HISTIDINE-REQUIRING MUTANTS OF *ESCHERICHIA COLI* K12

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IN *Salmonella typhimurium* a cluster of nine genes controlling histidine biosynthesis has been mapped in detail and correlated with enzymes in the biochemical pathway. Numerous observations are in accord with the concept of a unified control—or operon system—for the histidine genes in Salmonella (LOPER *et al.* 1964; AMES *et al.* 1967).

Mutations leading to a requirement for histidine are known in *Escherchia coli* and have been located on the *E. coli* K12 genetic map (TAYLOR and TROTTER 1967) in a position comparable to that of the histidine operon in *S. typhimurium* (SANDERSON 1967). Data presented here indicate that the *his* genes in *E. coli* are clustered and may constitute an operon very similar to that of Salmonella. The relative positions of two nearby genes in *E. coli* also are the same as in Salmonella. However, heterogeneity must be present between the two species, for *E. coli* F′ *his* recombines readily within *E. coli* but fails to recombine, or recombines only very rarely, with the chromosome when present in Salmonella. For reasons which are not understood, among *E. coli* histidine mutations (in comparison with previous studies in Salmonella) extended deletions are unusually common while point mutations, particularly in certain genes, are rare. The independent studies reported in the accompanying paper by GOLDSCHMIDT *et al.* (1970) extend these observations.

MATERIALS AND METHODS

Bacterial strains: Histidine-requiring mutants were obtained in several *E. coli* K12 sublines after screening through penicillin (Table 1). Mutants isolated in this laboratory were obtained by screening with 67 units/ml penicillin with or without prior treatment by diethylsulfate. Four regulatory mutants were obtained by selection for resistance to 1,2,4-triazole-3-alanine (ROTH, ANTÓN and HARTMAN 1966). We are grateful to Dr. R. G. JONES who furnished the triazole-alanine. Regulatory mutants *his-495* and *-496* were obtained as spontaneous mutants in strain UTH653 while mutants *his-497* and *-498* were obtained in strain UTH1038 after treatment with diethylsulfate. Additional bacterial strains are described under *Detection of nonsense mutations.*

Media: Difco nutrient broth and the E medium of VOGEL and BONNER (1956) were used, the latter with 0.2% glucose as carbon source. Solid media contained 1.5% agar. For enrichment of E medium, 1.25% v/v nutrient broth (EM) or 2.5% v/v nutrient broth (2 EM) was added.

Histidinol utilization: Cultures either were tested on individual E plates with crystals of L-histidinol or were inoculated onto E plates containing 120 μg/ml L-histidinol. Plates were scored after incubation at 37°C for 3 days.

Genetic mapping: E or EM plates were spread with about 2×10^8 recipient bacteria and spotted with cultures of donor strains. Twenty *E. coli* Hfr strains carrying histidine mutations

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TABLE 1

Sources of histidine-requiring E. coli *mutants*

Mutation	Strain	Parent strain	Mutagen	Source[a]
his-1	AB335	unknown	unknown	1
2[b]	AB381	AB352	ultraviolet	1
3	AB1111	AB265	"	1
4	AB1103	AB265	"	1
5[b]	AB438	AB312	"	1
33[c]	AB1917	AB352	"	2
34[c]	AB1918	AB352	"	2
35[c]	AB1919	AB352	"	2
36[c]	AB1920	AB352	"	2
37[c]	AB1921	AB352	"	2
323		UTH1038[d]	"	3
451-460		UTH653[d]	diethylsulfate	4
461-463		UTH1038[d]	"	4
464-465[c]		AB1117	spontaneous	4
466-476[c]		AB1117	diethylsulfate	4
477[c]		AB352	"	4
478[c]		AB352	spontaneous	4
479-494, 499-512	-	(See Table 2)		
750-930		UTH653[d]	ultraviolet	3

[a] Obtained through the courtesy of: (1) Dr. A. L. TAYLOR, (2) Dr. E. A. ADELBERG, (3) Drs. T. S. MATNEY and E. GOLDSCHMIDT, (4) Isolated in this laboratory.

