GENETIC ANALYSIS OF THE HISTIDINE OPERON IN *ESCHERICHIA COLI* K12

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IN *Salmonella typhimurium* a cluster of nine genes, behaving as a single operon (AMES and GARRY 1959) specify the enzymes which carry out the ten reactions involved in the biosynthesis of histidine; one of the nine enzymes is bifunctional (LOPER, GRABNAR, STAHL, HARTMAN and HARTMAN 1964). The histidine operon is contained in a linear segment of deoxyribonucleic acid (DNA) estimated by AMES, GOLDBERGER, HARTMAN, MARTIN and ROTH (1967) to be 11,000 base pairs in length (over three cell lengths). Fifteen cistrons have been sequenced and their enzyme products identified.

Mutations leading to a requirement for histidine (*his*) in another enteric organism, *Escherichia coli*, have been found to cluster on its genetic map (TAYLOR and TROTTER 1967) at a position comparable to that of the histidine operon in Salmonella (SANDERSON 1967). The high degree of correspondence in the locations and functions of genes in these two organisms (DEMEREC and OHTA 1964) prompted us to initiate an analysis of the histidine region in *E. coli* so that it might be compared in detail to the one in Salmonella.

Several aspects of this analysis were facilitated by the isolation in our laboratories of an F-merogenote or F′-episome (JACOB and ADELBERG 1959) in *E. coli* K12 that appeared to carry genetic information analogous to the histidine operon in Salmonella. Such a system makes possible (1) the construction of partially diploid derivatives that are heterozygous for specific *his* mutations and (2) the subsequent classification of the mutations into complementing groups.

Since F-merogenotes are transmissible from *E. coli* to *S. typhimurium* it was possible to identify the enzyme defect involved in each complementation group in *E. coli* by introducing an F-merogenote carrying a specific *his* mutation into a set of *S. typhimurium* recipients with known defects and then noting the complementation pattern. In addition, the partially diploid system permitted the construction of *E. coli* heterogenotes carrying three *his* mutations and the ordering of the *his* mutations by three-factor analysis.

MATERIALS AND METHODS

Media: Difco Antibiotic Medium #3 (Pen Assay broth) and Difco nutrient broth (supplemented with 0.5 g NaCl per liter) were employed as complete media. Hard agar contained 15 g Bacto-Agar per liter; soft agar contained 7.5 g per liter.

The DAVIS and MINGIOLI (1950) minimal medium was prepared by adding the following to 900 ml of sterile water or freshly autoclaved agar: 100 ml 10× salts (70 g K_2HPO_4, 30 g KH_2PO_4, 5 g Na citrate·$2H_2O$, 1 g $MgSO_4 \cdot 7H_2O$, and 10 g $(NH_4)_2SO_4$ are dissolved in water in sequence, brought to 1 liter volume and autoclaved), 5 ml of a thiamine stock solution (0.1 g/100 ml), and 4 ml of a 50% glucose solution. Additional growth requirements were met with the following final concentrations of supplements: histidine, 100 μg/ml; histidinol, 125 μg/ml; other amino acids and thymine, 20 μg/ml.

Curing cultures of F-merogenotes. Overnight Pen Assay broth cultures were diluted to 10^{-5} in 2× nutrient broth adjusted to pH 7.6 containing 12.5 μg/ml acridine orange (National Analine Corporation) and incubated at 37°C until turbidity developed. The cultures were serially diluted and spread onto the surface of nutrient agar plates. The loss of the F-merogenote was detected by velveteen replicating the colonies to a minimal agar plate containing a lawn (0.1 ml of an overnight Pen Assay broth culture spread to dryness) of an F^- carrying a *his* mutation complemented by the *his* mutation in the particular F-merogenote. Colonies which failed to F-duce the recipient to His+ were picked from the nutrient agar master plate and their cured status confirmed by demonstrating their insensitivity to F-specific phage.

