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# SPECIFIC HISTIDINE DECARBOXYLASES IN THE GASTRIC MUCOSA OF MAN AND OTHER MAMMALS

## DETERMINATION, LOCATION AND PROPERTIES

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**Abstract**—Relatively high activities of the specific histidine decarboxylases were found in the gastric mucosa of men, monkeys, pigs, cows, dogs, cats, guinea-pigs, rabbits and rats. Some improvements of the assay of specific histidine decarboxylase were necessary, before these enzymes could be demonstrated in all stomachs investigated. The specific histidine decarboxylase could also be shown in the human gastric carcinomas. By pH-optima, substrate optima, $K_m$, inhibition by α-methylhistidine but not by α-methyldopa, inhibition by benzene and activation by pyridoxal-5′-phosphate the histidine decarboxylases in the gastric mucosa could be characterized as specific histidine decarboxylases. The enzyme in the stomach of guinea-pigs has been purified 22-fold by ultracentrifugation and gelfiltration on Sephadex G 100. The demonstration of the specific histidine decarboxylases (isoenzymes) in the gastric mucosa of numerous mammals has some importance for the hypothesis of a physiological function of histamine as a chemostimulator of gastric secretion. Problems of the nomenclature of histidine decarboxylases are discussed. The terms "acid" and "alkaline" histidine decarboxylases are proposed.

THE QUESTION whether histamine is formed in the gastric mucosa *in vivo*, plays an important role in the discussion about histamine as a physiological stimulator of the gastric secretion. The lack[13] or extremely low activity[12] of the specific histidine decarboxylases (L-histidine carboxylyase EC 4.1.1.22) in the fundic and corpus region of most of the mammalian species is considered as a definite argument against such a function of histamine.[7] Only in the gastric mucosa of rats,[22] rabbits[34] and guinea-pigs[15] could a relatively high activity of these enzymes be demonstrated. An unspecific histidine decarboxylase according to Weissbach *et al.*[30] was found in the gastric mucosa of men, dogs, pigs, cats, rabbits and guinea-pigs by Werle[31] and Werle and Zeisberger.[32] But a participation of this enzyme in the formation of histamine *in vivo* was questioned because of its relatively high Michaelis-constant.[30]

However, by improvements of the assay of the specific histidine decarboxylases now for the first time these enzymes could be demonstrated in the gastric mucosa of many mammalian species.

## MATERIALS AND METHODS

Preparation and purification of histidine decarboxylase: Gastric tissue from patients (Surgery clinic of the University of Munich), chimpanzees (zoological gardens), pigs, cows (slaughterhouse), dogs, cats, rabbits, guinea-pigs and rats (laboratory animals)

have been frozen by $CO_2$ snow immediately after the withdrawal or death of the animal. The mucosa was separated from the muscular layer and homogenized with two volumes of 0·2 M phosphate buffer ($KH_2PO_4/Na_2PO_4$, pH 7·0). For 5 min the homogenate was centrifuged at 1800 *g* and thereafter for 30 min the supernatant at 0° and 100,000 *g*. This crude extract was used as source of histidine decarboxylase.

As a first step of purification 8–10 ml of the crude extract of the gastric mucosa from guinea-pigs were put on a column of Sephadex G 100 (3·5 × 100). A solution containing 0·16 M NaCl and 0·02 M phosphate buffer ($KH_2PO_4/Na_2HPO_4$, pH 7·0) was used as eluant. Fractions of 8–9 ml of the eluate were collected and used directly for the determination of the enzyme. The appearance of the proteins in the eluate was determined by measuring the light absorption at 280 nm with an Ultraviolet Absorptiometer LKB 8300 A Uvicord II.

*Reagents.* L-histidine (base) and pyridoxal-5′-phosphate (Fluka), aminoguanidine sulfate (Schuchardt), chlorpromazine (2-Chlor-10-(3-dimethylaminopropyl) phenothiazine chloride) (Bayer), nicotinamide (Merck, Darmstadt), α-methyldopa and α-methylhistidine (Merck, Sharp & Dohme, West Point, U.S.A.), Sephadex G 100 (Deutsche Pharmacia Gesellschaft), *o*-phthaldialdehyde (recrystalled from ligroin, b. p. 50–70°) (Fluka).