[b] F+ donors.

[c] Hfr donors with F-attachment site 0–12, the same site as for AB312 (TAYLOR and ADELBERG 1960).

[d] Strain description in Table 1 of GOLDSCHMIDT *et al.* (1970).

were crossed in such plate matings with one another and with 190 F⁻ histidine auxotrophs. In similar tests, Salmonella strains carrying extended deletions in the *his* operon and F′ *his* episomes of *E. coli* origin (Table 2) were crossed with various Salmonella and *E. coli* mutants. In the Salmonella crosses, essentially no crossing over was detected between the *E. coli* genetic material on the episome and the Salmonella chromosome; therefore, the tests were complementation tests. When Salmonella strains carrying *E. coli* F′ *his* elements were mated with *E. coli* mutants, both complementation and recombination were involved in production of prototrophs.

Detection of nonsense mutations: UAG and UGA nonsense mutations on *E. coli* F′ *his* were detected by spotting the F′ strains (Table 2) on minimal agar plates spread with Salmonella

TABLE 2

Salmonella strains carrying E. coli *F′* his

Strain*	Mutation
SB570	his+
SB571	hisA323
SB590;TA1	his+
SB591	hisB479
SB592	F480
SB593	C481
SB594	C482
SB595	A483
SB596	HAFIE484
SB597	C485
SB598	B486
SB599	C487
SB600	C488
SB601	C489
SB602	B490
SB603	F491
SB605	CB493
SB606	C494
SB607	C499
SB608	C500
SB609	F501
SB610	B502
SB611	F503
SB612	C504
SB613	C505
SB614	B506
SB615	Db507
SB616	I508
SB671	Da509
SB672	Dab510
SB673	Dab511
SB674	Dab512
TG5701	G2416
TR84	E2414

* Strains SB570 and SB571 are *S. typhimurium* LT2 *hisG70 trp-407* carrying F′ *his* of *E. coli* K12 (GOLDSCHMIDT *et al.* 1970). The other strains are *S. typhimurium* LT2 *hisDCBHAFIE712 ser-821* carrying an F′ *his* obtained by Dr. T. TAKANO in *E. coli* K12 strain AB311. The F′ *his*+ episome was transferred into Salmonella (strain SB590; also known as strain TA1), and *his*− mutations were obtained by treatment with diethylsulfate. Strains courtesy of Dr. G. R. FINK.

strain *hisGDCBHAF644* and on this same strain carrying either a relatively efficient UAG suppressor (*supW501*, in SB787) or a strong UGA suppressor (*sup-584*, in TR612; *sup-1002*, in TR613). Growth ensued when the mutation on the F′ *his* was transferred and suppressed by the suppressor in the recipient bacteria.

UAG and UAA mutations in *E. coli* F− recipient histidine-requiring bacteria were detected by spreading them on minimal agar plates and spotting with strain SL4040 (*met− trp− fla− str*^{r} *rfb− gal−*/F′ *gal+ att*λ *sup-812*). Strain SL4040 carries an F′ containing an ochre suppressor capable of suppressing many UAA and UAG mutations.

Reversion tests: Capacity for reversion to prototrophy was tested by plating about 2×10^8 bacteria on EM medium and adding mutagens directly to the plate (AMES and WHITFIELD 1966). Plates were observed for the presence and distribution of prototrophic colonies after 2 days incubation at 37°C and a subsequent 2 days at room temperature. Mutagens tested were diethylsulfate (DES) and N-methyl-N′-nitro-N-nitrosoguanidine (NG) from commercial sources and 2-chloro-6-methoxy-9-[3-(2-chloroethyl)-aminopropylamino] acridine dihydrochloride (ICR-191) which

TABLE 3

Classification of E. coli *histidine-requiring mutants by growth response and response to mutagens*

