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Mechanism of Inhibition of Histamine Synthesis in the Rat: Two Specific Gastric Histidine Decarboxylases

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SUMMARY

A comparison of the properties of histidine decarboxylase (EC 4.1.1.22) from the pylorus of the stomach and the fetus of rats indicated similarities in kinetic parameters, such as K_m values and pH optima. Certain acidic compounds, carbonyl reagents, and substrate analogues inhibited the enzymes competitively and displayed indentical inhibitor dissociation constants. Heat inactivation studies employing partially purified preparations indicated indistinguishable rates of loss of activity. The results indicate that, except for a difference in the degree of inhibition by bisulfite, the properties of the pyloric and fetal enzymes were indistinguishable by kinetic criteria.

Gel filtration experiments, however, permitted partial resolution of the pyloric and fetal histidine decarboxylases. An additional species of enzyme emerged in cochromatography experiments involving the pyloric and fetal enzymes and with preparations derived from pylorus that had been aged for 2 months at −15°. The observed differences in elution volume of the pyloric and fetal enzymes suggest that the two species are discrete catalysts.

A partially purified histidine decarboxylase from the fundic portion of rat stomach was characterized. Kinetic studies indicated that it represents a second histidine decarboxylase, distinct from aromatic L-amino acid decarboxylase. The resistance of fundic histidine decarboxylase to known histidine decarboxylase inhibitors is discussed in relation to the difficulties encountered in attempting to inhibit histamine formation *in vivo*.

INTRODUCTION

Histamine has been proposed as the final mediator of gastric acid secretion (1). Histidine decarboxylase (EC 4.1.1.22), the enzyme responsible for the biosynthesis of histamine, was shown to be inhibited *in vitro* by indomethacin and other acidic anti-inflammatory drugs (2, 3). A later report extended these findings to intact rats (4); the ability of indomethacin to produce inhibition of histidine decarboxylase *in vivo* was reported following the administration of high doses. No inhibition was found, however, when the drug was administered to fasted rats (4). The status of feeding is known to affect the level of gastric histidine decarboxylase (5); fasting markedly reduces the levels of histamine as well as of histidine decarboxylase (6). Radwan and West (6) demonstrated the presence of two histidine decarboxylase activities. One, localized in the pyloric region of the rat stomach, was found to be sensitive to inhibition by certain amino acids but was relatively insensitive to fasting. The histidine decarboxylase derived from the fundus was not readily inhibited, but disappeared following deprivation of food (6). It was hoped that better knowledge of the enzymes responsible for histamine

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synthesis in the rat stomach would contribute to our understanding of the factors leading to inhibition of histamine formation *in vivo*. The present report describes results obtained with partially purified histidine decarboxylase preparations from the fundic and pyloric regions of the stomach and the fetus of rats.

METHODS

Purification of histidine decarboxylase. Partially purified fetal histidine decarboxylase was prepared according to Håkanson (7), using modifications previously described (8). For the pyloric enzyme, the procedure described below was used.

Male rats, obtained from Carworth Farms, weighing 165–320 g, were employed as the source of the gastric enzymes. The animals were killed by a blow on the head. The stomachs were excised quickly. The fundus [thin part as used by Radwan and West (6); synonymous with cardiac portion] and pyloric region [thick part (6); acid-secreting portion] were collected separately. The tissues were rinsed carefully in cold 0.9% NaCl, blotted, and frozen. The tissues were homogenized with 2 volumes of 10 mM potassium phosphate buffer, pH 6.6, using a mortar and pestle. Grinding was aided by adding small amounts of purified sand. The homogenate was centrifuged for 20 min at 39,000 × *g* (max *g* force). The supernatant fluid was recentrifuged for 60 min at 160,000 × *g* (max *g* force). The protein concentration of the supernatant fluid was adjusted to 10 mg/ml. The extract was fractionated by addition of a neutralized, saturated solution of ammonium sulfate at 0°. Pyloric histidine decarboxylase was precipitated between 0 and 45% saturation, and the fundic histidine decarboxylase, between 55 and 85% saturation. The protein precipitates were dissolved in 0.1 M potassium phosphate buffer, pH 7.0, and dialyzed against 5 mM potassium phosphate buffer, pH 6.6. Precipitates that appeared during dialysis were removed by centrifugation. Purification was 4-fold, and the recovery was nearly quantitative. Additional purification was achieved by gel filtration, using a 1.3 × 101 cm column of Sephadex G-150, with 5 mM potassium phosphate buffer, pH 6.6. Two-milliliter samples of the ammonium sulfate fractions, each representing 5–73 mg of protein, were applied. Fractions exhibiting maximal activity were pooled. The purification was 7–8-fold relative to the supernatant fluid from the ultracentrifugation. Maximal reaction velocities of $^{14}CO_2$ evolution of fetal, pyloric, and fundic preparations thus obtained ranged from 0.2 to 0.3 nanomole min^{-1} mg^{-1} at the respective pH optima. The various preparations were stable at −15°.

