GAS CHROMATOGRAPHIC ANALYSIS OF HISTAMINE METABOLITES IN URINE

EXCRETION OF LABELLED MATERIAL IN DOGS*

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Studies by SCHAYER of urinary metabolites following injection of ^{14}C-labelled histamine have demonstrated that this compound is metabolized in various ways[1,2]. In man, ring-N-methylation constitutes one of the main catabolic pathways[3] (Fig. 1). The ring-N-methylated histamine undergoes further oxidative deamination in the side-chain to N-methylated imidazoleacetic acid. Identification of the urinary metabolites by isotope dilution technique has shown that ring-methylation takes place only at the nitrogen remote from the side-chain. In man, about 50 % of injected ^{14}C-labelled histamine can be recovered from urine as 1-methylimidazole-4-acetic acid (1,4-MeImAA). The isomeric 1-methylimidazole-5-acetic acid (1,5-MeImAA) could not be detected with this method[3].

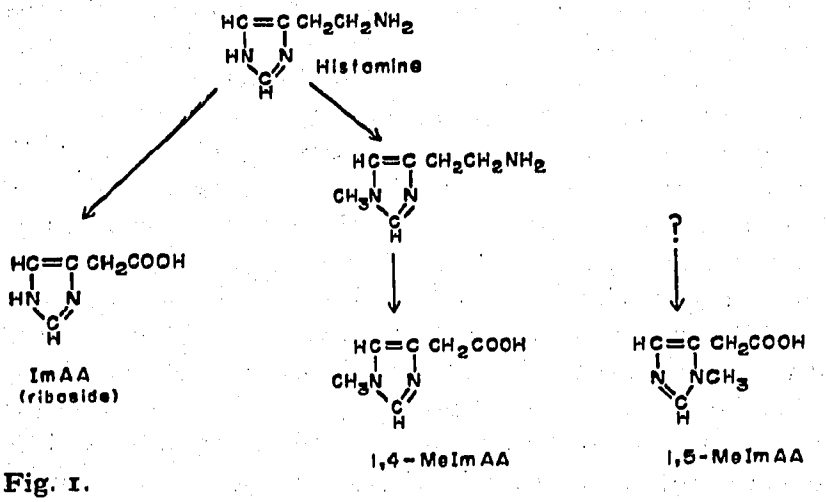

Fig. 1.

Studies in our laboratories on "endogenous" urinary metabolites gave results which are contradictory to these findings. Gas chromatographic analysis of human urine without previous administration of labelled histamine has shown that both isomers of ring-methylated imidazoleacetic acid are normally excreted[4].

A number of "biological" explanations for the difference between these results

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and those of SCHAYER *et al.* have been put forward in a previous paper[4]. One purpose of the present investigation has been to eliminate the possibility of experimental errors, since differences in the sensitivities of the two methods could also possibly explain the diversity of the results. Another purpose has been to find out if the intake of histamine or histidine in food is reflected in the excreted amount of 1,4-MeImAA and 1,5-MeImAA.

METHODS

2-^{14}C-Histamine dihydrochloride and 2-^{14}C-*l*-histidine were obtained from The Radiochemical Centre, Amersham, England. The specific activities were 192 μC/mg of free base and 226 μC/mg, respectively. The labelled histamine, dissolved in saline, was injected subcutaneously in dogs weighing 8–18 kg. In other experiments, labelled histamine or histidine was suspended in milk and given to the dogs perorally. No food restrictions were imposed on the animals before or during the experiments. Urine was collected in metabolic cages or by catheterization during 6 or 24 h after administration of the labelled compound.

An aliquot of the collected urine, corresponding to 50 mg creatinine, was made slightly alkaline and run through an anion ion exchange column. The imidazolic acids retained by the column were eluted with acetic acid, the eluate was dried, and the acids esterified with methanol containing hydrogen chloride. The esters were extracted with chloroform (for details see ref. 4).

In order to test the recovery, 0.1–0.5 ml of the crude urine and 5–10 μl of the final extract were each added to 15 ml of scintillation liquid (toluene–ethylene glycol monoethyl ether (1:1, v/v), containing 0.4% PPO and 0.01% dimethyl POPOP[5]) in plastic tubes, and the radioactivity counted in a Packard Tricarb liquid scintillation counter, Model 314 Ex-2. The values were corrected for background and for quenching by addition of internal standard. 1 mμC of authentic labelled histamine or histidine gave about 1000 c.p.m. under the given conditions.