Reversion pattern	Do not grow on L-histidinol		Grow on L-histidinol	
	Locus and isolation No.	No. of mutants	Locus and isolation No.	No. of mutants
Stable	2, 5, 33-37, 452-456, 458, 459, 461, 475, 750-753, 755-759, 761-764, 769, 771 772, 775, 777, 779, 781, 783-788, 792, 795, 801, 805, 807, 808, 811-813, 815, 824, 826-834, 836, 837, 840, 842, 844, 847, 848, 853, 857-859, 861, 864, 866-870, 874, 876, 878, 879, 882, 887, 890, 891, 893-898, 902, 905, 908-910, 912, 914, 916, 919, 920, 924, 926-930.	110	C463, 474, F860, 862* F892	5
Spontaneous only	Dab472	1	1, 464, C466, C470, 471 473, C476, C478, 774, F776, C782, F800, F802, F804, F817, 825*, F841 F852, B855, F881, C901, I903, C904, A915, C922, A925.	27
ICR191 +	Dab468	1	467, C477, C917	3
NG +		0	C3, 469, F793, A849, F856, F863, A872, A889, C906, F923.	10
DES +		0	4, F765, F820, C821.	4
NG +, DES +		0	F451, H457, 460, C462, 760*, 766*, A767, C768, F770, F773, 778*, C780, C794, F796, A797, A798, A799, C803, C806, C809, C810, 814*, A816, F818, 822, 823, F835, C838, F839, A843, 846*, C851, 854*, F871, C875, C877, C880, A884, C885, C886, 888, C899, C900, A907, C911, C913, C918.	47
ICR191 +, NG +, DES +	Dab921	1	A323, C465, C873	3
	Total mutants:	113		99

Abbreviations: ICR-191 is 2-chloro-6-methoxy-9-[3-(2-chloroethyl)aminopropylamino] acridine dihydrochloride; NG is N-methyl-N′-nitro-N-nitrosoguanidine; DES is diethylsulfate. A plus indicates a positive response to the mutagen; other tests are negative.

* Appear to map in loci *hisA* or *hisF*, or both, but data do not allow a clearcut locus designation. *his 774* and *823* should also bear an asterisk (*).

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Enzyme assays: Bacteria were grown in 25 ml aliquots of E medium containing 6 μg/ml D,L-histidine and harvested after rapid growth on the L-histidine ceased and the culture reached about 125 Klett units. Bacteria were sedimented by centrifugation in the cold. The pellet was either stored at —20°C or immediately resuspended in 2 ml Tris buffer (0.01 M, pH 7.5), and sonicated for 2 min (MSE, Model 160W). Assays were performed on supernatants cleared by centrifugation for 30 min at 31,000 × *g*.

Protein was measured by a Biuret method (LAYNE 1957). Measurements of histidinol phosphate phosphatase (*B* gene) activity followed the procedure of AMES, GARRY and HERZENBERG (1960). Imidazoleacetol phosphate: L-glutamate aminotransferase (*C* gene) activity was measured by the method of AMES, GARRY and HERZENBERG (1960) or, in most experiments, by the modified procedure of MARTIN and GOLDBERGER (1967). Assays for L-histidinol dehydrogenase were performed by the procedure of AMES, HARTMAN and JACOB (1963). Phosphoribosyl-ATP pyrophosphorylase (*G* gene) activity was measured by the assay of AMES, MARTIN and GARRY (1961) except that increase in absorbance was recorded at 295 nm rather than at 290 nm. The assay for 6-phosphogluconate dehydrogenase (*gnd* gene) was that of MURRAY and KLOPOTOWSKI (1968), and for TDP-glucose oxidoreductase (RHA-2) activity, the procedure of OKAZAKI *et al.* (1962). Strains are considered to lack RHA-2A activity when the total activity is between 1/3 and 1/2 of wild type (cf. NIKAIDO *et al.* 1967).

