Histidine Regulatory Mutants in *Salmonella typhimurium*

I. Isolation and General Properties

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(Received 8 August 1966, and in revised form 26 September 1966)

At least four genetically distinct classes of mutations give rise to triazolealanine resistance in *Salmonella typhimurium*. Each class studied affects regulation of the histidine operon.

Mutants of one class, hisO, are located at one end of the histidine operon. This class may be similar to operator constitutive mutants studied in other systems. At least two genetic sites are present in this region. A second class, hisS, is linked in transduction tests to the guaA and strB loci. This class contains a low specific activity of histidyl-tRNA synthetase and may represent mutations in the structural gene for this enzyme (Roth & Ames, 1966). Mutations of a third class, hisR, are linked in transduction tests to the ilv and metE loci (Roth & Hartman, 1965) and affect the levels of effective transfer RNA(his) (Silbert, Fink & Ames, 1966). A fourth class of mutants, hisT, is linked to the purF and aroD loci.

1. Introduction

The biosynthesis of histidine in *Salmonella typhimurium* involves ten enzymes. These are determined by a cluster of genes, the histidine operon, which is regulated as a unit (Ames & Garry, 1959; Ames, Garry & Herzenberg, 1960; Ames & Hartman, 1962). The level of each of the ten enzymes can vary from one, the repressed level, to about 25, under conditions of histidine starvation.

Regulatory mutants were obtained which are unable to repress fully the enzymes of the histidine operon. These mutants were obtained by selection for resistance to the histidine analogue, 1,2,4 triazole-3-alanine. This analogue has been shown to be incorporated into protein and to cause repression of the histidine operon (Levin & Hartman, 1963). Among the TRA-resistant mutants are regulatory mutants having a defective control mechanism for the histidine operon. Such mutants are not subject to repression by the analogue and thus escape growth inhibition. The regulatory mutants have been classified into at least four genetic groups. This report describes
the location of the regulatory mutations of each type on the Salmonella chromosome and some properties of the mutants. These results are discussed in terms of tentative models for regulation of the histidine operon.

2. Materials and Methods

(a) Bacterial strains

All strains used are derivatives of *S. typhimurium* LT-2, except hisH107 and its derivatives, which are derived from strain LT-7. The following strains were kindly donated by M. Demerec: araD5, guaA1, guaA9, purF145, and strD57. Strain SL751 (purC7 proA40 ile-405 rha-461 str-r iM-10 fla-56 fim-1) was received through the courtesy of B. A. D. Stocker. Strain metE338 ara-9 ileC401 has been described by Roth & Hartman (1966). Descriptions of the above genetic symbols and properties of the mutants have been summarized by Sanderson & Demerec (1965).

Histidine regulatory mutants and their means of selection are presented in Table 1. Procedures used in their isolation are described below in section 3(a). Mutants hisT1222, hisR1223, hisT1224, hisT1227 and hisT1230 were selected by B. N. Ames in partial revertants of frameshift mutation hisC202. Mutants hisR1300, hisT1501, hisT1503, his-1502, and his-1509 were selected by B. N. Ames and G. FerroLuzzi-Ames. Histidine-independent derivatives of some of the regulatory mutants isolated in hisH107 were constructed by transduction. The double mutants, hisO1202 hisG46, hisO1242 hisG46, and hisO1812 hisG46, were constructed as outlined below. Mutation hisO1242, originally obtained in strain SL751, was re-isolated in a prototroph by transduction into deletion his-63 and selection of a P22-sensitive, prototrophic recombinant clone (G. R. Fink, personal communication).

Table 1

<table>
<thead>
<tr>
<th>Regulatory mutation</th>
<th>Parent strain</th>
<th>Mutagen</th>
<th>Selection method†</th>
</tr>
</thead>
<tbody>
<tr>
<td>hisR1200</td>
<td>hisH107</td>
<td>spontaneous</td>
<td>TRA†</td>
</tr>
<tr>
<td>hisO1202, hisR1203</td>
<td>hisH107</td>
<td>ultraviolet</td>
<td>TRA</td>
</tr>
<tr>
<td>hisR1204, hisR1205</td>
<td>hisH107</td>
<td>spontaneous</td>
<td>TRA</td>
</tr>
<tr>
<td>his-1206, hisT1207</td>
<td></td>
<td>ultraviolet</td>
<td>TRA</td>
</tr>
<tr>
<td>hisR1208, hisR1209</td>
<td></td>
<td>spontaneous</td>
<td>TRA</td>
</tr>
<tr>
<td>hisS1210, hisS1211</td>
<td></td>
<td>TRA + AMT</td>
<td></td>
</tr>
<tr>
<td>hisS1213, hisS1219</td>
<td></td>
<td>TRA + AMT</td>
<td></td>
</tr>
<tr>
<td>hisT1814, hisT1815</td>
<td>hisH107</td>
<td>diethyl sulfate</td>
<td>TRA</td>
</tr>
<tr>
<td>hisS1816, hisR1826</td>
<td></td>
<td>spontaneous</td>
<td></td>
</tr>
<tr>
<td>hisR1223</td>
<td>hisC202, PR-2</td>
<td>spontaneous</td>
<td>2-methyl histidine</td>
</tr>
<tr>
<td>hisT1222, hisT1224</td>
<td>hisC202, PR-2</td>
<td>spontaneous</td>
<td>TRA</td>
</tr>
<tr>
<td>hisT1227, hisT1230</td>
<td>hisC202, PR-8</td>
<td>spontaneous</td>
<td>TRA</td>
</tr>
<tr>
<td>hisT1501, hisT1503</td>
<td>LT-2 wild type</td>
<td>spontaneous</td>
<td>TRA + AMT</td>
</tr>
<tr>
<td>his-1502, his-1509</td>
<td>SL751</td>
<td>spontaneous</td>
<td>TRA + AMT</td>
</tr>
<tr>
<td>hisO1849, hisS1859</td>
<td></td>
<td>TRA + AMT</td>
<td></td>
</tr>
<tr>
<td>hisO1812, hisR1813</td>
<td></td>
<td>TRA + AMT</td>
<td></td>
</tr>
<tr>
<td>his-1817, his-1818</td>
<td></td>
<td>TRA + AMT</td>
<td></td>
</tr>
<tr>
<td>his-1820, his-1821</td>
<td></td>
<td>TRA + AMT</td>
<td></td>
</tr>
<tr>
<td>his-1822</td>
<td></td>
<td>TRA + AMT</td>
<td></td>
</tr>
<tr>
<td>his-1819, his-1823</td>
<td>ara-9</td>
<td>diethyl sulfate</td>
<td>TRA + AMT</td>
</tr>
<tr>
<td>his-1824, his-1825</td>
<td>ara-9</td>
<td>spontaneous</td>
<td>TRA + AMT</td>
</tr>
</tbody>
</table>

† Described in section 3(a).
HISTIDINE REGULATORY MUTANTS

(b) Media

Difco nutrient broth was used as maximally supplemented liquid medium with 1.5% Difco agar as solid medium. The E medium of Vogel & Bonner (1956) was used, with 0.2% dextrose added, as minimal salts medium. Solid media used for scoring the wrinkled colony phenotype of the TRA-resistant mutants contained 2% dextrose. Amino acid supplements, when used, were added at a concentration of 50 µg/ml unless otherwise noted. Histidinol was added at a concentration of 125 µg/ml. Triazolesalane was obtained from R. G. Jones, Eli Lilly Co., Indianapolis, Indiana. Aminotriazole was obtained from the Aldrich Chemical Co.

