Histidine Decarboxylase in the Adult Rat

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Histamine is regarded as one of the mediators of the inflammatory response (3), presumably because of its effect on microcirculation (1). Local histidine decarboxylase, under the influence of bacterial products (e.g. endotoxin) or other traumatic experiences, supposedly produces histamine and increases nutritional circulation sufficiently to sustain or restore function and to mobilize defensive mechanisms (10).

In the conventional animal microflora and host experience, the most intensive encounter in the intestinal tract. Here the histamine levels are definitely higher than in the germfree animal and are influenced by the presence and composition of the microflora (2). In the conventional rat these levels are exceeded only by those found in the pyloric part of the stomach. However, all studies of histidine decarboxylase activity in rat intestine so far have indicated at best extremely low values (7). As the specific decarboxylation of histidine is known to be a relatively slow reaction (5), we investigated the *in vitro* conversion of histidine by rat intestinal tissue and invariably found a fast degradation to more acidic products that left no opportunity for a measurable histamine formation. *In vivo* studies, however, always indicated a slow but consistent formation of histamine in the small intestine of the conventional rat.

Materials and Methods

Germfree and conventional adult rats (Lobund strain of Wistar origin) were used in all experiments. The animals had been reared on sterilized practical type diets and were 3 to 6 months old.

For the determination of histamine, tissues were homogenized in 0.1 N HCl in 1% saline and, except in the case of intestine, the extract was purified via column chromatography on cation exchange resin Rexin 102 H[2] (9). After elution with 1 N HCl, histamine was determined according to the method of Shore (11) with the modification of Kremzner and Wilson (8).

Histidine decarboxylase was determined *in vitro* according to the principle described by Schayer (10), with histidine-C^{14} (ring) as substrate. Incubation usually was for 3 hours at 37°. Histamine-C^{14} in the incubation mixture was isolated via a cation exchange resin (see above) and further purified via organic solvent extraction as described by Shore (11). Histamine-C^{14} in the final acid extract was determined by scintillation counting. Histamine-T^3 was carried through all phases to determine the recovery of histamine formed during incubation.

Histidine decarboxylation *in vivo* was determined after i.c., i.v. or i.p. administration of histidine-C^{14}. Several hours later the rats were sacrificed. Tissues were homogenized in 1/15 molar phosphate pH 7.4

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and total C^{14} in the tissue was determined and expressed as a percentage of the total dose. Histamine-C^{14} was isolated via column chromatography and organic solvent extraction as described above for the *in vitro* determination. Histamine C^{14} in tissue was calculated as a percentage of the total amount of C^{14} present in the tissue at the time of sacrifice.

Paper chromatography of tissue incubates was carried out after termination of the reaction with semicarbazide and centrifugation for 15 minutes at 15,000 rpm in Spinco Ultracentrifuge Model L. Ascending chromatography on Whatman #1 filter paper was used (6), the developing mixture being a phenol ammonia mixture at pH 7.0 (12). Parallel samples of histidine-C^{14} in the incubation mixture were run with all tissue samples. Sample strips were cut and eluted with 0.01 N HCl, and the distribution of the label was determined with the scintillation counter.

Results and Discussion

Histamine contents of a number of tissues of germfree and conventional rats are given in Table 1. The concentrations found in the small intestine, spleen and blood of the germfree rat are lower than in the conventional animal. Of all the internal tissues tested the small intestine contained by far the highest total amount of histamine, although the concentration per gm tissue was highest in the stomach.

TABLE 1. Histamine in tissues of adult female germfree and conventional rats.
Diet 5010C. Approx. 10 animals/group

	Germfree		Conventional	
	μg/g	μg total/100 BW	μg/g	μg total/100 BW
Stomach	....	....	40.0	10
Small Intestine[1]	16.1[s]	36[s]	30.0	75
Liver	2.2	8.2	2.2	10.4
Spleen	1.6[s]	0.5[s]	2.9	0.8
Blood	0.085[s]	0.4[s]	0.183	1.1

[1] Actual determination in ileum, but values throughout small intestine are quite similar (2).
[s] Significantly different from concentration in conventional animal.

The high concentrations found in the stomach obviously express the high local metabolic activity underlying gastric digestion. The second highest concentration is found in the wall of the intestine, which normally forms the barrier between host and intestinal microflora and constitutes the tissue most intensively exposed to this flora and its metabolic products. The influence of bacterial stimulation on histamine concentration is obvious from the difference found between germfree and conventional animals and from the variation in this concentration found in conventional animals over the years (2).