The Sephadex column was calibrated with standards, using the following substances: blue dextran 2000, 6 mg; catalase, 2 mg; yeast alcohol dehydrogenase, 3 mg; bovine serum albumin, 10 mg; bovine hemoglobin, 6 mg; and tritiated water, 0.2 μCi. Stokes radii and the effective pore radius of the column (155 A) were calculated by the method of Ackers (9).

Assay of histidine decarboxylase. The standard reaction mixture contained the following in a total volume of 0.5 ml: 0.1 M potassium phosphate, pH 6.9; 0.01 mM pyridoxal phosphate; 0.75 mM L-histidine; 0.38 μCi of DL-histidine or 0.19 μCi of L-histidine (carboxyl-^{14}C); and 0.4–6 mg of protein. Histidine was added last to initiate the reaction. Prior incubation of carbonyl reagents with enzyme or pyridoxal phosphate was avoided. Incubation was conducted for 30, 45, or 60 min at 37°. The reaction vessel and estimation of $^{14}CO_2$ were described earlier (10). The proportionality of evolution of $^{14}CO_2$ to the time of incubation was established with the various preparations. Incubation of unfractionated dialyzed extracts from pylorus in excess of 45 min revealed a lack of linearity of the reaction rate. Partially purified pyloric enzyme as well as fundic and fetal preparations could be incubated for at least 90 min without detectable deviation of proportionality of product formation to the time of incubation. Histamine was assayed fluorometrically (11) following chromatographic purification on Cellex-P columns (12).

Statistics of estimates of Michaelis constants. Estimates of Michaelis constants were highly reproducible. The standard error of the K_m values was less than ±5% in 19 experiments, and less than ±10% in 10 experiments. In the remaining 10 cases values

of the standard error ranged from ±10 to ±30%.

Aromatic amino acid decarboxylase. Pyloric extracts, centrifuged at 160,000 × *g* and subsequently dialyzed, were used without further purification. Assay of aromatic L-amino acid decarboxylase activity was analogous to that of histidine decarboxylase, except that 0.05 μmole of DL-3,4-dihydroxyphenylalanine (DL-dopa) (0.19 μCi, carboxyl-^{14}C) was used as substrate. Incubation was conducted for 5 min at 37°, at pH 7.1. Protein was measured by the method of Lowry *et al.* (13), with bovine serum albumin serving as reference standard. Catalase and alcohol dehydrogenase were estimated by the methods of Beers and Sizer (14) and Racker (15), respectively.

Materials. Flufenamic acid was donated by Parke, Davis and Company; indomethacin, by Merck Sharp and Dohme, Inc.; phenylbutazone, by Geigy Pharmaceutical Company; and 3-hydroxybenzyloxyamine phosphate (NSD-1024) and 4-bromo-3-hydroxybenzyloxyamine phosphate (NSD-1055), by Smith and Nephew Research, Ltd. DL-α-Methylhistidine hydrochloride was purchased from Regis Chemical Company, Chicago; the supplier stated a melting point of 290–291° (with decomposition). Isotopically labeled dopa and histidine were obtained from New England Nuclear Corporation. Sigma Chemical Company provided bovine hemoglobin, bovine serum albumin, crystalline yeast alcohol dehydrogenase, and crystalline catalase. Sephadex G-150 and blue dextran 2000 were supplied by Pharmacia.

RESULTS AND DISCUSSION

Comparison of properties of fetal and pyloric histidine decarboxylases. Preliminary observations indicated that histidine decarboxylases from fetal and pyloric extracts displayed very similar characteristics. The pH optimum for both the fetal and pyloric histidine decarboxylases was 6.8.

The Michaelis constant for histidine decarboxylase is known to change as a function of the pH (7, 16). Håkanson (7) explained this relation by demonstrating that the enzyme requires the anionic form of the substrate. The present measurements showed this relation to obtain for both the fetal and pyloric histidine decarboxylases. Under optimal conditions of assay (0.75 mM histidine, pH 6.8) the K_m for the fetal enzyme was 0.32 ± 0.03 mM, and for the pyloric enzyme it was 0.27 ± 0.006 mM (standard error). A summary of the K_m values at various pH values is given in Fig. 1.