RESULTS

Growth and reversion tests: Table 3 shows that stable, nonrevertible mutations are frequently found in *E. coli his* mutants (115 out of 212 mutants examined). Such stable mutations are found in several *E. coli* K12 sublines after ultraviolet treatment (104 out of 175 mutants examined) or after diethylsulfate treatment (11 out of 25 analyzed). Most of these mutants (96%) fail to grow on L-histidinol and thus are assumed to lack *hisD* function (Table 3). Among 97 revertible mutants, 32 are classified as frameshifts that either fail to respond to the mutagens tested or respond to ICR-191 alone, 61 are classified as base substitutions that revert with nitrosoguanidine (NG) or diethylsulfate (DES) or both, and 4 are unclassified as to type since they respond to all three mutagens.

Behavior of E. coli *F′* his *in Salmonella:* F′ factors are not readily transferred from *E. coli* K12 to Salmonella, a result of restriction of foreign DNA by the recipient bacteria (ZINDER 1960; COLSON, COLSON and VAN PEL 1970). However, once the *E. coli* factor has escaped restriction and resides in Salmonella, it is readily transferred between Salmonella strains and back into *E. coli* K12.

Mutations on *E. coli* F′ *his* were passed from Salmonella donors (Table 2) into a variety of well mapped Salmonella histidine-requiring mutants. There was no evidence that crossing over took place between the *E. coli* episomal material and the chromosomal mutations; prototrophs appeared to form only by complementation. This allowed assignment of locus designations to the episomal mutations in parallel with locus designations used in Salmonella (Table 2) where the operator plus structural genes have been mapped with the sequence *O–G–D–C–B–H–A–F–I–E*. Mutations were found that affected homologues of each of the 9 known Salmonella genes, indicating that all the genes for histidine biosynthesis are contained on the *E. coli* episome, and thus clustered in *E. coli* as they are in

Salmonella. Indeed, the F′ *his*+ episomes complemented Salmonella strains lacking the entire histidine operon.

One mutant episome (*his-484*) lacks functions for genes *H*, *A*, *F*, *I*, and *E*. We interpret this as indicating that these 5 genes are adjacent on the *E. coli* chromosome. Another episome (*his-493*) lacks functions for genes *C* and *B*; this could be an extended mutation involving two adjacent genes, akin to similar mutations in Salmonella, or it could be a double mutation due to the diethylsulfate mutagenesis. Complementation patterns of *E. coli hisD* mutants *507*, *509*, and *510–512* (Table 2) are the same as previously found in Salmonella by LOPER *et al.* (1964). That is, the episomal *Da* mutant complements Salmonella *Db* mutants and the *Db* mutant complements Salmonella *Da* mutants. Mutants designated in Table 2 as *Dab* fail to complement all Salmonella *D* mutants tested. In the case of *hisD*, interspecies complementation appears to proceed similarly to intraspecies complementation.

Recombination tests in E. coli *with F′* his *episomes:* Table 3 includes gene locus designations in large measure based on frequency of prototroph formation with the F′ *his* strains listed in Table 2. The Salmonella strains carrying *E. coli* episomes contribute F′ *his* to *E. coli* with a frequency only slightly lower than the high frequency encountered between Salmonella strains. Upon entry into *E. coli*, the *E. coli*-derived genetic material on the episome forms prototrophs both by complementation and by crossing over. Representative data which enabled some of the mutations to be assigned to the *F* gene are given in Table 4, and additional data appear in GARRICK (1967). GOLDSCHMIDT *et al.* (1970) have confirmed the locus designations for six of the mutations in Table 3 by obtaining them as F′ *his* exogenotes and testing them for complementation in Salmonella strains with mutations in known loci. Other locus designations are supported by the enzyme analyses reported in a later section.

