Br. J. Pharmac. (1978), **64**, 109–122.

METABOLISM AND DISTRIBUTION OF EXOGENOUS HISTAMINE IN CATS

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1 The metabolism and disposition in blood and tissues of exogenous [^{14}C]-histamine was examined in cats.

2 The principal metabolites in blood of histamine instilled into the small intestine (directly or by transfer from the stomach) and colon were imidazoleacetic acid and *t*-methylimidazoleacetic acid, being present in approximately equal amounts although in individual cats one or other acid could predominate. Only small amounts of histamine entered the circulation although in two of four cats given the largest dose (82 μmol/kg) large amounts were recovered. The amount of ^{14}C radioactivity absorbed varied directly with the dose instilled. The chief metabolite in kidney and urine, whether histamine was instilled into the intestine or infused parenterally, was *t*-methylimidazoleacetic acid. Histamine was not absorbed from the stomach and its metabolism there was negligible.

3 In contrast, when histamine was infused into blood *leaving* the intestine (portal vein) the main metabolite in blood and tissues was *t*-methylimidazoleacetic acid being found in approximately 5-fold the concentration of imidazoleacetic acid. The small amount of histamine which eluded inactivation/uptake by liver, lungs, heart during the infusion was halved on circulation through the intestine. When histamine was infused into blood *supplying* the intestine, (cranial mesenteric artery) *t*-methylimidazoleacetic acid while still the major metabolite in blood was now only 1.4 times the concentration of imidazoleacetic acid. Additionally, the blood concentration of histamine during the infusion exceeded that of the metabolites.

4 *t*-Methylimidazoleacetic acid was also the principal metabolite in blood and tissues following histamine infusion into a cannula carrying a replacement venous blood supply to the liver of abdominally eviscerated cats. Imidazoleacetic acid and *t*-methylhistamine were present in equal concentrations and in one-quarter to one-third that of the methylated acid. The latter was also the principal metabolite following intra-arterial histamine infusion to abdominally eviscerated cats without a hepatic blood supply, although initially *t*-methylhistamine predominated: a large peak of histamine was present during the infusion period. When additionally the renal vessels were ligated, *t*-methylhistamine predominated throughout the experiment.

5 In conclusion, intraduodenally instilled histamine was metabolized equally by diamine oxidase and imidazole *N*-methyltransferase (followed by deamination by monoamine oxidase). In contrast, imidazole *N*-methyltransferase was the principal inactivator of parenterally infused histamine, deamination of *t*-methylhistamine by monoamine oxidase becoming progressively less efficient with the cumulative exclusion of the intestines, liver and kidney from the circulation.

Introduction

Histamine may be metabolized to imidazoleacetic acid by diamine oxidase (DAO, EC 1.4.3.6) and/or be methylated by imidazole *N*-methyltransferase (EC 2.1.1.8) to *tele*-methylhistamine (*t*-methylhistamine; for this nomenclature see Black & Ganellin, 1974) before being further degraded by monoamine oxidase (MAO, EC 1.4.3.4) to *t*-methylimidazoleacetic acid. In cats, Blackwell & Marley (1966) noted that duodenally instilled histamine was without systemic effect unless the animal had been pretreated with an amine oxidase inhibitor when histamine entered the circulation. This indicated that some MAO inhibitors (now known to be non-selective) interfere with histamine metabolism. The appearance of circulating histamine was possibly a consequence of diamine oxidase or imidazole *N*-methyltransferase inhibition in the intestinal wall. Inhibition of the latter enzyme might occur through the accumulation of *t*-methylhistamine

(Reilly & Schayer, 1970) although the rapid entry of histamine into the circulation precluded this. DAO inhibition appears a possibility but Schayer (1956) construed from a study of cat urinary metabolites that orally or subcutaneously administered histamine was predominantly metabolized by the alternative route, namely methylation to *t*-methylhistamine and then deamination to *t*-methylimidazoleacetic acid.

To determine the relative importance of the two metabolic routes the *in vivo* metabolism of histamine in cats has been investigated. A preliminary account of part of this work has been given to the Physiological Society (Marley & Thomas, 1976).

Methods

Experiments were performed on cats (2.5 to 5.5 kg) of either sex. Food, apart from milk and water, was withheld overnight (18 h). The cats were anaesthetized with chloralose (80 mg/kg i.v.), the trachea cannulated and the animal artificially ventilated; also cannulated were a femoral vein, for intravenous injections and a femoral artery for recording arterial blood pressure. Unless otherwise stated, [^{14}C]-histamine (5 μCi) was given with non-radioactive histamine dihydrochloride in experiments. The nonradioactive histamine carrier was given proportional to body weight and therefore the specific activity of the administered histamine differed with body weight. Consequently, results are expressed as mol ^{14}C-compounds.

Instillation of histamine

Instillation of histamine into the intestine from the stomach A polyethylene cannula (5 mm o.d., 3 mm i.d.) was inserted via the oesophagus into the stomach which was then ligated at the cardiac sphincter. A second similar cannula was inserted retrogradely into the stomach via an incision in the 'first' part of the duodenum and secured with a ligature. The distal end of this cannula was then introduced anterogradely into the 'second' part of the duodenum and tied in position. The intervening portion of this cannula exterior to the duodenum was clamped. Portal blood samples were obtained via a scalp infusion needle (Butterfly-23 0.6 mm, Abbott Ireland, Ltd., Sligo, Eire) inserted into the portal vein and cemented in position with cyanoacrylate adhesive ('Permabond', R.S. Components Ltd.) so as not to interrupt blood flow. A second infusion needle was inserted retrogradely into the cranial mesenteric artery and cemented *in situ* about 20 mm from the aorta, to obtain arterial blood samples. Radioactive histamine (5 μCi with 1.7 μmol/kg) was instilled into the stomach in 10 ml of 0.9% w/v NaCl solution (saline) via the oesophageal cannula and portal blood samples taken at 5 min intervals for 2 h. The clamp was next removed from the second cannula and the stomach contents gently massaged into the duodenum; transfer was facilitated by passing saline (10 ml) via the oesophageal cannula into the stomach. The gastroduodenal cannula was then reclamped and serial blood samples taken at 5 min intervals from the cranial mesenteric artery and portal vein.

Instillation of histamine into the small intestine One cannula (5 mm o.d., 3 mm i.d.) was inserted anterogradely into the duodenum and another retrogradely into the jejenum with 180 mm of intestine separating the cannula tips; the cannulae were tied in, leaving the intestinal blood supply intact. A 60 to 100 mm length of the adjacent jejenum ('distal segment') was isolated from the proximal segment by ligatures, taking care to preserve its blood supply, to control for uptake of ^{14}C-compounds from the blood into the intestinal wall. Histamine (5 μCi with 1.7 and 5.0 μmol/kg but 10 μCi with 82 μmol/kg) was instilled into the duodenum/jejunum via the proximal cannula. Blood samples were taken as above. Luminal contents were collected at the end of the experiments from the distal cannula by flushing saline (30 ml) via the proximal cannula. The above procedures were also carried out in 2 spinal cats; in these, halothane anaesthesia was maintained until the cats had been made spinal by destroying the brain through the approach for the *encéphale isolé* as described by Bradley & Key (1958).