Bacterial strains and bacteriophage: Table 1 lists the His+ stocks that were mutagenized in various experiments to obtain the *his* mutations listed in Table 2. The prototroph UTH 653 was derived from a cross of Hfr G5 (MATNEY, GOLDSCHMIDT, ERWIN and SCROGGS 1964) and a prolineless mutant of UTH 432, UTH 452, in which the selected prototroph was found to differ from UTH 432 by being λ^- and nonpermissive to T4 amber mutants.

TABLE 1

E. coli *stocks used to derive histidine requiring mutants*

UTH No.*	Strain	Genotype	Source
321	C	F^- *thy-321*	J. C. Suit
432	K12	F^- *ara, gal, malA, xyl, mtl,* λ^+/λ^r, *sup*	J. Lederberg (W2979)
444	K12	F^- *try-444*	mutant of UTH432
452	K12	F^- *pro-455, strA*	mutant of UTH455
455	K12	F^- *pro-455*	mutant of UTH432
653	K12	F^- λ^-/λ^r, *strA* (*pro*$^+$, *sup*$^+$)	recombinant of UTH452
952	K12	HfrG6 *try-678*	J. C. Suit
1038	K12	F^- λ^-/λ^s	S. E. Luria (K12S)
4349	K12	F' *his*$^+$/*hisG-E870, ile-2774*	derived in this study
4462	K12	F^- *nadB29, thi, mtl, xyl, ara, lac, gal,*$^+$	ATCC 9723b
4662	K12	F^- *nadB3*	E. A. Adelberg

*UTH prefix designates University of Texas/Houston stock culture collection. Symbols indicate the nutritional requirement for thymine (*thy*), tryptophan (*try*), proline (*pro*), histidine (*his*), isoleucine (*ile*), thiamine (*thi*), and nicotinate (*nad*); inability to utilize arabinose (*ara*), galactose (*gal*), maltose (*mal*), xylose (*xyl*), mannitol (*mtl*), and lactose (*lac*); the presence ($\lambda+$) or absence (λ^-) of the lambda prophage and the ability (λ^s) or inability (λ^r) to absorb λ phage; permissiveness to a T4-amber phage (*sup*).

TABLE 2

The genetic analysis of histidineless mutations isolated after various mutagenic treatments

Mutagen	Parent Strains UTH No.	G2743	D4086	D3861	C780	B747	B855	B4087	H1767	A323	F504	I903	Extended Deletions GDCBHAFI
UV	653		921		768 901		855			767	765 871	903	750 781 828 866 905
					780 904					797	770 881		751 783 829 867 908
					782 906					798	773 884		752 784 830 868 909
					794 911					799	776 892		753 785 831 869 910
					803 913					816	793 923		754 786 832 870 912
					806 917					843	796		755 787 833 874 914
					809 918					849	800		756 788 834 876 916
					810 922					872	802		757 792 836 878 919
					821					889	804		758 795 837 879 920
					822					907	817		759 801 840 882 924
					838					915	818		761 805 842 887 926
					851					925	820		762 807 844 890 927
					873						823		763 808 847 891 928
					875						835		764 811 848 893 929
					877						839		769 812 853 894 930
					880						841		771 813 857 895
					885						852		772 815 858 896
					886						856		775 824 859 897
					899						860		777 826 861 898
					900						863		779 827 864 902
										760	825		
										766	846		
										774	854		
										778	862		
										814	888		
	452	4602		4597	4595					4600	4599		4596 : 1–56
					4598								
	432										504		Not Determined
	952								1767				Not Determined
	1038									323			Not Determined
X-Ray	321												4315
			4314										
DES	1038	3745		3861	3597								
		3857		3734	3603								
MNNG	1038	4762	4202		3452	4188				3465	3467		
			4758				4189				4192		
			4203								4210		
			4204								4761		
	432				4031					4029	4032		
										4033	4702		
										4701			
	444				4045	4046					4044		
											4066		
	4349		4086					4087					
	4462			4731					4744	4730	4736		
				4735						4733	4737		
										4743			
	4662	4768			4740				4770	4742	4741		
					4767				4771	4772	4756		
									4773		4769		
Spontaneous	452										4612		11 Isolates, Not Stocked
Obtained from	2743	2743											
other	747					747							
Laboratories	4183	4183											
	1164	1164											

S. typhimurium stocks bearing the mutations listed in Table 3 were described by LOPER *et al.* (1964).

An F-specific bacteriophage (RNA-3) isolated by E. P. GOLDSCHMIDT, was employed to test for the presence of F in various derivatives. It has an advantage over MS2 in not requiring Ca^{++} and thus effecting visible lysis of sensitive cells on ordinary nutrient agar plates.