Gas chromatographic analysis of the urine extract was performed with an F & M gas chromatograph, Model 400, equipped with a flame ionization detector and a splitter system. Two columns with different stationary phases–10% ethylene glycol adipate (EGA) and 7% neopentyl glycol succinate (NGS)–were used (for details, see ref. 4). The splitter divides the gas stream at the outlet side of the column into two parts, one of which passes to the detector. The other part passes to a port in which teflon tubes can be inserted. The compounds emerging in the effluent from the column were allowed to condense on the walls of the teflon tube. At suitable time intervals the teflon tube was removed, and a new one inserted into the splitter port. The teflon tubes were then connected to a burette and 15 ml of scintillation liquid was allowed to drop slowly through the tubes into vials, the radioactivity of which was then counted. The values were corrected for background activity and quenching. The method for measuring the radioactivity in the effluent from the gas chromatograph was tested with authentic ^{14}C-1,4-MeImAA (methyl ester). Samples containing 1–10 μg, with a total radioactivity of 50–2000 c.p.m., were injected into the gas chromatograph. The effluent was collected when the ester peak appeared. With a splitter ratio of 1:1, about 35% of the injected radioactivity could be recovered (50% escaped through the detector).

RESULTS AND DISCUSSION

Previous investigators[6] have pointed to methylation of the ring-nitrogen as the major route of catabolism of injected histamine in dogs. Gas chromatographic analysis of imidazolic acids in the urine now demonstrated that dogs like humans excreted both isomers of methylimidazoleacetic acid (Fig. 2). The identity of the two isomers was established on two columns as described in a previous publication[4]. Using a high sensitivity adjustment, a peak with a retention time coinciding with that of authentic imidazoleacetic acid (ImAA, methyl ester) could also be identified (Fig. 2). The excretion pattern of imidazoleacetic acids in the dog thus seems to be very similar to that of man.