Ninety-five of the F⁻, stable, histidinol-negative mutants (Table 3) failed to complement or to recombine with any of the F′ *his* strains having SB numbers (*hisD*, *C*, *B*, *A*, *F*, and *I* mutants in Table 2), but did form prototrophs in combination with the two F′ *his*+ testers. We conclude that all of these strains carry extended deletions lacking at least the six genes *hisD*, *C*, *B*, *A*, *F*, and *I*. A number of the strains also were tested against TR84 (*hisE*) and TG5701 (*hisG*) and again failed to produce prototrophs, indicating that the entire gene cluster is deleted. The deletions are not all identical, however, as revealed by enzyme analyses reported later.

Prototrophs appeared at two days when a polar (see *Survey of histidine enzymes*, below) *hisD* mutant (*921*) was infected with F′ *his* carrying mutations in the *D*, *C*, *B*, *A*, and *F* genes but appeared in one day in combination with F′ *his* carrying mutations in the *G*, *E*, and *I* genes or in combination with F′ *his*+. Similar tests could not be performed with other *D* and with *C* polar mutations since all are derivatives of AB1117 and fail readily to accept F′ *his*+ episomes. Earlier appearance of colonies may be due to complementation, and this may be decreased by the strongly polar *hisD* mutation. Genes *I* and *E* are proposed to be at the end of the operon based on the results of GOLDSCHMIDT *et al.* (1970).

TABLE 4

Determination as F *gene mutations based on frequency of prototroph formation with the F' tester strains listed in Table 2*

Histidine mutant	F' his tester strains:				
	HAFIE484	F480	F491	F501	Others
his-451	0	100	100	60	+[a]
765	0	0	0	1	+
770	0	2	10	10	+
773	0	12	40	10	+
776	0	0	20	10	+
793	0	0	0	5	+
796	0	3	25	12	+
800	0	200	60	70	+
802	0	15	25	15	+
804	0	30	15	45	+
817	0	0	0	0	+
818	0	35	100	10	+
820	0	0	0	14	+
835	0	10	40	4	+
839	0	7	40	0	+
841	0	0	0	6	+
852	0	15	70	45	+
856	0	12	60	30	+
860	0	25	NT	NT	+
863	0	0	0	30	+
871	0	80	150	40	+
881	0	15	50	60	+
884	0	0	0	4	+
892	0	0	15	2	+
923	0	25	60	50	+

NT—not tested.
[a] + indicates confluent growth in the test spot (greater than 300 colonies).

Successful complementation of *I* and *E* episomes might be due to a gene dosage phenomenon or due to the presence of an internal promoter in the *E. coli* operon in analogy with a similarly located internal promotor found in the Salmonella operon (ATKINS and LOPER 1970). We infer that the order of the genes in the proposed operon is similar to that in Salmonella, that is, *O–G–D-(CBHAF)–(IE)*. If complementation facilitates opportunity for recombination on minimal medium, a parallel behavior by polar *hisA* mutants would lead to low recombination levels both with *hisA* and *hisF* episomal tester strains. Mutants with such

behavior are marked with an asterisk in Table 3 and also have been noted by GOLDSCHMIDT *et al.* (1970).

Fifteen of the stable histidinol-negative mutants (*his-2, 5, 33–37, 751, 759, 762, 763, 833, 894, 908, 930*) produced prototrophs in tests with four of the F′ *his* episomes listed in Table 2. Prototrophs were recovered in crosses with SB593, SB671, SB672, and SB674. Tests for nonsense suppressors (see METHODS) demonstrated that the four aberrant F′ factors carried amber mutations whereas none of the other F′ mutations were ambers (SB612 and TG5701 carry UGA mutations). We conclude that each of the 110 stable histidinol non-growers (Table 3) is an extended deletion covering the entire cluster of histidine genes. Fifteen of the stable strains also bear suppressors for amber mutations.