The possibility was considered that pyloric and fetal histidine decarboxylases are identical. To test this postulate, the effects of known inhibitors on unfractionated histidine decarboxylases from pylorus and fetus were compared. The inhibitors employed belong to the following classes: (a) α-amino acids; (b) carbonyl reagents, including hydroxylamines, hydrazines, cyanide, and bisulfite; and (c) acidic compounds, including several anti-inflammatory drugs. The agreement be-

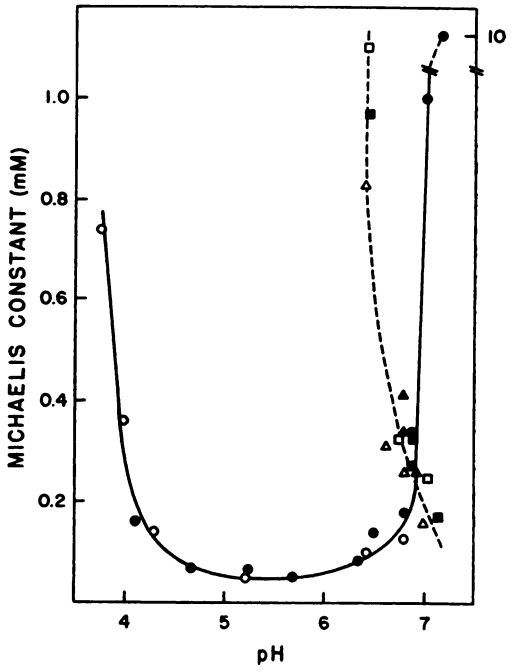

FIG. 1. *Michaelis constants of fundic, pyloric, and fetal histidine decarboxylases as a function of pH*

Circles, fundic; triangles, pyloric; squares, fetal histidine decarboxylase. Open symbols, purified enzyme from gel filtration experiments; solid symbols, dialyzed crude preparations or $(NH_4)_2SO_4$ fractions. Solid line, fundic; dashed line, pyloric and fetal histidine decarboxylases. Values below pH 6, 0.1 M acetate; above pH 6, 0.1 M phosphate buffer.

TABLE 1

Inhibition of fetal, pyloric, and fundic histidine decarboxylases and pyloric dopa decarboxylase

Dialyzed crude extracts (see METHODS) were used as enzyme, 3–7 mg of protein each; 0.1 M potassium phosphate (pH 6.8); 0.01 mM pyridoxal phosphate; and 0.75 mM L-histidine or 1.1 mM DL-dopa. The incubation period was 45 min with histidine as substrate; 5 min with dopa.

Inhibitor	Inhibitor concentration	Inhibition			
		Fetal histidine decarboxylase	Pyloric histidine decarboxylase	Fundic histidine decarboxylase	Pyloric dopa decarboxylase
	mM	%	%	%	%
4-Toluenesulfonic acid hydrazide	0.003	75	76	2	2
	1.5			29	96
3-Hydroxybenzyloxyamine	0.0008	65	69	1	28
	0.2			13	100
Hydroxylamine	0.01	18	20	21	6
	0.1	97	100	91	55
2-Hydroxybenzoic acid	10.0	82	84	0	99
Indomethacin	1.0	66	69	7	69
Flufenamic acid	1.0	86	85	9	98
3,4,5-Trihydroxybenzoic acid methyl ester	1.0	53	60	0	78
DL-Dopa	0.4	23	29	0	
α-Methyldopa	1.0	11	8	3	78
DL-α-Methylhistidine	21.9	99	99	7	1
Potassium cyanide	1.0	95	94	4	92
Sodium bisulfite	0.5	60	15	0	52

tween the values for percentage inhibition of the enzymes from the two sources is very close (Table 1). One exception was noted. The effect of bisulfite on pyloric histidine decarboxylase was reproducibly lower than for the fetal enzyme. These efforts were extended, using partially purified preparations. One representative of each of the three classes of inhibitors was selected to determine the type of inhibition. As may be seen in Fig. 2, indomethacin inhibits fetal histidine decarboxylase competitively with respect to the substrate. A similar experiment was performed with 4-toluenesulfonic acid hydrazide, an effective inhibitor of gastric histamine synthesis *in vivo* (17), and α-methylhistidine; the inhibition by both the hydrazide and α-methylhistidine was competitive with histidine. The inhibitor dissociation constants for pyloric and fetal histidine decarboxylases were in excellent agreement for each of the three inhibitors (Table 2). Except for the discrepancy in the response to inhibition by bisulfite, the experimental data thus far presented favor the view that pyloric and fetal histidine decarboxylases are identical.

