	Recipient	No. *purF*$^+$ *aroD*$^+$ *str* recombinants scored	%S
hisW1821 (F'*lac*$^+$)	SB562	1820	1·7
hisW1825 (F'*lac*$^+$)	SB562	320	< 0·3
Controls			
hisW1824 (F'*lac*$^+$)	SB562	184	< 0·5
SB394	SB562	72	32·0

Strain SB562 carries mutations *hisW1824 purF145 aroD5 metG319 str*; strain SB394 carries *trp-109* (F'*lac*$^+$). S, smooth.

4. Discussion

The data presented in this paper demonstrate the existence of two new genetic classes of histidine regulatory mutants. They raise to six the number of known histidine regulatory genes. At present, most of the histidine regulatory mutants isolated for TRA resistance have been mapped. The appearance of additional genetic classes does not seem probable, since more than 40 mutants have been studied (Roth *et al.*, 1966) and even the less frequent classes (*hisO* and *hisW*) are represented by at least three mutants each.

Except for some *hisS* mutants, mutants of the six classes are very similar regarding their effects on the control of the histidine operon (Roth *et al.*, 1966). They synthesize histidine enzymes in the presence of histidine at levels 4- to 15-fold higher than the wild-type repressed level. Moreover, they are able to increase the synthesis even further when the supply of histidine becomes limiting. It is not clear, however, whether this increase is produced by true de-repression or is an effect of the reduction in the growth rate, since in the *hisT*, *hisR* and *hisO* classes a decreased growth rate, brought about by using different carbon sources, causes a marked increase in the level of histidine biosynthetic enzymes (Klopotowski & Ames, unpublished results).

As for *hisT* mutants, the biochemical alteration of *hisU* and *hisW* mutants is still unknown. In merodiploid cells they are, as well as *hisR* and *hisT* mutations, recessive to the wild-type allele (Fink & Roth, 1968). It has been found that *hisU1817*, *hisU1819* and *hisW1825* mutants have normal levels of histidyl-tRNA synthetase (J. Roth, unpublished results); tRNA(His) acceptor capacity in the same mutants is at present being investigated (G. Fink, personal communication).

As long as the biochemical function of *hisU*, *hisW* and *hisT* genes remains unsolved, it will be difficult to ascertain their role in the control of the histidine operon, since they could either work directly on repression, or exert their action, indirectly, by affecting the true active elements. In any event, the complexity of the histidine regulatory system is now evident.

Note added in proof: Results recently obtained indicate that the "homeless" mutant *his–1509* (Roth *et al.*, 1966) belongs to the *hisW* class: in F'*lac*-mediated conjugation crosses it showed linkage to the *purF* marker (63·1%) but not to the *metE* gene (<1·0%). Since it has been demonstrated by Silbert *et. al.* (1966) that mutant *hisW1509* has only 60% of the wild-type histidine-specific tRNA acceptor activity, this result indicates that *hisW* gene, like *hisR*, is concerned with the synthesis of functionally active tRNA for histidine.

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