Instillation of histamine into the large intestine Two cannulae were inserted as for the small intestine but into the colon, with 80 mm of intestine separating the cannulae tips. Otherwise the procedures were as described above.

Infusions of histamine

Abdominal viscera intact In these cats, radioactive histamine was infused (5 μCi with 14 nmol kg^{-1} min^{-1} for 20 min) into the portal vein. Portal blood was sampled from a cannula cemented in at the confluence of the splenic and cranial mesenteric veins; hepatic outflow was sampled from a cannula inserted into a femoral vein so that its tip lay in the inferior vena cava immediately above the entry of the hepatic veins and arterial blood was sampled from a carotid artery.

Abdominal viscera removed, hepatic arterial supply intact and replacement venous flow to the liver The abdominal viscera were removed after ligation of all their arterial inputs and then ligation of the venous outflows; the hepatic arterial supply to the liver was left intact. A replacement hepatic venous supply was

obtained by retrograde cannulation of a common iliac vein, the other end of the cannula being tied into the stump of the cranial mesenteric vein. This cannula contained heparin (10 mg/ml) and had proximal and distal side arms for infusion of radioactive histamine (5 μCi with 14 nmol kg^{-1} min^{-1} for 20 min) and for withdrawal of blood, respectively.

Abdominal evisceration but without blood supply to the liver Cats were abdominally eviscerated and radioactive histamine (5 μCi with 14 nmol kg^{-1} min^{-1} for 20 min) infused intra-arterially into a femoral artery in the direction of flow; blood samples were taken from a carotid artery.

Isolation of radioactive histamine and its metabolites

The method for the isolation and measurement of radioactive histamine and its metabolites has been described elsewhere (Thomas & Marley, 1978). Briefly, blood samples and tissues (the latter removed at the end of experiments) were deproteinized with perchloric acid. Aliquots of the supernatant were removed for determination of total radioactivity and for one-dimensional two-stage paper chromatography. The following compounds were separated by chromatography and measured by liquid scintillation spectrometry: histamine, imidazoleacetic acid, its riboside, *t*-methylimidazoleacetic acid, *t*-methylhistamine and acetylhistamine. Complete chromatographic separation of [^{14}C]-histamine standard from *t*-methylhistamine was not obtained, approximately 10% of the radioactive histamine being recovered with *t*-methylhistamine (Table 1). However, apart from the intestinal washings (which contained a large proportion of histamine), the net effect of this correction would be negligible and it was therefore not applied; overlap of [^{14}C]-*t*-methylimidazoleacetic acid into the imidazoleacetic acid zone was only 4.4% (as ascertained in experiments with aminoguanidine, an inhibitor of DAO; Marley & Thomas, unpublished data).

In certain experiments, sufficient blood (2 ml) was removed to assay histamine by the radiochromatographic procedure described above and also by the spectrophotofluorimetric method of Shore, Burkhalter & Cohn (1959).

Measurement of portal blood flow

In 3 cats a cuff, connected to a S.E.M. 275 flow meter (S.E. Laboratories Engineering, Ltd., Feltham, Middlesex) was fitted closely around the portal vein. The flow meter/cuff was calibrated during experiments by clamping the vessel briefly to produce zero flow and at the end of the experiments, by measuring the volume of blood collected/unit time from the distal end of the severed vein. Blood flow measurements and the total radioactivity determinations were used to calculate the rate of absorption from the intestine of ^{14}C-compounds by Fick's principle, viz.

$$Q_A = F(C_{PV} - C_{CMA})$$

where Q_A = the quantity absorbed, F = the blood flow and C_{PV} = concentration in portal venous blood and C_{CMA} = concentration in cranial mesenteric arterial blood.

Since blood concentrations varied with time, the integrated form of the above expression was used,

$$Q_A = F\int_{t_1}^{t_2}(C_{PV} - C_{CMA})$$

The rate of absorption was therefore obtained by subtracting the blood concentration (mol ^{14}C-compounds) against time (method of least squares) in cranial mesenteric arterial blood from that of portal blood and correcting for blood flow.

Drugs used were [ring-2-^{14}C]-histamine (59.7 μCi/μmol, The Radiochemical Centre, Amersham) and histamine dihydrochloride (BDH). Chromatographic reference compounds were *t*-methylhistamine hydrochloride, acetylhistamine and *t*-methylimidazoleacetic acid hydrochloride (Calbiochem), imidazoleacetic acid hydrochloride (Sigma).

Results

Instillation of histamine into the stomach followed by transfer into the small intestine

In the three cats studied, during the 2 h histamine (5 μCi with 1.7 μmol/kg) was within the stomach, radioactivity was not detected in portal blood indicating that histamine was not absorbed from the stomach. However, on displacing the stomach contents into the duodenum/jejunum, radioactivity appeared within 15 min in the portal venous and cranial mesenteric arterial blood.

Following transfer, between 3% and 7% of the total radioactivity recovered in portal blood was [^{14}C]-histamine; the greater part of the radioactivity was associated with histamine metabolites of which imidazoleacetic acid and *t*-methylimidazoleacetic acid predominated. Their mean concentrations in portal venous blood were approximately equal at all times following transfer of histamine from the stomach to the duodenum/jejunum (Figure 1a) and reached a peak some 50 to 60 min after transfer at which time they accounted for 80 to 90% of the total ^{14}C-compounds recovered. The distribution in cranial mesenteric arterial blood (i.e. blood supplying the duodenum/jejunum), was similar although dilution by mixing with the total blood compartment reduced the absolute concentrations by about 40% (Figure 1b). Imidazoleacetic acid riboside, acetylhistamine and

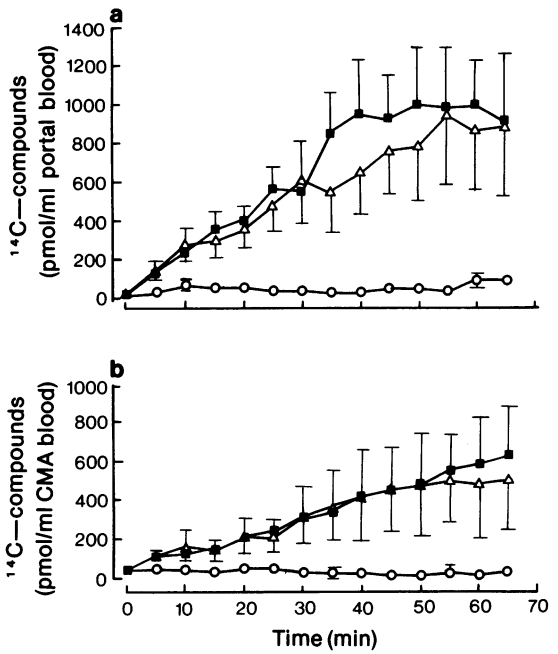

Figure 1 Mean concentrations for three cats of histamine (○), imidazoleacetic acid (△) and *t*-methylimidazoleacetic acid (■) in (a) portal blood and (b) cranial mesenteric arterial (CMA) blood at intervals after transfer of [^{14}C]-histamine (5 μCi with 1.7 μmol/kg) from the stomach to the proximal duodenum/jejunum (at zero time), the histamine having been instilled into the stomach 2 h earlier. (Some of the values are coincident.) Vertical lines show s.e. means.