(c) Transduction tests

Phage was prepared and stored as reported by Hartman (1956). Transduction tests were performed directly on solid medium with no prior adsorption. Phage and cells were spread together on selection medium at a multiplicity of infection dependent on the chromosome region for which recombinants were selected; this adjustment compensated for different transduction frequencies found for various chromosomal regions.

In crosses for which a TRA-resistant mutation served as an unselected marker, the transduction was performed on solid medium containing 2% glucose. On this medium, TRA-resistant clones form characteristic wrinkled colonies which can be scored with the aid of a dissection microscope. TRA-sensitive clones form normal smooth-appearing colonies (Roth & Hartman, 1965).

P22-sensitive (non-lysogenic) recombinants from phage-mediated crosses were recovered using the magnesium-hypersensitive phage of N. D. Zinder (personal communication). The procedure was modified from that of Frank D. Vasington (personal communication). E medium was made up with one-tenth of the normal K₂HPO₄ concentration and the pH was adjusted to 7.0. Just prior to the pouring of plates, the medium was made 0.05 M in MgCl₂. Mg-hypersensitive phage was grown on the donor strain in broth and irradiated with ultraviolet light to approximately 10% survival. The transduction tests were performed and recombinants were picked after only 24 to 36 hr incubation. The tiny recombinant colonies were restreaked several times on high-magnesium medium. In each case, colonies were picked while still small. Finally, clones were tested for sensitivity to P22 phage.

(d) Construction of hisG46 hisO double mutants

HisG46 hisO double mutants were used in the mapping of the hisO mutations. The strains were constructed by transducing the recipient hisD¹ hisG46 to growth on histidinol (hisD⁺) with phage grown on a hisO mutant. The recombinants were tested for growth on histidine and for colony morphology. Recombinants which required histidine and formed wrinkled colonies on medium containing 2% glucose proved to be the hisG46 hisO double mutants.

(c) Growth and preparation of cells for enzyme assays

Cells were grown in 100 ml. of minimal medium on a rotating shaker bath at 37°C. At intervals, 1.0-ml. samples were removed and the optical density determined at 650 mµ in a Beckman DU spectrophotometer. When the optical density of the culture reached 0.5 to 0.6, the cells were harvested by centrifugation for 20 min at 10,000 g in a Servall refrigerated centrifuge. The cells were resuspended in 30 ml. of 0.05 M-Tris-HCl buffer (pH 7.5) containing 0.1 mM-ATP and 2 mM-MgCl₂. In the initial experiments, 1.4 mM-mercaptoethanol was also included. Since this had no noticeable effect on the activities measured, it was omitted in later experiments. The cell suspension was then centrifuged as before; the cells were resuspended in the same buffer and sonicated for 2 min in a sonic oscillator (Measuring & Scientific Equipment, Ltd; model 160W). The sonicate was then centrifuged 1 hr at 30,000 g to remove cell debris. The supernatant fraction was passed through small columns of Sephadex gel (G25 or G50) to remove any remaining small molecules, and the resulting crude extract was used directly in the enzyme assays. Protein content of the extracts was estimated by the biuret method.
Assay of enzymes

The histidyl tRNA synthetase was assayed by following the attachment of \([^{14}C]\)histidine to tRNA. The reaction mixture of 0.25 ml. contained the following: E. coli tRNA, 5 o.d.\textsubscript{260} units or 0.2 mg (General Biochemicals Corp.); cacodylic acid (pH 7) 20 \mu moles; ATP, 0.5 \mu moles; MgCl\textsubscript{2}, 0.5 \mu moles; mercaptoethanol, 1.5 \mu moles; L-\([^{14}C]\)histidine (35 mc/mM; Nuclear Chicago), 0.01 \mu mole; gelatin, 0.1 mg. For the experiments presented in Table 10, L-\([^{14}C]\)leucine (22 mc/mM) replaced histidine. The reaction was initiated by the addition of crude extract and was stopped by the addition of 1.0 ml. of 5\% trichloroacetic acid. The reaction tubes were chilled in an ice-bath and the precipitate removed by filtration of the mixture with suction through a Millipore filter (pore size 0.45\mu; 25 mm diameter). The filter disc carrying the precipitate was washed repeatedly with 5\% trichloroacetic acid, dried, fastened to an aluminium planchet with rubber cement and assayed for radioactivity in a gas-flow counter (Nuclear Chicago).

Histidinol phosphate phosphatase was assayed (as an index of the degree of de-repression of the histidine operon) using the method of Ames, Garry & Herzenberg (1960). One unit of activity is the amount of enzyme which frees 1 \mu mole of PO\textsubscript{4} from histidinol phosphate in 1 hr under the conditions used. No MgCl\textsubscript{2} was added to the reaction mixture. Duplicates were run and the results averaged for each determination. In some experiments the toluenized cell assay and specific activity calculations of Ames, Hartman & Jacob (1963) were used.

3. Results

(a) Isolation of TRA-resistant mutants

Histidine regulatory mutants were selected for resistance to the histidine analogue, 1,2,4-triazole-3-alanine (TRA), which acts as a false co-repressor of the histidine operon (Levin & Hartman, 1963). Since wild-type Salmonella growing on minimal medium have almost fully repressed levels of the histidine biosynthetic enzymes (Ames & Garry, 1959), addition of a false co-repressor molecule, such as TRA, does not severely restrict growth. Salmonella having a partial block in the histidine pathway are able to grow on minimal medium only when they produce high levels of the histidine biosynthetic enzymes. Such cells require de-repression for growth under these conditions, and thus show sensitivity to the presence of the false repressor.