*Determination of histidine decarboxylase*

The incubation was performed in the Warburg apparatus at 37° and in a $N_2$ atmosphere. In the main flask there were 0·8 ml of crude extract or eluate, 1·5–1·3 ml

TABLE 1. OPTIMAL SUBSTRATE CONCENTRATIONS, pH-VALUES AND COMPOSITIONS OF THE INHIBITORY MEDIUM FOR THE ASSAY OF HISTIDINE DECARBOXYLASE IN THE GASTRIC MUCOSA OF DIFFERENT SPECIES

| Species | Substrate concentration (M) | pH-value | Composition of the inhibitory medium | | |
|---|---|---|---|---|---|
| | | | amino-guanidine ($10^{-4}$ M) | chlor-promazine ($10^{-4}$ M) | nicotin-amide (N) |
| man | $5 \times 10^{-4}$ | 6·8 | 1·3 | — | $10^{-2}$ |
| monkey | $1 \times 10^{-4}$ | 7·0 | — | 5·0 | $10^{-2}$ |
| dog | $1 \times 10^{-4}$ | 7·0 | — | 5·0 | $10^{-2}$ |
| pig | $1 \times 10^{-4}$ | 6·9 | 1·3 | — | $10^{-2}$ |
| cow | $1 \times 10^{-4}$ | 7·2 | 1·3 | — | $10^{-2}$ |
| rabbit | $1 \times 10^{-3}$ | 6·4 | 5·0 | — | $10^{-2}$ |
| guinea-pig | $5 \times 10^{-4}$ | 6·3 | 1·3 | 5·0 | $10^{-2}$ |
| rat | $1 \times 10^{-3}$ | 5·7 | 1·3 | — | $10^{-2}$ |

Mean values from three determinations. Concentrations of the inhibitors as final concentrations.

of phosphate buffer (0·2 M $KH_2PO_4/Na_2HPO_4$), pH according to the pH-optimum of the enzyme (Table 1) and 0·1–0·3 ml of a solution containing the inhibitors of histamine degradation according to Table 1. For the determination of the unspecific histidine decarboxylase 25 m-moles of benzene were added to the incubation mixture, which activates this type of enzyme.[28] Furthermore, to the reaction mixtures with the purified enzyme 0·1 ml of pyridoxal-5′-phosphate was added in a final concentration of $6{\cdot}25 \times 10^{-5}$ M (cf. 35).

In the side arm there was 0·5 ml of L-histidine in a concentration which gave, when added to the incubation mixture, an optimal substrate concentration according to Table 1. The final volume of the incubation mixture was 3·0 ml; all the substances were dissolved in 0·2 M phosphate buffer. The reaction was started by tipping the substrate into the main flask of the Warburg vessels and stopped after definite times (see below) by the addition of 0·5 ml of 3 N perchloric acid. Thereafter histamine in the reaction mixtures was measured spectrofluorometrically after the method of Shore *et al.*[26] as modified by Burkhalter.[4] The recovery of histamine, which had been added before the perchloric acid to the incubation mixture in the cold, was 70 per cent in the average. The specificity of the fluorometric assay was controlled by the biological assay on the isolated guinea-pig's ileum. The results obtained by the two methods corresponded within a difference of $\pm 5$ per cent. 0·1–0·5 $\mu$g antazoline per ml Tyrode solution in the bath inhibited the contraction of the ileum by the extracts completely. Furthermore, the fluorescence spectra of the tissue extracts in 0·1 N HCl were identical with that of standard histamine.

The enzyme activity was expressed in pmoles of histamine formed per minute and mg protein. Three histamine values and two blanks were used for the determination of the histamine formation: The histamine values were obtained from reaction mixtures with the intact histidine decarboxylase, the first of which was incubated for 5 min, the second for 10 min and the third for 15 min. The two blanks were obtained from incubation mixtures in one of which the enzyme had been inactivated by perchloric acid before the incubation whereas in the other the enzyme had been completely inhibited by $5 \times 10^{-3}$ aminoguanidine.[16] The formation of histamine per minute was calculated as an average from the differences between the histamine values in the three reaction mixtures and one blank with the inactivated enzyme, since no differences could be found between blanks obtained from incubation mixtures with the inactivated enzyme, which had been incubated for different times. The second blank had to control the completeness of the inhibition of histamine degradation, which could be assumed whenever the two blanks gave the same histamine values. Only if this condition was satisfied, was the histamine formation calculated.

*Determination of histidine and protein.* Histidine was determined spectrofluorometrically by the method of Pisano *et al.*[20], the protein concentration by the biuret method of Weichselbaum.[29]

## RESULTS

### 1. *Determination of the specific histidine decarboxylases*

1.1. *Composition of the inhibitory medium against a degradation of histamine.* In different tissues of mammals histamine is inactivated by different enzymes:[17] According to the organ especially diamine oxidase (diamine: $O_2$-oxidoreductase EC 1.4.3.6), histamine methyltransferase (EC 2.1.1.8) and diphosphopyridine nucleotidase [NAD(P)-glycohydrolase EC 3.2.2.6][1] are involved. In the assay of histidine decarboxylases these enzymes had to be eliminated by the addition of inhibitors: The diamine oxidase by aminoguanidine, the histamine methyltransferase by chlorpromazine and the diphosphopyridine nucleotidase by nicotinamide.

