efficient enzyme systems – glutamate dehydrogenase and asparagine synthetase – are also present[11,16,17]. In spite of the presence of these enzymes, substantial quantities of ammonia are reported to escape from the foliage into the atmosphere[10]. It is possible that limitations of acceptor molecules and/or energy may be the factors responsible for the accumulation of ammonia and its release from the foliage, and the process may be considered as a part of the ammonia detoxification mechanism.

1 This work was done under the National Fellowship Project (ICAR) on 'Nitrogen Assimilation in Crop Plants'.

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0014-4754/83/111302-02$1.50+0.20/0

Effect of prolonged inhibition of histidine decarboxylase on tissue histamine concentrations[1]

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Summary. In rats, chronic infusion of α-fluoromethyl histidine, a selective irreversible inhibitor of mammalian histidine decarboxylase, caused a marked depletion of histamine in all tissues examined. There were no gross pharmacological effects associated with this depletion.

Histamine is formed by decarboxylation of histidine by the specific histidine decarboxylase (E.C. 4.1.1.22)[3] and is present at varying concentration in almost all mammalian tissues[4]. One of the possible approaches to studying the role of histamine in mammalian physiology is by blocking its synthesis using specific inhibitors of histidine decarboxylase. We report here that α-fluoromethyl histidine, a selective enzyme-activated inhibitor of histidine decarboxylase[5–8], given to rats by infusion over 3 weeks, decreases the concentration of histamine in all tissues examined.

Materials and methods. Materials. α-Fluoromethyl histidine was synthesized in our laboratory by a method to be published elsewhere. Histamine, histidine and S-adenosyl methionine were purchased from Merck, Darmstadt, FRG. L-[1-^{14}C]histidine (50 mCi/mmole) came from New England Nuclear Corporation, Boston, USA. L-[2,5-^{3}H]histidine (40–50 Ci/mmole) and S-adenosyl-L-[methyl-^{3}H]-methionine (80 Ci/mmole) were supplied by the Radiochemical Center, Amersham, England.

Animals and treatment. Male rats of the Sprague-Dawley strain were used in this experiment. For chronic infusion of α-fluoromethyl histidine, Alzet 2001 minipumps loaded with 100 mg of coumpound in 200 µl of water were implanted in the peritoneal cavity under light ether anesthesia. The minipumps delivered a flow rate of 1 µl/h for 1 week. Every 7 days the minipumps were renewed. Control animals were sham operated but no minipumps were implanted. The animals were weighed daily and rectal temperature (Appelab thermosonde) was measured at intervals. Gastric secretion was measured on given days by a technique which consisted of washing the stomach with 2 ml of warm physiological saline via a gastric sonde[9]. The pH of the recovered solution was recorded. The animals were killed on day 21.

Tissue preparations. At the end of the chronic treatment rats were killed by decapitation and different organs were removed. All the tissues used were homogenized in 5 vols of phosphate buffer (2×10^{-3} M, pH 7.9 containing 0.1% Triton X100). The crude homogenates were used for determination of histidine decarboxylase activity where appropriate. The supernatants obtained after centrifugation were used for measurement of histamine.

Analysis. Tissue histamine content was determined by a modification of the enzymatic isotopic assay described by Taylor and Snyder[10]. Histidine decarboxylase activity in gastric mucosa was determined by the measurement of $^{14}CO_2$ libeerated from L-[1-^{14}C]histidine. Both methods have been described in detail in a previous publication[8].

Hypothalamus histidine decarboxylase activity was measured by a modification of the radiochromatographic procedure described by Baudry et al[11]. The incubation mixture (70 µl) consisted of 50 µl of tissue homogenate, 1 µCi of purified L-[2,5-^{3}H]histidine (about 2×10^{-7} M), 10^{-5} M of pyridoxal phosphate, 10^{-4} M of histamine and 5×10^{-2} M of phosphate buffer pH 7. After 1 h incubation at 37 °C, the enzymatic reaction was stopped by addition of 10 µl of 2.4 M perchloric acid. After addition of 600 µl of 0.2 M Tris-HCl buffer pH 8, the [^{3}H]histamine formed was isolated by ion-exchange chromatography on Amberlite CG 50 (200–400 mesh).