The partial block in the biosynthetic pathway, required for TRA sensitivity, can be introduced genetically by a "leaky" histidine mutation or physiologically by addition of the histidine analogue, 3-amino-1,2,4-triazole, to wild-type cells. This analogue is an inhibitor of imidazole glycerol phosphate dehydrase (Hilton, Kearney & Ames, 1965). Wild-type cells can overcome inhibition by AMT by increasing their levels of histidine biosynthetic enzymes. In the presence of AMT, wild-type cells grow with obligately de-repressed enzyme levels and are sensitive to inhibition by TRA. Either of these partial blocks allowed direct selection of TRA-resistant mutants. 2-Methyl histidine-resistant mutants may be selected by analogous techniques.

(b) Detection and properties of de-repressed mutants

Several classes of mutations could be responsible for resistance to TRA. One such class would be represented by mutants, the histidine operon of which is no longer repressed by TRA. Some of these regulatory mutants might be expected to be insensitive also to repression by histidine and thus be unable to repress fully the levels of histidine biosynthetic enzymes. Another class would be represented by mutants in which the partial block in the pathway has been removed, either by reversion of the leaky mutation, or by mutation of the dehydrase gene to AMT resistance. A third class would be represented by permease mutants that are unable to take up TRA or AMT.
Colonies formed by TRA-resistant regulatory mutants were distinguished from other TRA-resistant mutants by their characteristic morphology. Cells growing with de-repressed histidine enzyme levels form wrinkled-appearing colonies (B. N. Ames, personal communication). This characteristic morphology was found to depend on

### Table 2

**Effect of glucose concentration on enzyme level**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Histidinol phosphate phosphatase (specific activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2% glucose</td>
</tr>
<tr>
<td>hisR1203</td>
<td>2.04</td>
</tr>
<tr>
<td>hisH107</td>
<td></td>
</tr>
<tr>
<td>hisO1203</td>
<td>3.32</td>
</tr>
<tr>
<td>hisH107</td>
<td>0.51</td>
</tr>
</tbody>
</table>

All strains grown on minimal salts medium supplemented with 40 μg L-histidine per ml. Carbon sources are as indicated above. The histidinol phosphate phosphatase assay of Ames, Garry & Herzenberg (1960) was used.

### Table 3

**Doubling times and repressed enzyme levels in some TRA-resistant mutants**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Doubling time (min)</th>
<th>Histidinol phosphate phosphatase (specific activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hisH107</td>
<td>47(0)†</td>
<td>0.48(19)†</td>
</tr>
<tr>
<td>hisR1201</td>
<td>63(1)</td>
<td>4.44(1)</td>
</tr>
<tr>
<td>hisO1202</td>
<td>48(4)</td>
<td>3.36(5)*</td>
</tr>
<tr>
<td>hisR1203</td>
<td>50(4)</td>
<td>3.28(1)</td>
</tr>
<tr>
<td>hisR1204</td>
<td>55(1)</td>
<td>2.88(1)</td>
</tr>
<tr>
<td>hisR1205</td>
<td>50(3)</td>
<td>2.25(1)</td>
</tr>
<tr>
<td>his-1206</td>
<td>89(2)</td>
<td>3.32(2)</td>
</tr>
<tr>
<td>hisT1207</td>
<td>61(3)</td>
<td>3.4(1)</td>
</tr>
<tr>
<td>hisR1208</td>
<td>56(7)</td>
<td>4.63(8)</td>
</tr>
<tr>
<td>hisS1209</td>
<td>46(3)</td>
<td>0.36(7)*</td>
</tr>
<tr>
<td>hisS1210</td>
<td>54(3)</td>
<td>0.86(5)*</td>
</tr>
<tr>
<td>hisS1211</td>
<td>55(8)</td>
<td>3.0(7)*</td>
</tr>
<tr>
<td>hisS1213</td>
<td>56(2)</td>
<td>3.43(1)</td>
</tr>
<tr>
<td>hisS1219</td>
<td>52(2)</td>
<td>1.79(1)</td>
</tr>
<tr>
<td>hisO1242</td>
<td>8.9(6)*</td>
<td></td>
</tr>
<tr>
<td>hisT1501</td>
<td></td>
<td>5.93(1)</td>
</tr>
<tr>
<td>his-1509</td>
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<td>2.6(1)</td>
</tr>
<tr>
<td>hisO1812</td>
<td></td>
<td>4.54(1)*</td>
</tr>
<tr>
<td>hisK1813</td>
<td></td>
<td>3.65(1)*</td>
</tr>
<tr>
<td>hisT1814</td>
<td></td>
<td>6.11(1)*</td>
</tr>
<tr>
<td>hisT1815</td>
<td></td>
<td>5.94(1)*</td>
</tr>
<tr>
<td>hisS1816</td>
<td></td>
<td>5.83(1)*</td>
</tr>
</tbody>
</table>

† Numbers in parentheses indicate how many values have been averaged.

* Includes some values determined for hisH107* derivative grown on minimal medium plus 50 μg histidine/ml.

b Isoleucine added to medium for growth.

c All hisS mutants tested de-repress further when grown on nutrient broth. See Table 9.

d Phosphatase activities for these mutants were determined using the toluenized cell assay, and the values converted to the comparable degree of de-repression in terms of mmpoles phosphate per mg protein per hour of experimental and control cultures. All other assays were performed according to Ames, Garry & Herzenberg (1960). Specific activities were assayed by the method of Ames, Garry & Herzenberg (1960). All cells were grown in the presence of excess histidine.
elevated concentrations (2%) of glucose or other fermentable carbon sources in the medium (Fig. 1 in Roth & Hartman, 1965). The wrinkled phenotype is not expressed on 0.2% glucose, although the concentration of glucose has no significant effect on the levels of histidine biosynthetic enzymes (Table 2). Mutants selected for resistance to TRA were examined for colony morphology on medium containing 2% glucose. Only those mutants which formed wrinkled colonies were studied further. In every case tested, wrinkled colony formers had high enzyme levels for the histidine biosynthetic enzymes and presumably carried an altered regulatory element for the histidine operon.

Repressed enzyme levels of histidinol phosphate phosphatase and doubling times for some TRA-resistant, de-repressed mutants are presented in Table 3. All mutants tested were able to de-repress further when grown on limiting histidine (Table 4). This suggested that the mutants tested possessed altered regulatory mechanisms and that in none of these cases had a regulatory element been completely destroyed.