The optimal composition of the inhibitory medium and the concentrations of

inhibitors were different from species to species, as shown in Table 1. In this concentrations the three prementioned enzymes, which inactivate histamine, were inhibited completely.[16,19] Aminoguanidine and chlorpromazine in higher concentrations also inhibited the histidine decarboxylases (cf. Lorenz and Werle,[16]) contrary to nicotinamide, which was without any influence on histidine decarboxylase even in a final concentration of $5 \times 10^{-2}$ M.

In about 20 per cent of our experiments an inactivation of histamine took place, which could not be explained by the action of diamine oxidase, histamine methyltransferase and diphosphopyridine nucleotidase. This inactivation was detected by experiments in which the specific histidine decarboxylase was completely inhibited by $5 \times 10^{-3}$ M aminoguanidine,[19] $10^{-3}$ M 2-mercaptobenzimidazol-1,3-dimethylol[18] or $1 \times 10^{-2}$ M α-methyldopa.[34] A further pathway of histamine degradation such as a transamination,[11] which was not, or not fully eliminated in each experiment by the

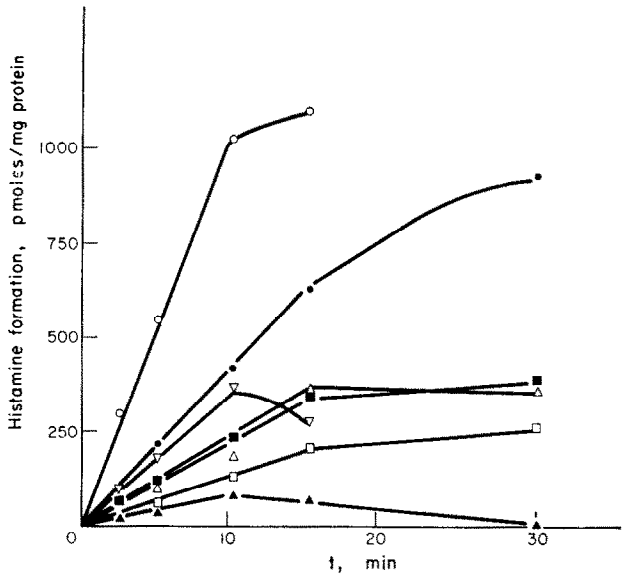


FIG. 1. Kinetics of histidine decarboxylation in crude extracts of the gastric mucosa from different species. For the conditions of the incubation see methods and Table 1.
○——○ pig; ●——● rat; ▽——▽ cow; ■——■ man;
△——△ guinea-pig; □——□ rabbit; ▲——▲ monkey.

inhibitors added, could explain these findings. But very little is known about histamine transamination[11] and nothing about inhibitors of this reaction. Since we were not able at this time to exclude this histamine degradation in 20 per cent of our experiments, the histamine formation was not calculated in these cases (c.f. Methods).

1.2. *Reaction kinetics.* In the crude extracts L-histidine is decarboxylated only for few minutes with a constant velocity (Fig. 1). But this time is very different from species to species and to a smaller extent also from experiment to experiment. Therefore reaction kinetics should be followed in each determination of the activity of

histidine decarboxylase. The formation of an inhibitory complex between pyridoxal-5'-phosphate and substrate seems to be responsible for the decrease of reaction velocity.[16,34,35] Although inhibitors of the degradation of histamine have been added, in some species already after 10–30 min there is a loss of newly formed histamine in the incubation mixtures (Fig. 1). In the thyroid glands of pigs we could show that a degradation of aminoguanidine and chlorpromazine was responsible for this phenomenon.[19] The reaction kinetics can explain in part, why other authors have found no[13] or a very low[12] activity of the specific histidine decarboxylase in the stomachs of most of the mammalian species. These authors have determined the activity of the enzyme after an incubation time of 3 hr.

2. *Activity of the specific histidine decarboxylases in the gastric mucosa*

2.1. *Normal gastric tissues of different mammals.* In the gastric mucosa of man and all mammals as far as investigated, specific histidine decarboxylases with relatively high activities could be demonstrated (Table 2). The most rapid formation of hista-

TABLE 2. ACTIVITY OF THE SPECIFIC HISTIDINE DECARBOXYLASES IN CRUDE EXTRACTS OF THE GASTRIC MUCOSA

| Species | Histidine decarboxylation in pmoles histamine formation/min and mg protein | | | | | |
|---|---|---|---|---|---|---|
| | *n* | fundus | *n* | corpus | *n* | antrum |
| man | 7 | 10·6 ± 2·6 | 12 | 7·1 ± 1·4 | 3 | 4·9 ± 1·2 |
| monkey | 3 | 18·7 ± 7·7 | 5 | 4·6 ± 1·8 | 3 | 7·2 ± 1·7 |
| dog | 2 | 38·4 | 2 | 46·5 | 2 | 26 |
| cat | 1 | 7·4 | — | — | — | — |
| pig | 4 | 202 ± 105 | 4 | 60 ± 14 | 4 | 77 ± 18 |
| cow | — | — | 2 | 44·7 | 2 | 24·0 |
| rabbit | 2 | 15·2 | 2 | 5·1 | 2 | 2·9 |
| guinea-pig | 3 | 16·4 ± 9·1 | 3 | 13·8 ± 1·5 | 3 | 13·1 ± 0·8 |
| rat | — | — | 10 | 29·3 ± 6·8 | — | — |

Mean values ± standard deviation. In cows the rennet bag has been used, in rats the glandular portion of the stomach after removing the rumen. For the conditions of the assay see Methods and Table 1.