t-methylhistamine were present in both samples but in low concentrations (< 50 pmol ^{14}C-compound/ml blood) as was histamine. Histamine was not metabolized in the stomach since an aliquot (1 ml) of stomach contents, removed via the gastro-duodenal cannula at the time (2 h) of their transfer to the duodenum, contained mainly histamine with insignificant traces of metabolites (Table 1). Stomach washings 2 h later at the end of experiments contained 10.3 ± 1.9% of the instilled ^{14}C-radioactivity. The proportions of histamine and its metabolites were slightly modified, there being a small decrease (11%) in histamine and small increases in its metabolites. Duodenal/jejunal washings at 4 h also contained 6.5 ± 2% of the ^{14}C-radioactivity originally instilled into the stomach but contained proportionally less histamine and more of the acid metabolites, imidazoleacetic acid and *t*-methylimidazoleacetic acid than stomach washings (Table 1).

The ^{14}C-compounds in various tissues were also determined at the end of experiments. The duodenal-jejunal wall contained approximately twice the concentration of ^{14}C-compounds present in the stomach wall, metabolites predominating in the duodenal-jejunal wall and histamine predominating in the stomach wall (Table 1). The amounts of ^{14}C-compounds in liver and kidney were considerably less than in duodenal/jejunal tissue, the predominant metabolite in each case being *t*-methylimidazoleacetic acid (Table 1). This and the fact that the acid metabolites were present in equal proportions in portal venous and cranial mesenteric arterial blood, suggested that these tissues, particularly the kidney derived *t*-methylimidazoleacetic acid directly from histamine and *t*-methylhistamine rather than by selectively removing it from the blood; urinary concentration of the methylated acid was five times that of imidazoleacetic acid (Table 1).

Instillation of histamine into the small intestine

Radioactivity appeared in the portal and cranial mesenteric arterial blood within 15 min of instilling histamine (1.7, 5.0, 82 μmol/kg) into the proximal duodenum/jejunum (14 cats).

As in preceding experiments, imidazoleacetic acid and *t*-methylimidazoleacetic acid predominated in both portal and cranial mesenteric arterial blood following instillation of histamine (1.7 μmol/kg) and again there was a reduction in the concentration of ^{14}C-compounds in cranial mesenteric arterial blood compared with portal blood. During the initial 30 min, histamine accounted for 15% (100 pmol/ml blood) of the ^{14}C-compounds, thereafter declining (approx. 50 pmol/ml blood). Similar findings were obtained after intraduodenal instillation of a larger dose of histamine (5 μmol/kg), the acid metabolites again predominating. Maximum concentrations of imidazoleacetic acid and *t*-methylimidazoleacetic acid were reached approximately 50 min after instillation and were maintained at about 600 pmol/ml in portal blood and approximately half this value in cranial mesenteric arterial blood with the smaller dose (1.7 μmol/kg) and about double these values with the larger dose (5 μmol/kg). Inconsistent results were obtained after the largest intraduodenal dose of histamine (82 μmol/kg). In 2 of the 4 cats, the acid metabolites of histamine again predominated in portal blood samples, imidazoleacetic acid being present in greater quantities than the methylated acid (Figure 2a); histamine accounted for approximately 14% of total ^{14}C-compounds recovered during the first 30 min. However, in the other 2 cats, large amounts of histamine (60% of total ^{14}C-compounds) were recovered in portal blood (Figure 2b), findings corroborated by spectrofluorimetric assay.

Corrected rates for absorption of radioactivity (see Methods) for the three doses of histamine are shown

Table 1 Terminal distribution of histamine and its metabolites in tissues, gastric contents, gastric washings and urine after transfer of [^{14}C]-histamine (1.7 μmol/kg) from the gastric lumen to the proximal duodenum/jejunum in cats

	^{14}C-compound (nmol/g)	*Residue of total dose (%)*	*ImAA riboside** (%)*	*ImAA (%)*	*tMeImAA (%)*	*AcHis (%)*	*tMeHis (%)*	*His (%)*	n
[^{14}C]-histamine*			1.4 ± 0.2	0.9 ± 0.0	2.0 ± 0.2	0.8 ± 0.3	9.1 ± 3.3	85.8 ± 4.4	3
Gastric contents (2 h)	134.4 ± 27.3		1.0 ± 0.0	0.8 ± 0.1	1.6 ± 0.3	0.6 ± 0.2	11.8 ± 5.3	84.1 ± 5.1	3
Gastric washings (4 h)	—	10.3 ± 1.9	1.9 ± 0.0	4.5 ± 2.1	3.2 ± 1.4	2.3 ± 0.9	14.9 ± 5.7	73.1 ± 5.5	3
Duodenal/jejunum washings (4 h)	—	6.5 ± 2.0	4.9 ± 2.3	18.9 ± 9.0	8.6 ± 1.9	2.8 ± 0.9	14.9 ± 4.5	49.9 ± 9.6	3
Stomach wall	12.2 ± 1.7		4.0 ± 0.8	6.0 ± 1.0	7.9 ± 1.3	4.8 ± 3.5	21.8 ± 6.9	55.3 ± 8.4	3
Proximal duodenum/ jejunum wall	26.4 ± 9.7		10.3 ± 3.9	38.9 ± 7.2	22.4 ± 5.1	2.2 ± 0.7	12.3 ± 4.4	13.9 ± 4.4	3
Kidney	8.1 ± 3.7		13.3 ± 2.6	32.9 ± 6.3	48.5 ± 4.4	0.6 ± 0.3	3.8 ± 3.3	0.9 ± 0.6	3
Liver	3.0 ± 1.6		11.7 ± 1.7	34.8 ± 9.2	44.3 ± 6.4	0.2 ± 0.2	4.5 ± 3.4	4.4 ± 1.6	3
Urine	43.9 ± 24.2		3.4 ± 2.3	15.5 ± 7.4	74.5 ± 7.1	0.7 ± 0.3	4.9 ± 1.0	1.1 ± 0.1	3

Results are expressed as mean ± s.e. mean with *n*, the number of experiments.* Recoveries of ^{14}C-radioactivity on chromatography of [^{14}C]-histamine also included for reference.

** Abbreviations used in Tables 1–3: ImAA riboside = imidazoleacetic acid riboside, ImAA = imidazoleacetic acid, *t*MeImAA = *t*-methylimidazoleacetic acid, AcHis = Acetylhistamine, *t*MeHis = *t*-Methylhistamine and His = Histamine.