Nomenclature: The inability to grow without histidine will be designated phenotypically as His^- or genotypically as *his* (DEMEREC *et al.* 1966). A new mutation is assigned an isolation number that usually corresponds to the lowest available stock collection number, e.g., *his-780, his-870, his-3734.* When the defective function(s) has been identified, the appropriate capital letter(s) (gene) replaces the hyphen, i.e., *hisC780, hisG-E870, hisD3734.* In describing partial diploids, the mutational content of the F-merogenote will be given first, e.g., 4631 is F'*hisC780/ hisG-E870, ile-2774* signifying that the episome carried the *780* mutation in its *C* gene while the endogenote has a deletion of the entire histidine operon as well as a second mutation in a gene involving isoleucine biosynthesis.

Since the symbol His^- signifies the nutritional requirement for histidine, the inability of a mutant to substitute histidinol for this requirement will be designated Hol^-, indicating a defect in the ability to convert histidinol to histidine and thus, by convention, a mutation involving the *D* gene.

Mutagenesis: Mutants isolated following treatment with ultraviolet light or X rays were obtained by the membrane filter/penicillin enrichment procedure detailed by MATNEY and GOLDSCHMIDT (1962). Treatment by diethyl sulfate (DES) consisted in adding 0.1 ml of mutagen to 4.9 ml of log phase Pen Assay broth culture (about 4×10^8 bacteria/ml) and holding the tube at 37°C without shaking for 30 min. The treated culture was diluted 1:100 into 50 to 100 tubes of fresh broth and incubated overnight. The cultures were serially diluted and plated onto nutrient agar plates. His^- mutants were found by velveteen replicating the resulting colonies to minimal agar with and without histidine. Mutants occurring in different culture tubes were considered to have arisen from unique mutational events induced by the DES treatment.

A mutagenic treatment with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (Aldrich Chemical Co.) was developed. A freshly prepared saturated (at 26°C) MNNG solution (0.05 ml) in 0.8% NaCl was added to a 5 ml Pen Assay log phase culture (about 2×10^8 bacteria/ml), and incubation was continued with aeration at 37°C for 45 to 60 min. The culture was serially diluted and plated onto nutrient agar plates. The resulting colonies were velveteen replicated to minimal agar plates with and without histidine.

Mating procedures: Qualitative F-duction tests to observe complementation were done in two ways. A cross-streak procedure consisted of spreading bacteria of one mating type from a large 3 mm diameter loop evenly in a 1 cm band across the center of a minimal agar plate. Single loopfuls of cells of the opposite mating type were drawn through the band once; cells deposited above the juncture and between cross-streaks served as nutritional marker controls and as areas for the appearance of His^+ "revertant" colonies after two days of incubation.

Conjugation occurred when cells of opposite mating type came in contact. If the *his* mutation in the F-merogenote complemented the *his* mutation in the F^- recipient, overnight incubation resulted in a confluent streak of His^+ growth.

If the two *his* mutations involved a cistron in common then no His^+ growth would be evident after 18 hr of incubation. If the noncomplementing mutations did not overlap, scattered His^+ recombinant colonies made their appearance after 24 hr of incubation but well in advance of spontaneous "revertants". With practice the cross-streaking procedure permitted one to determine (1) if two mutations complemented one another (2) if either mutation gave rise spontaneously to His^+ "revertants" and (3) if two noncomplementing mutations overlapped.

If large numbers of isolates were involved in an experiment, a spot test was employed in which a small loopful (1 mm) of cells of one parental type could be spread over a designated square centimeter area of a lawn plate. This procedure permitted as many as 25 isolates to be tested per lawn plate but an additional plate had to be spotted for the nutritional marker control.