*n* = number of animals tested, but in cats, rats and guinea-pigs the organs of ten animals have been pooled.

mine was measured in the region of fundus and corpus, where the greatest density of acid forming parietal cells had been demonstrated. But the activity of histidine decarboxylases could be measured in the antrum, too. The highest activity of histidine decarboxylases of all gastric tissues was found in the region of fundus and corpus of the pig. Like that of the placenta of hamsters[22] it belongs to the highest activities of histidine decarboxylase of all normal tissues, as far as known.

As in the rat[5] and in the rabbit[34] a correlation could be shown between the content of histamine and the activity of histidine decarboxylase in the gastric mucosa of the cow, but not in that of the pig and the monkey (Fig. 2).

2.2. *Human gastric carcinoma.* We could also demonstrate the presence of histidine decarboxylase in human gastric carcinomas (Table 3). Compared with the results in Table 2 the activities of the enzyme were lower than in normal tissues, but without any exception the activity of the enzyme was measurable. No proportion-

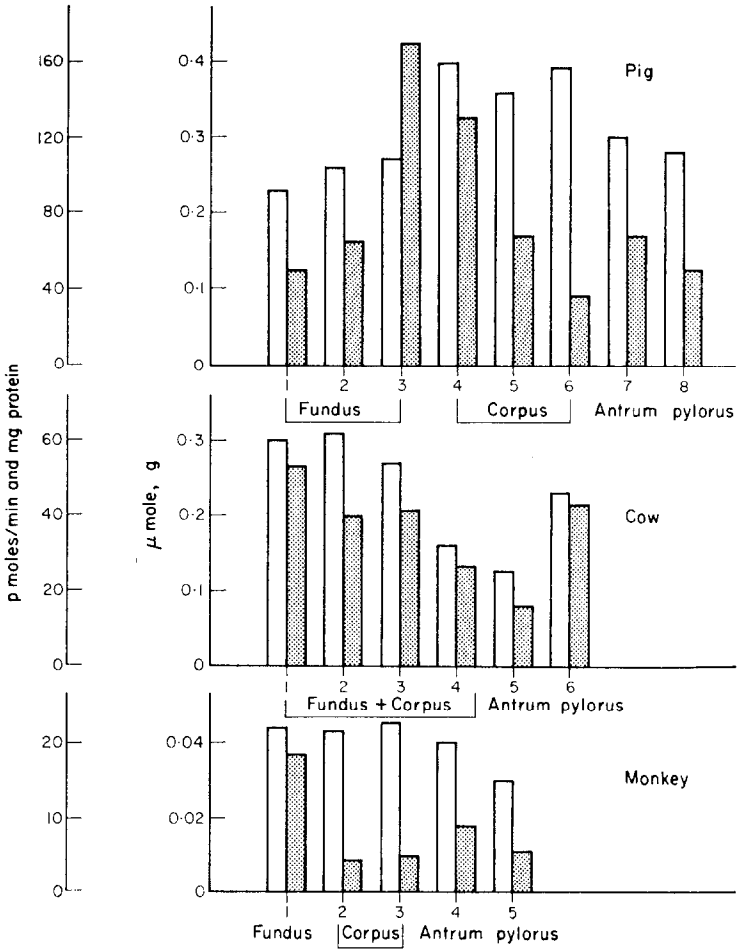


FIG. 2. Regional distribution of histamine and the specific histidine decarboxylase in the gastric mucosa of pig, cow and monkey. Mean values from two determinations. □ histamine in μmole/g wet weight, ▦ activity of the specific histidine decarboxylase in pmoles/min and mg protein. The gastric mucosa has been dissected in equal strips of 3–5 cm, along to the circular muscular layer. Correlation coefficients between the histamine content and the activity of the enzyme. pig: $r = 0{\cdot}08$; cow: $r = 0{\cdot}86$, $P < 0{\cdot}05$; monkey: 0·1.

ality could be shown between the histamine content, which was higher in the inflammatory area around the carcinoma than in the tumor itself, and the activity of the specific histidine decarboxylase.