Results and discussion. In mice, α-fluoromethyl histidine given acutely decreased the concentration of histamine in

brain and stomach[6,7] but did not affect the histamine content in skin[7], a tissue rich in mast cells. We confirmed that a single dose of α-fluoromethyl histidine administered to rats inhibits histidine decarboxylase activity in brain (hypothalamus) and stomach and decreases histamine concentration in brain, the effect being the greatest in the hypothalamus. However, in rats we found no significant depletion of histamine content in gastric mucosa and in the other tissues examined (spleen, lung, thymus, liver and heart, table 1). It is clear that inhibition of synthesis over short time periods will only affect tissues in which the turnover rate of histamine is of the same order of magnitude as the duration of enzyme inhibition.

Three types of histamine storage systems with extremely different turnover rates can be distinguished; the neuronal histamine which has a half life of a few minutes[12,13], the gastric histamine which turns over at a relatively rapid rate (several hours[14]) and the mast cell histamine which has a very slow turnover[15].

Therefore we administered α-fluoromethyl histidine by continuous i.p. infusion for 21 days. Histidine decarboxylase activity, measured at the end of the 3 weeks treatment in hypothalamus and gastric mucosa, represented 5–10% of enzyme activity in the control. Intermediate points are not available, but it can be assumed that this degreee of inhibition was attained rapidly since a single administration of the inhibitor (10 mg/kg i.p.) produced an 80% inhibition of histidine decarboxylase activity in brain and stomach within 90 min (unpublished). Histamine concentration was measured in a number of tissues (table 2). Significant depletion of histamine was found in all tissues; it ranged from over 90% for the hypothalamus to about 40% in heart. Thus, prolonged inhibition of histamine biosynthesis will lower the concentration of the amine even in tissues rich in mast cells.

An almost total depletion was found in the hypothalamus, in which histamine may function as a neurotransmitter. On the basis of micro injections of histamine in hypothalamic areas, a role of hypothalamic histamine in body water homeostasis has been suggested[16]. Food and water intake were not measured in the present experiment; it is, however, unlikely that they were grossly altered by monofluoromethyl histidine infusion as there was no difference in weight gain between the experimental and the control group (table 3). Similarly there was no change in rectal temperature over the whole period of the treatment (table 3). This however does not mean that hypothalamic functions such as hormone secretion and regulation of corticosteroid biosynthesis are not changed and this possibility deserves investigation.

Many authors have reported that histamine plays a key role in gastric acid secretion. We have reported before that a single dose does not affect basal acid secretion but reduces the total acid output due to pentagastrin injection[9]. The concentration of histamine in the gastric mucosa is not changed significantly by a single injection of monofluoromethyl histidine (table 1)[9]. Prolonged infusion of the histidine decarboxylase inhibitor depletes the mucosal histamine content by 73% (table 2). Despite the reduced concentration of histamine, the rate of unstimulated acid secretion as measured by the pH of a stomach washing is still unchanged (table 3). It may be that the 30% of histamine remaining represent the physiologically active pool and that almost total depletion has to be achieved before acid secretion is affected.

Histamine plays a role in physiological processes such as allergy, inflammation and anaphylactic shock. It will be of interest to determine whether the depletion of histamine

Table 1. Effect of a single dose of α-fluoromethyl histidine (50 mg/kg, i.p.) on histidine decarboxylase activity and histamine content in various tissues

