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REFERENCE

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On the presence of histidine decarboxylase activity in rat forestomach

Recent reports on the presence of histidine decarboxylase (L-histidine-carboxy-lyase, EC 4.1.1.22) in the rat fundus (forestomach, thin membranous or ruminal portion) and the pylorus (glandular or muscular portion) have been contradictory. Radwan & West (1967) claimed preparations from the rat fundus to have many of the enzymatic properties of the specific histidine decarboxylase found in rat foetal liver and rat hepatoma; the enzymatic activity of the fundic preparations, however, was slightly inhibited by α-methyl histidine while preparations from the pylorus displayed many of the enzymatic properties of the aromatic-L-amino acid decarboxylase (EC 4.1.1.26). Håkanson & Owman (1966) and Aures, Håkanson & Schauer (1968) found that the ruminal portion had only a trace of the enzyme activity compared with the oxyntic gland area of the pyloric portion. Leinweber & Braun (1970) concluded that, in addition to the pylorus, the fundic portion also contains histidine decarboxylase activity which is resistant to most known inhibitors. The lack of response to inhibition was used to explain the failure to obtain a reduction of histamine formation *in vivo*. These authors also suggested that the activity of the fundic portion bore a striking similarity to that of the *Lactobacillus* enzyme purified by Rosenthaler, Guirard & others (1965). Kobayashi & Maudsley (1969) believed that the enzyme activity of the fundus was weak and in an insoluble form, and was present only in male rats. Isaac (1970) and Beavens, Horáková & Severs (1970) presented evidence from studies in germ-free rats that the fundus activity was of bacterial origin.

These contradictory communications prompted us to describe our early investigations on this subject which indicate that the non-enzymatic activity of the fundus offers an alternative explanation to these conflicting reports.

Adult, male Sprague-Dawley rats (CFE, Carworth Farms, New City, New York), 200 to 250 g, and fed Purina rat pellets *ad libitum* were killed by decapitation and the whole stomach immediately excised. The thin forestomach was separated from the muscular pyloric portion by cutting along the line of demarcation. Both portions were washed free of stomach contents in 0·9% cold saline and homogenized in an all-glass homogenizer with 3 volumes of cold saline. The homogenates were centrifuged at 35 000 *g* for 1 h at 4° and the resulting supernatant frozen in 2 ml aliquots at −20°, if not used immediately.

Histidine decarboxylase was assayed (Ellenbogen, Markley & Taylor, 1969). Unless otherwise noted, incubation mixtures contained 0·2 ml of 0·1M sodium phosphate buffer (pH 6·8), 0·2 ml of 1×10^{-5}M pyridoxal phosphate, 0·5 ml of enzyme, 0·1 ml of 1×10^{-2} M L-histidine-^{14}COOH (New England Nuclear or Calbiochem) and water to a final volume of 2·0 ml. After the addition of the enzyme and cofactor, the mixture was allowed to incubate for 10 min. The reaction was started by the addition of substrate, incubated at 37° for 1 h and corrected for a boiled enzyme control (boiled 30 min). All assays were made in duplicate and the results averaged.

Table 1. *Comparative properties of preparations from fundus and pylorus.*

Source of material	Treatment*	Activity (% of native material)
Fundus	Boiled	100
	Perchloric acid	92
	Dialysed	0
Pyloric	Boiled	17
	Perchloric acid	33
	Dialysed	70

* See text for details.

When incubation mixtures were corrected for boiled enzyme controls from the same rat stomach preparation, the following observations were made (Table 1): (1) Boiling the supernatants for 30 min usually did not decrease the activity of the fundus, and frequently produced an increase; the activity of the supernatant from the pyloric portion was decreased by boiling for 2 min and further boiling for up to 90 min caused no further drop in activity. (2) With preparations from the fundus, approximately 92% of the activity was recovered after precipitation in 5% perchloric acid and neutralization to pH 6·5, 10 min later; similar treatment markedly reduced the activity of the preparations from the pylorus. (3) Activity of the fundus was completely eliminated by dialysis whereas dialysis of the pylorus preparations caused a small decrease in activity.