Recombination tests with Hfr and F$^+$ donors: Conjugation experiments with *E. coli* donors confirmed results from the F′ conjugation tests. Of the 110 stable histidinol-negative strains (Table 3), none showed detectable recombination with 19 *E. coli his*$^-$ Hfr strains and two F$^+$ strains (labeled with superscripts b and c, respectively, in Table 1). While eight of the male strains contain extended deletions leading to a histidinol-negative phenotype, some contain point mutations in the *hisD* (*468, 472*) and *hisC* (*465, 466, 470, 476–478*) genes and recombine well with other point mutations. *hisC* mutants in Hfr strains were shown to serve as competent donors of the histidinol-positive phenotype to several deletion mutant recipients (*his-753, 756, 757,* and *761*) on histidinol-containing plates. Fertility in these latter crosses was about 100-fold less than expected from fertility in tests with recipients carrying point mutations. These results confirm the absence of genes *hisD* and *hisC* in the extended deletion strains.

Similarly, the eight stable histidinol-negative Hfr and F$^+$ donor strains (*his-2, 5, 33–37, 475*) failed to form prototrophs with almost all of the 100 point mutations in the *hisD, C, B, A, F,* and *I* genes, indicating that these genes were absent in the stable Hfr donors. Exceptional prototrophs were noted when the male strains were crossed with a few NG-revertible mutant strains: *460, 803, 822, 838, 877, 880, 900, 918,* and *921*. Since each of these strains except *921* shows a *his*$^+$ phenotype when mated with SL4040 (carries an F′ with an ochre–amber suppressor), we suppose that these are nonsense mutations that are suppressed in crosses with the eight male strains listed above. As we noted in the preceding section, the presence of amber suppressors in these male strains accounts for additional otherwise discordant data in crosses with strains bearing F′ *his* episomes with amber mutations.

Survey of histidine enzymes: An enzyme survey (Table 5) supports the genetically determined locus designations for a number of mutations. In addition, the data reveal that three mutants classified genetically as *hisC* mutants (*C466, C470,* and *C476*) are deficient both in *C* and in *B* activity, and two mutants classified genetically as frameshifts in gene *hisD* (*D468* and *D921*) lack *D* activity and contain low *C* and *B* activities. In parallel with similar results in Salmonella (cf. FINK and MARTIN 1967) we assume that these mutations are strongly polar and that genes *D, C,* and *B* follow operator in that order in an *E. coli his* operon with the same gene arrangement as its Salmonella counterpart.

Basal repressed levels of three histidine biosynthetic enzymes are comparable in two *E. coli* sublines and in Salmonella (Table 6). The same is true of the *hisG* enzyme (pyrophosphorylase), not shown in the table. The enzymes were assayed by procedures developed for Salmonella; no attempts were made to modify or check the reactions to see if they proceeded optimally other than to demonstrate proportionality with enzyme concentration and with time of reaction.

Triazolealanine-resistant mutants of *E. coli* contain high constitutive enzyme levels (Table 6), similar to Salmonella mutants (ROTH, ANTÓN and HARTMAN 1966). Enzymes dictated by the *E. coli hisD* and *hisC* genes are coordinately affected (Table 6). Our assays on *hisB* activity in repressed bacteria showed variation sufficient to prevent a decision as to the coordinacy of phosphatase with the other two enzymes.

Other functions: To determine the extent of the large deletions outside the histidine region, functions were examined for genes mapping nearby. In *E. coli* K12, genes for flagella and motility and a locus (*uvrC*) affecting resistance to ultraviolet irradiation lie close to *his* (reviewed by TAYLOR and TROTTER 1967). Ten *his* extended deletions (*his-33*, *34*, *35*, *36*, *37*, *461*, *475*, *750*, *751*, and *752*) were observed to be motile on microscopic examination and form swarms in semisolid medium. The latter three mutants were tested and exhibit sensitivities to ultraviolet light no different from the wild type. We conclude that the deletions measured do not extend into the respective *fla*, *mot*, and *uvrC* loci.