**Table 4**

*Further de-repression of constitutive mutants*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Strain</th>
<th>De-repression of the histidine operon (relative specific activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Excess histidine</td>
</tr>
<tr>
<td>1</td>
<td>hisH107 (control)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>hisO1202 hisH107</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>hisR1203 hisH107</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>hisR1208 hisH107</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>hisS1209 hisH107</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>hisS1211 hisH107</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>hisG46 (control)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>hisO1812 hisG46</td>
<td>10.2</td>
</tr>
<tr>
<td>3</td>
<td>hisH107 (control)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>hisT1207 hisH107</td>
<td>8.6</td>
</tr>
</tbody>
</table>

All strains were grown on minimal medium containing 0.2% glucose as carbon source. Repressed enzyme levels were determined in the presence of excess histidine, usually 50 μg/ml. Additional de-repression was measured as follows:

- **Experiment 1:** Cultures were started with 4 μg L-histidine/ml and cells harvested 45 min after histidine was exhausted.
- **Experiment 2:** Cultures were grown on 150 μg histidinol/ml.
- **Experiment 3:** Cultures were grown on 0.4 mM adenine.

Since hisH107 is a "leaky" histidine mutation, strains carrying this lesion can de-repress in the absence of exogenous histidine; such de-repression is enhanced by adenine. In each case activities are presented as the specific activity of histidinol phosphate phosphatase relative to the repressed level of the control culture run with each experiment. In experiment 1, the assay of Ames, Garry & Herzenberg (1960) was used; in experiments 2 and 3, the toluenized cell assay of Ames, Hartman & Jacob (1963) was used.

(c) The hisO mutants

Three of the TRA-resistant mutants have been designated hisO on the basis of their high levels of repressed enzyme and map position adjacent to the structural genes of the histidine operon.
(i) Deletion mapping

The map positions of hisO mutations were first determined by selecting prototrophic recombinants from transductional crosses between recipients carrying a histidine deletion mutation and donors carrying only the hisO mutation. Positions of the deletions tested and results of the crosses are indicated in Fig. 1 for mutant hisO1202. If hisO1202 were located within the material homologous to that deleted in a recipient, all prototrophic recombinants were expected to carry the hisO1202 mutation of the donor and, consequently, to form wrinkled colonies. If, on the other hand, hisO1202 were located outside of the deleted region, some of the his\(^+\) recombinants which received the deleted region from the hisO1202 donor would fail to receive also the hisO1202 mutation and would form smooth colonies. All of the deletion mutants numbered 1300 or larger are derived from hisG203 and presumably represent extensions at the ends of the parental deletion (Ames, Hartman & Jacob, 1963). The single smooth colony found on the selection plates of the cross with hisG1300 is assumed to be either a revertant of the hisO mutation or a modifying mutation in the recipient which prevents expression of the rough phenotype. This assumption is based on the fact that no such colonies arose from the cross with hisG203, a smaller deletion mapping within the deleted region of hisG1300. From the data presented in Fig. 1, it was inferred that hisO1202 lies within the hisG203 deletion and thus must be located in the right-hand end of the hisG gene or else outside of the structural genes of the histidine operon.

![Fig. 1. Transduction tests with phage grown on hisO1202. Tests performed as described in section 2(a). Presence of 100% smooth (hisO1202\(^+\)) colonies in tests with metE and ilvC recipients (\(\dagger\) = controls) and presence of wrinkled colonies (hisO1202) in tests with his\(^-\) deletion mutants show that hisO1202 is unlinked to the former loci and linked with the latter. The results obtained with various his\(^-\) deletion mutants further confine the location of hisO1202 to the "right" end of the histidine operon. The dagger (\(\dagger\)) indicates one probable spontaneous mutant (see text).]
(ii) Three-point crosses

The his\textit{O} mutations lie to the right of all known histidine mutations, that is, very close to those sites at the extreme right end of the histidine region as the map is generally drawn. This conclusion is based on three-point transduction tests. Recipients were his\textit{G46} his\textit{O} double mutants; donors were various point mutants in the his\textit{G} gene. Results of such crosses and the cross-over positions which best account for these results are presented in Fig. 2. With donor lesions located to the right of his\textit{G46}, the percentage of the recombinants which received the donor's wild-type operator allele (through quadruple cross-overs) was much lower than the percentage which received the same allele (through double cross-over) when the donor lesion was located to the left of his\textit{G46}. From these results, it was inferred that all three operator mutations were located either to the right of all known histidine mutations or else

\[\text{\textbf{F}ig. 2. Location of his\textit{O} mutations through three-point transduction tests.}\]

Phage grown in auxotropic mutants defective in the his\textit{G} gene was used in transduction tests with various his\textit{G46} his\textit{O} double-mutant recipients. Prototrophic recombinants were scored for the frequency of appearance of the unselected his\textit{O} mutation (wrinkled colony formation) as opposed to his\textit{O}\textsuperscript{+} (smooth colony formation). The map of the his\textit{G} locus is that of Loper, Grabnar, Stahl, Hartman & Hartman (1964). The order of mutant sites in the "right" end of his\textit{G} is now known to be: (70-638) ([611-205-200] 255 638 70). Sites in brackets are not separable by transduction tests; sites in parentheses have not been ordered.

\[\text{\textsuperscript{†}, Includes spontaneous revertants of his\textit{G46} all of which carry his\textit{O} and form wrinkled colonies;}\]

\[\text{\textsuperscript{‡}, percentage presented may be lower than true values since some spontaneous revertants are scored as wrinkled recombinants; NT, not tested.}\]
very close to those known sites lying at the extreme right-hand end of the gene hisG. Thus, results are consistent with a location of all three hisO mutations at the extreme right-hand end of the operon either within or beyond the structural gene.

Evidence that wrinkled colony-forming recombinants scored in mapping hisO were in fact de-repressed is presented in Table 5. High enzyme levels accompany wrinkled colony morphology in each case.

### Table 5

*Enzyme levels in recombinants*

<table>
<thead>
<tr>
<th>Cross</th>
<th>Colony genotype selected</th>
<th>Colony morphology</th>
<th>Histidinol phosphate phosphatase (specific activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hisG203 × hisO1202</td>
<td>hisG203+</td>
<td>W</td>
<td>2.8</td>
</tr>
<tr>
<td>hisG203 × hisO1202</td>
<td>hisG+</td>
<td>W</td>
<td>2.4</td>
</tr>
<tr>
<td>hisD1 hisG46 × hisO1202</td>
<td>hisD+</td>
<td>W</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>hisG+</td>
<td>W</td>
<td>2.2</td>
</tr>
<tr>
<td>hisD1 hisG46 × hisO1202</td>
<td>hisD+</td>
<td>W</td>
<td>2.7</td>
</tr>
<tr>
<td>hisD1 hisG46 × hisO1202</td>
<td>hisD+</td>
<td>S</td>
<td>0.1</td>
</tr>
<tr>
<td>hisD1 hisG46 × hisO1202</td>
<td>hisG+</td>
<td>S</td>
<td>0.1</td>
</tr>
</tbody>
</table>

In each cross the selected recombinant received donor material for the histidine region. The recipient strains are listed first in presenting the cross. Cells for enzyme assays were grown on minimal medium containing 50 μg L-histidine/ml. The histidinol phosphate phosphatase assay of Ames, Garry & Herzenberg (1960) was used. (W, wrinkled; S, smooth.)