Tissue tested	Histidine decarboxylase activity (%)		Histamine content ng/mg tissue (%)	
	Control n = 5	Treated n = 5	Control n = 5	Treated n = 5
Brain			0.080 ± 0.008 (100 ± 10)	0.051 ± 0.005* (64 ± 6)
Hypothalamus	100 ± 15	7 ± 4*	0.31 ± 0.02 (100 ± 6)	0.072 ± 0.01* (23 ± 3)
Gastric mucosa	100 ± 12	10 ± 8*	39 ± 3 (100 ± 8)	40 ± 6 (102 ± 15)
Spleen			2.5 ± 0.5 (100 ± 20)	2.0 ± 0.6 (80 ± 20)
Lung			6.1 ± 0.8 (100 ± 10)	6.0 ± 0.5 (98 ± 8)
Thymus			8.4 ± 0.9 (100 ± 10)	7.3 ± 0.4 (87 ± 5)
Liver			0.9 ± 0.2 (100 ± 20)	0.8 ± 0.3 (90 ± 30)
Heart			4.2 ± 0.2 (100 ± 5)	3.6 ± 0.5 (90 ± 10)

Rats were killed 6 h after i.p. administration of α-fluoromethyl histidine (treated) or saline (control). Histidine decarboxylase activities in the control group of rats are 12 ± 3 nmoles/g tissue/h in the gastric mucosa and 170 ± 20 cpm/mg tissue/h in the hypothalamus. * $p < 0.5\%$.

Table 2. Effect of prolonged infusion of α-fluoromethyl histidine (5 mg/kg/h for 21 days) on histidine decarboxylase activity and histamine content in various tissues

Tissue tested	Histidine decarboxylase activity (%)		Histamine content ng/mg tissue (%)	
	Control n = 7	Treated n = 5	Control n = 7	Treated n = 5
Hypothalamus	100 ± 20	4 ± 1*	0.31 ± 0.06 (100 ± 19)	0.02 ± 0.01* (6 ± 4)
Gastric mucosa	100 ± 10	12 ± 7*	41 ± 4 (100 ± 10)	11 ± 1* (27 ± 3)
Spleen			3 ± 0.6 (100 ± 20)	0.59 ± 0.04* (20 ± 2)
Lung			5.5 ± 0.9 (100 ± 16)	1.4 ± 0.4* (25 ± 7)
Thymus			8.9 ± 0.5 (100 ± 11)	3.9 ± 0.6* (44 ± 7)
Liver			0.9 ± 0.1 (100 ± 11)	0.5 ± 0.04* (56 ± 5)
Heart			4.5 ± 0.2 (100 ± 5)	2.8 ± 0.3* (62 ± 7)

Histidine decarboxylase activities in the control group of rats are 9 ± 1 nmoles/g tissue/h in the gastric mucosa and 150 ± 30 cpm/mg tissue/h in the hypothalamus. * $p < 0.5\%$.

Table 3. Effect of prolonged infusion of α-fluoromethyl histidine on weight gain, rectal temperature and gastric acid secretion in rats

		Day 3	Day 7	Day 14	Day 21
Body weight (g)	Control	150 ± 2	171 ± 3	215 ± 3	229 ± 7
	Treated	151 ± 1	176 ± 3	214 ± 6	240 ± 4
Calculated drug intake (mg/kg/day)		84	69	61	57
Rectal temperature (°C)	Control	36.1	36.0	–	36.5
	Treated	36.7	36.3	–	36.7
pH of stomach washing	Control	2.6 ± 0.1	2.6 ± 0.2	2.8 ± 0.3	2.7 ± 0.2
	Treated	2.9 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.7 ± 0.1

Each value is the mean ± SEM of 5 animals. None of the values is statistically different from that of the matched control.

reported in table 2 has any effect on these pathological or physiological conditions. In conclusion, monofluoromethyl histidine represents a safe nontoxic means to deplete whole body histamine stores. More will be cerntainly be heard about the pharmacology of this compound.