3, *Properties of the specific histidine decarboxylases in the gastric mucosa*

3.1. *Enzymatic activity at different pH-values.* By means of pH-activity curves (Fig. 3) the following pH-optima have been determined: pig pH 6·9–7·3, cow pH 6·4 and 7·2, men pH 6·8–7·0 and in contrast to these values guinea-pig 6·4, rabbit pH 6·3 and rat pH 5·7. Therefore the specific histidine decarboxylases of rodents differ from those of other mammals. A second peak of the pH-activity curve of the

histidine decarboxylase from the rat's stomach (pH 7·9–8·1) is due to the unspecific histidine decarboxylase (see below). Surprisingly enough this enzyme showed a very high activity. The two peaks in the pH-activity curve of the histidine decarboxylase from the gastric mucosa of cows belong to the specific histidine decarboxylase (see below). The incubations in the following experiments have been performed at the pH-values shown in Table 1.

3.2. *Enzymatic activity at different substrate concentrations.* In crude extracts of the gastric mucosa the histamine formation was enhanced by increasing concentrations of L-histidine (Fig. 4). The substrate optimum was different from species to species

TABLE 3. HISTAMINE AND SPECIFIC HISTIDINE DECARBOXYLASE IN THE CARCINOMOUS TISSUE OF HUMAN STOMACHS

| Diagnosis* | Histamine content† | Activity of the histidine decarboxylase† |
|---|---|---|
| (1) adenocarcinoma, scirrhus (fundus) | 0·062 | 5·8 |
| (2) adenocarcinoma (corpus) | 0·072 | 0·4 |
| (3) adenocarcinoma, polypous (anastomosis after Billroth II wall) | 0·060 | 2·2 |
| (4) adenocarcinoma, l.c.3,polypus | 0·031 | 3·0 |
| (5) adenocarcinoma, l.c.3, metastasis (mesenteric lymph node) | 0·031 | 6·0 |
| (6) adenocarcinoma (cardia) | 0·054 | 9·6 |
| (7) adenocarcinoma, l.c.6 (adjacent fundic mucosa) | 0·051 | 5·5 |

* The diagnoses have been performed by professor Dr. A. Schauer, Department of Pathology, University of Munich.

† Histamine content in μmole/g wet weight, activity of the histidine decarboxylase in pmoles histamine formation/min and mg protein.

by about one order of magnitude (Table 1). The highest concentrations of L-histidine were necessary for the enzymes from rats and rabbits, the lowest for those from pigs and cows. *Supraoptimal* concentrations of L-histidine at first inhibit the specific histidine decarboxylases of the gastric mucosa (Fig. 5): 80 per cent in the cow, 60 in men, 50 in the pig, 40 in the guinea-pig and 35 per cent in the rat (cf. Fig. 4). But by a further enhancement of the substrate concentration this inhibition of the enzyme was diminished in incubations with crude extracts of the gastric mucosa from cows, pigs and guinea-pigs. At this time these results are difficult to explain. But they seem to be very important, since most of the other workers, who tried to demonstrate histidine decarboxylase in the gastric mucosa, used concentrations of L-histidine in a range of $10^{-3}$ up to $10^{-2}$ M.

The *Michaelis constants* of gastric histidine decarboxylases from different species derived from Lineweaver–Burk-plots[14] (Fig. 4), showed the following values: Man $5·7 \times 10^{-5}$ M, pig $2·0 \times 10^{-5}$ M, cow $1·3 \times 10^{-5}$ M, guinea-pig $3·3 \times 10^{-5}$ M, rabbit $1·0 \times 10^{-4}$ M and rat $2·5 \times 10^{-4}$ M (at ph 7·0). Compared with the $K_m$ of specific histidine decarboxylases from other tissues[17,25] they are relatively low, but our value of $K_m$ in the rat's stomach agrees very well with that of other workers.[10,27] The endogenous concentration of free histidine in the crude extracts measured by the spectro-