(iii) Recombination between hisO mutations

Since all three hisO mutations seemed to be located at the same place, the right-hand end of the hisG gene, crosses were made to determine whether recombination between hisO mutations could be detected. Such recombinants were first sought using the hisO mutations as unselected markers in transductional crosses between a double-mutant recipient, hisG46 hisO, and a donor strain carrying a different hisO lesion. The frequency of hisO+ (smooth) recombinants (~0.01%) was too low for convenient analysis. In order to facilitate detection of recombinants, use was made of an observation by M. J. Voll (personal communication) that constitutive mutants grow poorly at 42°C whereas wild-type cells grow well at this temperature. This temperature sensitivity seems to be a consequence of de-repression of the histidine operon.

Croses were performed using a double mutant, hisG46 hisO, as recipient and a second hisO mutant as donor. Results of these crosses are presented in Table 6. The data in Table 7 indicate that the smooth colony recombinants exhibit the hisO+ phenotype. It is concluded that recombination can occur between hisO1812 and the other two hisO mutations, but not between hisO1202 and hisO1242. Thus, at least two sites are present in the hisO region; if the lesions in these strains are deletions, they do not overlap.

The basal (repressed) enzyme levels for hisO1202 and hisO1242 are quite different (Table 3). Therefore, all three hisO mutations can be differentiated either on genetic or on biochemical grounds.
### Table 6

**Percentage of hisO⁺ recombinants in transduction tests between hisO mutations**

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hisO1202</td>
</tr>
<tr>
<td>G46 O1202</td>
<td>&lt;0.02% (0/5310)</td>
</tr>
<tr>
<td>G46 O1242</td>
<td>&lt;0.02% (0/4535)</td>
</tr>
<tr>
<td>G46 O1812</td>
<td>0.05% (1/1983)</td>
</tr>
</tbody>
</table>

Histidine-requiring (hisG₄₆)-hisO double mutants were infected with phage grown on hisO mutants. Bacteria were plated on minimal medium containing 2% glucose. The percentage of smooth (hisO⁺) recombinants among prototrophic (k⁺46*) clones is shown for each test. In parentheses the ratio hisO⁺ hisG₄₆⁺/total hisG₄₆⁺ is indicated. The results are pooled for replicate experiments. In some experiments the transduction plates were incubated at 37°C. In other experiments, the plates were incubated at 20°C for 16 hr to allow expression of the hisO⁺ genotype and then incubated for two more days, either at 37°C or at 42°C. The three methods produced concordant results.

The data indicate that the order of mutant sites may be G46 (O1202 O1242) O1812.

### Table 7

**Repressed enzyme levels in smooth (hisO⁺) recombinants of crosses between hisO mutants**

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>Fold de-repression (relative specific activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G46 O1242</td>
<td>O1812</td>
<td>1.1</td>
</tr>
<tr>
<td>G46 O1202</td>
<td>O1812</td>
<td>0.9</td>
</tr>
<tr>
<td>G46 O1812</td>
<td>O1242</td>
<td>0.7</td>
</tr>
<tr>
<td>G46 O1812</td>
<td>O1202</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Controls

- G46: 1
- G46 O1812: 7.1
- G46 O1902: 5.0
- G46 O1242: 20.9

Cultures of hisG₄₆⁺ hisO⁺ recombinants were grown on minimal medium containing 50 μg L-histidine/ml. Cells were harvested while still in log-phase and histidinol phosphate phosphatase was assayed with the toluenised cell assay of Ames, Hartman & Jacob (1963).

Fold de-repression is the specific activity of phosphatase relative to that of hisG₄₆ and is indicated separately for each recombinant clone examined.
HISTIDINE REGULATORY MUTANTS

(d) The hisR mutants

A second class of TRA-resistant mutants, hisR, has been mapped previously (Roth & Hartman, 1965; Roth & Sanderson, 1966) and found to lie between the metE and the ilvA loci (Fig. 3). HisR mutants, like hisO, have abnormally high repressed levels of the histidine biosynthetic enzymes and are able to de-repress further these levels when grown under conditions of limiting histidine (Table 4).

![Chromosome map of Salmonella typhimurium showing approximate locations of his loci. The map is based on that of Sanderson & Demerec (1966) with additions described in this report. Approximate locations of several markers are included for purposes of orientation: thr (threonine), pro (proline), gal (galactose) and try (tryptophan).](image)

The nature of the hisR gene product and its role in repression have been investigated by Silbert et al. (1966). They find that hisR mutants have approximately 55% of the normal level of histidine-specific tRNA acceptor activity, whereas none of the other classes of constitutives shows such a reduction. This suggests that the hisR gene is involved, directly or indirectly, in the production of histidine-specific tRNA.

(i) General properties

The class of mutants designated hisS was distinguished from other classes by:
(1) requirement of abnormally high histidine concentrations for maximal repression;
(2) possession of low specific activities of histidyl tRNA synthetase; and (3) a distinct location on the genetic map. It was first noted that several hisS mutants formed wrinkled colonies on nutrient agar containing 2% glucose, but normal, smooth-appearing colonies on minimal medium supplemented with histidine. After hisH107* derivatives were made of the original hisH107 hisS double mutants, it was possible to measure the enzyme levels of these strains on histidine-free medium. This study revealed that hisS mutants required abnormally high levels of histidine in order to repress the histidine biosynthetic enzymes (Table 8; see also Table 1 in Roth & Ames, 1966). Nutrient agar medium apparently did not contain sufficient histidine to cause repression in these mutants.

Wild-type cells grow on minimal medium with repressed levels of the histidine biosynthetic enzymes. In contrast, the above experiments show that hisS mutants, hisS1210, hisS1211 and hisS1520 require exogenous histidine for maximal repression, although each has an intact histidine region. Apparently the normal internal pool of histidine is not sufficient to result in repression of these mutants. In the case of hisS1211, even excess exogenous histidine cannot repress enzyme levels completely.
Cells were grown on minimal medium with the indicated histidine concentration. Histidinol phosphate phosphatase was measured by the toluenized cell assay of Ames, Hartman & Jacob (1963).

