Proceedings of the National Academy of Sciences
Vol. 66, No. 4, pp. 1096–1103, August 1970

Components of Histidine Transport: Histidine-Binding Proteins and *hisP* Protein*

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Communicated by W. Z. Hassid, May 28, 1970

Abstract. The high-affinity ($K_m = 3 \times 10^{-8}$ M) transport system for histidine in *Salmonella typhimurium* has been resolved into three components: J, K, and P. J, which is a histidine-binding protein released by osmotic shock, is specified by the *hisJ* gene: *hisJ* mutants lack the binding protein and are defective in histidine transport. Another class of mutants—*dhuA*, which is closely linked to *hisJ*—has five times the normal level of binding protein and has an increased rate of histidine transport. P, which is a protein specified by the *hisP* gene, is required for the J binding protein to be operative in transport. *hisP* mutants, though defective in transport, have normal levels of J binding protein. K, a third transport component, works in parallel to J, and also requires the P protein in order to be operative in transport. A second histidine-binding protein has been found but its relation to K is unclear. *hisJ*, *dhuA*, and *hisP* have been mapped and are in a cluster (near *purF*) on the *S. typhimurium* chromosome.

Introduction. Previous work from this laboratory[1,2] on histidine transport in *Salmonella typhimurium* had demonstrated the presence of two systems involved in histidine uptake: the histidine specific permease ($K_m \cong 10^{-8}$ M, involving the *hisP* gene) and the general aromatic permease ($K_m \cong 10^{-4}$ M; *aroP* gene). Mutants defective in each of these systems were described. Here we show that uptake through the histidine-specific permease occurs (as shown in Fig. 1)

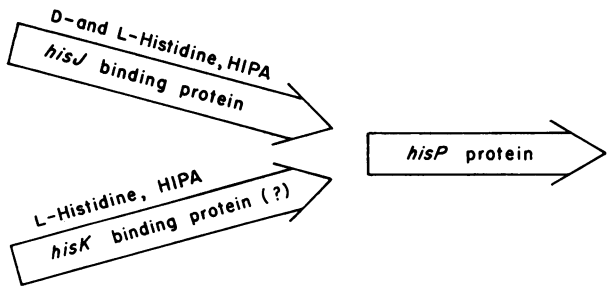

FIG. 1.—Schematic representation of transport through the high-affinity transport system.

through at least two components, J and K, functioning in parallel, and that transport through either of these components is dependent upon the presence of a protein specified by the *hisP* gene. Transport through the J component requires an intact *hisJ* gene, which codes for a histidine-binding protein.

A class of proteins located on the surface of gram-negative bacteria and easily released by mild osmotic shock[3] binds a variety of small molecules. After the

pioneering work from the laboratories of Pardee, Heppel, and Oxender,[4] these "binding proteins" have been implicated in the active transport of those small molecules (recently reviewed[5]). Among these, the amino acid-binding proteins have been indirectly linked to amino acid transport. This paper demonstrates that one of these proteins which binds histidine is an indispensable part of a histidine transport system, thus confirming the relationship between transport and binding proteins, at least as far as histidine is concerned.

Materials and Methods. Strains and genetic tests: All strains used were derived from *S. typhimurium* strain LT-2 and are described in Table 1. All strains, except

TABLE 1. *Bacterial strains.*

Strain	Genotype*	D-histidine growth	HIPA-resistance	J binding protein
TA831	*hisF645*	−	Sensitive	+
TA271	*hisF645 dhuA1*	+	Supersensitive	+ (increased)
TA1014	*dhuA1*		Supersensiive	+ (increased)
TA1646	*hisF645 dhuA1 hisJ5601*	−	Sensitive	−
TA1650	*dhuA1 hisJ5601*		Sensitive	−
TA1647	*hisF645 dhuA1 hisJ5602*	−	Sensitive	−
TA1651	*dhuA1 hisJ5602*		Sensitive	−
TA1648	*hisF645 dhuA1 hisJ5603*	−	Sensitive	−
TA1652	*dhuA1 hisJ5603*		Sensitive	−
TA1649	*hisF645 dhuA1 hisJ5604*	−	Sensitive	−
TA1653	*dhuA1 hisJ5604*		Sensitive	−
TA1008	*hisF645 dhuA1 hisP5503*	−	Resistant	not assayed
TA1195	*dhuA1 hisP5503*		Resistant	+ (increased)
hisP1661	*hisP1661*		Resistant	+
TA1613	*purF145 hisHB22*	−	Sensitive	not assayed
Wild type	histidine-independent transductant of TA831 or LT-2		Sensitive	+

* All *his* mutations except *hisJ* and *hisP* are defective in histidine biosynthesis. Only the histidine-requiring strains, but not their prototrophic transductants, can be assayed for D-histidine growth.