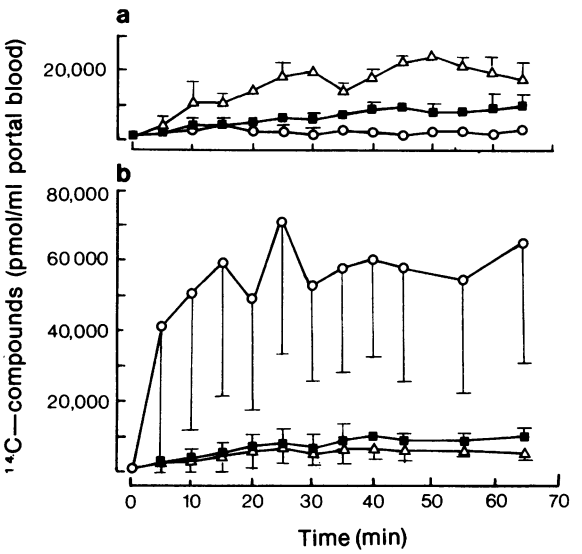

Figure 2 Mean concentrations of histamine (○), imidazoleacetic acid (△) and *t*-methylimidazoleacetic acid (■) in portal blood at intervals following the instillation of [^{14}C]-histamine (10 μCi with 82 μmol/kg) into the proximal duodenum/jejunum at zero time. (a) Results obtained with the slowest rates (two cats) of absorption and (b) the fastest rates (two cats). Vertical lines show ranges.

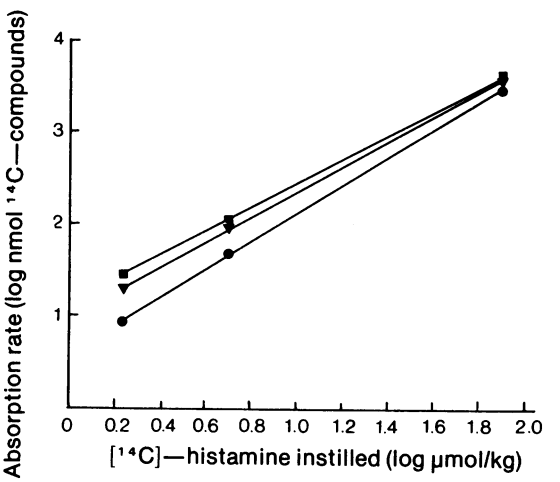

Figure 3 Absorption rates (log nmol ^{14}C-compounds) at 20 min (●), 40 min (▼) and 60 min (■) after instillation of various doses of [^{14}C]-histamine (5 μCi with 1.7 and 5.0 μmol/kg and 10 μCi with 82 μmol/kg) into the proximal duodenum/jejunum. Values are the means obtained from four cats at each of the doses.

in Figure 3. Absorption was dose-dependent and increased with time. The mean results encompassed wide individual variations. Thus in the 4 cats receiving the largest doses of histamine, rates increased, decreased or remained constant with time (Figure 4). The fastest rates occurred in cats from which large amounts of [^{14}C]-histamine were recovered in portal blood (Figure 2b).

There were also individual variations in the metabolism of instilled histamine. Thus whereas the mean results suggested that the two routes were equally important, in about half the cats ring *N*-methylation predominated, deamination by DAO being the preferred route in the remainder. Typical of cats in which ring *N*-methylation predominated is the experiment illustrated in Figure 5. Thus *t*-methylimidazoleacetic acid was the main metabolite in portal blood, appearing some 30 min after instillation of histamine. However, large amounts of imidazoleacetic acid were also present indicating diamine oxidase activity. Histamine and *t*-methylhistamine could usually be detected in portal blood during the first 30 to 40 min after histamine instillation, this being evident in Figure 5 (and 6) in which small peaks of these compounds were seen. In contrast, Figure 6 shows the result of an ex-

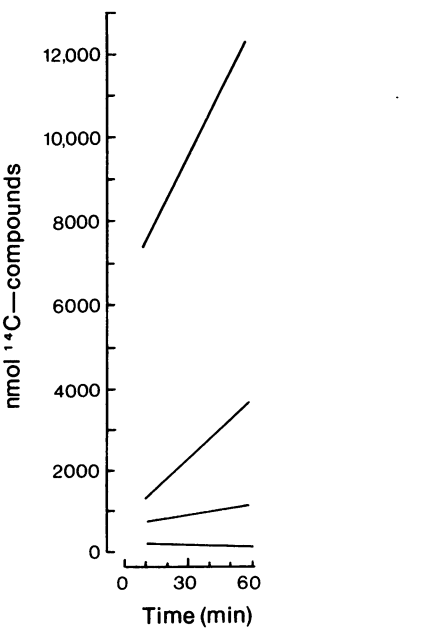

Figure 4 Absorption rates (nmol ^{14}C-compounds) after instillation of [^{14}C]-histamine (10 μCi with 82 μmol/kg) into the proximal duodenum/jejunum. Each slope represents the result obtained from one cat. For calculation of absorption rates see Methods.

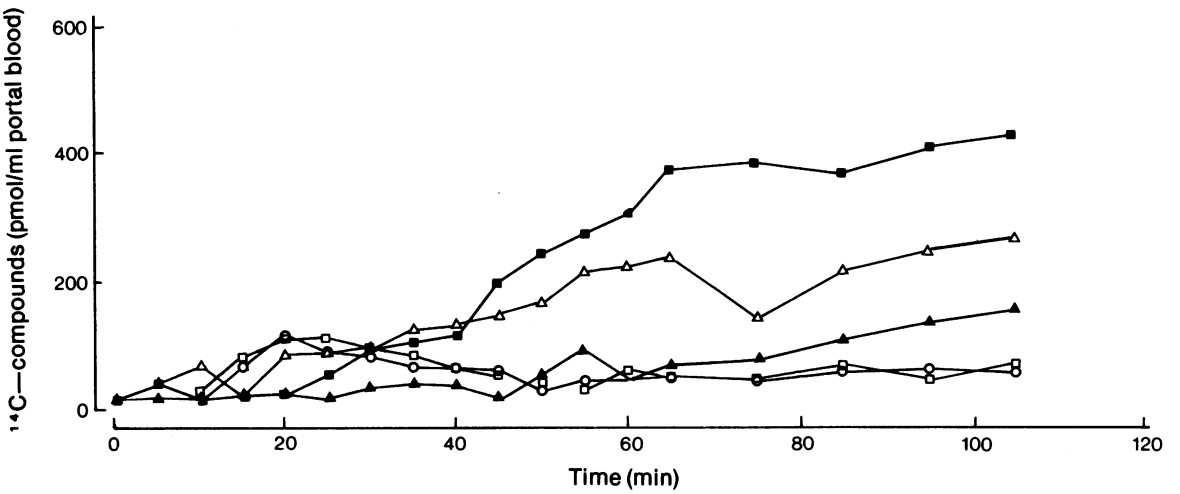

Figure 5 Concentrations of histamine (○), *t*-methylhistamine (□), imidazoleacetic acid, (△) its riboside. (▲) and *t*-methylimidazoleacetic acid (■) in portal blood at intervals following instillation of [^{14}C]-histamine (5 μCi with 1.7 μmol/kg) into the proximal duodenum of one cat (2.8 kg) at zero time.

