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# GASTRIC HISTAMINE METABOLISM IN THE AGING RAT

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**Abstract**—Rat glandular stomach histamine concentration increases from 15.5 μg/g at 25 days of age to 78 μg/g at 179 days of age, whereas histamine levels in the forestomach do not change with aging. This increase in histamine occurs primarily in the muscular layer of the glandular stomach but not the mucosal and serosal layers of this organ. There is little change in the histamine-forming capacity of the stomach in this age group, nor does Compound 48/80 lower histamine levels *in vivo* in glandular stomach ; it does lower levels in the forestomach and diaphragm. These data suggest that changes in rat stomach histamine storage capacity but not storage properties occur with aging.

It has been shown in rats that both gastric histamine concentration and histidine decarboxylase activity increase with age from birth until approximately 40 days of age when levels do not differ from "adults" (the authors do not specify the age of "adults") [1, 2]. Moreover, Aures and Hakanson [2] report that the increase with age in gastric histamine levels "seems" to correlate with the change in histidine decarboxylase activity.

During the course of studies on this phenomenon, we observed that histamine levels in stomach increased linearly until rats reached 180 days of age. This report documents these observations. Further, we have attempted to define the change in histamine metabolism occurring with age which may account for this increase. Considering that the level of gastric histamine is dependent upon the level of gastric histidine decarboxylase as well as the release of histamine from gastric tissue, we investigated the relationship between age and histidine decarboxylase activity in rat stomach. In addition, the ability of Compound 48/80 to influence histamine levels in glandular stomach was studied to determine whether changes in histamine storage properties are affected by aging.

## MATERIALS AND METHODS

Male Sprague–Dawley rats obtained from ARS/Sprague–Dawley (Madison, WI) were used for all experiments. Histamine was assayed according to the extraction and spectrophotofluorometric method of Shore *et al.* [3]. Histidine decarboxylase activity (HDA) in gastric homogenates was measured as previously described [4] in which the $^{14}CO_2$ resulting from the enzymatic decarboxylation of histidine-[carboxyl-$^{14}$C] was collected and measured. Enzymic acitivity is expressed as μg histamine formed/hr/g of stomach.

Statistical methods were performed according to Snedecor and Cochran [5].

Hyamine hydroxide, L- and D-histidine-$^{14}$COOH were purchased from New England Nuclear Corp. Pyridoxal phosphate monohydrate, *o*-phthalaldehyde and histamine dihydrochloride monohydrate were obtained from CalBiochem; L- and D-histidine monohydrochloride monohydrate were obtained from Mann Research Laboratories, and Trizma base and Compound 48/80 were obtained from Sigma Chemical Co. Solution concentrations are expressed with corrections for salt and water content of the powdered chemicals.

## RESULTS

*Relationship between glandular stomach histamine concentration, histidine decarboxylase activity, and age.* A study was undertaken when it was noticed that, in older rats, considerably higher glandular stomach histamine levels were present than those previously reported in adult rats [1]. The histamine concentration and histidine decarboxylase activity of the rat glandular stomach were measured in animals 25–179 days of age (Fig. 1). A 5-fold increase in histamine concentration from 15.5 to 78.0 μg/g was found with a correlation coefficient of 0.90. The histidine decarboxylase activity in these rats increased 2.7-fold, from 5.6 to 15.3 μg/g/hr; however, only a low correlation exists between age and histidine decarboxylase activity (Fig. 1). As can be seen in Fig. 1, the HDA actually represents a biphasic curve. From days 25 to 81, the slope of the line is 0.04 and the slope of the line from day 83 to 179 is 0.006. These data show that enzymic activity increased 2.7-fold until 81 days of age and then plateaued, while the histamine concentration continued to increase with a slope of 0.36 μg/g/day. Neither the histamine concentration nor the enzymic activity in the forestomach changed with age (4.66 ± 0.341 μg/g and 5.5 ± 1.6 μg/g/hr, respectively; mean ± S. E.; N = 63). Thus, the increase in histamine concentration with age is exclusively in the glandular stomach. Besides age, this relationship is valid also for body weight of the animal ($r = 0.82$; N = 88; slope = 0.11896 μg/g/g) and the weight of the glandular stomach ($r = 0.8$; N = 91; slope = 46.05 μg/g/g).