TA831 and TA271, obtained from P. E. Hartman and T. Klopotowski respectively, were constructed in this laboratory, grown, and analyzed genetically by transduction with phage P22-L4 as described earlier.[2] The selection and test for *hisP* mutations has been described previously.[2] Strains carrying *hisJ* mutations were isolated from TA271 (*hisF645 dhuA1*, a strain capable of utilizing D-histidine as the source of L-histidine) as D-histidine nonutilizers which were still sensitive to 2-hydrazino-3-(4-imidazolyl) propionic acid (HIPA)[6,7] (gift of F. A. Kuehl, Jr., of Merck, Sharp and Dohme Research Laboratories, Rahway, N.J.). TA271 was mutagenized with ICR 191,[8] then exposed to penicillin in minimal medium containing 2×10^{-5} M D-histidine: about 15% of the D-histidine nonutilizers thus obtained (assayed by radial streak[2] against 1 μmol of D-histidine) were HIPA-sensitive, i.e., *hisJ* mutants.

Column chromatography: DEAE-Sephadex (Pharmacia, Piscataway, N.J.) columns were prepared according to the Pharmacia instruction manual and equilibrated in 0.01 M Tris·HCl, pH 7.4. The shock fluid was dialyzed overnight against the equilibrating buffer and applied to the column. After washing the column with two bed volumes of equilibrating buffer, the protein was eluted with a linear NaCl gradient from 0 to 0.2 M, with a flow rate of 20 ml/hr.

Assays: The uptake of ^{3}H-histidine (New England Nuclear Corp., Boston, Mass., 2 Ci/mmol) was determined by the "growing-cells" method[1] (about 2 μg of dry weight of cells per milliliter). The trichloroacetic acid precipitates were filtered on glass fiber filters (Gelman, Ann Arbor, Mich., type A), washed with 10% trichloroacetic acid, then

with 95% ethanol, air dried, and counted in POP-toluene (Spectrafluor, Amersham/Searle) with an efficiency of 30%. Linear initial rates of uptake are expressed as μmoles of ^{3}H-histidine incorporated per minute per gram dry weight.

Shock fluid was prepared[3] from cells harvested at a density of 5×10^8 cells/ml. The shock fluid was concentrated 10- to 60-fold by ultrafiltration (Union Carbide no. 8 dialysis tubing), and then filtered through a Millipore filter type HA, 0.45 μm (Millipore Filter Corp., New Bedford, Mass.). Histidine-binding activity was assayed by dialyzing (for 18 hr at 4°C) 0.3 ml of the shock fluid against a large volume of 0.1 M NaCl–0.01 M Tris·HCl, pH 7.4, containing 1×10^{-8} M ^{3}H-L-histidine. Small dialysis sacs were made from Union Carbide dialysis tubing (no. 8) preheated in water at 60°C for 3 hr. Aliquots of 0.2 ml from sacs and external buffer were counted in 10 ml Bray's solution[9] with an efficiency of 20%. One unit of binding activity is defined as 1 pmole of histidine bound at 1×10^{-8} M histidine. This assay allows proportionality of activity between 0.5 and 140 units of histidine-binding activity. Acid phosphatase[10] and protein concentration[11] were also measured.

Results. Mutations affecting the high-affinity histidine transport system: (1) *hisP*. This class of mutants has been described previously[2,7] as being resistant to the inhibitory histidine analogue HIPA. Their resistance is caused by a defect in the high-affinity L-histidine transport system. The *hisP* gene codes for a protein, as shown by the isolation of amber mutants in this gene.[2]

(2) *dhuA*. Histidine-requiring strains (e.g., deletion *hisF645*) are unable to utilize D-histidine as the source of L-histidine, but mutations arise at high frequency which confer the ability to utilize it. A class of these mutants allowing D-histidine growth (*dhuA*) has been isolated by Klopotowski and his collaborators.[12] They showed that the *dhuA* mutations are adjacent to the known *hisP* gene, and cause an increased D- and L-histidine uptake. The *dhuA* mutants have an increased sensitivity to HIPA (Table 1), as a consequence of the increased transport of this inhibitor.

(3) *hisJ*. These mutants have been selected (as described in *Materials and Methods*) in *dhuA1 hisF645* for the loss of the ability to grow on D-histidine, while still retaining HIPA sensitivity (Table 1).