Preliminary studies, comparing the two types of preparations, indicated very similar rates of enzyme inactivation by heat treatment (18). A more detailed investigation, however, revealed conditions under which pyloric and fetal histidine decarboxylases displayed different rates of heat inactivation; markedly differing rates of heat inactivation were noted in the presence of 0.1 mM pyridoxal phosphate, using unfractionated extracts or the 0–45% ammonium sulfate fractions. As pyloric histidine decarboxylase appeared to be the less stable enzyme, the presence of proteolytic enzymes was considered. The majority of proteolytic enzymes possess relatively low molecular weights (19). Fractionation by gel filtration with Sephadex G-150 was therefore employed to achieve additional purification. Pooled eluate fractions of maximal activity were incubated in parallel at 53°. Aliquots were withdrawn at certain time intervals and assayed for surviving activity. As shown in Fig. 3, iden-

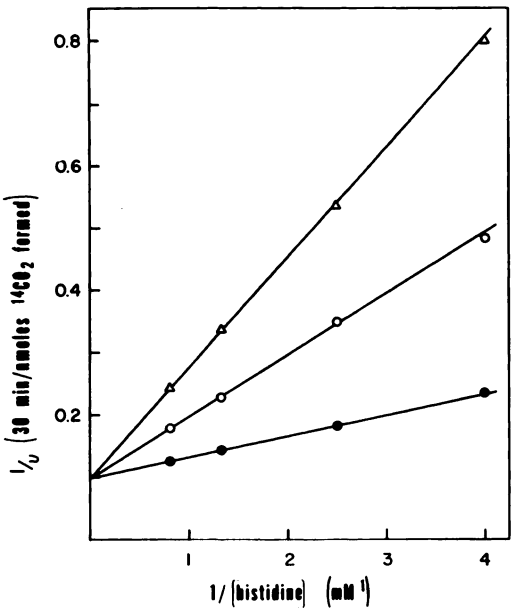

FIG. 2. *Competitive inhibition of fetal histidine decarboxylase by indomethacin*

Histidine concentrations varied from 0.25 to 1.25 mM. Conditions of assay were those described under METHODS. △——△, 0.7 mM indomethacin; ○——○, 0.35 mM indomethacin; ●——●, no indomethacin. The enzyme preparation was the 25–43% $(NH_4)_2SO_4$ fraction.

tical rates of heat destruction are demonstrable, in both the absence and presence of pyridoxal phosphate. Addition of pyridoxal phosphate resulted in a 6-fold increase in stability (half-lives, 12 min and 71 min, respectively). The parallel loss of activity with the most purified preparations suggests that the two enzymes are identical.

Although preparations that were completely inactive in the absence of added pyridoxal phosphate have not been obtained, an attempt was made to estimate the Michaelis constants for pyridoxal phosphate. In the presence of 0.75 mM L-histidine at pH 6.9, the K_m value for the fetal enzyme was 0.046 ± 0.014 μM, and for pyloric histidine decarboxylase it was 0.040 ± 0.0048 μM (standard error), using pooled fractions from gel filtration experiments. The agreement of these values further supports the concept of a common enzyme.

Gel filtration studies. Additional purification of pyloric and fetal histidine decarboxylases was achieved by means of gel filtration on Sephadex G-150. It was noted that the relative positions of emergence of the two enzyme activities from the column differed (Fig. 4, upper). It is well known that resolution of proteins by Sephadex requires differences in molecular shape. In the case of globular proteins the elution position has been correlated with the molecular weight (9).

In order to verify the discrepancy in elution volume between the pyloric and fetal histidine decarboxylases, equal amounts of the two enzymes were combined and cochromatographed. It was apparent that the two peaks appeared farther apart than was predicted (Fig. 4, lower). Subsequently it was noted that pyloric histidine decarboxylase, when chromatographed singly, yielded diverging results. On gel filtration, histidine decarboxylase prepared from fresh pylorus emerged as a single peak. A different pattern was observed when the preparation was chromatographed after storage for 1 month at −15°. The result is shown in Fig. 4 (upper). The elution profile exhibited a small shoulder; its relative position is in agreement with the displaced peak obtained in the cochromatography experiment.