F-duction was quantitated by a membrane filter mating procedure described by MATNEY and

ACHENBACH (1962). In crosses involving Salmonella donors, a two hr log phase Pen Assay broth culture was found to be critical to obtain optimal yields of F-ductants.

RESULTS

Isolation of F-merogenotes carrying the histidine operon. Ten derivatives with polarized chromosome donating properties were isolated from a histidineless (*his-323*) F^+ strain of *E. coli* without benefit of acridine orange treatment (MATNEY *et al.* 1964). Four of these donors (G1, G3, G8, and G9) had their origin of chromosome transmission near the histidine operon and transferred markers with a counterclockwise sequence (*O-try, pro . . . purG*). Since all four strains had relatively poor donating ability and were sensitive to curing by acridine orange, an effort was made to isolate more fertile derivatives. In one experiment, colonies of G8 were velveteen replicated to an F^- lawn whose cells carried a different histidineless mutation (*his-504*). Several G8 colonies gave rise to intense patches of His^+ growth on the lawn plates; they were picked from the master plate and proved to be both His^- and capable of giving rise to intense "recombinant" growth when re-streaked against F^-*his-504*. The His^+ "recombinants" became His^- when grown in medium containing acridine orange. The fertile colonies of G8 thus were shown to be His^- homogenotes, F′ *his-323/his-323* (UTH2727), while the His^+ F-ductants of F^- *his-504* were, therefore, *trans* heterogenotes bearing complementary mutations, F′ *his-323/his-504*. Subsequent analysis showed that the *his-323* mutation resided in the *A* gene (*hisA323*) and the *his-504* resided in the *F* gene (*hisF504*).

Comparable F-merogenotes have been obtained from G1, G3 and G9 populations (the G2 F′ promotes chromosome donation at a point some distance from the histidine operon). Several hundred colonies have been tested in which the F-merogenote carried the newly acquired histidine region and all appear to be homogenotic for the *hisA323* mutation. Thus the original F′-episome appears to acquire the histidine operon by a process which does not leave a corresponding deletion in the chromosome.

Derivation of F-merogenotes bearing various histidineless mutations: His^- derivatives appearing in His^+ heterogenotic populations were identified by velveteen replication of colonies to minimal agar plates with and without histidine. Two types of derivatives had been expected: those which carried at least one of the two mutations in both the F-merogenote and chromosome and those which had simply failed to inherit an F-merogenote and reverted to the F^- state. Since only one F^- segregant has been found that arose spontaneously in the thousands of His^- colonies analyzed in this study, it is clear that this F-merogenote is inherited at division by both daughter cells with remarkable fidelity.

His^- homogenotes arising from a complementing *trans* heterogenote, e.g., F′ *hisA323/hisF504*, display seven genotypes: F′ *hisA323/hisA323*; F′ *hisF504/hisF504*; F′ *hisA323, hisF504/hisA323, hisF504*; F′ *hisA323/hisA323, hisF504*; F′ *hisF504/hisA323, hisF504*; F′ *hisA323, hisF504/hisF504*; and F′ *hisA323, hisF504/hisA323*. The mutational composition of the F-merogenote is readily

established by cross-streaking the His$^-$ homogenote against three F$^-$ recipients on minimal agar: F$^-$ *hisA323*; F$^-$ *hisF504*; and F$^-$ *hisC780*. All of the above homogenotes should F-duce the last recipient strain to His$^+$ since this complementary mutation is not involved in the original heterogenote. No His$^+$ growth arising from a cross-streak indicates that the donor strain is homozygous for the mutation carried in the recipient strain. His$^+$ growth will be greatly reduced when the donor carries the mutation in its F-merogenote but not on its chromosome; the small number of His$^+$ F-ductants observed is the result of a minority population of secondary recombinant F-merogenotes which carry the wild-type allele.