*Influence of age on the Michaelis constants of histi-*

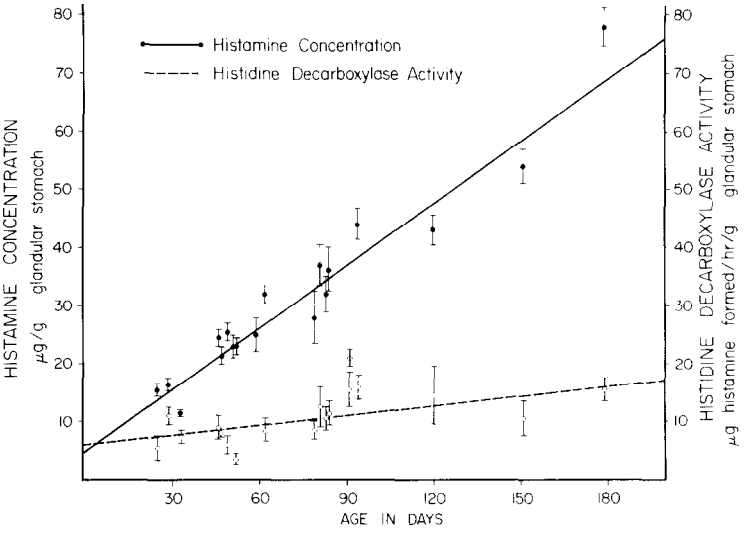


Fig. 1. Relationship between glandular stomach histamine concentration and histidine decarboxylase activity with age. Histamine concentration is on the left y-axis and histidine decarboxylase activity is on the right y-axis. A correlation coefficient of 0.90, $P < 0.001$, exists between age and histamine concentration measured in 99 rats. A correlation coefficient of 0.39, $P < 0.001$, exists between age and histidine decarboxylase activity measured in 88 rats. Each value is the mean of four to eight animals. Vertical lines represent S. E.

*dine decarboxylase*. The previous results suggested that, with age, there was an increased capacity for the storage of histamine, or a change in the properties of histidine decarboxylase in the rat glandular stomach. The marked increase in histamine concentration that occurred with increasing age did not correlate with the magnitude or the time course of the increase in the amount of enzyme (if the assumption is made that histidine decarboxylase activity reflects the amount of enzyme). However, it is possible that a change in the Michaelis constants occurred with age and was not detected using our method for measuring HDA. Experiments were designed to determine the Michaelis constants for histidine decarboxylase in stomach homogenates from rats 31 and 179 days of age. A Lineweaver–Burk plot was constructed to determine the apparant $K_m$ and $V_{max}$ by linear regression analysis for each age; these data are shown in Table 1. There is no significant difference in either $K_m$ or $V_{max}$ for the enzyme histidine decarboxylase between these two age groups. These data lend support to the lack of relationship between histamine levels and HDA activity.

*Relationship between age and distribution of histamine in the rat glandular stomach.* We investigated the distribution of histamine in the mucosal, muscular and serosal layers of the rat glandular stomach in an attempt to further clarify the site at which the increased amine concentration occurs. In all experiments, the mucosal histamine concentrations were lower than the concentrations in muscle, combined muscle and serosa, or serosa alone (Table 2). Moreover, mucosal histamine concentrations did not correlate highly with age and serosal concentrations were not significantly different. A high correlation with age existed in the combined muscle and serosal histamine concentration, $r = 0.96$. These data demonstrate that the increase in histamine occurring with age is specifically located in the muscular layer of the rat glandular stomach which is a layer with a low HDA (6).

*Effect of Compound 48/80 on histamine release* in vivo. Feldberg and Talesnik [7] have shown that the stomach of rats 30–40 days of age is resistant to the histamine-depleting action of Compound 48/80. To determine whether histamine storage characteristics change with increasing age, we treated rats 170–200 days of age with Compound 48/80.

A dose of 1 mg/kg of Compound 48/80 dissolved in saline (1 mg/ml) or saline alone was injected into the tail vein of rats. After the injection, the animals were decapitated, and 1.0-ml blood samples were collected for histamine analysis. Diaphragm and forestomach tissues were studied for comparison with the glandular stomach because they are known to release histamine after the injection of Compound 48/80 [7, 8]. Within 5 min of injection, the following changes occurred (Fig. 2): diaphragm histamine concentration decreased 53 per cent, forestomach histamine concentration decreased 70 per cent, and blood levels of this amine increased 1890 per cent. At no

Table 1. Relationship between age and kinetic constants of histidine decarboxylase*

| Age (days) | Apparent $K_m \times 10^{-4}$ M | Apparent $V_{max}$ (μg/g/hr) | No. of animals |
|---|---|---|---|
| 31 | 4.0 ± 0.21 | 23.9 ± 3.9 | 7 |
| 179 | 3.5 ± 0.23 | 21.4 ± 4.1 | 5 |

* Each value is the mean ± S. E.