When histidine decarboxylase was prepared from pylorus that had been stored for 2 months at −15°, a distinctly biphasic elution profile was obtained. The larger peak coincided with that shown in Fig. 4 (upper). A second peak, amounting to approximately 33% of the total activity, appeared in the same position as the displaced peak obtained in the cochromatography experiment. These findings indicate the presence of several species of histidine decarboxylase in rat tissues that are indistinguishable by most kinetic criteria (the fundic enzyme will be excluded from present consideration). Combining partially purified preparations of the pyloric and fetal enzymes resulted in the formation of a distinct, catalytically active, new species of histidine decarboxylase, exhibiting an increase in Stokes radius (83 A; cf. additional data of Table 2). The latter form of the enzyme was indistinguishable from that arising during storage of pylorus at −15°; this species of enzyme may represent an artifact resulting from prolonged storage. The postu-

TABLE 2

Characteristics of histidine and dopa decarboxylases of rat

The following preparations were used: pyloric histidine decarboxylase, 0–45% $(NH_4)_2SO_4$ fraction; pyloric dopa decarboxylase, supernatant fluid (160,000 × *g*) of dialyzed, crude extract; fetal histidine decarboxylase, 25–43% $(NH_4)_2SO_4$ fraction, type A or B (8); fundic histidine decarboxylase, 55–85% $(NH_4)_2SO_4$ fraction. Experiments with α-methylhistidine were done with pooled fractions from Sephadex G-150 purification experiments. Values of "maximal capacity of decarboxylation *in vivo*" were derived from measurements *in vitro* (expressed as nanomoles per minute per milligram of protein) with dialyzed crude extracts, converted to the maximal capacity of that organ to produce $^{14}CO_2$ under optimal conditions (nanomoles per hour per organ). Values for maximal rate of $^{14}CO_2$ formation (V_{max}) were derived from double-reciprocal plots. Inhibition experiments involving histidine decarboxylase and hydroxylamine employed incubation periods of 10 min.

Variable	Pyloric histidine decarboxylase	Pyloric dopa decarboxylase	Fetal histidine decarboxylase	Fundic histidine decarboxylase
Inhibitor dissociation constants (M)				
DL-α-Methylhistidine	3.9×10^{-4}[a]		4.8×10^{-4}	
4-Toluenesulfonic acid hydrazide	3.3×10^{-7}	6.8×10^{-5}	5.1×10^{-7}	
Indomethacin	1.4×10^{-4}	7.0×10^{-5}	1.8×10^{-4}	
Hydroxylamine	3.4×10^{-6}	5.2×10^{-5}	5.2×10^{-6}	4.7×10^{-5}
Maximal rate of decarboxylation *in vitro* (nanomoles/min/mg protein)	0.031	0.393	0.023	0.016
Maximal capacity of decarboxylation *in vivo* (nanomoles/hr/organ)	20–23	623–762		6–11
pH optimum	6.8	7.1	6.8	4.6–6.4
Stokes radius (A)	48	36	57	48

[a] The pH of the reaction mixture was 6.8 (see METHODS).

late that pyloric and fetal histidine decarboxylases are identical was not supported by the present gel filtration data.

Fundic histidine decarboxylase. Radwan and West (6) suggested the presence of two histidine decarboxylases in rat stomach. They noted a considerable discrepancy in pH optima for the pyloric and fundic histidine decarboxylases. The pH optimum for partially purified histidine decarboxylase from the fundic region is shown in Fig. 5. Radwan and West reported a pH optimum of 5.6. In the present experiment, optimal rates were obtained over the broad pH range of 4.6–6.4.

The Michaelis constant did not change in this pH range (Fig. 1). In contrast to fetal and pyloric histidine decarboxylases, an increase in pH above 6.4 resulted in a marked rise in K_m. At pH values lower than 4.7, the K_m increased markedly. The effect of pH on the K_m of histidine parallels closely the shape of the pH optimum curve (Fig. 5). It was observed that V_{max} values did not change measurably between pH 3.8 and 6.5. Between pH 6.9 and 7.2 the V_{max} increased more than 10-fold. The marked effect of pH upon the K_m of fetal histidine decarboxylase has been attributed to the role of the anionic form of histidine as the substrate (7). A different interpretation was advanced to account for the change in K_m of histidine decarboxylase from *Lactobacillus* (20). The pure enzyme displayed a K_m 6 times lower at the optimal pH than at neutral pH. This pH effect was ascribed to the participation of an imidazole group of the substrate or the enzyme in substrate binding (20). In this respect, fundic histidine decarboxylase bears a similarity to the bacterial enzyme.