The mutational composition of all F-merogenotes carrying more than one mutation was preserved by introducing them into strain UTH2774, an F$^-$ which contained the *hisG–E870* deletion of the entire operon and a nutritional requirement for isoleucine (induced in UTH870 by MNNG). F-ductants were selected on minimal agar supplemented with histidinol and isoleucine unless the F-merogenote carried a mutation in the *D* gene, in which case UTH2774 cells were retrieved after a broth mating by growing on minimal agar supplemented with histidine and isoleucine; the acquisition of the F-merogenote was determined by velveteen replication to an F$^-$ lawn carrying a complementary *his* mutation.

Mutant isolation and characterization. The first effort to isolate large numbers of His$^-$ mutants involved the treatment of UTH653 with UV. As shown in Table 2, this experiment yielded 180 mutants which were numbered consecutively from *his-750* to *his-930*. It was soon discovered that 96 of these *His*$^-$ mutants were unable to utilize histidinol and therefore carried mutations that affected the *D* gene function. Subsequent tests revealed that 95 of these Hol$^-$ mutants were not complemented by any *his* mutation and that none of these mutants reverted to His$^+$ spontaneously. They were regarded, therefore, as being extended deletions involving *G*, *D*, *C*, *B*, *H*, *A*, *F*, and *I* gene functions. Since the 95 extended deletions isolated in this experiment were apparently identical, the *his* mutations isolated in subsequent experiments were not assigned isolation/allele numbers until this category of mutation had been identified.

The *his* mutations isolated to date involve eleven cistrons as shown in Table 2. All of the mutations listed in a given column failed to be complemented by the mutation cited as the column heading but were complemented by the mutations appearing at the heading of the other columns. Fourteen mutations involving adjacent cistrons are enclosed in horizontal lines. Three Hol$^-$ mutants, e.g., *hisD4314*, were not complemented by either *hisD4086* or *hisD3861* while the latter complemented one another. Ten mutations were found to involve both *A* and *F* gene functions. The *hisB4189* mutation was not complemented by any of the three intercomplementing *B* mutations.

The data presented in Table 2 reveal a drastic difference in distribution of mutations among the histidine cistrons following UV and MNNG mutagenic treatments. In addition no extended deletions have been induced by MNNG. The 28 *C* mutations recovered from UTH653 were examined to see if they were identical, i.e., descendants of a spontaneous mutation and present in the population prior to UV treatment. Two mutations were leaky; two were unstable and

yielded large numbers of His$^+$ revertants spontaneously; four gave no recombinants with the standard mutation *hisC780*, eight mutants gave a large number of recombinants and the rest yielded a few recombinants with *hisC790*. Thus, at least five unique mutations were present among the 28 isolates.

Interspecific complementation: S. typhimurium received F-merogenotes from *E. coli* K12 with low efficiency. Salmonella derivatives carrying mutation *hisDa-208* and mutation *hisG70* with approximately 100-fold better receptivity were prepared by selecting rare His$^+$ F-ductants after *E. coli* F′ × *S. typhimurium* F$^-$ crosses, and then curing them of their F-merogenotes by exposure to ultraviolet light (attempts to use standard acridine orange curing procedures were unsuccessful). MNNG-induced tryptophanless mutations were selected in both for contraselective purposes.

The interspecific complementation procedure consisted of F-ducing one of the two improved Salmonella recipients to His$^+$ by admitting an F-merogenote carrying a specific *his* mutation in its *E. coli* operon. This *E. coli* F′/*S typhimurium* hybrid donor was then cross-streaked against a set of *S. typhimurium* strains carrying known mutations. The analysis of 14 *E. coli* mutations is presented in Table 3. Eight complementing groups are identified. The three mutations involving the *B* gene functions, *747*, *855* and *3650*, although failing to give unique complementation patterns against the Salmonella mutations (*hisBa12*, *hisBb61*, *hisBc59* and *hisBd40*), are intercomplementary and thus probably involve different cistrons of the *E. coli hisB* gene. Likewise the two *D* mutations cited in Table 3 involved the *b* cistron. However, of the twelve *D* mutants listed in Table 2, four (*921*, *4086*, *4202*, and *4204*) involve one cistron, five involve a second cistron (*3734*, *3861*, *4597*, *4731* and *4735*) and three mutants impair the functioning of both *D* cistrons (*4203*, *4314* and *4758*). Correspondence to the *a* and *b* cistrons of the *D* gene in Salmonella has not been firmly established.