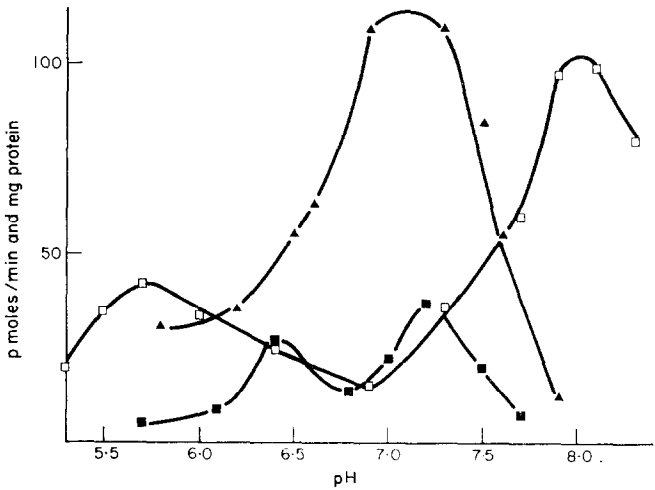


FIG. 3a

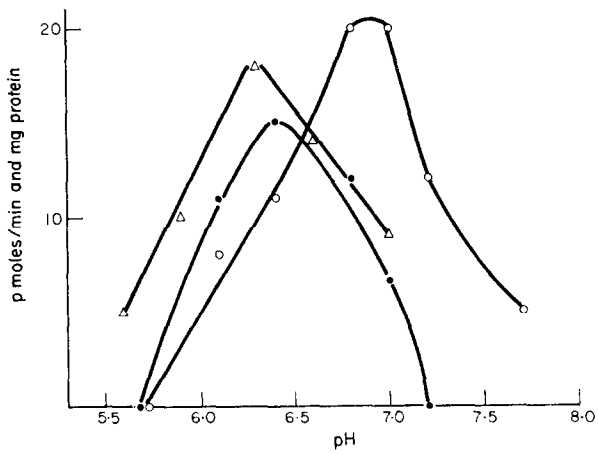


FIG. 3b

FIG. 3. pH-activity curves of the specific histidine decarboxylases of the gastric mucosa from different species. Mean values from 3–4 determinations. For conditions of the incubation see Methods and Table 1, the unspecific histidine decarboxylase of the rat's stomach has been incubated with benzene from pH 7·3 up to pH 8·3.

Part (a) ▲——▲ pig; □——□ rat; ■——■ cow;
Part (b) ○——○ man; ●——● guinea-pig; △——△ rabbit.

Activity of the histidine decarboxylase given in pmoles/min and mg protein.

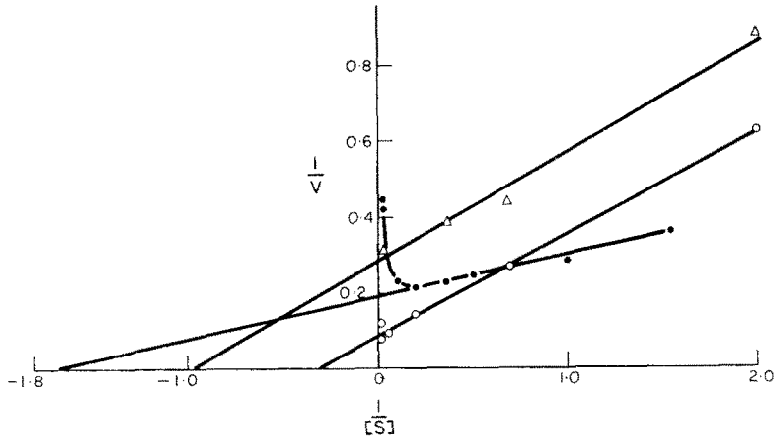


FIG. 4a

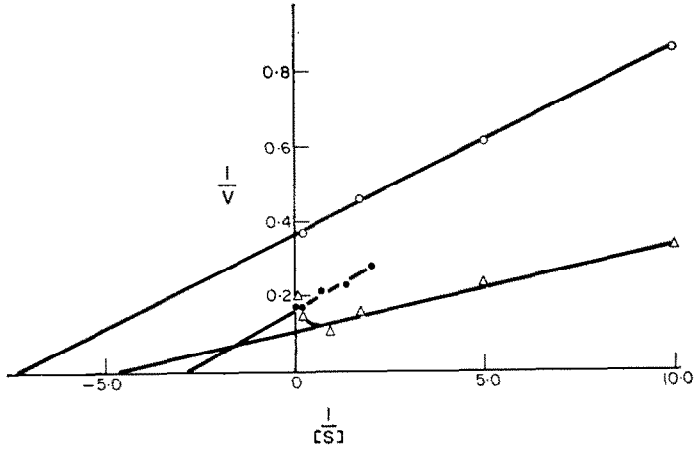


FIG. 4b

FIG. 4. Michaelis constants of the specific histidine decarboxylases of the gastric mucosa from different species (after Lineweaver and Burk.)[27] Mean values from 2–3 determinations. v = nmole/min, [s] = a × $10^{-4}$ (M), enzyme solution 25–45 mg protein per incubation sample. For further conditions see Methods and Table 1.

Part (a) ●——● man; △——△ rabbit; ○——○ rat;
Part (b) ●——● guinea-pig ○——○ cow; △——△ pig.

fluorometric assay of Pisano *et al.*[20] has been 2–5 $\times$ $10^{-5}$ M. Therefore the histamine formation *in vivo* by the specific histidine decarboxylases of the gastric mucosas should be possible. The $K_m$ value of the unspecific histidine decarboxylase of the rat's stomach is 1·0 $\times$ $10^{-3}$ M, which is in agreement with the value estimated by Radwan and West,[21] but it is about 15-fold lower than the value found by Weissbach *et al.*[30]