Schayer has pointed out that local formation of histamine would cause an increase in nutritional circulation whenever and wherever needed, presumably to support function (10). The above picture is suggestive of substantial histidine decarboxylating activity, not only

in the stomach, but also in the small intestine. However, when histidine decarboxylase activity was tested *in vitro* the stomach tissue showed high values, but intestinal tissue assayed according to this method failed to show any activity. This confirmed results obtained by others (7). Further studies showed that intestinal tissue homogenate, added to a histidine decarboxylating stomach preparation, would stop all further formation of histamine (Figure 1).

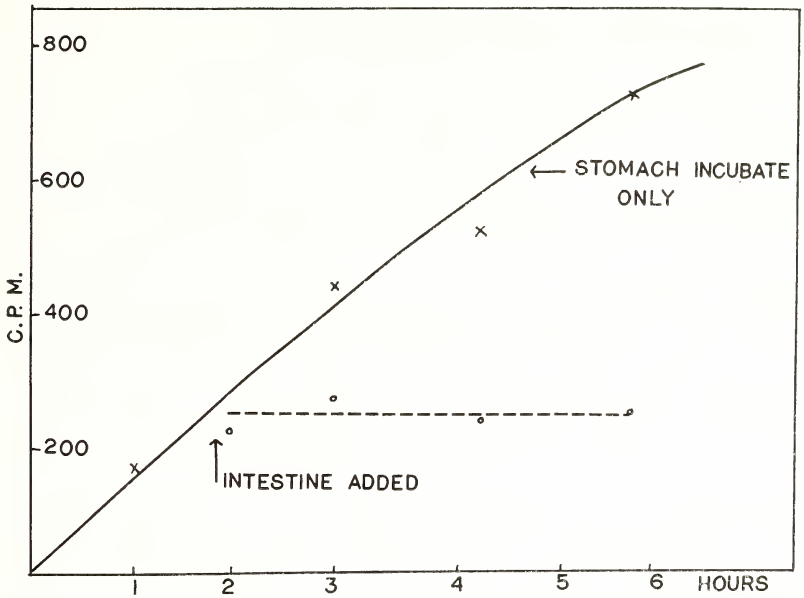

Figure 1. Formation of histamine-C^{14} in vitro from histadine-C^{14} after incubation with stomach tissue (50 mg) only (x———x), and with stomach tissue to which intestinal tissue (100 mg) was added two hours after start of incubation (o— — —o).

It was speculated that substrate deprivation caused by a rapid oxidative deamination of histidine might totally overshadow decarboxylation in intestinal tissue. Three hour tissue incubates, originally containing approximately 80 μg histidine and histidine-C^{14} (2.2 x 10^6 dpm) were analyzed by paper chromatography to determine the character of the labeled compounds present. Analysis of liver, spleen, stomach and intestine indicated that in all instances except in the case of the stomach, a substantial portion of the original histidine-C^{14} had been converted to more acidic products. Intestinal tissue contained hardly any histidine-C^{14} after incubation, but in incubates containing stomach tissue, a substantial part of the original histidine-C^{14} was still available (Figure 2).

Thus it appeared that under these experimental conditions no valid estimate could be made of the histidine decarboxylating capacity of rat small intestinal tissue. It was hoped, however, that *in vivo* experimentation, involving the activity of the integrated tissue, would yield more meaningful results. As this required administration of the labeled

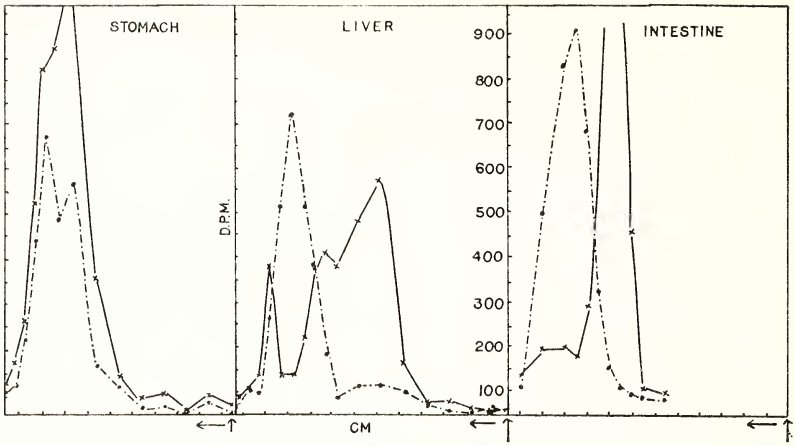

Figure 2. Ascending chromatography on paper of histidine-C^{14} + tissue incubate (x———x) and of histadine-C^{14} incubated in reagent mixture (o— - —o). Vertical arrow indicates site of application (see text).