(4) *dhuA hisP*. The *hisP* mutation has been introduced also in the *dhuA* mutant strain by selecting for resistance to HIPA. The resulting double mutants have lost the capacity to grow on D-histidine simultaneously to the loss of sensitivity to HIPA (Table 1).[12] In agreement with the properties of these double mutants, 30 *hisP* mutants (containing a histidine operon deletion) were tested and found to be unable to mutate to D-histidine utilization. Thus the *hisP* gene is required for transport of both D- and L-histidine and the analogue HIPA.

Genetic mapping: Both *dhuA*[12] and *hisP*[2] mutations were confirmed to be about 40% cotransducible with the *purF* locus, by transducing on L-histidine medium the recipient TA1613 (*purF145 hisHB22*), with phage prepared either on TA271 (*hisF645 dhuA1*) or on 30 independently isolated *hisP*-carrying strains. The *Pur*$^+$ *His*$^-$ recombinants were tested for D-histidine growth and HIPA-resistance respectively.

Moreover, the *dhuA* and *hisP* loci can be shown to be very close together by exposing TA1613 (on L-histidine medium) to phage grown on TA1008 (*hisF645 dhuA1hisP5503*). Only 2% of the *Pur*$^+$ *His*$^-$ recombinants inherit *dhuA1* alone from the donor: this rare type requires a recombinational event *between dhuA* and *hisP* because the *hisP* mutation eliminates D-histidine growth.

In a similar cross the *dhuA* and *hisJ* mutations can be shown to be closely linked. Strain TA1613 was exposed (on L-histidine medium) to phage grown on *dhuA hisJ* double mutants TA1646, TA1647, TA1648, and TA1649. Only 2% of the *Pur*$^+$ *His*$^-$ recombinants inherit the *dhuA* mutation without *hisJ*, i.e., are D-histidine growers. The *hisP*, *hisJ*, and *dhuA* sites are therefore very closely linked to each other.

Histidine-binding proteins in the wild type and in mutant strains: By giving bacterial cells a mild osmotic shock treatment,[3] histidine-binding activity was liberated in the shock fluid. Fractionation of the wild type shock fluid on DEAE-Sephadex revealed that 95% of the binding activity (at 10^{-8} M L-histidine) was eluted as a peak (J protein) at 0.04 M NaCl. A second peak (tentatively designated as K protein), representing about 5% of the activity, was eluted at 0.15 M NaCl.

The histidine-binding activity in the shock fluid of the various mutants is shown in Table 2. It is clear that the level of J binding protein is increased in

TABLE 2. *Levels of J binding protein.**

Strain	Genotype	No. of expts.	Units of J Binding Protein per: Unit of acid phosphatase	Protein released (mg)	Dry weight of bacteria (g)
	Wild type	6	8 (1)†	42 (1)	302 (1)
TA1014	*dhuA1*	5	42 (5)	165 (4)	1,520 (5)
TA1650	*dhuA1 hisJ5601*	3	<0.45 (<0.06)	<2.4 (<0.06)	<26 (<0.08)
TA1651	*dhuA1 hisJ5602*	3	<0.45 (<0.06)	<2.4 (<0.06)	<26 (<0.08)
TA1652	*dhuA1 hisJ5603*	4	<0.45 (<0.06)	<2.4 (<0.06)	<26 (<0.08)
TA1653	*dhuA1 hisJ5604*	1	<0.45 (<0.06)	<2.4 (<0.06)	<26 (<0.08)
hisP1661	*hisP1661*	4	8 (1)	37 (0.9)	375 (1)
TA1195	*dhuA1 hisP5503*	4	39 (5)	158 (4)	3,592 (12)

* In all strains, except the *hisJ* containing strains, 95% or more of the binding activity has been shown by chromatography to be caused by the J binding protein. Therefore all quantitative assays in this table were done on the total shock fluid. Because of variability in the efficiency of shocking, the data are presented relative to the weight of protein released and the activity of acid phosphatase released, in addition to the weight of starting bacteria. The results averaged in this table were from completely independent experiments.