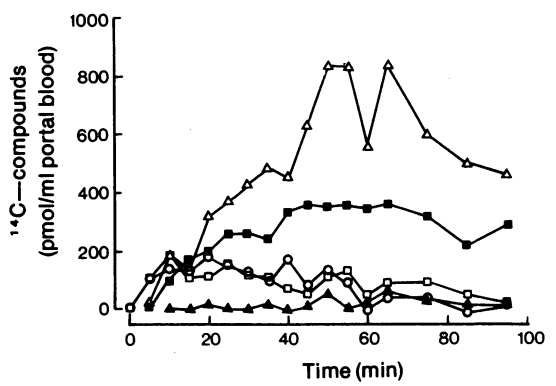

Figure 6 Concentrations of histamine (○), *t*-methylhistamine (□), imidazoleacetic acid (△), its riboside (▲), and *t*-methylimidazoleacetic acid (■) in portal blood at intervals following instillation of [^{14}C]-histamine (5 μCi with 1.7 μmol/kg) into the proximal duodenum of one cat (2.7 kg) at zero time.

periment in which imidazoleacetic acid was the major metabolite, which together with its riboside conjugate accounted for over 50% of the recovered radioactivity after 1 h. Again, the minor metabolites, imidazoleacetic acid riboside and acetylhistamine were present but these together accounted for less than 10% of total ^{14}C-compounds recovered.

^{14}C-compounds in various tissues, intestinal washings and urine taken at termination of the experiments are shown in Table 2. The wall of the proximal duodenal/jejunal segment contained the acid metabolites, *t*-methylimidazoleacetic acid and imidazoleacetic acid, which together accounted for approximately 60% of the radioactivity recovered from this site after instillation of histamine (1.7 and 5.0 μmol/kg). Histamine and *t*-methylhistamine together accounted for about one quarter of the total ^{14}C-compounds with the smaller doses (1.7, 5.0 μmol/kg) but about 70% after the largest dose (82 μmol/kg). The proportion of [^{14}C]-histamine to its metabolites in duodenal/jejunal washings also increased as the dose of histamine increased. The ratio of total radioactivity in proximal duodenum/jejunum tissue to that in the wall of the contiguous distal segment varied between 11 and 35:1 indicating that the metabolites in the proximal intestinal site had not arisen from the circulation. In the distal segment, acid metabolites again predominated, there being a relative reduction in the proportion of *t*-methylimidazoleacetic acid compared with imidazoleacetic acid as the dose increased. Recovery of significant amounts of [^{14}C]-histamine in the distal segment showed that histamine entering the circulation from the duodenal/jejunal segment could be reabsorbed from the circulation. No consistent pattern emerged from the liver samples, *t*-methylimidazoleacetic acid predominating after histamine, 1.7 μmol/kg, whereas imidazoleacetic acid was the major metabolite after the larger doses. In contrast, *t*-methylimidazoleacetic acid was the major metabolite in the kidney and urine, constituting 39 to 66% of the total [^{14}C]-compounds irrespective of the dose of histamine instilled. The ability of the kidney to

Table 2 Terminal distribution of histamine and its metabolites in tissues, luminal washings and urine after the instillation of [^{14}C]-histamine, (a) 5 μCi with 1.7 μmol/kg, (b) 5 μCi with 5 μmol/kg and (c) 10 μCi with 82 μmol/kg into the proximal duodenum/jejunum of cat.

	^{14}C-compounds (nmol/g)	*Residue of total dose (%)*	*ImAA riboside (%)*	*ImAA (%)*	*tMeImAA (%)*	*AcHis (%)*	*tMeHis (%)*	*His (%)*	n
(a)									
Proximal duodenum/jejunum wall	36.7 ± 11.1		10.8 ± 2.5	29.5 ± 6.7	30.2 ± 4.8	5.4 ± 1.6	9.4 ± 2.4	14.6 ± 4.4	6
Distal jejunum wall	1.3 ± 0.2		10.3 ± 2.3	31.6 ± 7.1	39.0 ± 5.3	3.7 ± 1.5	8.0 ± 3.0	7.4 ± 2.4	6
Duodenal/jejunal washings	—	7.5 ± 2.6	10.4 ± 2.1	16.0 ± 2.7	14.6 ± 4.2	4.9 ± 1.7	10.7 ± 1.0	43.2 ± 6.3	6
Liver	3.3	—	4.2	32.8	44.4	4.0	9.2	5.4	1
Kidney	26.2 ± 15.2	—	6.2 ± 2.3	36.6 ± 2.6	40.0 ± 5.4	5.1 ± 1.3	7.4 ± 7.4	4.7 ± 1.5	3
Urine	—	9.0 ± 4.5	0.9 ± 0.6	19.9 ± 5.0	39.5 ± 6.9	6.8 ± 1.6	22.8 ± 1.7	10.1 ± 1.9	3
(b)									
Proximal duodenum/jejunum wall	99.6 ± 62	—	7.9 ± 2.1	35.5 ± 7.4	26.9 ± 7.5	5.3 ± 1.7	12.1 ± 4.1	12.2 ± 3.4	4
Distal jejunum wall	9.0 ± 4.3	—	10.1 ± 4.4	36.8 ± 5.0	29.6 ± 4.1	4.1 ± 1.6	7.3 ± 2.3	12.0 ± 5.1	4
Duodenal/jejunal washings	—	15.3 ± 4.9	5.3 ± 2.8	8.8 ± 1.1	14.9 ± 6.0	4.3 ± 0.5	9.6 ± 3.7	57.1 ± 5.7	4
Liver	5.6 ± 2.4	—	13.1 ± 2.2	38.6 ± 2.9	21.8 ± 0.8	9.3 ± 2.6	10.4 ± 1.9	6.8 ± 0.1	2
Kidney	40.3 ± 14.1	—	7.4 ± 5.5	34.8 ± 7.3	42.7 ± 10.7	3.3 ± 0.4	6.2 ± 2.3	5.6 ± 0.5	3
Urine	—	9.3 ± 5.7	0.6 ± 0.5	24.3 ± 6.2	53.4 ± 18.5	5.8 ± 2.2	7.5 ± 6.0	8.4 ± 8.0	2
(c)									
Proximal duodenum/jejunum wall	1184.8 ± 495	—	5.8 ± 2.5	13.3 ± 6.4	4.1 ± 0.9	2.0 ± 0.5	12.4 ± 2.9	62.3 ± 8.2	4
Distal jejunum wall	33.5 ± 10.1	—	9.5 ± 4.2	35.9 ± 13.9	20.4 ± 7.8	1.1 ± 0.8	12.5 ± 3.8	20.6 ± 10.6	3
Duodenal/jejunal washings	—	48 ± 9	1.7 ± 0.3	1.9 ± 0.5	2.1 ± 0.7	0.7 ± 0.1	8.6 ± 3.1	84.9 ± 2.6	4
Liver	32 ± 14	—	11.5 ± 4.1	31.4 ± 14	24.3 ± 4.0	8.9 ± 3.9	15.7 ± 3.9	8.2 ± 3.1	4
Kidney	244.9 ± 81.3	—	12.9 ± 5.2	30.4 ± 11.7	42.7 ± 13.2	3.4 ± 1.7	6.0 ± 4.4	4.6 ± 3.9	4
Urine	—	1.3 ± 1	7.2 ± 4.4	15.3 ± 10.9	66.4 ± 15.5	3.1 ± 1.6	3.4 ± 1.2	4.5 ± 0.2	2

Results are expressed as mean ± s.e. mean, or the range when *n*, the number of experiments, was two.

metabolize and sequester ^{14}C-metabolites of histamine is implied by the much larger concentration of ^{14}C-compounds/g tissue compared with the distal intestine or liver.