The K_m values of the fundic enzyme (Fig. 1) were used for plotting pK_m against pH. By observing established rules (21), this plot may facilitate the graphic estimation of pK values for the enzyme, substrate, and enzyme-substrate complex. Two values for the fundic enzyme were thus obtained, i.e., pK 4.5 and 6.7. Both values are associated

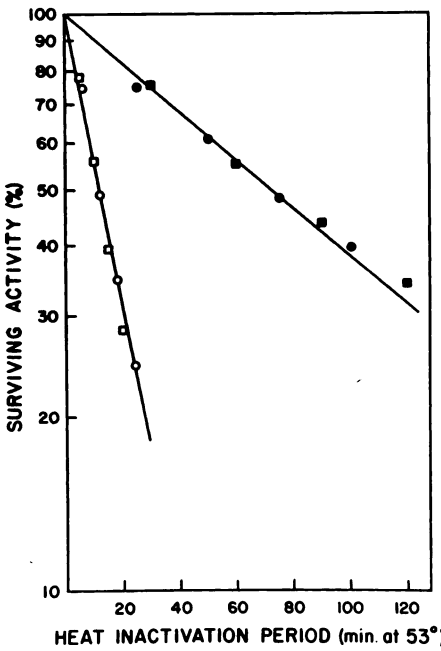

FIG. 3. *Stability of fetal and pyloric histidine decarboxylases during incubation at 53°*

Squares, fetal; circles, pyloric histidine decarboxylase. Solid symbols, 0.01 mM pyridoxal phosphate present; open symbols, no pyridoxal phosphate present during heat inactivation. The enzyme, 0.4 mg/ml, was purified by gel filtration. The buffer was 0.1 M potassium phosphate, pH 6.8.

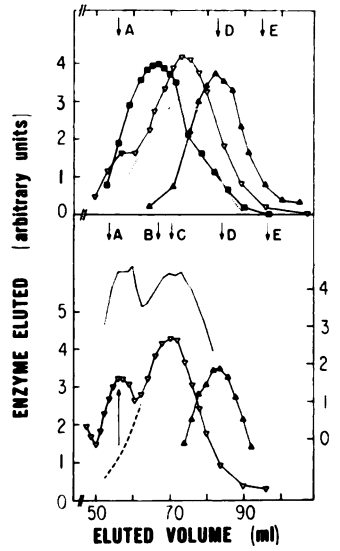

FIG. 4. *Gel filtration of histidine decarboxylases of the rat on Sephadex G-150*

Upper: fetal (■——■), pyloric (▽——▽), and fundic (····) histidine decarboxylases, successive experiments. Dopa decarboxylase (▲——▲) assays were done with fractions of pyloric extracts. Lower: chromatography of a 0–45% $(NH_4)_2SO_4$ preparation from pylorus (▽——▽) that had been stored for 2 months at −15°; dopa decarboxylase (▲——▲). The dashed line represents the extrapolated line used to estimate the amount of enzyme under the secondary peak (↑). The superimposed curve (——) represents histidine decarboxylase, cochromatography of combined fetal and pyloric 0–45% $(NH_4)_2SO_4$ fractions; note that the scale on the right-hand ordinate is displaced. Arrows at the top denote appearance of peaks of standards: *A*, blue dextran 2000; *B*, catalase; *C*, alcohol dehydrogenase; *D*, bovine serum albumin; *E*, bovine hemoglobin. The elution volume of tritiated water was 146 ml.

with a decrease in slope; neither value would thus be attributable to the enzyme-substrate complex. A pK value of 4.5 may be interpreted as indicating an effect displayed by the free enzyme. On the other hand, a pK of 6.7 may reflect an imidazole function of either enzyme or substrate. It is interesting to compare these constants with values derived for the fetal enzyme (which would be expected to behave like pyloric histidine decarboxylase). Estimates may be derived from data published by Håkanson (22); one pK value, 5.1, is associated with an increase in slope, suggesting the dissociation of the enzyme-substrate complex. A second pK value (>8) is associated with a decrease in slope. The comparison of the pK values of fundic and fetal histidine decarboxylases suggests differences in their mechanisms of decarboxylation.

Additional evidence that fundic histidine decarboxylase is a separate enzyme is provided by inhibition studies. Among the 11 effective inhibitors of pyloric histidine decarboxylase (Table 1), only hydroxylamine proved markedly inhibitory. The kinetics of inhibition was noncompetitive with histidine, employing 10-min incubation periods. This finding was surprising, as the analogous experiment with fetal enzyme indicated competitive inhibition (8). Toluenesulfonic acid hydrazide was moderately active at concentrations exceeding 1 mM. Surprisingly, the other carbonyl reagents were not inhibi-