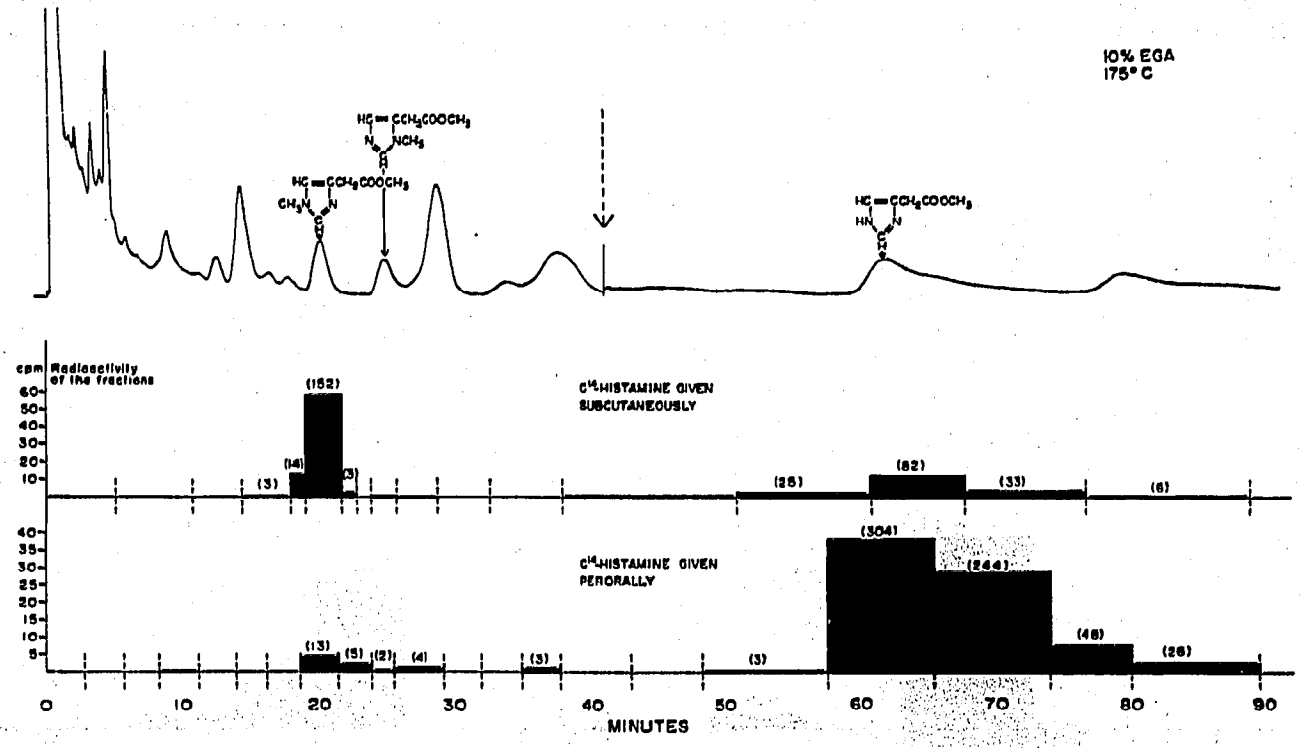

Fig. 2. Upper panel: Gas chromatographic analysis of urine extract from a dog. The dotted arrow represents an adjustment of a 4 times higher sensitivity of the recorder. Middle panel: Radioactivity in the gas chromatographic effluent from a urine extract of the same dog. Labelled histamine in μg amounts had been given to the dog subcutaneously. The dotted lines represent changes of trapping tubes. The total radioactivity in c.p.m. trapped in each tube is seen within the parentheses. The ordinate scale represents the radioactivity trapped per min. The total radioactivity of the injected sample was 880 c.p.m. Splitter ratio: 1:1. Lower panel: Radioactivity of the gas chromatographic effluent from a urine extract of the same dog. Labelled histamine in μg amounts had been given to the dog perorally. The radioactivity is expressed as above. The total radioactivity of the injected sample was 2120 c.p.m. Splitter ratio: 1:1.

After subcutaneous administration of labelled histamine (0.2 μg/kg) to a dog, about 45 % of the total radioactivity could be recovered during the next 6 h. Discounting losses in the procedure, 75 % of this radioactivity corresponds to aromatic acids and other compounds, which are retained by an anionic resin and could be extracted by chloroform after esterification (see Methods). Gas chromatographic analysis of the chloroform extract with simultaneous recording of the radioactivity in the

effluent demonstrated that the radioactivity is confined to the peaks representing 1,4-MeImAA (methyl ester) and ImAA (methyl ester) (Fig. 2). No radioactivity is recovered in the peak corresponding to 1,5-MeImAA (methyl ester). It is thus evident that in spite of the normal occurrence of 1,5-MeImAA in urine, subcutaneously administered histamine is methylated only at the nitrogen remote from the side-chain. The experiments are in agreement with previous investigations by SCHAYER *et al.*

The same amount of labelled histamine, with or without addition of non-labelled carrier (46 mg/kg), was also given to dogs perorally. Upon collection of urine during the next 6 or 24 h, 50 % and 90 % of the given total radioactivity was recovered respectively. Gas chromatography with simultaneous radioactivity analysis showed that most of the administrated histamine is excreted as non-methylated ImAA (Figs. 2 and 3). In these experiments, where comparatively large amounts of non-labelled histamine were given, the gas chromatographic peak corresponding to ImAA