Three-factor analysis of E. coli *mutations.* The frequency with which *his*$^+$ recombinant F-merogenotes arise in a heterogenote having two complementing *his* mutations on the F-merogenote and a third complementing mutation on the chromosome should depend upon whether the chromosomal mutation mapped between or outside the exogenotic mutations. The frequency of *his*$^+$ recombinant F-merogenotes in the heterogenotic population was determined by 30 min membrane filter matings of the donor with the F$^-$ containing the *hisG-E870* deletion. All F-merogenotes tested contained D^+ genes and, therefore, all F-ductants would develop colonies on minimal agar supplemented with histidinol (Hol$^+$) while only *his*$^+$ recombinant F-merogenotes could F-duce this recipient to the ability to form colonies on unsupplemented minimal agar (His$^+$). The ratio of His$^+$ to Hol$^+$ colonies was, therefore, a direct measure of the frequency of *his*$^+$ recombinant F-merogenotes in the parental F′ population.

It may be seen from the data presented in Table 4 that *his*$^+$ recombinant F-merogenotes arose in the 4503 heterogenotic population some 100 times more frequently than in heterogenote 4507. Gene *A*, therefore, resides between *G* and *F* while *G* lies outside *A* and *F*, permitting construction of the gene sequence *GAF*. A comparison between the frequencies of *his*$^+$ recombinant F-merogenotes

TABLE 3

Derivatives involved in the interspecific complementation analysis of E. coli His⁻ *mutations*

E. coli his Allele	*E. coli* homogenote UTH number	*E. coli* F/ *S. typhimurium* hybrid number	Non-complementing *Salmonella* mutation
G3857	3886	190	*G46, 70, 204*
G2743	3894	194	*G46, 70, 204*
D3734	3749	192	*Db108*
D3861	3897	196	*Db108*
C780	3058	183	*C2, 524*
C810	3308	185	*C2, 524*
B3650	3883	189	*Babcd22*
B747	3057	182	*Babcd22*
B855	3314	188	*Babcd22*
H457	3766	195	*H32, 107, 610*
H1767	3790	197	*H32, 107, 610*
A323	2727	181	*A30*
F504	3059	184	*F45, 197*
I903	3306	186	*La125*

in the next three populations, 4512, 4511 and 4513 permits gene *C* to be positioned in an analogous manner. Since the *hisH1767* mutation yielded only a five-fold difference in the frequency of his^+ recombinants when inside (UTH 4062 and UTH 4064) as opposed to outside (UTH 4061), its position is somewhat questionable. An F-merogenote carrying *hisA323* and *hisI903*, necessary for the definite positioning of the *I* gene, has not been constructed. The recombinant frequencies obtained with UTH 4806 and 4807 are similar and are interpreted to mean that the *I* gene is located close to *F* and outside the *G–F* segment.

It is interesting that the frequencies of recombination obtained within each of the six groups listed in Table 4 are consistent with the results anticipated in a three-factor analysis but that frequencies obtained in one group cannot be compared with those of another to establish the map distance between markers. For example, the map distance from *hisD4314* to *hisA323* must be considerably