The data in Table 5, however, indicate that many of the deletions are greatly extended to one side of the histidine map position, covering not only the *his* genes but also a locus for 6-phosphogluconate dehydrogenase (*gnd*) and a gene eliciting RHA-2A activity. Mutant *his-461* is deficient in the entire *his* cluster and in GND but contains RHA-2A activity, suggesting the gene order *histidine–gnd–RHA2A*. This gene order parallels that found in Salmonella (NIKAIDO *et al.* 1967; MURRAY and KLOPOTOWSKI 1968).

DISCUSSION

Our genetic mapping results and enzyme data are most easily interpreted if the order of genes in *E. coli* K12 is: (*hisO–G*)–*D–C–B*–(*H–A,F*)–(*I,E*)–*gnd–RHA-2A*. These preliminary data and those in the accompanying paper (GOLDSCHMIDT *et al.* 1970) jibe well in according the K12 genes functional and relative spatial identities to the extensively studied *his* operon of *S. typhimurium*. Furthermore, our data and work of J. GREEB, J. F. ATKINS, and J. C. LOPER (personal communication) indicate that the two-unit complementation pattern within gene *hisD* found in Salmonella (LOPER *et al.* 1964), *Da* and *Db*, also is detected in *E. coli hisD* mutants. The *hisD* enzymes (L-histidinol dehydrogenase) of *E. coli* K12 and of *S. typhimurium* are electrophoretically identical (J. R. ROTH, personal communication). In a genetically similar situation, four *hisI* mutations on the TAKANO F′ *his* from *E. coli* fall either into the *Ia* complementation type (*his-2409*, *2410*, and *2411*) or into the *Ib* type (*his-2413*) when tested against a spectrum

TABLE 5

Enzyme content of histidine auxotrophs

Mutant	Enzyme activity*					
	his^G	his^D	his^C	his^B	GND	RHA-2A
1		+	+	+		
2		0	0	0	0	
C3		+	0	+	+	
4		+	+	+		
5		0	0	0	0	
33		0	0	0	0	
34		0	0	0	0	
35		0	0	0	0	
36		0	0	0	0	
37		0	0	0	0	
A323		+	+	+		
461	0				0	+
C465		+	0	+		
C466		+	0	0		
467		+	+	+		
D468		0	low	low	+	
469		+	+	+		
C470		+	0	0		
471		+	+	+		
D472		0	0	0	+	
473		+	+	+		
474		+	+	+		
475		0	0	0		
C476		+	0	0		

of Salmonella *hisI* mutants of known (LOPER *et al.* 1964) complementation type (P. E. HARTMAN and K. LEVINE, unpublished). GOLDSCHMIDT *et al.* (1970) have found that *E. coli* mutant *hisI903* is of the *Ia* type, an observation we have confirmed. The orientation in the chromosome of the genes for histidine biosynthesis (*his*), 6-phosphogluconate (*gnd*) and TDP-glucose oxidoreductase (RHA-2A) activity are the same in *E. coli* (our data; PEYRU and FRAENKEL 1968) and Salmonella (NIKAIDO *et al.* 1967; MURRAY and KLOPOTOWSKI 1968; HARTMAN, RUSGIS and STAHL 1965).

Both the F′ *his* episome obtained by GOLDSCHMIDT *et al.* (1970) and the F′ *his*

TABLE 5—Continued

Cl477		+	0	+		
Cl478		+	0	+		
750	0	0	0	0	0	0
751		0	0	0	0	
752		0	0	0		
753	0				0	0
762					0	
763					0	
C803	+					
F818	+	+	+	+		
822	+					
833					0	
838	+					
C875		+	0	+		
877	+					
880	+				+	
883					0	
C900	+					
I903					+	
C906		+	0	+		
D921		0	low	low		
C922		low	0	+		
928					0	

* Enzyme activities as defined and described in MATERIALS AND METHODS. Symbols for enzyme activity: 0 = no activity detected; + = activity comparable to wild type or above; low = activity lower than wild type.

