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URINARY EXCRETION OF FREE HISTAMINE IN GUINEA-PIGS AFTER ORAL HISTIDINE

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Two observations show that histamine may enter the circulation following bacterial decarboxylation of histidine in the digestive tract. In rats, Wilson (1954) obtained some decrease in urinary excretion of free and conjugated histamine after oral antibiotics. In dogs, Irvine, Duthie & Waton (1959) found that sterilization of the gastro-intestinal tract caused a great reduction in the acid gastric secretion from a Heidenhain pouch, as well as in the urinary excretion of free histamine, which follows a meal of meat or ingestion of histidine. In contrast to these results the present experiments on the guinea-pig show that in this species sterilization of the intestinal tract does not affect the urinary excretion of free histamine which follows oral administration of histidine.

METHODS

Female guinea-pigs of 400–600 g were used for the experiments. During each experiment they were given water (5 % body weight) by stomach tube every 2 hr for 8 hr. When the effect of histidine on the urinary excretion of histamine was examined, histidine monohydrochloride was added to the second oral water load.

The guinea-pigs were placed in individual metabolism cages and the urine was collected in 2 hr periods following each dose of water. The spout of the funnel under the metabolism cage was placed about 1 in. above the point of a stainless-steel gauze cone, whose diameter was such that it just fitted the top of a small funnel leading to the collection bottle containing 1·0 ml. N-HCl. In this way the urine passed into the bottle while faeces rolled down the cone and fell clear of the collecting bottle.

The guinea-pigs were not starved because it was found that the variations in urinary histamine were smaller in fed than in starved animals; moreover the faeces were firmer and diarrhoea seldom occurred.

The urinary free histamine was determined by assay of the urine, without preliminary extraction, on the atropinized guinea-pig ileum suspended in 5 ml. oxygenated Tyrode solution. If the urinary samples were strongly acid, owing to the small volume of urine excreted, sufficient sodium bicarbonate was added to make the urine faintly acid, neutralization being avoided because of precipitation. The identity of the gut-contracting substance with histamine was established by showing that the contractions were abolished by minute doses of mepyramine maleate and recovered in parallel with histamine. This according to Reuse (1948) is good evidence for the identification of histamine.

To sterilize the gut the guinea-pigs were given 24 mg neomycin sulphate in water by stomach tube in the morning and 40 mg in the evening for 6 days. Since this treatment was found only to reduce the bacterial content of the faeces, but not to make them sterile, treatment was continued by giving twice daily, by stomach tube, doses of the following mixture: 8 g neomycin sulphate, 4 g chlortetracycline, 4 g chloramphenicol and 16 g sulphadimidine, water to 1000 ml. The mixture was shaken vigorously immediately before removal by syringe for administration. The guinea-pigs received 3 ml. of this mixture in the morning and 5 ml. in the evening. Faecal bacterial swabs were taken daily from the centre of freshly expelled faeces, and after they had been sterile for at least 4 days the experiments were performed to determine the urinary excretion of free histamine before and during three successive 2 hr periods, after oral administration of histidine. The animals were then killed and bacterial swabs were taken from each part of the intestine. All swabs were plated aerobically on McConkey and agar media and broth culture. Swabs from upper caecum and lower colon were, in addition, incubated anaerobically in broth cultures.

RESULTS

Urinary free histamine after oral histidine

The urinary excretion of free histamine in a 2 hr period following an oral administration of water (5 % body weight) varied not only from animal to animal but also in the same animal during different days. Some 2 hr samples contained 0·05–0·1 μg, the threshold values which could be assayed, a few samples contained > 2 μg, and one sample as much as 3·6 μg. The mean value of thirty-two samples obtained from eight animals was 0·83 μg.

TABLE 1. Effect of the oral administration of histidine on the urinary excretion of free histamine (μg/2 hr). All guinea-pigs were given an oral water load (5 % body weight) every 2 hr for 8 hr with or without the addition to the second water load of 12·5–100 mg histidine monohydrochloride/100 g body weight. Each result is the mean, together with upper and lower limits, from eight guinea-pigs

	2 hr control values	Histamine (μg) in successive 2 hr samples		
		0–2 hr	2–4 hr	4–6 hr
Water alone	0·76	0·78 (0·48– 1·05)	0·92 (0·63– 1·44)	0·84 (0·42– 1·50)
Water + histidine 12·5 mg/100 g	0·56	2·36 (1·80– 3·30)	1·01 (0·68– 1·45)	0·65 (0·16– 1·10)
Water + histidine 25 mg/100 g	0·74	8·49 (2·08–13·8)	1·72 (0·96– 2·80)	0·52 (0·10– 0·96)
Water + histidine 100 mg/100 g	1·36	20·6 (9·30–56·0)	31·8 (18·0 –50·9)	21·3 (6·80–50·9)

The first line of Table 1 shows that if the oral administration of water (5 % body weight) is repeated every 2 hr there is no significant increase in the urinary excretion of free histamine. On the other hand, the second, third and fourth lines show that the oral administration of increasing doses of histidine given with the second water load caused an increasing rise in urinary free histamine. After 12·5 and 25 mg/100 g the increase in urinary histamine returned to normal by the 4–6 hr sample, whereas after 100 mg/100 g these samples still contained large amounts of histamine.

From these results it would appear that in guinea-pigs part of the histidine given orally is decarboxylated and appears as free histamine in the urine. But the results do not indicate whether decarboxylation occurs in the lumen of the intestine or in the tissues after absorption.