Table 2. Distribution of histamine in layers of the rat glandular stomach according to age

| Age of rats (days) | Mucosal histamine* | N† | Muscle histamine* | N† | Combined muscle and serosal histamine* | N† | Serosal histamine* |
|---|---|---|---|---|---|---|---|
| 31 | 3.75 ± 0.03 | 4 | | | 19.8 ± 0.9 | 12 | |
| 54 | 5.33 ± 0.51 | 6 | | | 31.5 ± 1.4 | 12 | |
| 70 | 8.15 ± 1.49 | 10 | 44.6 ± 1.8 | 10 | | | 20.2 ± 1.1 |
| 190 | 12.10 ± 0.90 | 3 | | | 90.5 ± 6.2 | 6 | |
| 200 | | | 118 ± 21.0 | 3 | | | 22.0 ± 2.2 |
| 387 | 9.58 ± 1.04 | 6 | 164 ± 10.7 | 6 | | | 29.7 ± 2.9 |

* Mean histamine concentration expressed as μg/g ± S. E.
† Number of animals.

time was the glandular stomach histamine concentration significantly different from control values. After 4 hr, diaphragm and forestomach histamine concentrations were still depressed, while blood histamine concentration returned to control levels. It was possible that histamine was released from glandular stomach but levels were not lowered because a concurrent increase in histidine decarboxylase activity maintained the histamine level constant. To test this hypothesis, rats were fasted for 24 hr, because fasting is known to lower gastric histidine decarboxylase activity [4], and the above experiment was then repeated using 214-day-old rats.

Glandular stomach histidine decarboxylase activities in these rats was low (1.55 ± 0.16 μg/hr/g) compared to non-fasted controls and remained low after Compound 48/80 (15 min and 4 hr after Compound 48/80 the enzymic activity was 1.19 ± 0.25 and 2.09 ± 0.46 μg/hr/g respectively). The response to Compound 48/80 in these animals was similar to that reported in Fig. 2. Thus, under conditions in which histidine decarboxylase activity is reduced, Compound 48/80 still failed to lower glandular stomach histamine levels.

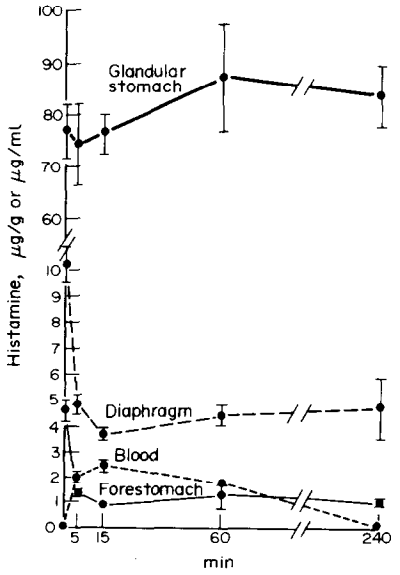


Fig. 2. Effect of Compound 48/80 on histamine release *in vivo*. The histamine level in rat tissues at various times after administration of Compound 48/80 (1 mg/kg, i.v.) is shown. Each point represents the mean value of three to four rats except control values which represent nine animals. Vertical lines depict S. E. Standard errors of the mean are not depicted if less than ±0.01 μg/g or μg/ml of histamine. Blood is expressed as μg/ml; all other tissues are expressed as μg/g. At all observed time periods, control histamine levels were not different from zero time.

## DISCUSSION

There is a direct relationship between the age of a rat and the gastric concentration of histamine (Fig. 1). This relationship is valid not only for age but also for the body weight of the animal and the weight of the glandular stomach. This relationship exists for that part of the rat stomach considered physiologically functional [9], the fundic portion, whereas the histamine levels in the forestomach do not vary with age. Observations of changes in histamine levels with age in rat lung and skin have been reported [10, 11]; however, the establishment of a functional significance has proven difficult.

Our objective was to determine whether the increase in gastric histamine content was related to a change in histamine synthesis, a change in histamine storage characteristics or a change in the capacity of the storage sites. Kahlson and Rosengren [12] have suggested a functional relationship between rate of growth and "nascent" histamine. Their criteria imply that nascent histamine is utilized at the same rate as it is formed and thus, storage capacity of the tissue is low while the histidine decarboxylase activity is high; that is, nascent histamine is non-mast cell in location. We must conclude from our data that we are not dealing with nascent histamine for the following reasons. First, the histidine decarboxylase activity is relatively low compared to the histamine content. From our data in Fig. 1, we could predict that only prior to the birth of the rat is the histidine decarboxylase activity of the gastric tissue greater than the histamine content. Second, the enzymatic characteristics of histidine decarboxylase do not change as the age of the animals increases, and third, the major increase in histamine content occurs in the muscular layer of the glandular stomach which is a layer thought not to contain nascent histamine [1, 6].

Finally, our data demonstrating the inability of Compound 48/80 to reduce the levels of histamine in glandular stomach support the belief that the storage sites in this tissue are different from those in other

tissues, namely, diaphragm and forestomach. The fact that Compound 48/80 does not influence gastric histamine levels in older rats (Fig. 2) or in young rats [7] demonstrates that Compound 48/80 resistant storage sites do not change with aging. It is conceivable that the number of storage sites in glandular stomach increases with age to account for the increasing histamine content rather than a change in storage or synthesis characteristics. This phenomenon has been reported for histamine metabolism in peritoneal mast cells [13].

It is interesting, but unexplainable, that the rat forestomach differs in its storage and synthesis of histamine from that in the glandular stomach. Compound 48/80 releases amine from the forestomach (Fig. 2) and the histidine decarboxylase activity in this tissue is of bacterial origin [14, 15].

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