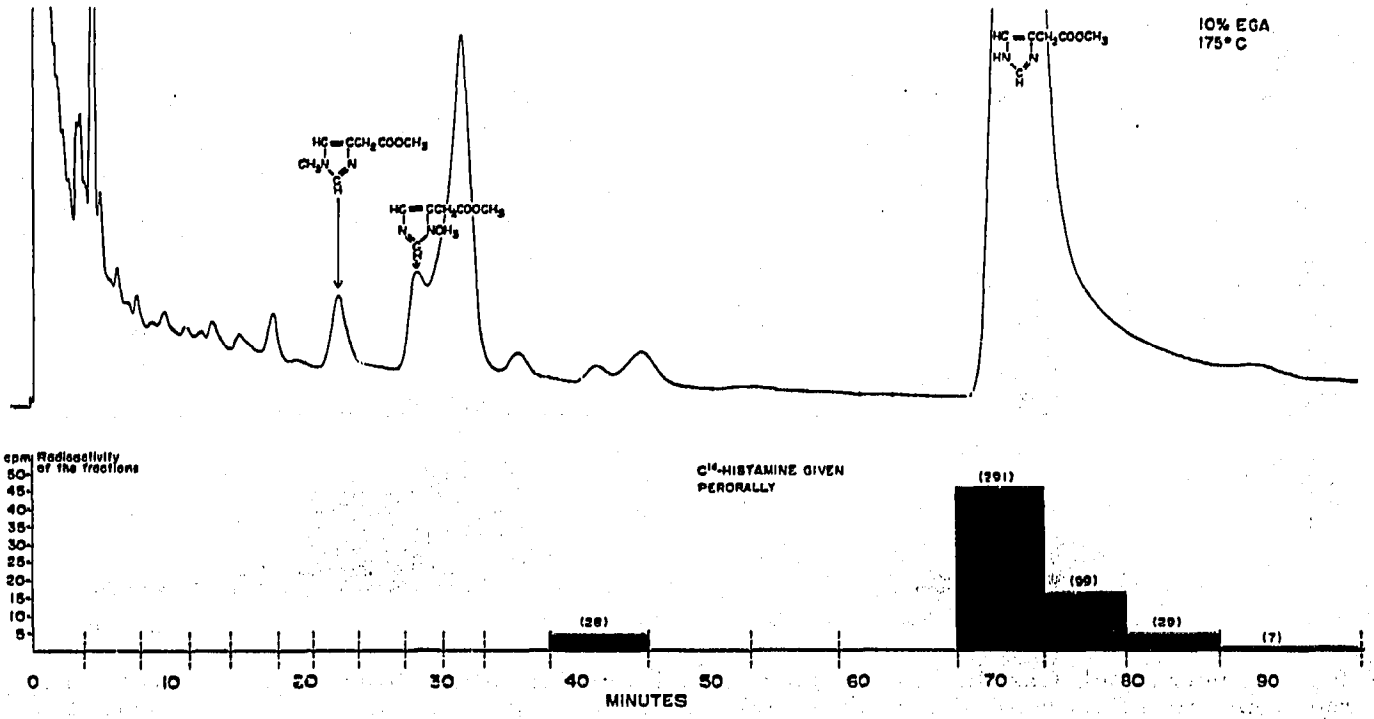

Fig. 3. Gas chromatographic analysis of urine extract from a dog given mg amounts of labelled histamine. Gas chromatogram: upper panel. Radioactivity in the effluent: lower panel. The radioactivity is expressed as in Fig. 2. The total radioactivity of the injected sample was 450 c.p.m. Splitter ratio: 1:1.

(methyl ester) was very high (Fig. 3). No radioactivity corresponding to 1,5-MeImAA (methyl ester) could be detected in the above experiments (Fig. 3). It is thus unlikely that the metabolic origin of 1,5-MeImAA is histamine absorbed from the intestine.

The fact that most of the histamine administered perorally is excreted as ImAA may be attributed to the high concentration of diamineoxidase (histaminase) in the intestinal mucosa[7,8]. Previous investigators have shown that *in vitro* histamine is degraded by intestinal mucosa of dogs mainly to ImAA[8]. It is notable that ^{14}C-labelled histamine given perorally to man is excreted mainly as 1,4-MeImAA[6].

In the above experiments, no attempts were made to demonstrate the ex-

cretion of the riboside of ImAA. According to previous investigators, however, dogs do not conjugate appreciable quantities of ImAA with ribose[6,9].

^{14}C-Labelled histidine (11 μg/kg) mixed with a large amount of the non-labelled amino acid (256 mg/kg) was also administered to dogs. About 25 % of the radioactivity could be recovered in urine during 24 h. About 1 % of this represented compounds which under the given conditions were retained by an anion exchanger column and could be extracted by chloroform after esterification. The gas chromatogram revealed a peak corresponding to ImAA (methyl ester), which was much higher than normal (Fig. 4). A part of the radioactivity was also confined to this peak (Fig. 4). No radioactivity was found corresponding to the peaks of 1,4-MeImAA (methyl ester) and 1,5-MeImAA (methyl ester). Radioactivity was also found in a peak the identity of which is at present unknown.