In spinal cats given intraduodenally instilled histamine, the rates of absorption of ^{14}C-labelled compounds and the metabolites recovered were similar to those for anaesthetized cats viz., imidazoleacetic acid and *t*-methylimidazoleacetic acid were the predominant metabolites.

Instillation of histamine into the large intestine

Radioactivity was recovered in portal and cranial mesenteric arterial blood following histamine (1.7 μmol/kg) instillation into the large bowel. The mean rate of absorption for the three cats of ^{14}C-compounds (1.09, 1.33 and 1.48 log nmol ^{14}C-compounds at 20, 40 and 60 min respectively) was similar to that obtained for the same dose in the small intestine (Figure 3). However, as with the latter, there was wide individual variation. The cat with the fastest rate of absorption chiefly deaminated the instilled histamine while of the cats with the slower absorption rates the principal metabolite in blood was *t*-methylimidazoleacetic acid in one while the small amounts of radioactivity from the other precluded characterization.

Infusions of histamine

Abdominal viscera intact Ring *N*-methylation followed by deamination (MAO) was the predominant route of inactivation of histamine infused into the portal vein of the three cats tested. Thus *t*-methylimidazoleacetic acid was the main metabolite recovered from blood taken from vessels in which blood had circulated respectively through the liver (inferior vena cava), through the liver and lungs (carotid artery: Figure 7) and through the liver, lungs and intestine (cranial mesenteric vein): imidazoleacetic acid was also present but in about one-fifth the concentration of *t*-methylimidazoleacetic acid. Concentrations (e.g. 72 ± 35 pmol/ml at 10 min) of histamine in blood from the inferior vena cava and carotid artery were greater than concentrations (31 ± 5 pmol/ml at 10 min) in cranial mesenteric venous blood, indicating that during its circulation histamine was taken up or metabolized by the intestine. Concentrations of *t*-methylhistamine were less than that of histamine during the infusion but about equal after it and so were considerably less than those of *t*-methylimidazoleacetic acid at all times.

The action of the intestine on circulating histamine was further investigated with a modification of the above technique. Histamine was instead infused into the blood supplying the intestine (cranial mesenteric artery). In this instance and in contrast to the above

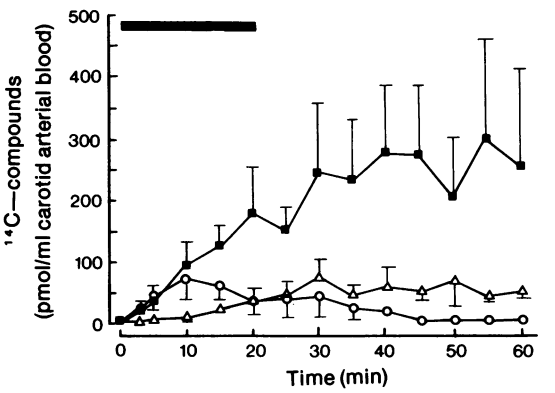

Figure 7 Mean concentrations for three cats of histamine (○), imidazoleacetic acid (△) and *t*-methylimidazoleacetic acid (■) in carotid arterial blood at different intervals during (solid bar) and following a 20 min infusion of [^{14}C]-histamine (5 μCi with 14 nmol kg^{-1} min^{-1}) into the portal vein. Vertical lines are the s.e. means.

results the concentrations of *t*-methylimidazoleacetic acid (peak of 980 pmol/ml portal blood) were only about 1.4 times greater than those of imidazoleacetic acid in portal venous (peak of 770 pmol/ml) and carotid arterial blood i.e. results similar to those obtained when histamine was instilled into the small intestine. However, unlike either the portal vein infusions or duodenal instillations (apart from the 82 μmol/kg dose), large peaks of [^{14}C]-histamine and *t*-methylhistamine were present in portal (1120 and 700 pmol/ml blood respectively at 15 min) and carotid arterial blood (280 and 420 pmol/ml blood respectively at 15 min) during the infusion. In tissues *t*-methylimidazoleacetic acid predominated (Table 3a). Kidney contained the greatest concentration of ^{14}C-compounds e.g. 4.9:1 compared to the intestine and 3.4:1 compared to the liver.

Abdominal evisceration but without blood supply to the liver In two cats which were abdominally eviscerated, histamine was infused via a femoral artery in the direction of flow; *t*-methylimidazoleacetic acid was still the principal metabolite in carotid arterial blood. However, there were differences from the preceding infusion experiments viz., a pronounced peak of histamine during the first 20 min, a large peak of *t*-methylhistamine at about 25 min and the relatively slower appearance of *t*-methylimidazoleacetic acid such that it only equalled *t*-methylhistamine at 45 min but finally accounted for approximately 60% of the total ^{14}C-compounds (Figure 8).

In a cat which had been abdominally eviscerated and the renal arteries ligated, the fate of infused hista-

Table 3 Terminal distribution of histamine and its metabolites in cat tissues after infusion of [^{14}C]-histamine (5 μCi with 14 nmol kg^{-1} min^{-1} for 20 min) (a) via the portal vein and (b) via the iliac-portal vein cannula

	^{14}C-compounds (nmol/g)	*ImAA riboside* (%)	*ImAA* (%)	*tMeImAA* (%)	*AcHis* (%)	*tMeHis* (%)	*His* (%)	n
(a)								
Duodenum/ jejunum wall	0.44 ± 0.1	9.6 ± 4.0	17.8 ± 4.4	58.8 ± 11.9	4.4 ± 3.2	7.8 ± 5.3	1.5 ± 1.5	3
Liver	0.9 ± 0.5	11.4 ± 7.4	12.4 ± 3.9	68.9 ± 12.6	2.6 ± 1.3	2.5 ± 1.8	2.1 ± 1.5	3
Kidney	2.78 ± 1.1	10.9 ± 5.8	14.5 ± 4.8	65.6 ± 12.7	2.1 ± 0.9	4.4 ± 0.8	2.5 ± 1.1	3
(b)								
Liver	1.22 ± 0.6	5.7 ± 1.7	7.0 ± 1.9	75.2 ± 7.6	3.1 ± 1.5	6.3 ± 1	2.7 ± 1.4	3
Kidney	6.63 ± 1.2	3.6 ± 0.9	7.8 ± 0.1	84.5 ± 3.2	1.4 ± 0.8	1.8 ± 1.1	0.9 ± 0.6	3

Results are expressed as mean ± s.e. mean with *n*, the number of experiments.