Intestinal sterilization. Sterilization of the intestinal tract of guinea-pigs does not alter the urinary excretion of free histamine after oral histidine. This is shown in Table 2. The large increase in urinary free histamine produced after an oral dose of 100 mg histidine monohydrochloride/100 g body weight was obtained also after sterilization of the intestine. The values of Table 2 referring to the urinary free histamine after sterilization were obtained after faeces had been sterile for at least 4 days. The bacterial

TABLE 2. Urinary excretion of free histamine (μg/2 hr urine) in guinea-pigs given an oral water load (5 % body weight) every 2 hr with the addition to the second water load of 100 mg histidine monohydrochloride/100 g body weight, before and after sterilization of the intestinal tract with an antibiotic mixture

		Before sterilization*	After sterilization†
2 hr before oral histidine		0·79 (0·01– 2·2)	0·50 (0·5– 0·6)
Time (hr) after oral histidine	0–2	20·6 (6·4 –45·7)	19·6 (15·9–23·4)
	2–4	28·3 (8·0 –67·6)	36·7 (25·2–57·0)
	4–6	16·4 (2·8 –30·0)	15·3 (8·5–20·8)

* Mean of nine values obtained with three guinea-pigs.

† Mean of three values obtained with three guinea-pigs, after faeces had been sterile for 4 days.

spectrum was obtained from the various parts of the intestine of two guinea-pigs killed after the last urinary sample had been collected. Samples from the caecum, colon and rectum, taken before sterilization, produced, in each medium used, heavy growths, particularly of lactobacilli, coli and faecalis. Relatively few bacteria, chiefly subtilis, were found in the small intestine. After sterilization, there was no growth of lactobacilli, faecalis or other micro-organisms, except sometimes a slight growth of coli which was insignificant compared with the growth before sterilization, and may have been due to contamination during sampling.

This result shows that in guinea-pigs intestinal bacteria are not responsible for the decarboxylation of histidine to histamine, since almost complete sterilization of the bowel has had no significant effect on the urinary excretion of free histamine after oral histidine. It is therefore likely that in this species histidine is first absorbed and then decarboxylated.

DISCUSSION

The finding by Wilson (1954) in rats and by Irvine *et al.* (1959) in dogs that sterilization of the intestinal tract reduces the urinary excretion of free histamine has been attributed to the fact that in these species in-

testinal bacteria decarboxylate histidine which may be derived from the food or administered orally, the histamine then being absorbed through the intestinal wall. From the results of the present experiments it would appear that in guinea-pigs bacterial decarboxylation of histidine does not take place in the intestine, and that the histamine excreted in their urine after oral histidine is formed in the tissues from absorbed histidine. In different species different mechanisms may therefore be responsible for the source of the tissue histamine, and in guinea-pigs, in contrast to dogs and rats, bacterial decarboxylation of histidine in the intestine does not contribute to the body histamine.

The difference in the results obtained with sterilization of the intestinal tract between guinea-pigs and dogs recalls the differences, which have been found in *in vitro* work on histamine formation in these species (Waton, 1956). In tissues of guinea-pigs, and in fact of all the rodents tested, the rat included, the presence of histidine decarboxylase was easily demonstrated with normal incubation methods. By similar methods it has not, however, been possible to show the presence of the enzyme in the dog. This may not necessarily mean that the enzyme is absent in this species, but only that the *in vitro* methods are inadequate when applied to its tissues; but it suggests that in dogs the bacterial decarboxylation of histidine in the intestine is a major source of the body histamine. The same may apply to cat and man, since no histamine-forming enzyme has been found in their tissues.

The rat may take up an intermediary position, since the histidine-decarboxylating enzyme is present in its tissues, but the results of Wilson (1954) also suggest bacterial decarboxylation of histidine in the intestine: yet the effect of sterilization seems to be less pronounced than in the dog, since Wilson found that sterilization of the intestinal tract of the rat produced a reduction of only 45 % in urinary free histamine after normal diet.

It is possible that in all species histamine can be absorbed from the intestine and also formed in the tissues from absorbed histidine. But, because of dietary differences, the herbivorous guinea-pig would be unable to obtain sufficient histamine from the intestine and so may form more histamine in its tissues than the carnivores cat, dog and man, which obtain sufficient histamine from their diet, whereas the rat, being omnivorous, takes up an intermediary position. It may, in fact, be that in cat, dog and man the body is protected against excess of absorbed histamine by the histamine-destroying enzyme histaminase, present in large quantities in the kidneys.

In the present experiments, the highest dose of histidine monohydrochloride given orally was 400–500 mg, which corresponds to 320–400 mg free amino acid. This amount of histidine may appear large, but it is not

unphysiological, because it is just above the normal daily intake of histidine for laboratory animals. It has been estimated that a guinea-pig of 400–500 g weight eats 40–50 g pelleted diet SG 1 per day, and the histidine content of this diet according to the makers (Oxo Ltd.) is approximately 0·44 %, i.e. 180–220 mg histidine (free amino acid). In the present experiments doses of histidine as low as 45–50 mg free amino acid were sufficient to produce an increase in urinary free histamine.

The finding that the herbivorous guinea-pig, in contrast to the carnivorous dog, does not decarboxylate histidine in the intestine emphasizes also the caution that is necessary when drawing conclusions from results obtained in one species on the formation of histamine to that in other species.

SUMMARY

1. The urinary excretion of free histamine has been measured in guinea-pigs before and after the oral administration of histidine.

2. Oral administration of histidine 12·5–100 mg/100 g body weight produces a rise in the urinary excretion of free histamine.

3. Sterilization of the intestinal lumen, shown by necropsy and bacteriological test of the intestinal contents, does not affect the increased urinary excretion of free histamine after oral histidine.

4. The results suggest that in guinea-pigs intestinal histamine produced by bacteria is not the source of the increased histamine excreted in the urine after oral histidine, the histamine probably being formed in the tissues from absorbed histidine.

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