precursor, either i.c., i.v., i.p., or i.m. to the live animal, it was recognized that the exposure of the specific tissue to the labeled histidine could only be approximated and that values thus obtained could at best be regarded as semi-quantitative. However, all nine conventional animals which received histidine-C^{14} i.c., i.v. or i.p. showed low but fairly consistent concentrations of histamine-C^{14} in the wall of the small intestine. Repeated i.v. administration of histidine-C^{14} gave the most consistent results. Apparently this minimized the effect of substrate depletion in intestinal tissue by oxidative deamination and transamination reactions. The values are given in Table 2, and are compared to values found in the pyloric portion of the stomach. In each case the amount of histamine-C^{14} found in a tissue was expressed as a percentage of the total amount of C^{14} found in that tissue at the end of the experimental period.

TABLE 2. Histamine-C^{14} in tissues after histidine-C^{14} administration via carotid artery of 4 conventional rats[1]

	C^{14}/gm[2]	$\frac{\text{Histamine } C^{14}}{\text{Total } C^{14}} \times 100$
Stomach	0.55	11.7
Small intestine	1.16	0.21
Spleen	0.37	+[3]
Lung	0.28	0.35
Liver	1.67	....

[1] 4 injections at 0, 30, 60 and 90 minutes, sacrificed at 150 minutes.
[2] Percent of total dose.
[3] Spread in data, but almost always significant formation of histamine-C^{14}.

The histamine-C^{14} found in the wall of the small intestine appeared to be of true local origin. The histamine concentration in the blood is quite low, and it was considered to be extremely doubtful that histamine-C^{14} formed in other organs could have accumulated in the wall of the small intestine via the blood to the extent found in these experiments. Nevertheless, histamine-C^{14} in amounts much larger than found in the stomach during the experimental period was injected intravenously. No specific accumulation of histamine-C^{14} was seen in the wall of the small intestine.

In part of the experiments the duodenum was ligated just beyond the pylorus to prevent a possible direct transfer of histamine-C^{14} formed in the pyloric part of the stomach to adjacent parts of the duodenum. This procedure appeared to have no effect on the outcome of the experiments and confirmed the "local" character of the histidine decarboxylating enzyme.

Based on the data in Table 2 one can estimate that within a given time period the stomach produces in the order of 60 times more histamine than the small intestine. This estimate is based on the assumption that the total amount of C^{14} found in the tissue at the end of the experimental period is a measure of the exposure of that tissue to histidine-C^{14}. Obviously this last assumption represents only a rather crude approximation.

Lung and spleen were found to have histidine decarboxylating activity of the same order as found in the small intestine. Neither *in vivo* nor *in vitro* experiments ever showed any histidine decarboxylating activity in the liver of the adult rat.

The above experiments indicate the presence of a specific histidine decarboxylase in the small intestine of the rat. The rate of histamine formation appears slow, especially when compared to that in the stomach. These data are in agreement with the results of studies which indicated a rapid formation and fast turnover of histamine in the stomach, but a slow formation and slow turnover in the intestinal tissue (7). Apparently in both tissues the balance between formation and catabolism is such that the steady state concentrations are of a similar order.

It thus appears that the small intestine has a low but definite histamine forming capacity. The presence of a conventional intestinal microflora results in higher levels of intestinal histamine which seem to reflect the state of "physiological inflammation" of this tissue in the conventional rat (4).

Summary

The relatively high concentration of histamine in the small intestine of the rat indicated local formation from histidine, presumably under the influence of the intestinal microflora. *In vitro* techniques could not demonstrate this formation, but revealed the much more rapid conversion of histidine to more acidic materials. *In vivo* administration of histidine-C^{14} showed, however, that a slow but consistent formation of histamine takes place in the wall of the small intestine of the rat.

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