### Table 8

**Effect of histidine on enzyme levels of hisS mutants**

<table>
<thead>
<tr>
<th>Strain</th>
<th>L-Histidine concentration (mm)</th>
<th>Histidinol phosphate phosphatase (specific activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hisS1210 hisH107+</td>
<td>0</td>
<td>13-8</td>
</tr>
<tr>
<td></td>
<td>0-01</td>
<td>16-4</td>
</tr>
<tr>
<td></td>
<td>0-04</td>
<td>2-6</td>
</tr>
<tr>
<td></td>
<td>0-07</td>
<td>1-9</td>
</tr>
<tr>
<td></td>
<td>0-10</td>
<td>2-8</td>
</tr>
<tr>
<td></td>
<td>0-15</td>
<td>2-9</td>
</tr>
<tr>
<td></td>
<td>1-00</td>
<td>1-0</td>
</tr>
<tr>
<td>hisS1211 hisH107+</td>
<td>0</td>
<td>24-9</td>
</tr>
<tr>
<td></td>
<td>0-3</td>
<td>9-9</td>
</tr>
<tr>
<td></td>
<td>3-0</td>
<td>10-0</td>
</tr>
<tr>
<td></td>
<td>10-0</td>
<td>9-4</td>
</tr>
<tr>
<td>LT-2 wild type</td>
<td>0</td>
<td>2-6</td>
</tr>
<tr>
<td></td>
<td>0-15</td>
<td>1-8</td>
</tr>
</tbody>
</table>

Numbers in parentheses are the number of times the particular assay has been made.

W, S, these letters stand for wrinkled (W) and smooth (S) colony former. The hisS mutants have low specific activity of the histidyl tRNA synthetase and have higher basal levels of histidinol phosphate phosphatase on nutrient broth medium than on minimal medium.

†Supplemented with 50 μg L-histidine/ml. Strains were sometimes used from which the parental hisH107 mutation had been removed. This difference had no effect on the activities measured. The histidinol phosphate phosphatase assay of Ames, Garry & Herzenberg (1960) was used.

‡Pool medium contains 17 amino acids plus thiamine.
(ii) **Involvement of histidyl tRNA synthetase**

Another property unique to hisS mutants is their low specific activity of the histidyl tRNA synthetase when measured in the transfer reaction (Table 9). In the two hisS mutants tested, this decrease is due to an altered synthetase having a greatly increased $K_m$ value for histidine (Roth & Ames, 1966). Alternative explanations of the decreased activity, such as: (1) presence of an internal synthetase inhibitor, and (2) a regulatory effect on all aminoacyl tRNA synthetases, are deemed unlikely for mutant hisS1209. In an extract-mixing experiment, the presence of mutant extract had no effect on the activity of wild-type enzyme (Fig. 4). This argues against the presence of an inhibitor in the mutant extract. The possibility of a regulatory effect on all synthetases is made unlikely by the observation that hisS1209 has normal activity of the leucyl tRNA synthetase (Table 10). More information on the nature of the hisS mutations is presented in an accompanying paper (Roth & Ames, 1966).

![Graph of histidyl tRNA synthetase activity](image)

**Fig. 4. Tests for the presence of an inhibitor in hisS1209 extracts.**

The transfer of $[^{14}C]$histidine to tRNA was examined. Each assay was incubated 7 min. Gelatin (0.04 mg) was added to increasing amounts of extract from hisH107 in order to keep protein concentrations approximately constant.

The extract of hisH107 catalyzed transfer of $[^{14}C]$histidine to tRNA, whereas the extract of hisS1209 promoted no detectable transfer. The negative slope of the hisS1209 curve may reflect destruction of tRNA by nucleases in the crude extract. A fixed amount (0.039 mg protein) of hisS1209 extract was added to increasing amounts of hisH107 extract. No inhibition of the hisH107 enzyme activity was noted, indicating that the low specific activity of histidyl-tRNA synthetase activity in hisS1209 extracts is not due to the presence of an internal inhibitor.

(iii) **Map position**

All hisS mutations map near the strB locus and thus comprise a particular genetic class. This locus was mapped first through conjugational crosses involving Hfr donor strains (Roth, 1965). These crosses indicated that the hisS locus was located between cysC and the histidine region, and very close to the purC locus. It was then attempted.
to identify a transducing fragment which might cotransduce both hisS and other genes known to be located within this region of the chromosome. For this purpose, phage grown on hisS mutants was used to transduce various auxotrophic mutations to prototrophy. The mutants used as recipients were indicated as located between the cysC locus and the histidine region (Sanderson & Demerec, 1966). Recombinant colonies were inspected for development of wrinkled colonies on minimal medium containing 2% glucose. If the hisS gene were cotransducible with the auxotrophic mutation carried by the recipient, then some of the recombinant colonies would be expected to express the wrinkled colony phenotype of the donor hisS mutation. Wrinkled colonies appeared among the recombinants from crosses between guaA and hisS and crosses between strB and hisS (Table 11). Several of these recombinants were isolated, grown up on minimal medium and checked for enzyme levels. All were found to have de-repressed levels of histidinol phosphate phosphatase and abnormally low specific activities of histidyl tRNA synthetase (Table 12). Since these properties are characteristic of the donor strains in each case, these colonies were identified as true recombinants in which the mutant hisS allele had been co-transduced with the guaA+ or the strB+ allele.

**Table 10**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Histidyl tRNA synthetase (mM/mg × min)</th>
<th>Leucyl tRNA synthetase (mM/mg × min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT2</td>
<td>0.754</td>
<td>1.84</td>
</tr>
<tr>
<td>hisS1209</td>
<td>0.02</td>
<td>1.83</td>
</tr>
<tr>
<td>hisH107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ileA155</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 11**

<table>
<thead>
<tr>
<th>Donor</th>
<th>strB57 Colonies (% W) scored</th>
<th>guaA1 Colonies (% W) scored</th>
<th>Recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>guaA9 Colonies (% W) scored</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>aroD5 (control) Colonies scored</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>purF145 (control) Colonies scored</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT2 (control)</td>
<td>180 0 3625 &lt;0.03</td>
<td>1720 &lt;0.06 200 &lt;0.5 1118 &lt;0.09</td>
<td></td>
</tr>
<tr>
<td>hisS1209</td>
<td>835 8.3 429 &lt;0.23</td>
<td>366 &lt;0.27 30 &lt;3.3 — —</td>
<td></td>
</tr>
<tr>
<td>hisS1210</td>
<td>614 7.8 1220 0.98</td>
<td>307 0.65 200 &lt;0.5 — —</td>
<td></td>
</tr>
<tr>
<td>hisS1211</td>
<td>205 5.4 547 1.65</td>
<td>328 1.53 100 &lt;1.0 1660 &lt;0.06</td>
<td></td>
</tr>
<tr>
<td>hisS1213</td>
<td>273 17.5 1622 0.19</td>
<td>1214 0.88 — — — —</td>
<td></td>
</tr>
<tr>
<td>hisS1219</td>
<td>96 9.4 1602 0.20</td>
<td>1353 0.66 523 &lt;0.2 2877 &lt;0.03</td>
<td></td>
</tr>
<tr>
<td>hisS1520</td>
<td>749 9.7 3060 0.06</td>
<td>— — — — — —</td>
<td></td>
</tr>
</tbody>
</table>