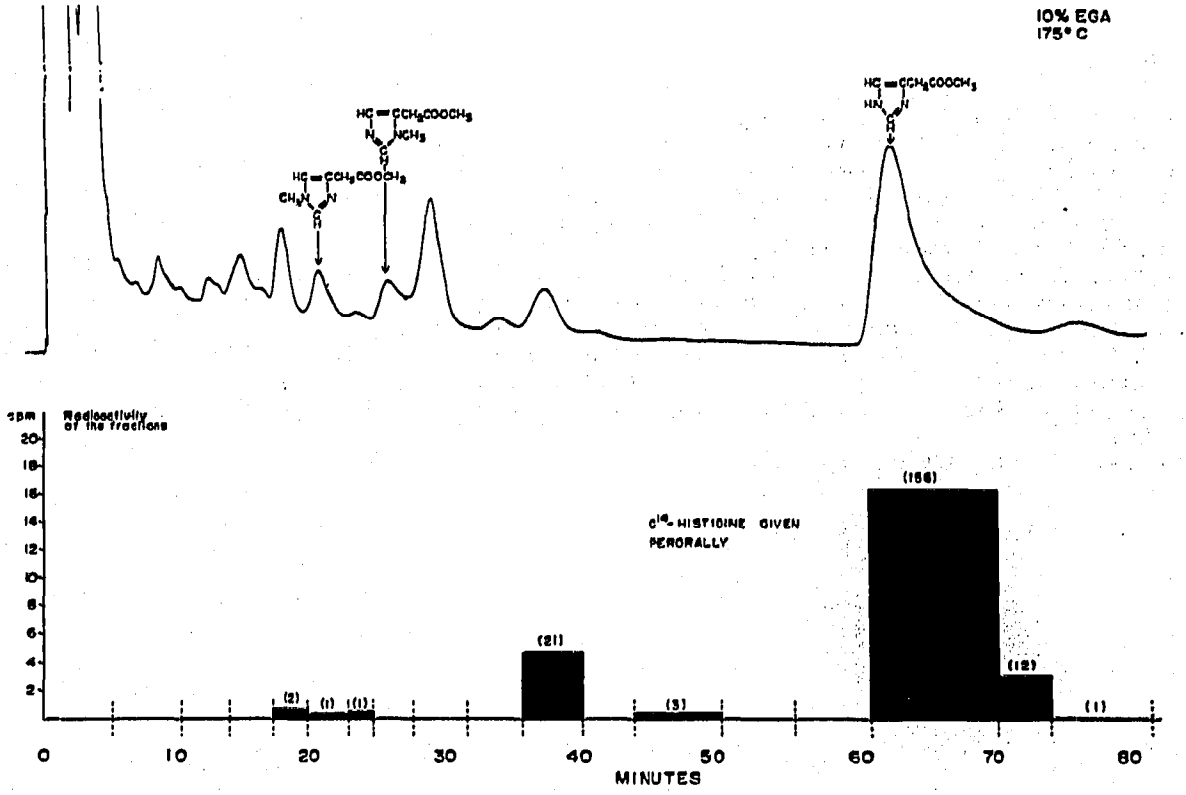

Fig. 4. Gas chromatographic analysis of a urine extract from a dog given labelled histidine perorally. Gas chromatogram: upper panel. Radioactivity in the effluent: lower panel. The radioactivity is expressed as in Fig. 2. The total radioactivity of the injected sample was 1240 c.p.m. Splitter ratio: 1 : 1.

Several investigators have reported the identification of ImAA as a metabolite of histidine[10–12]. It has been suggested that histidine is transformed into imidazole-pyruvic acid, part of which is then oxidized to ImAA. Ingested histidine may also be decarboxylated to histamine, which secondarily may be deaminated to ImAA. This histamine production appears to be insignificant relative to other reactions in histidine catabolism[12,13]. The excretion of ImAA thus depends on the histidine intake in food and will not specifically reflect the endogenous liberation of histamine. From the above experiments it can also be concluded that the excretion of 1,4-MeImAA is

independent of histidine intake. This result is in agreement with studies by BROWN *et al.*[12] on urinary metabolites of labelled histidine in monkeys, humans, and rats. Neither does histidine in food seem to be the metabolic origin of 1,5-MeImAA.

SUMMARY

Gas chromatographic analysis of urine has demonstrated that 1-methylimidazole-4-acetic acid as well as 1-methylimidazole-5-acetic acid is excreted by dogs. Subcutaneously administered labelled histamine in microgram quantities is excreted as 1-methylimidazole-4-acetic acid and imidazoleacetic acid, but does not contribute to the urine content of 1-methylimidazole-5-acetic acid. Histamine or histidine absorbed from the intestine do not seem to contribute to the next 24 hours' excretion of 1-methylimidazole-5-acetic or 1-methylimidazole-4-acetic acid.

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