3.3. *Action of α-methyldopa, α-methylhistidine and benzene.* The specific histidine decarboxylase is inhibited by α-methylhistidine (1–5 $\times$ $10^{-2}$ M final concentration), but not by α-methyldopa (1 $\times$ $10^{-3}$ M). Benzene activates the unspecific enzyme, but, dependent on the tissue, inhibits the specific histidine decarboxylase or is without influence on this enzyme. Therefore a differentiation between these two types of enzymes can be performed by these three substances.[17]

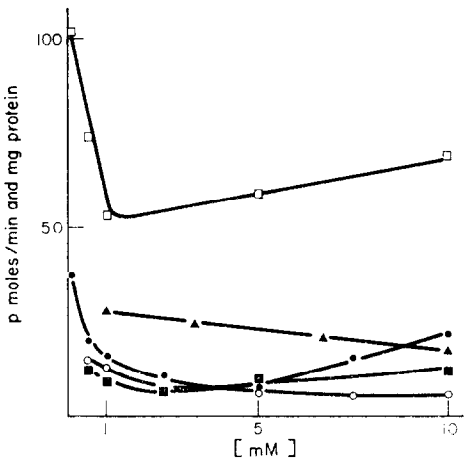


FIG. 5. Inhibition of the specific histidine decarboxylase of the gastric mucosa by supraoptimal histidine concentrations. Mean values from 2 to 3 determinations. Activity of the enzyme in pmoles/min and mg protein, substrate concentration in mM final concentration in the incubation mixture. The curves begin at the left side with the optimal substrate concentration.
○——○ man; ⊡——□ pig; ●——● cow; ▲——▲ rat; ■——■ guinea-pig.

*None* of the specific histidine decarboxylases of the gastric mucosa was activated by benzene, but the enzymes of the guinea-pig, rat and pig were inhibited to 60–100, 40 and 20–80 per cent respectively. *None* of these enzymes was inhibited by α-methyldopa, but all were blocked by α-methylhistidine to 70–100 per cent. Only the unspecific histidine decarboxylase of the rat's stomach (pH 7·9–8·1) was inhibited to 100 per cent by α-methyldopa and was activated 105 per cent by benzene. Therefore the histidine decarboxylases of the gastric mucosa are characterized as specific histidine decarboxylases[30] by pH-optimum, $K_m$, inhibition by α-methylhistidine and benzene, but not by α-methyldopa.

3.4. *Activation by pyridoxal-5'-phosphate.* By pyridoxal-5'-phosphate, known as coenzyme of both types of histidine decarboxylases, the enzymes of the gastric mucosa were activated to a different extent, in man to 55 per cent, in the pig to 220

per cent, in the rabbit to 110 per cent and in the rat to 100 per cent. The optimal concentrations of coenzyme found necessary to be added to the incubation mixtures were different from species to species and also often from one experiment to another. In man they were $3{\cdot}13 \times 10^{-5}$ M (as final concentration), in the pig $3{\cdot}13 \times 10^{-5}$ M, in the rabbit $1{\cdot}25 \times 10^{-5}$ M, in the rat $6{\cdot}25 \times 10^{-5}$ M. On the other hand supraoptimal concentrations of pyridoxal-5'-phosphate inhibited the enzyme by the formation of an inhibitory complex between coenzyme and substrate[34,35] in the human and hog gastric mucosa in a final concentration of $6{\cdot}25 \times 10^{-5}$ M to 60–100 per cent. Therefore we have omitted the addition of pyridoxal-5'-phosphate to all incubation mixtures with crude extracts. But in samples with Sephadex filtrates of the enzyme, an addition of coenzyme was found to be necessary. It was optimal in the final concentration of $6{\cdot}25 \times 10^{-5}$ M (cf. 35).

4. *Purification of the histidine decarboxylases of the gastric mucosa and kidney of guinea-pigs by ultracentrifugation and gelfiltration on Sephadex G* 100

As in the case of histidine decarboxylases of the pig's thyroid gland[35] we achieved an 8-fold purification of the specific histidine decarboxylase of the gastric mucosa from guinea-pigs and a 6-fold purification of the unspecific histidine decarboxylase of the kidney from the same animal by gelfiltration on Sephadex G 100. The loss of enzyme activity was only 20 and 30 per cent respectively (Table 4). Both of the histidine

TABLE 4. PURIFICATION OF THE SPECIFIC HISTIDINE DECARBOXYLASE OF THE STOMACH AND THE UNSPECIFIC HISTIDINE DECARBOXYLASE OF THE KIDNEY OF GUINEA-PIGS BY ULTRACENTRIFUGATION AND GELFILTRATION ON SEPHADEX G 100

| Enzyme material | Specific activity | | Whole activity in per cent | |
|---|---|---|---|---|
| | stomach | kidney | stomach | kidney |
| homogenate | 6·0 (0) | 40 (0) | 100 | 100 |
| (1) supernatant (1800 *g*) | 7·5 (1·25) | 45 (1·13) | 100 | 80 |
| (2) supernatant (100,000 *g*) | 15·7 (2·6) | 78 (1·95) | 90 | 70 |
| Sephadex G 100 | 132 (22) | 490 (12·3) | 70 | 60 |

Specific activity in pmoles histamine formation/min and mg protein. The whole activity of the homogenate is expressed as 100 per cent, the factor of purification is added in parentheses.

decarboxylases appeared in the eluate between the middle and last part of the peak of proteins.