Wrinkled (W) recombinant colonies result from crosses between hisS donors and guaA or strB recipients. All hisS mutants except hisS1209 show such linkage to guaA1; none is linked to aroD5 or purF145. StrB is a mutation which may be selected either for resistance to low concentrations of streptomycin or for a requirement for thiamine + nicotinic acid (Demerec & Lahr, 1960). In these experiments, strB57 (formerly designated nic-5) was transduced to prototrophy.
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TABLE 12

Enzyme content of recombinants of hisS transductional crosses

<table>
<thead>
<tr>
<th>Strain</th>
<th>Histidinol phosphate phosphatase</th>
<th>Histidyl tRNA synthetase†</th>
</tr>
</thead>
<tbody>
<tr>
<td>guaA1</td>
<td>1-2</td>
<td>1</td>
</tr>
<tr>
<td>guaA9</td>
<td>0-62</td>
<td>0-10</td>
</tr>
<tr>
<td>LT-2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>hisS1210 hisH107+</td>
<td>5-3</td>
<td>0-10</td>
</tr>
<tr>
<td>guaA1 x hisS1210</td>
<td>6-7</td>
<td>0-06</td>
</tr>
<tr>
<td>(wrinkled recombinant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>guaA9 x hisS1210</td>
<td>5-6</td>
<td>0-13</td>
</tr>
<tr>
<td>(wrinkled recombinant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>guaA9 x hisS1210</td>
<td>5-9</td>
<td>0-16</td>
</tr>
<tr>
<td>(wrinkled recombinant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hisS1211 hisH107+</td>
<td>9-6</td>
<td>0-15</td>
</tr>
<tr>
<td>guaA9 x hisS1211</td>
<td>12-0</td>
<td>0-15</td>
</tr>
<tr>
<td>(wrinkled recombinant)</td>
<td>‡</td>
<td>0-05</td>
</tr>
<tr>
<td>hisS1213 hisH107†</td>
<td>8-3</td>
<td>‡</td>
</tr>
<tr>
<td>guaA1 x hisS1213</td>
<td>11-7</td>
<td>0-21</td>
</tr>
<tr>
<td>(wrinkled recombinant)</td>
<td>‡</td>
<td></td>
</tr>
<tr>
<td>hisS1219 hisH107</td>
<td>‡</td>
<td>0-08</td>
</tr>
<tr>
<td>guaA9 x hisS1219</td>
<td>6-1</td>
<td>0-31</td>
</tr>
<tr>
<td>(wrinkled recombinant)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Recombinants were derived from crosses between recipient guaA mutants and donor hisS mutants. Selection was made for gua+ and wrinkled colonies were chosen. GuaA mutants were grown on 50 μg guanosine/ml.; hisS1213, hisH107 and hisS1219, hisH107 were grown on 50 μg histidine/ml.; all other strains were grown on minimal medium. The histidinol phosphate phosphatase assay of Ames, Garry & Harzenberg (1960) was used.

† Activities are expressed relative to parental strain in each case.
‡ hisH107+ derivatives of these strains were not available, so their enzyme levels on minimal medium could not be determined.

The transduction tests showed that the hisS gene is co-transducible with the guaA and strB loci and, thus, is located very close to these loci on the genetic map (Fig. 5, below). This conclusion is supported by the finding that all of the hisS mutants tested were found linked to guaA or strB, whereas no linkage of hisS to other nearby loci (Sanderson & Demerec, 1965) was detected: try (tyrosine), <0-045%; phe (phenylalanine), <0-008%; purG (purine), <0-06%; purI (purine), <0-097%; purG (purine), <0-050%.

(f) The hisT mutants

The hisT mutants have high repressed levels of the histidine biosynthetic enzymes and normal specific activity of histidyl tRNA synthetase. The hisT locus maps between the aroD and the purF loci on the Salmonella chromosome. Transduction data for these mutants are presented in Table 13. The data in Table 14 give evidence that the wrinkled colonies scored in the transduction tests are indeed histidine-regulatory mutants. Since the linkage between aroD5 and purF145 (Table 15, 8-1%) is weaker than the linkage of any hisT mutant to either of them, we infer that the
### Table 13

**Transductional crosses with hisT mutants**

<table>
<thead>
<tr>
<th>Donor</th>
<th>purF145</th>
<th>aroD</th>
<th>Recipient</th>
<th>guaA1 (control)</th>
<th>metE333 (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colonies scored (%) W</td>
<td>Colonies scored (%) W</td>
<td>Colonies scored (%) W</td>
<td>Colonies scored (%) W</td>
<td>Colonies scored (%) W</td>
</tr>
<tr>
<td>hisT1207</td>
<td>2225</td>
<td>38.9</td>
<td>1068</td>
<td>19.1</td>
<td>1013 &lt; 0.10</td>
</tr>
<tr>
<td>hisT1222</td>
<td>495</td>
<td>38.4</td>
<td>—</td>
<td>—</td>
<td>395 &lt; 0.25</td>
</tr>
<tr>
<td>hisT1224</td>
<td>348</td>
<td>42.8</td>
<td>—</td>
<td>—</td>
<td>650 &lt; 0.15</td>
</tr>
<tr>
<td>hisT1227</td>
<td>405</td>
<td>38.6</td>
<td>—</td>
<td>—</td>
<td>352 &lt; 0.28</td>
</tr>
<tr>
<td>hisT1230</td>
<td>917</td>
<td>38.6</td>
<td>—</td>
<td>—</td>
<td>238 &lt; 0.42</td>
</tr>
<tr>
<td>hisT1501</td>
<td>—</td>
<td>—</td>
<td>1252</td>
<td>45.0</td>
<td>1081 &lt; 0.09</td>
</tr>
<tr>
<td>hisT1503</td>
<td>36</td>
<td>19.4</td>
<td>—</td>
<td>—</td>
<td>4400 &lt; 0.03</td>
</tr>
<tr>
<td>hidS1211</td>
<td>1660</td>
<td>&lt; 0.06</td>
<td>100</td>
<td>&lt; 1.0</td>
<td>547 1.6</td>
</tr>
<tr>
<td>LT2 (control)</td>
<td>1118</td>
<td>&lt; 0.09</td>
<td>200</td>
<td>&lt; 0.5</td>
<td>3625 &lt; 0.03</td>
</tr>
</tbody>
</table>

W, Percentage of transductant colonies which have wrinkled morphology characteristic of de-repressed strains.