TABLE 4

Determination of the gene sequence in the histidine operon of E. coli *K12*

UTH no.	Mutations in heterogenotes*	Frequency $(\times 10^{-4})$ of F'*his*$^{+}$ recombinants	Indicated gene sequence
4507	$\frac{F'\ G + F/}{+ A +}$ *met*	0.7, 0.6	
			GAF
4503	$\frac{F'\ + A\ F/}{G + +}$ *met*	80, 70	
4512	$\frac{F'\ G + F/}{+ C +}$ *met*	2, 6	
4511	$\frac{F'\ + A\ F/}{C + +}$ *met*	13, 29	*GCAF*
4513	$\frac{F'\ G + A/}{+ C +}$ *met*	1, 3	
4557	$\frac{F'\ G + C/}{+ D +}$ *arg,met,leu*	0.1	
			GDCAF
4558	$\frac{F'\ + A\ F/}{D + +}$ *arg,met,leu*	2	
4014	$\frac{F'\ + A\ F/}{B + +}$ *pro*	2	
4015	$\frac{F'\ G\ C\ +/}{+ + B}$ *pro*	3	*GDCBAF*
4040	$\frac{F'\ C + A/}{+ B +}$ *pro*	0.2	
4062	$\frac{F'\ B + A/}{+ H +}$ *try*	4.4	
4064	$\frac{F'\ C + A/}{+ H +}$ *try*	4.6	*GDCB(H?)AF*
4061	$\frac{F'\ + A\ F/}{H + +}$ *try*	19	
4806	$\frac{F'\ G\ F\ +/}{+ + I}$ *pro*	3.6	
			GDCB(H?)AF(I?)
4807	$\frac{F'\ A\ F\ +/}{+ + I}$ *pro*	8.2	

* His^{-} mutations in order of appearance: *hisF504, hisG2743, hisA323, hisC780, hisD4314, hisB747, hisH1767, hisI903.*

greater than from *hisH1767* to *hisA323*, yet the frequencies of recombination of 2×10^{-4} and 19×10^{-4}, respectively, do not reflect the true distances. In explanation it should be noted that recombination in the 4557 and 4558 heterogenotes appears to be 20 times less frequent than in the 4062, 4064 and 4061 group. The unique endogenotic background of each group may exert considerable influence on recombination mechanisms. In addition, the *hisD4314* mutation does not revert to His$^+$ spontaneously and since it impairs the function of both *D* cistrons it is probably a deletion of some magnitude. The *hisH1767* mutation on the other hand, is a point mutation since it reverts to His$^+$ spontaneously and is subject to extragenic suppression.

DISCUSSION

Eleven complementing groups of mutations have been identified as affecting histidine biosynthesis in *E. coli*. They correspond to eleven of the fifteen cistrons previously identified in Salmonella. Intragenic complementation has been observed with mutations involving genes *D* (two cistrons) and gene *B* (three cistrons). In Salmonella genes *E* and *I* also contain two cistrons each; thus far no *E* mutants and only one *I* mutant have been isolated in *E. coli*. No intragenic complementation has been observed for mutations involving genes *G*, *C*, *H*, *A*, and *F*. The number of mutations affecting each of these five genes (Table 2) is sufficiently large to have detected intragenic complementation with the possible exception of gene *H* (six mutations).

The gene order in *E. coli* has been established by three-factor analysis to be the same as Salmonella: *G*, *D*, *C*, *H*, *A*, *F*, *I* (no *E* mutants). Thus the genetic information involved with histidine biosynthesis in *E. coli* would appear to have a similar if not identical arrangement as the histidine operon in *S. typhimurium*. The orientation of the *his* operon in *E. coli* has not been determined.

During the course of these studies several observations were made which indicated that structural differences may exist either within the histidine regions themselves or in adjacent flanking information. The first stemmed from the failure to retrieve histidineless recombinant products in hybrid heterogenotic populations in which one mutation was carried within the histidine operon on the *E. coli* F-merogenote and a complementary mutation resided in the histidine operon on the *S. typhimurium* chromosome.

The second observation that gave evidence of dissimilarity between the two histidine regions was the frequent occurrence of the extended deletion type of mutation in *E. coli*, which appeared to involve the loss of the entire histidine region. A more detailed analysis of these mutants and a discussion of their significance appears in the accompanying paper (GARRICK-SILVERSMITH and HARTMAN 1970).

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SUMMARY

Histidineless mutations in *E. coli* involving eleven cistrons and corresponding to eight of the nine gene functions in *S. typhimurium* have been identified. The gene sequence is similar if not identical in the two operons: *G, D, C, B, H, A, F, I, (E)*. Dissimilarities were found in that no recombination was observed in interspecific hybrids and an extended deletion of the entire operon was the most prevalent type of mutation in *E. coli*.

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