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episome independently obtained by TAKANO from *E. coli* K12 contain the entire *his* cluster and a closely linked gene for 6-phosphogluconate (*gnd*). Crossing over appears to be frequent when these episomes are transferred from Salmonella back into *E. coli* K12; yet, crossing over is very rare or absent when these episomes are contained in Salmonella. This indicates that a strong genetic divergence has taken place between the *his* regions of the two bacterial species in spite of the gross structural and functional similarities noted above. Such structural divergence previously was indicated in the failure of ST. PIERRE and DEMEREC (1968) to obtain stable *E. coli–S. typhimurium* hybrids for the *his* region although such

TABLE 6

Enzyme levels in repressed S. typhimurium *and* E. coli *K12 strains and in some constitutive* E. coli *mutants*

Strain	Enzyme activity					
	D		C*		B	
	Specific activity	Relative specific activity	Specific activity	Relative specific activity	Specific activity	Relative specific activity
Salmonella LT2	3.9	---	0.48	---	0.65	---
E. coli						
UTH653 (his$^+$)	4.5	≅1	0.36	≅1	0.33	≅1
his-495 (constitutive)	121	27	7.3	20	5.1	15
his-496 (constitutive)	119	26	7.7	21	4.0	12
UTH1038 (his$^+$)	5.3	≅1	0.37	≅1	0.50	≅1
his-497 (constitutive)	123	23	5.4	15	3.6	7
his-498 (constitutive)	98	19	7.8	21	3.2	6

Values are averages of independent assays on two or more extracts.
* Assay of AMES *et al.* (1960).

hybrids were obtained for other chromosome regions. MARY ANN JANKOWSKI (personal communication) has found that the *E. coli* and Salmonella 6-phosphogluconate dehydrogenases (GND) differ electrophoretically, and even more extreme differences are probably imposed nearby in the respective somatic antigen (*rfb*) regions (cf. ØRSKOV and ØRSKOV 1962; NIKAIDO *et al.* 1967). These known genetic differences, located some 4–12 genes away from the *his* operon, might be expected to depress recombination frequencies in adjacent regions in interspecies crosses (INO and DEMEREC 1968), but the magnitude of the effect appears surprising to us. Perhaps the different segments of *E. coli* and *Salmonella* histidine polypeptide chains are incompatible, as found for the *pyrF* product by INO and DEMEREC (1968).

Extended deletions are much more common among the *E. coli* mutants than among Salmonella mutants (LOPER *et al.* 1964; P. E. HARTMAN, unpublished). This abundance of extended deletions was first noted with ultraviolet-induced mutants and then with mutants induced by diethylsulfate (GOLDSCHMIDT *et al.* 1970; Table 3). There also is an unexplained paucity of point mutations in genes *hisD* and *hisB* among the *E. coli* mutants when compared with similar material from Salmonella. We have no substantial explanation to offer for these phenomena; however, extended histidinol-negative deletion mutations occur frequently when P2 phage is situated near the histidine gene cluster (KELLY and SUNSHINE 1967). We noted (GARRICK 1967) that some of the "extended deletion" strains upon initial tests gave several to 1000 colonies on histidinol plates; retests

of the same stock cultures several months later revealed no such colonies on histidinol. A similar behavior was noted for one mutant by KELLY and SUNSHINE (1967). Examination of our strains carrying histidinol-positive point mutations failed to reveal the presence of histidinol-negative bacteria in such clones. If there is a propensity for formation of extended deletions among point mutants, it is not apparent in these stocks.

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SUMMARY

Nine genes for histidine biosynthesis and genes specifying 6-phosphogluconate and TDP-glucose oxidoreductase activities in *E. coli* K12 are clustered on the chromosome in an arrangement that is similar, if not identical, to that previously observed in Salmonella. In spite of the gross similarity, interspecies crossing over is rare or absent in this chromosome region. Extended deletion mutations constitute about half of the chromosomal mutations to histidine-requirement after ultraviolet or diethylsulfate treatment of the *E. coli* K12 strains examined.

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