† The numbers in parentheses are relative to the wild type.

strains containing a *dhuA* mutation, and absent in strains containing a *hisJ* mutation. The binding protein from *dhuA1* has the same chromatographic properties and binding affinity ($K_D \sim 2 \times 10^{-7}$ M at 4°C) for histidine as the protein from the wild type. We exclude the possibility that the *hisJ* and *dhuA* mutations have caused a change in the total amount of protein released by shocking because all the strains assayed release about the same amount of either total protein or acid phosphatase per gram dry weight. Introduction of a *hisP* mutation in either the wild type or in a *dhuA*-containing strain (see *hisP1661* and TA1195 in Table 2), does not affect the level of J binding protein. The level of the K binding protein appears to be unchanged in the strains assayed in Table 2, but these experiments will be performed on a larger scale for accurate quantification.

Numerous D-histidine utilizing revertants of *hisJ* mutants have been isolated and all have recovered the J binding protein. The binding protein from one of these revertants[13] appears to be temperature sensitive, as compared to the wild

type protein (from *dhuA1*). The mutant protein loses about 61% of the histidine-binding activity after being heated at 100°C for 50 min, while the wild type protein loses about 20% of the activity (average value of several experiments). Further analysis of this mutant protein is in progress.

Uptake of L-histidine in the wild type and in transport mutants: The rate of uptake and the affinity of the transport system for L-histidine in the wild type, in *dhuA1 hisJ5601* (missing the J binding protein), and in *hisP1661* (with a normal level of J binding protein) are shown in Figures 2*A* and 2*B*. Both *hisJ* and *hisP*

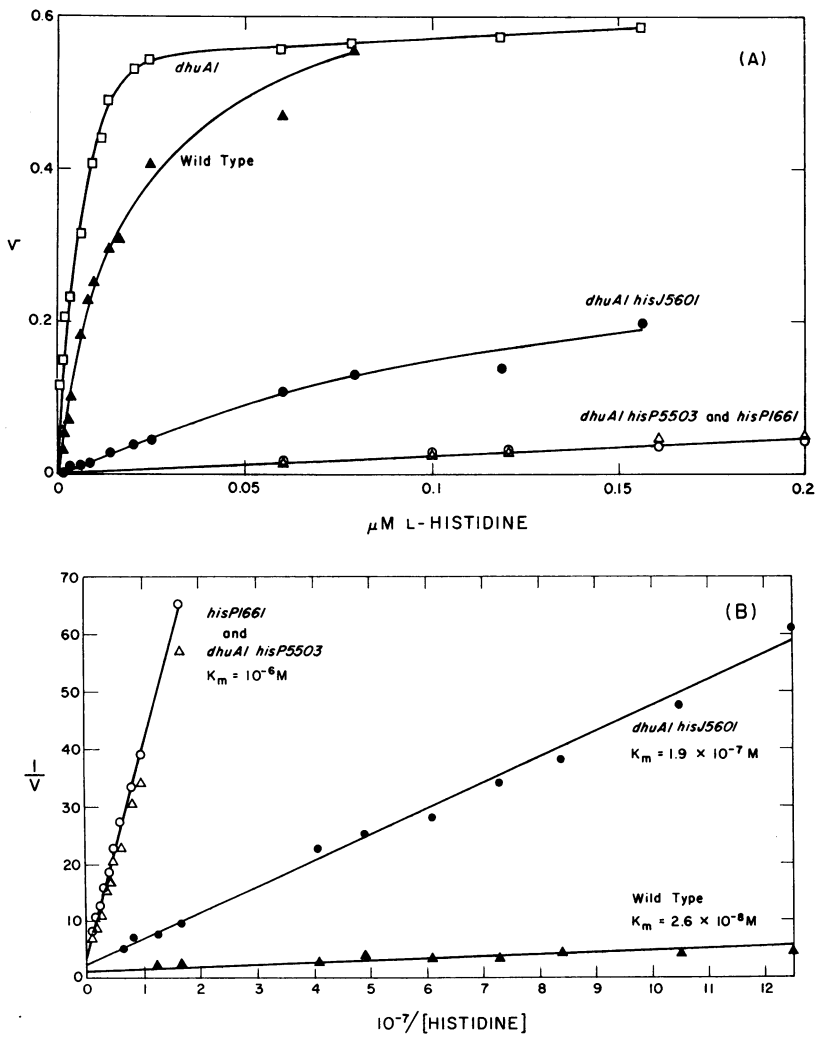

FIG. 2.—(*A*) Initial rates of uptake of L-histidine as a function of histidine concentration. (*B*) Lineweaver-Burk plots of L-histidine uptake. The K_m for *dhuA1* is 6.6×10^{-9} M and has been calculated, together with the K_m for the wild type, from a separate plot at an appropriate scale. *dhuA1* (TAI014, □); wild type (▲); *dhuA1 hisJ5601* (TA1650, ●); *dhuA1 hisP5503* ((TA1195, △); *hisP1661* (○).