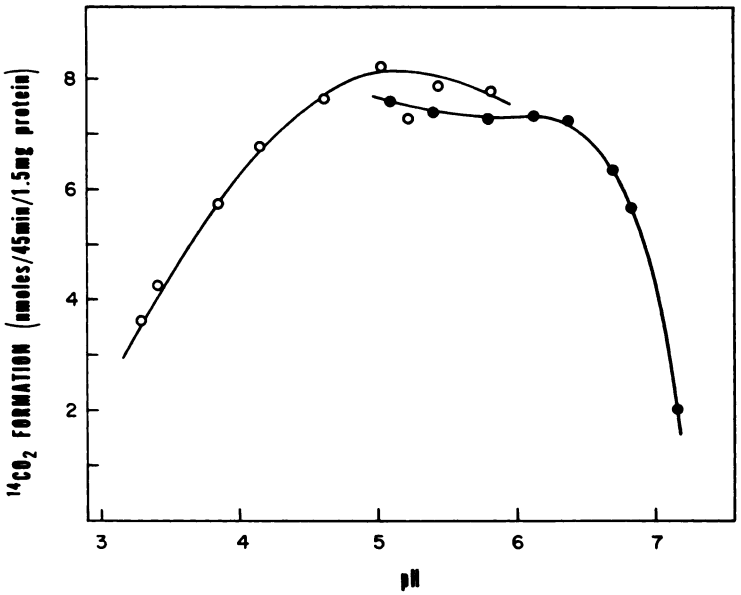

FIG. 5. *pH optimum of fundic histidine decarboxylase*
The buffer was 0.1 M potassium phosphate (●——●) or 0.1 M potassium acetate (○——○). The enzyme preparation was the 55–85% $(NH_4)_2SO_4$ fraction.

tory. No stimulation by addition of 0.01 mM pyridoxal phosphate has been observed with any preparation of fundic histidine decarboxylase. Pyridoxal phosphate (0.01 mM) was present in all routine assays. Inhibition experiments with the carbonyl reagents listed in Table 1 were therefore repeated, but without the coenzyme. The results with 7-fold purified enzyme, obtained in gel filtration experiments, were essentially identical with those shown in Table 1. Fundic and pyloric histidine decarboxylases furthermore differ in their response to salt fractionation: the fundic enzyme precipitates at 55–85%, and the pyloric enzyme, at 0–45%, saturation with ammonium sulfate.

In view of the marked differences between pyloric and fetal histidine decarboxylases, on one hand, and the fundic enzyme, on the other hand, the ability of the latter enzyme to form histamine was tested. Under optimal conditions of assay the formation of nearly stoichiometric amounts of histamine and $^{14}CO_2$ was observed (ratio of histamine to $^{14}CO_2$ = 1.26; 1.21).

Aromatic L-amino acid decarboxylase. Aromatic L-amino acid decarboxylase (also referred to as dopa decarboxylase, EC 4.1.1.26) is present abundantly in the pyloric region. This enzyme was strongly inhibited by the carbonyl reagents and acidic compounds listed in Table 1. α-Methyldopa inhibited dopa decarboxylase but was without effect on the three types of histidine decarboxylase preparations. Conversely, α-methylhistidine inhibited only pyloric and fetal histidine decarboxylases, not dopa decarboxylase; surprisingly, this substrate analogue was without effect on the fundic enzyme. These findings indicate that pyloric dopa decarboxylase is distinguishable from pyloric histidine decarboxylase. This postulate was tested further, as earlier workers had suggested that, at high concentrations of histidine, dopa decarboxylase was capable of histamine formation (the "nonspecific" histidine decarboxylase of the older literature; for a review, see ref. 23). The kinetics of inhibition by 4-toluenesulfonic acid hydrazide was therefore analyzed. This compound inhibited both decarboxylases competitively with respect to the substrate. This type of inhibition kinetics might have been expected, since hydroxylamines are known to act as competitive inhibitors of rat fetal histidine decarboxylase (8). Although the mechanisms of inhibition

with both decarboxylases are identical, it is significant that the inhibitor dissociation constants differ more than 100-fold (Table 2). On the other hand, the K_i values for pyloric and fetal histidine decarboxylases agreed within the limits of experimental error.

Among the inhibitors of dopa decarboxylase as well as fetal and pyloric histidine decarboxylases, the 3,4,5-trihydroxybenzoic acid methyl ester is of interest. This compound (propylgallate), a food additive approved for human consumption (24), was recently reported to be an effective tyrosine hydroxylase inhibitor (25).

Gel filtration studies provided further evidence that pyloric dopa and histidine decarboxylases are separate enzymes. The elution peaks of these decarboxylases differed significantly (Fig. 4). Dopa decarboxylase from the pyloric and the fetal preparations emerged as a single peak, the positions of which agreed well.