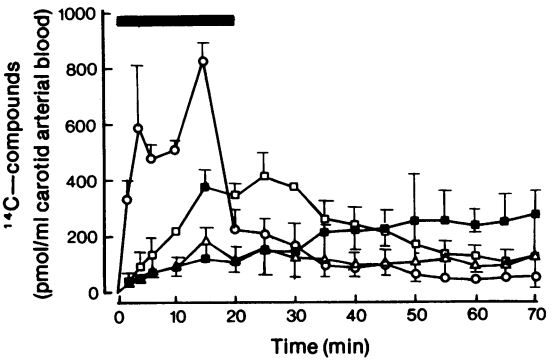

Figure 8 Mean concentrations for two eviscerated cats of histamine (○), *t*-methylhistamine (□), imidazoleacetic acid (△) and *t*-methylimidazoleacetic acid (■) in carotid arterial blood at intervals during (solid bar) and following a 20 min infusion of [^{14}C]-histamine (5 μCi with 14 nmol kg^{-1} min^{-1}) into a femoral artery. Vertical lines give the ranges.

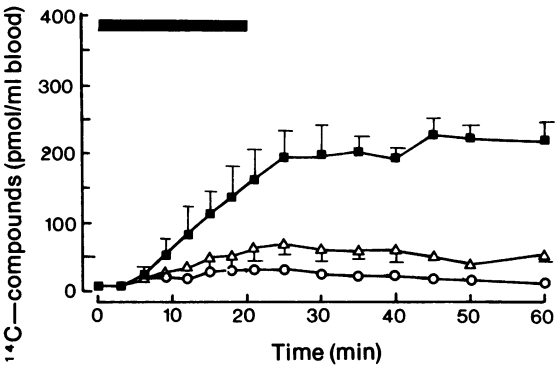

Figure 9 Mean concentration from three cats of histamine (○), imidazoleacetic acid (△) and *t*-methylimidazoleacetic acid (■) in blood taken from the distal sidearm of an iliac-portal vein cannula at intervals during (solid bar) and following a 20 min infusion of [^{14}C]-histamine (5 μCi with 14 nmol kg^{-1} min^{-1}) into the proximal side-arm of the iliac-portal vein cannula, the intestines having been removed. Vertical lines give the s.e. means.

mine (5 μCi with 14 nmol min^{-1} kg^{-1} for 20 min) was considerably modified. The predominant metabolite, accounting for about 45% of the ^{14}C-compounds in carotid blood, was *t*-methylhistamine and not *t*-methylimidazoleacetic acid for the duration of the experiment.

Intravenous infusion: abdominal viscera removed, hepatic arterial supply intact and replacement venous flow to liver The main radioactive metabolite in blood taken from the iliac-portal vein cannula at all stages in the three cats tested was *t*-methylimidazoleacetic acid (Figure 9); at the end of the experiment, this metabolite accounted for 64% of the total radioactivity. *t*-Methylhistamine (not shown), unlike in portal vein infusions in intact cats, was equal in concentration to imidazoleacetic acid, each contributing 11% of total radioactivity. However, this was less than that found in eviscerated cats demonstrating that while the intestine deaminates some *t*-methylhistamine, the liver is the more important. This was also the case for the removal/inactivation of histamine, since while histamine persisted beyond the infusion period, its concentrations were only between 20 and 40 pmol/ml blood.

There was 6.5-fold more radioactivity per g tissue in the kidney compared with liver (Table 3b).

Discussion

When [^{14}C]-histamine was instilled into the cat small intestine or colon, large amounts of radioactivity appeared in portal blood although not after instillation into the stomach. Despite there being wide variation between cats in the rates at which labelled compounds were absorbed, there was a direct dose-dependent relation. These results are consistent with the role played by the small intestine in amine absorption. In contrast, Duncan & Waton (1968) found radioactive histamine in blood from both the small intestine and stomach following instillation of [^{14}C]-histamine into these sites in dogs. Even allowing for species variation this is a surprising result since in the acidic *milieu* of the stomach, histamine would be mainly ionized and therefore not expected to cross the stomach wall.

In portal blood from intact cats, most of the radioactivity was accounted for by roughly equal amounts of imidazoleacetic acid and *t*-methylimidazoleacetic acid irrespective of whether histamine had or had not passed via the stomach to the small intestine. This indicates deamination (DAO) to imidazoleacetic acid to be as important as *N*-methylation followed by deamination (MAO) to *t*-methylimidazoleacetic acid and contrasts with Schayer's (1956) conclusions based on urine analyses, that the predominant metabolic route of ingested histamine in cats was N-methylation followed by (MAO) deamination.

These *in vivo* results are compatible with *in vitro* observations showing that diamine oxidase activity in cat intestinal wall, although the lowest of various species (Kim, Backus, Harris & Rourke, 1969), is still greater than *N*-methylating activity (Kim *et al.*, 1969; Backus & Kim, 1970). That the metabolites originated in the intestinal wall and not by uptake from blood,

was verified by the much greater amounts of radioactivity in the proximal compared to the distal small intestinal segments.

The ratio of [^{14}C]-histamine to its metabolites in portal blood depended partly on the amount of [^{14}C]-histamine instilled. Thus with the smaller doses (1.7 and 5 μmol/kg), histamine accounted for 12 to 15% of the total radioactivity during the 30 min after instillation compared with about 40% (4 cats) after the largest dose (82 μmol/kg). Absorption of small amounts of histamine into portal blood, also described by Duncan & Waton (1968) in dogs, may be of importance since the quantities absorbed were sufficient to elicit acid secretion from a pentagastrin-sensitized stomach (Waton, 1973). Certainly, absorption of these amounts in cats is feasible because of the quantities of histamine in cat and dog foods (Duncan & Waton, 1968). The large amounts of [^{14}C]-histamine found in portal blood of two cats after histamine (82 μmol/kg), 70% of total radioactivity recovered, may explain the toxic effects occasionally seen in man after ingestion of histamine-containing foods and tentatively attributed to histamine (Ienistea, 1971).

Small percentages of the total radioactivity recovered were associated with imidazoleacetic acid riboside (1 to 8%) and acetylhistamine (3 to 10%). The delay in appearance of the riboside compound (Figures 5 and 6) is consistent with conjugation occurring, subsequent to the deamination of histamine by DAO (Tabor & Hayaishi, 1955). Rabbit liver and kidney slices can form ribosides (Crowley, 1964) and our results do not preclude the existence of this capability in cat duodenal wall. Action of bacterial flora in the alimentary canal (Urbach, 1949; Tabor, 1954) probably contributed to the small amounts of acetylhistamine recovered since its formation was less in histamine infusion experiments; rats bred under bacteria-free conditions do not produce acetylhistamine (Gustafson, Kahlson & Rosengren, 1957). Schayer (1956) did not find acetylhistamine in cat urine although in the present study it varied between 0.7 and 6.8% of the total ^{14}C-activity recovered (Tables 1–3). Cats, unlike some other species, do not form side-chain methylated histamine derivatives (Bunce & Lake, 1975) and therefore N^{α} methyl- and $N^{\alpha}N^{\alpha}$ dimethylhistamine were unlikely to interfere with the estimation of *t*-methylhistamine (Thomas & Marley, 1978).