mutant strains are defective in uptake *although to a different extent.* The affinities of these residual transport activities for L-histidine are 2×10^{-7} M and 10^{-6} M respectively. It is clear that these differences in residual rates and affinities are significant and characteristic of completely defective mutants in *hisJ* or *hisP*, as the same results were obtained upon analysis of three *hisJ* frameshift mutants and two *hisP* mutants, one of which is an amber mutant. We conclude that the loss of the J binding protein in *hisJ* mutants results in the loss of a transport component, J, with a very high affinity ($K_m \sim 10^{-8}$ M, as assayed in either the wild type or in the *dhuA* mutant).

Despite the loss of this component, *hisJ* mutants can still transport histidine very efficiently ($K_m = 2 \times 10^{-7}$ M, Fig. 2), presumably through additional transport components. Thus, the K_m of 2×10^{-7} M in *hisJ* mutants is ascribed to a component tentatively designated K. Mutations eliminating transport through K *only* have not yet been obtained. Mutations in *hisP* have apparently eliminated transport through both the J and the K components: this is indicated by the finding that the sum of the residual rates in *hisJ* and *hisP* mutants is less than the wild type rate (Fig. 2*A*). Therefore, J and K are acting in parallel, and apparently both require the *hisP* product in order to transport histidine.

The *dhuA1* mutant, which increased binding protein fivefold, shows a marked increase in transport (Fig. 2*A*), and has a higher affinity ($K_m = 6.6 \times 10^{-9}$ M) than the wild type ($K_m = 2.6 \times 10^{-8}$ M). If the wild type rate is truly the sum of the K and J activities, the preponderance of the J in the *dhuA* mutant could be responsible for this shift in the apparent affinity.

A double mutant, *dhuA1 hisP5503*, has the same rate of transport and poor affinity for histidine uptake as *hisP* mutant alone.

Additional transport systems of low affinity: As shown in Figures 2*A* and 2*B*, *hisP* mutants have a greatly decreased rate of L-histidine uptake. The remaining activity still gives Michaelis-Menten kinetics with a K_m of about 10^{-6} M. This has been shown by kinetic and genetic analysis to be caused by at least three more transport systems of low affinity, one of which is the general aromatic permease.[1] A complete study of these systems will be reported at a later date.

Discussion. It has been demonstrated previously that mutants in the *hisP* gene are lacking the high-affinity ($K_m = 10^{-8}$ M) transport system for L-histidine in *S. typhimurium.*[2,7] In this paper we show: (*a*) that this is a complex transport system made up of three components distinguishable kinetically and genetically; (*b*) that a histidine-binding protein, J (coded for by the *hisJ* gene), is one of the components of this system; (*c*) a number of aspects of D- and L-histidine transport through these components. These observations led us to propose the scheme of Figure 1.

J binding protein is involved in transport: Osmotic shock of the bacteria releases from the cell surface two proteins that bind L-histidine: J (about 95% of total) and K (about 5% of total). Mutants (*dhuA*), selected for increased utilization of D-histidine, have a fivefold increase in the J binding protein, thereby causing an increased transport for both D- and L-histidine, and an increased sensitivity to the histidine analogue HIPA. The *dhuA* mutation, which maps adjacent to the *hisJ* gene, increases the level of the J protein, possibly by affecting

the regulation of its synthesis. In agreement with this, the J protein appears to be the same in *dhuA1* and in the wild type on the basis of affinity for histidine and chromatographic properties.

The *hisJ* mutants, which were selected from strains carrying a *dhuA* mutation, have lost the ability to utilize D-histidine and completely lack the J binding protein. As a consequence they have a decreased transport for L-histidine and have lost the extra sensitivity to HIPA.

Further evidence that the *hisJ* gene is the structural gene for the J binding protein, rather than a regulatory gene, is that a D-histidine-utilizing revertant of a *hisJ* mutant appears to have an altered J binding protein.

The large difference in affinity for L-histidine between the transport *in vivo* ($K_m = 6.6 \times 10^{-9}$ M in *dhuA1*) and the J binding protein ($K_D = 2 \times 10^{-7}$M)can be rationalized as caused by the conditions of the binding assay. This is done at 4°C on a protein removed from its *in vivo* environment and under arbitrary salt and pH conditions.