1 Part of this work had been presented at the 11th Annual Meeting of the European Histamine Research Society, Bled, Yugoslavia, May 9–12, 1982. The authors wish to thank C. Grauffel and I. Fornieles for their excellent technical assistance.
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0014-4754/83/111303-03$1.50+0.20/0

Synergistic effect of AMP and fructose 2,6-bisphosphate on the protection of fructose 1,6-bisphosphatase against inactivation by trypsin[1]

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Summary. The rate of inactivation of chicken liver fructose 1,6-bisphosphatase by trypsin is reduced if the digestive reaction is conducted in the presence of AMP or fructose 2,6-bisphosphate. The effects of these 2 compounds are synergistic. Although fructose 1,6-bisphosphate does not protect the enzyme against tryptic inactivation, it can enhance the effect of AMP. Selective modification of the AMP allosteric site of fructose 1,6-bisphosphatase with pyridoxal-P and $NaBH_4$ renders the enzyme more resistant to tryptic inactivation, but the modified enzyme is no longer responsive to the protective effect of AMP.

Fructose 1,6-bisphosphatase (Fru-P_2ase) (EC 3.1.3.11) is a key enzyme in gluconeogenesis. This enzyme is extremely sensitive to allosteric inhibition by AMP as well as competitive inhibition by fructose 2,6-bisphosphate (Fru-2,6-P_2) and the inhibitory effects of these 2 compounds are synergistic[2,3]. It has been previously reported that the rate of inactivation of chicken liver Fru-P_2ase by trypsin was significantly reduced if the digestive reaction was carried out in the presence of AMP[4]. We now report that Fru-2,6-P_2 can also protect this enzyme against tryptic inactivation and that the protective effects of AMP and Fru-2,6-P_2 are synergistic.

Materials and methods. Bovine pancreatic trypsin, yeast glucose-6-P dehydrogenase and phosphoglucose isomerase, and other chemicals were all purchased from Sigma Chemical Company, Saint Louis, MO, USA. Fru-P_2ase was purified from chicken livers by the method previously described[5]. The activity of Fru-P_2ase was measured spectrophotometrically by following the formation of NADPH at 340 nm in a coupled reaction. Unless otherwise stated, the assay mixture (1 ml) contained 50 mM Tris-HCl buffer (pH 7.4), 0.1 mM EDTA, 0.15 mM $NADP^+$, 0.1 M KCl, 2 mM $MgCl_2$, 0.1 mM Fru-1,6-P_2, 1 unit each of phosphoglucose isomerase and glucose-6-P dehydrogenase, and an appropriate amount of Fru-P_2ase. The assay mixture without substrate was preincubated in a cuvette at 25 °C for 2 min. The reaction was initiated by the addition of substrate. The concentration of purified Fru-P_2ase was determined by its extinction coefficient at 280 nm[5]. Fru-2,6-P_2 was chemically synthesized by the method of Pilkis et al.[6]. The concentration of Fru-2,6-P_2 was determined by incubating an aliquot of Fru-2,6-P_2 stock solution at pH 2.5 for 30 min at 28 °C and assaying the amount of fructose-6-P formed using glucose-6-P dehydrogenase and phosphoglucose isomerase[6]. The Fru-2,6-P_2 preparation used in this study contained no detectable fructose-6-P, glucose-6-P, and Fru-1,6-P_2.

Results. Figure 1 shows the time course of changes of Fru-P_2ase activity on modification with trypsin. Under the condition described in the legend of this figure, the time required for inactivation of 50% of Fru-P_2ase activity was approximately 30 min. This increased to about 60 min or 80 min if the digestive reaction was carried out in the presence of 0.12 mM AMP or 0.12 mM Fru-2,6-P_2, respectively. If digestion with trypsin was performed in the presence of both 0.12 mM AMP and 0.12 mM Fru-2,6-P_2, more than 80% of Fru-P_2ase activity still remained even after 200 min. Table 1 shows that the protective effects of both AMP and Fru-2,6-P_2 decreased markedly if digestion with trypsin was carried out at higher pH. It also shows that Fru-1,6-P_2 failed to protect Fru-P_2ase against tryptic inactivation, but it significantly enhanced the protective effect of AMP.

Figure 2 shows that treatment of Fru-P_2ase with pyridoxal-P in the presence of Fru-1,6-P_2 followed by reduction with $NaBH_4$ resulted in irreversible desensitization of the enzyme to allosteric inhibition by AMP with only slight loss