### Table 14

**Repressed enzyme levels and colony morphology of parental strains and recombinants of hisT transductional crosses**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Histidinol phosphate phosphatase (specific activity)</th>
<th>Colony morphology†</th>
<th>Additions to medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental strains:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hisT1207 hisH107</td>
<td>3.4</td>
<td>W</td>
<td>50 µg L-histidine/ml.</td>
</tr>
<tr>
<td>purF145</td>
<td>0.5</td>
<td>S</td>
<td>20 µg adenosine/ml.</td>
</tr>
<tr>
<td>aroD5</td>
<td>NT</td>
<td>S</td>
<td>20 µg L-tyrosine +</td>
</tr>
<tr>
<td>hisH107</td>
<td>0.5</td>
<td>S</td>
<td>50 µg L-phenylalanine/ml.</td>
</tr>
<tr>
<td>Wrinkled recombinants:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>purF145 × hisT1207 hisH107</td>
<td>4.4</td>
<td>W</td>
<td>None</td>
</tr>
<tr>
<td>aroD5 × hisT1207 hisH107</td>
<td>5.5</td>
<td>W</td>
<td>None</td>
</tr>
</tbody>
</table>

Bacteria were grown on minimal medium to which the supplements listed in the last column were added. The histidinol phosphate phosphatase assay of Ames, Garry & Herzenberg (1960) was used. In the transduction tests from which wrinkled recombinants were obtained, the recipient strain is listed first and the donor strain second.

† Colony morphology: W, wrinkled, S, smooth. NT, not tested.

hisT gene is located between the aroD and the purF loci. This conclusion is substantiated by three-point tests (see also section (h) below). As yet, no biochemical alteration has been detected in hisT mutants. All strains tested (hisT1207, 1222, 1227, 1501) have normal levels of histidyl tRNA synthetase. No change in the amount of histidine-specific tRNA acceptor capacity has been detected in a hisT mutant (Silbert, et al., 1966).
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TABLE 15
Co-transduction of the aroD and purF loci

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>aroD5</th>
<th>% with unselected</th>
<th>purF145</th>
<th>No. tested</th>
<th>% with unselected</th>
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<td>5.6</td>
<td>488</td>
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</table>

(g) Unclassified mutants

Only four genetically distinct classes of TRA-resistant histidine regulatory mutants, hisO, hisR, hisS and hisT, have been found. However, several mutants remain which do not, at present, fit into the four categories delineated by transduction tests (i.e., show joint transduction with other appropriate markers). This may mean that additional classes remain to be found. Mutant his-1509 may belong to the hisR class since, like some hisR mutants, it has a decreased amount of histidine-specific tRNA acceptor capacity (Silbert et al., 1966). Several other possible explanations may apply to other unclassified mutants: (i) they may represent non-transducible chromosomal aberrations; (ii) they may fail to elicit de-repression in the new genetic background; (iii) they may elicit high basal enzyme levels in the new genetic background but are unable to show the wrinkled colony phenotype; and (iv) they may be lethal in the new genetic background. Material is now available to map genetically several of these mutations by conjugation tests. Location of these mutations may reveal whether or not additional genetic classes of TRA-resistant mutations exist. The currently unclassified mutants are: his-1206, -1502, -1817, -1818, -1819, -1820, -1821, -1822, -1823, -1824 and -1825.

(h) The purG-metG region of the Salmonella chromosome

During studies on the map locations of hisS and hisT mutations, the genetic map of Sanderson & Demerec (1965) has been partially refined (Fig. 5). The orientation of purG-glyA was established by Demerec & Ohta (1964). The map also includes

![Diagram](image_url)

FIG. 5. Revision of a portion of the Salmonella typhimurium linkage map.

The map depicts the region of the chromosome designated 65 to 80 by Sanderson & Demerec (1965). Values are approximate co-transduction frequencies. 0 means less than 1% co-transduction. Parentheses indicate that the relative order of markers has not been established. Abbreviations for the markers may be found in Sanderson & Demerec (1965), Shifrin, Ames & Ferro-Luzzi-Ames (1966), or are introduced in this report.
unpublished data of M. Demerec (purG-glyA interval), G. FerroLuzzi-Ames (hisP-aroD interval) and J. S. Gots (intervals for guaA-guaB-puri-purC in extension of data of Ozeki (1959)). Gene locus cysA, formerly indicated as being located approximately in this region of the Salmonella chromosome (Sanderson & Demerec, 1965), is not linked by P22-mediated transduction tests (less than 1% co-transduction) with purG, strB, guaA, guaB, purI, purC, purF, aroD or metG (Maxine Levinthal, personal communication). The 05 and rouB loci, indicated in Sanderson & Demerec (1965) as in this region, are now thought to be located between metG and the his operon, closer to the his operon than to metG (Mäkelä, 1965; Johnson, Krauskopf & Baron, 1965; N. Nikaido & Mark Levinthal, unpublished work).

4. Discussion

A search for regulatory mutants for the histidine operon has yielded four genetically distinct classes. These mutants, similar to mutants in other systems, have been called regulatory mutants because of their inability to control normally the function of the related operon. Data presented in this paper and in the two accompanying papers indicate that two of the presumed regulatory genes, hisS and hisR, are involved respectively in the production of histidyl-tRNA synthetase and histidine-specific tRNA. Thus, these two genes may function solely to produce the “true” end-product of the pathway, histidyl-tRNA. They may not, therefore, have a direct role in the regulatory mechanism and not be true regulatory genes. The finding of so many regulatory genes, and the possibility that two of these may not have a singular regulatory function, emphasizes the difficulty of identifying a true regulatory gene without a knowledge of its biochemical function.

Available data on the regulatory mutants are consistent with several models of the control mechanism. If one considers histidyl-tRNA the co-repressor and hisT the regulator gene producing the aporepressor, then the data can be reconciled with the operon model of Jacob & Monod. It should be noted, however, that several hisT mutants manifest slower growth rates on excess histidine, a fact inconsistent with a purely regulatory role for the hisT gene. Furthermore, hisT mutants are not maximally de-repressed, suggesting that none of these mutations completely destroys the capacity to repress the histidine operon.

Several alternative mechanisms for the regulation of the histidine operon have been previously discussed (Roth et al., 1966). We now favor a control mechanism acting at the level of messenger RNA translation in which histidyl-tRNA, or a derivative of it, acts directly to block translation of the histidine operon messenger.

This work was supported, in part, by research grant 5 ROI AI01660 of the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service. One of us (D.N.A.) held a fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina, while another (J.R.R.) held U.S. Public Health Service Fellowship 1-F1-GM-19,520.

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