***hisP* protein is necessary for the J binding protein to function in transport:** *hisP* mutants selected by HIPA resistance (presumably unable to transport HIPA) completely lack high-affinity transport for L-histidine. These mutants have normal levels of histidine-binding proteins that, because of the defect in the *hisP* protein, are not functional in the *in vivo* transport. That the *hisP* gene codes for a protein has been deduced by the finding of *hisP* amber mutants.[2]

A functional *hisP* protein is also necessary for the transport mediated through the increased level of the J binding protein in *dhuA* strains. Thus, a *dhuA hisP* double mutant still has the expected fivefold-increased level of binding protein associated with the *dhuA* mutation, yet it lacks the high-affinity transport of L-histidine. This double mutant no longer utilizes D-histidine, nor is it HIPA sensitive, which gives additional evidence that the J binding protein is nonfunctional in transport in the absence of the *hisP* protein. In accordance with our interpretation of the nature of this double mutant, it has been found that *hisP* mutants will not mutate to D-histidine utilization.

***hisP* protein is necessary for the function of another transport component, K:** All *hisJ* mutants have residual high-affinity L-histidine transport ($K_m = 2 \times 10^{-7}$ M), unlike the *hisP* mutants which have no high-affinity L-histidine transport. We define the residual activity in *hisJ* mutants as the K component of transport, which we deduce also to be dependent on a functional *hisP* protein, because *hisP* mutations eliminate this activity. The K component must also transport HIPA, as *hisJ* mutants are still HIPA sensitive, while *hisP* mutants are not. In support of the existence of two components J and K, both dependent on a functional *hisP* protein, is the fact that all HIPA-resistant mutants selected from the wild type are *hisP* mutants. One possibility is that the second, minor, L-histidine-binding protein, which is still present in *hisJ* and *hisP* mutants, is the K component. We have selected for HIPA resistance in *hisJ* mutants and these double mutants are being analyzed for the K binding protein to see whether they are *hisJ hisK* or *hisJ hisP* double mutants.

Other transport systems for L-histidine: The *hisP* mutations eliminate all high-affinity histidine transport and the Michaelis constant of the remaining

transport is about 10^{-6} M. This remaining transport is completely inhibited by aromatic amino acids, unlike the *hisP* system, and has been resolved into at least three components, one of which is the previously described *aroP* system.

We thank B. N. Ames for his constant interest and advice, T. Klopotowski for discovering and supplying us with the key strain, TA271, and M. A. Liggett for excellent technical assistance in the kinetic experiments.

* Supported by USPHS grant AM12121, to C. E. Ballou, whom we thank for support.

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[1] Ames, G. F., *Arch. Biochem. Biophys.*, **104**, 1 (1964).

[2] Ames, G. F., and J. R. Roth, *J. Bacteriol.*, **96**, 1742 (1968).

[3] Nossal, N. G., and L. A. Heppel, *J. Biol. Chem.*, **241**, 3055 (1966); Heppel, L. A., *Science*, **156**, 1451 (1967).

[4] Pardee, A. B., and L. S. Prestidge, these PROCEEDINGS, **55**, 189 (1966); Anraku, Y., and L. A. Heppel, *J. Biol. Chem.*, **242**, 2561 (1967); Piperno, J. R., and D. L. Oxender, *J. Biol. Chem.*, **241**, 5732 (1966).

[5] Pardee, A. B., *Science*, **162**, 632 (1968); Kaback, H. R., *Ann. Rev. Biochem.*, in press.

[6] Abbreviations used: HIPA, 2-hydrazino-3-(4-imidazolyl) propionic acid; K_m, Michaelis constant; K_D, dissociation constant.

[7] Shifrin, S., B. N. Ames, and G. F. Ames, *J. Biol. Chem.*, **241**, 3424 (1966).

[8] Ames, B. N., and H. J. Whitfield, *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 31, (1966), p. 211; Oeschger, N. S., and P. E. Hartman, *J. Bacteriol.*, **101**, 490 (1970).

[9] Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).

[10] Neu, H. C., *J. Biol. Chem.*, **242**, 3896 (1967).

[11] Lowry, O. H., N. J. Rosenbrough, N. C. Farn, and R. J. Randal, *J. Biol. Chem.*, **193**, 265 (1951).

[12] Krajewska-Grynkiewicz, K., W. Walczak, and T. Klopotowski, *J. Bact.*, in preparation.

[13] This revertant, induced by ICR 191 in a strain carrying a *hisJ* mutation which was also induced by ICR 191, does not grow on D-histidine as well as the *dhuA* grandparent. Presumably it is a frameshift revertant of the "+ −" type in *hisJ*.