With preparations from the pylorus, a linear response was obtained with enzyme amounts in excess of 10 mg and incubation times up to 2 h; a linear response could never be obtained beyond 1 mg of protein prepared from the fundus at a pH of 6·8 (Fig. 1). Activity was difficult to measure much below 1 mg of protein at pH 6·8 and only trace amounts were detectable at pH 5·6.

The above characteristics would indicate that much of the fundic activity could be non-enzymatic and is likely to be related to a heat-stable residual activity found adjacent to, or contaminated by, the pylorus. The studies by Aures & others (1968) on the

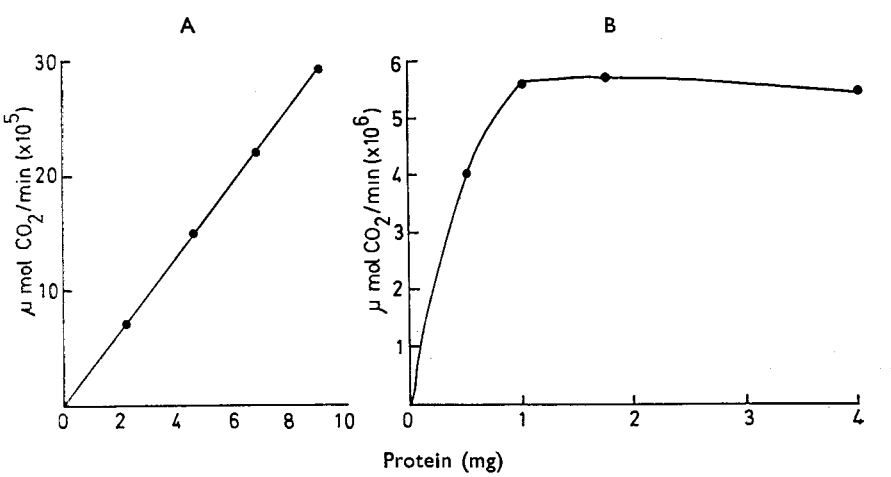

FIG. 1. The effect of activity of preparations from (A) the pylorus and (B) fundus *vs.* protein concentration. A 0·05–0·5 ml sample of 35 000 *g* supernatant was used as the source of "enzyme". Assay is described in text. Each point represents the average of 2 assays.

regional distribution of histidine decarboxylase indicate that enzyme activity occurs at the distal portion of the pyloric glandular area (oxyntic gland area) which is adjacent to the fundic portion. Thus, contamination by traces of the pyloric area could explain our occasional finding of some erratic activity in the forestomach. The highest specific activity observed with fundic preparations was 10% of that observed with material from the pylorus. The non-enzymatic decarboxylation could also explain the formation of nearly stoichiometric amounts of histamine and $^{14}CO_2$ observed by Leinweber & Braun (1970). The suggestion (Leinweber, 1968) that the forestomach activity is similar to that of the enzyme purified from *Lactobacillus* (Rosenthaler & others, 1965) is contradicted by the fact that cyanide does not inhibit the mammalian activity. The variable results seen by the different investigators were attributed to the variable amount of bacteria found in the stomach of the rats (Isaac, 1970; Beavens & others, 1970). The findings reported herein, however, offer an alternative explanation. Furthermore, decarboxylation catalysed by pyridoxal phosphate or pyridoxal itself occurs in the absence of any apoenzyme, and almost all of these reactions proceed by the same mechanism (Metzler, Ikawa & Snell, 1954; Snell, 1958). In addition, Lippmann (1969) has recently shown that production of $^{14}CO_2$ from carboxyl-labelled histidine can occur in the absence of enzyme and pyridoxal phosphate but in the presence of an organic compound, AY-17,224. Finally, our studies using a boiled enzyme blank and the studies of Levine & Watts (1967) who used a potent inhibitor of histidine decarboxylase emphasize the fact that estimation of enzyme blanks by the omission of enzyme is not always satisfactory.

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