Fundic extracts contain little dopa decarboxylase. The insensitivity of fundic histidine decarboxylase toward inhibitors of dopa decarboxylase (Table 1) precludes the possibility that the two activities are due to a single catalyst. Heat inactivation experiments of unfractionated, dialyzed fundic extracts supported this notion: incubation for 5 min at 65° at pH 6.8 resulted in complete destruction of dopa decarboxylase; histidine decarboxylase activity remained unchanged. Fundic histidine decarboxylase may be rapidly inactivated by exposure to 80° (69% loss of activity after 5 min).

Physiological significance of the presence of two gastric histidine decarboxylases. The rat stomach is unusual in that it contains two specific histidine decarboxylases. The search for a specific histidine decarboxylase in the stomach of nonrodents has not been successful (1). A recent report claimed the presence of a specific histidine decarboxylase in the gastric mucosa of monkey and man (26). On the other hand, extensive efforts in this laboratory to detect this enzyme in the canine stomach were unsuccessful.

The main purpose of the present study was the characterization of the decarboxylases of the rat stomach. Neither fundic nor pyloric histidine decarboxylase is identical with aromatic L-amino acid decarboxylase. The differences in the characteristics of fundic and pyloric histidine decarboxylases might well represent the basis, in part, of a regulatory mechanism in the gastric physiology of the rat. The K_m of pyloric histidine decarboxylase is sufficiently low at neutral pH to permit effective histamine synthesis; a change in pH toward the acid side renders this mechanism ineffective. At lower pH a second enzyme, fundic histidine decarboxylase, becomes operative. By virtue of a low K_m, this fundic enzyme can synthesize histamine at pH values as low as pH 3. A control of the activity of fundic and pyloric enzymes via intracellular changes in pH might conceivably be affected by changes in the pH in the gastric lumen. Evidence in support of such a mechanism does not appear to be available.

Fundic histidine decarboxylase is unusually insensitive to inhibition. It is inviting to consider the implications of this lack of response to inhibitors. Levine and Watts (27) reported that dopa, dopamine, and norepinephrine inhibit fetal and gastric histidine decarboxylases. The present study indicated that dopa and norepinephrine inhibit the pyloric but not the fundic enzyme. On the basis of observations made *in vitro*, norepinephrine and certain related compounds (27) offer themselves as candidates for the role as physiological regulators of histamine production at neutral pH. Fundic histidine decarboxylase would be independent of such a regulatory mechanism. Fundic histidine decarboxylase, on the other hand, might be subject to a separate and independent type of regulation; observations by Radwan and West (6) indicated that fasting is followed by complete loss of activity in the fundus. That finding was confirmed in this laboratory.

If both enzymes are part of the mechanism of supplying gastric histamine, it is of interest to consider the relative enzymatic capabilities of the fundic and pyloric enzymes. Based on calculations derived from values of maximal velocities under optimal conditions *in vitro*, the maximal capacity for histamine formation of the fundic and pyloric portions of the stomach were compared. As may be seen in Table 2, the values for the

pylorus are 3 times greater than those for the fundus. Thus, either section of the stomach is capable of contributing significant amounts of histamine. An evaluation of the actual rates of synthesis of histamine *in vivo* was not attempted.

The fundic histidine decarboxylase bears a striking similarity to the enzyme purified from *Lactobacillus* (20). The possibility that the fundic enzyme is of bacterial origin was not investigated in this study

The present findings suggest a clue to the difficulties experienced in this laboratory in obtaining a reduction in tissue levels of gastric histamine in the female rat following administration of inhibitors of histidine decarboxylase.[2] The repeated failure to observe a reduction of gastric histamine levels or urinary histamine excretion was especially striking following the administration of 4-bromo-3-hydroxybenzyloxyamine, since this compound has been documented to be an inhibitor of histidine decarboxylase both *in vitro* and *in vivo* (28). Another laboratory was unable to detect reductions in urinary excretion of histamine following administration of 4-bromo-3-hydroxybenzyloxyamine (29). It is felt that the resistance of fundic histidine decarboxylase to inhibition *in vitro* may be a prominent reason for the failure to observe inhibition of histamine formation *in vivo*. This consideration alone, however, would not explain the failure of indomethacin to inhibit histidine decarboxylase in fasted rats. Differences in the response to the administration of inhibitors *in vivo* by various strains of rats have been reported by Levine (30).

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[2] F.-J. Leinweber, L. A. Walker and G. A. Braun, unpublished observations.