Large amounts of [^{14}C]-histamine were recovered from stomach washings at 2 and 4 h (Table 1) indicating that ingested histamine was not metabolized in the stomach. This was consistent with both the absence of radioactivity in portal blood after instillation of histamine into the stomach since acidic compounds would be freely diffusible across the stomach wall, and the similarity of ^{14}C-metabolite compositions of blood on absorption from the small intestine, irrespective of whether or not histamine had previously been in the stomach. Since absorption did not take place from the stomach, the presence of radioactivity within the wall was unexpected. It is likely that some, if not all, of this was due to radioactivity trapped between villi and in mucus. This probably also contributed to radioactivity from the intestinal wall so affecting the ratio of radioactivity (2.5 : 1) recovered from these two tissues, viz. the small intestine and stomach. The metabolites recovered from small intestinal washings probably derived from metabolism within the intestinal wall rather than from intraluminal metabolism, since [^{14}C]-histamine incubated with intraluminal fluid was not metabolized (Thomas and Marley, unpublished results).

The predominant urinary metabolite was *t*-methylimidazoleacetic acid accounting for approximately 53% of total radioactivity recovered which was less than the 77% described by Schayer (1956). There are possible explanations for this discrepancy other than attribution to individual animal variation. First, in the present study histamine was instilled into the duodenum and urine collected for 2 h whereas Schayer (1956) gave histamine orally and collected urine over 6 h. Second, the isotope dilution technique (Schayer, 1956) probably underestimates the amount of imidazoleacetic acid riboside (Bergmark & Granerus, 1974; Sjaastad & Sjaastad, 1974), admittedly a minor metabolite in this study, and overestimates *t*-methylimidazoleacetic acid (Sjaastad & Sjaastad, 1974), thus exaggerating the importance of *N*-methylation. The differences between Schayer's and the present study could not be attributed to anaesthesia since results from spinal non-anaesthetized preparations and cats anaesthetized with chloralose were similar.

A large proportion of the urinary *t*-methylimidazoleacetic acid was renal in origin since the ratios of the concentrations of *t*-methylimidazoleacetic acid and imidazoleacetic acid in portal venous blood and in cranial mesenteric arterial blood were similar. Comparison with metabolite patterns in other tissues showed that there was more radioactivity in the kidney and a much greater proportion of *t*-methylimidazoleacetic acid. This could only have arisen by the methylation and subsequent deamination of circulating histamine and deamination of *t*-methylhistamine by renal imidazole *N*-methyltransferase and monoamine oxidase. Large amounts of imidazole *N*-methyltransferase are present in cat kidney (Backus & Kim, 1970).

Whereas intraduodenally instilled histamine was metabolized by both routes equally, intravenously infused histamine was preferentially *N*-methylated indicating the importance of intestinal DAO activity for deaminating ingested histamine. A small peak of histamine was detected in the inferior vena cava and carotid arterial blood, but not in cranial mesenteric

arterial blood, demonstrating that any infused histamine which escapes inactivation by single passage through the liver, heart and lungs is removed or inactivated by the intestines. Incomplete clearance of radioactive histamine has also been observed with the cat isolated heart-lung-liver preparation (Lilja & Lindell, 1961). The absence of a peak of *t*-methylhistamine, and its lower concentration compared to *t*-methylimidazoleacetic acid, in blood samples after intravenous infusion of histamines illustrates that MAO deamination closely follows *N*-methylation.

The fate of histamine infused into blood supplying the intestine (cranial mesenteric artery) differed markedly. The large peak of histamine in portal blood meant that the intestine dealt less ably than the liver with circulating histamine. Since imidazoleacetic acid was almost as important a metabolite as *t*-methylimidazoleacetic acid, intestinal DAO and MAO were of equal consequence although the amounts of *t*-methylimidazoleacetic acid and *t*-methylhistamine demonstrated the overall predominance of ring *N*-methylation for metabolizing histamine even when infused by this route. However, a lower ratio in blood of methylated to non-methylated histamine derivatives was obtained with this technique compared to portal vein infusions, hence approximating results obtained when histamine was instilled into intestine.

Removal of the liver and intestines from the circulation dramatically increased the size of the histamine peak during infusions, emphasizing the great importance of these organs to histamine metabolism. A larger concentration of *t*-methylhistamine compared to *t*-methylimidazoleacetic acid appeared during the first 40 min of experiments (the acid appearing in the blood at a slightly slower rate than usual) because of the absence of liver and intestinal MAO. However, the large peak of histamine present during the infusion was still rapidly metabolized by the remaining tissues, particularly the kidneys. When the renal vessels were ligated, following abdominal evisceration, the concentration of the deaminated metabolite, *t*-methylimidazoleacetic acid, was severely reduced.

To assess the relative importance of the liver and intestines, experiments were performed in which the intestines were removed and histamine was infused into an iliac vein-portal vein cannula. As when histamine was infused into the portal vein, *t*-methylimidazoleacetic acid was the predominant metabolite. However, the concentration of *t*-methylhistamine, its precursor, remained equal to that of imidazoleacetic acid throughout the experiment demonstrating the contribution of intestinal MAO to histamine metabolism. In these experiments, the small peak of histamine found in carotid arterial and inferior vena caval blood but absent from cranial mesenteric venous blood now persisted (although much reduced) beyond the infusion period, confirming that the intestine normally removed the small amounts of histamine eluding inactivation by the liver, etc. These results are compatible with high MAO activity in intestine, liver and kidney (Blaschko, 1952). Clearly, imidazole *N*-methyl transferase is widely distributed since *N*-methylation was not significantly altered although the reduced amounts of *t*-methylimidazoleacetic acid suggests that MAO is less closely associated with the *N*-methyltransferase outside the intestine, liver and kidney.

In summary, deamination by DAO was as important in cats as ring *N*-methylation (followed by MAO deamination) for instilled and (by implication) ingested histamine. For parenterally administered (and presumably for endogenous histamine other than of gut origin), ring *N*-methylation predominated, although deamination by DAO still contributed. Certainly for cats, conclusions based on urine analysis as to the metabolism of ingested histamine were misleading since more than one metabolic route was involved. The same could apply for man, for although products of DAO and imidazole-*N*-methyl transferase activities are present in equal concentrations in the urine (Bergmark & Granerus, 1974; Sjaastad & Sjaastad, 1974; see Schayer, 1956 for a dissenting view), DAO may nevertheless predominantly inactivate dietary histamine; evidence for this could be obtained by analysing venous blood.

In the cat experiments, some histamine invariably entered portal blood. Should this apply also for man, it would be hazardous if there was abnormal sensitivity to the substance, or renal and hepatic insufficiency. Such a consequence is also likely for subjects receiving drugs that interfere with histamine metabolism, since experiments in progress reveal that they enhance the proportion of histamine entering the circulation from the intestine.

The project was generously financed by the Wellcome Trust, and the salary of P.R.I. was defrayed by the Bethlem Royal and Maudsley Hospitals Research Fund.

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(Received February 21, 1978.)