As after some other steps of purification (ammonium sulfate fractionation, heat step[8] etc.) the enzyme preparations were only stable for one day at 4°, whereas deep freezing at −15° or lyophilization destroyed the activity completely. Experiments on the stabilisation of the enzymes are going on. In studies with the specific histidine decarboxylase of the stomach of guinea-pigs after centrifugation all of the activity could be shown in the supernatant. But in the experiments with the unspecific histidine decarboxylase of the guinea-pig's kidney we found about 40 per cent of the activity in the 1800 *g* sediment and about 20 per cent in the 100,000 *g* sediment.

Therefore, the unspecific histidine decarboxylase of the kidney seems to be less soluble and to a greater extent bound to structures than the specific histidine decarboxylase of the stomach.

## DISCUSSION

All arguments, which support the hypothesis of the mediator function of histamine in gastric secretion induced by acetylcholine and gastrin have been proved nearly exclusively in the rat: adequate location, formation and release of histamine and the induction of the specific histidine decarboxylase during the phases of secretion; furthermore the inhibition of secretion by inhibitors of the specific histidine decarboxylase, by infusion of diamine oxidase and by application of substances which form complexes with histamine. Therefore, the existence of a physiological function of histamine as a chemostimulator of the gastric secretion seems to be convincing only in the case of rat,[17] in all of the other species this theory has only the value of an hypothesis. Furthermore, since the rat is the "maverick"[6] between the mammals regarding to the metabolism of histamine and the sensitivity to histamine, the results gained in this species can be transferred to other species only to a small extent. Therefore the demonstration of specific histidine decarboxylases in the gastric mucosa of men and numerous other mammalian species is of some importance in two aspects: The hypothesis of histamine as a physiological chemostimulator of the gastric secretion has gained more probability. Furthermore, the determination of the specific histidine decarboxylase in pathological processes of gastric mucosa, especially gastric and duodenal ulcer, will perhaps open new ways in pathochemistry and pathophysiology of these diseases.

Weissbach *et al.*[30] differentiated two kinds of histidine decarboxylases according to some properties: A specific enzyme (substrate only L-histidine, pH-optimum 7·0, relatively low $K_m$ (about $10^{-4}$ M), inhibition by α-methylhistidine, but not by α-methyldopa and no activation by benzene) and an unspecific enzyme (substrates all of the naturally occurring aromatic L-amino acids, relatively high $K_m$ ($10^{-2}$ — $10^{-1}$ M), inhibition by α-methyldopa, but not by α-methylhistidine, activation by benzene). But some of the histidine decarboxylases found in the last years show deviations from these two types of enzymes especially regarding to pH-optimum and $K_m$. Therefore the existence of isoenzymes belonging to the one or the other type of histidine decarboxylase according to Weissbach *et al.*[30] can be assumed.[9] All the specific histidine decarboxylases found in the gastric mucosa, have a relatively low $K_m$, but its value differs from one species to another in one order of magnitude. All these enzymes show a pH-optimum around or below pH 7·0, but, compared with the other species, it shifted in rodents more to acid values. Thus isoenzymes of the specific histidine decarboxylase seem to occur in stomachs of different mammals. The unspecific histidine decarboxylase seems to have such isoenzymes, too.[19,35] The differentiation between specific and unspecific histidine decarboxylase or (in terms of other investigators) between histidine decarboxylase and dopadecarboxylase, whereby the latter enzyme decarboxylates L-histidine only *in vitro*, but not *in vivo*, has been questioned recently:[18,19,35] The specific histidine decarboxylase was shown to decarboxylate also DOPA and 5-HTP in some cases.[2] The DOPA decarboxylase in higher degrees of purity does not decarboxylate L-histidine *in vitro*.[3,33] The $K_m$ values of the unspecific histidine decarboxylases of the kidney of guinea-pigs and the stomach of rats are 1 ×

$10^{-3}$ M, those of the parotid and thyroid glands of rats are $1 \times 10^{-4}$ M.[19] Furthermore, a decarboxylation of L-histidine by the unspecific histidine decarboxylase of guinea-pig's kidney *in vivo* could be demonstrated by Schayer and Sestokas.[24] It seems therefore necessary to change the nomenclature of histidine decarboxylases. We propose the terms "acid" and "alkaline" histidine decarboxylase, since no histidine decarboxylating enzyme is known in literature, which could not be placed into one of these two categories (Lorenz and Werle in preparation).

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