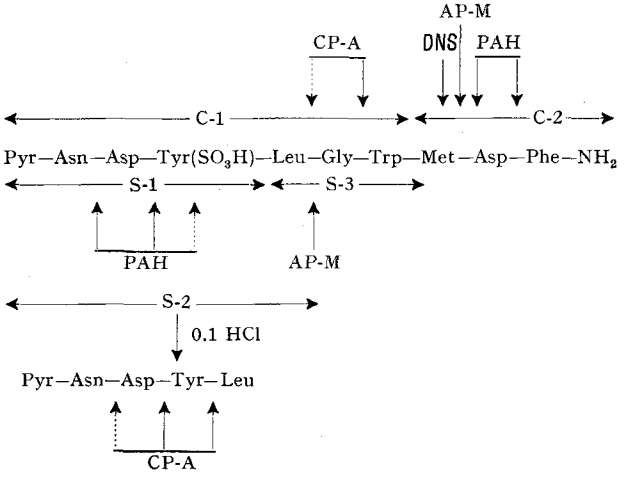
times the weight of the tissue. The extracts were combined and filtered.

Isolation procedure. An aliquot of extract corresponding to 100 g of fresh skin was evaporated to dryness, the residue washed with petroleum ether and then taken up in water plus 99% ethanol to give a final ethanol concentration of 95%. The liquid was passed through 2 columns of alkaline alumina Merck 90 (activity grade 1), each of 170 g, which were then eluted with ethanol-water mixtures of decreasing concentrations of ethanol, each of 200 ml. The peak of caerulein-like activity (guinea-pig gall bladder) emerged in the 2 50% ethanol eluates, which contained altogether approximately 35 mg of peptide, expressed as caerulein, and 50% of the activity put on the column.

The suspicion that Hylambates-caerulein might be different from caerulein was aroused by the fact, that in comparison to caerulein, Hylambates-caerulein was eluted by higher concentrations of ethanol and with considerably better yields.



Hylambates-caerulein present in the 50% ethanol eluates was further purified by preparative electrophoresis. The single biologically active peptide spot was positive to chlorine, to the Ehrlich reagent for tryptophan, to the α-nitroso-β-naphthol reagent for tyrosine, to the jodoplatinate reagent for sulphur aminoacids, but it was negative to ninhydrin. On ascending thin layer chromatography on silica gel, Hylambates-caerulein had an R_f 0.35 in the solvent system n-butanol-acetic acid-water (4:1:1), and on high voltage electrophoresis the peptide spot migrated toward the anode at neutral and acid pHs, its position being 0.5 relative to glutamic acid at pH 5.8 and 0.55 relative to cysteic acid at pH 1.9.

Structure. The structure of Hylambates-caerulein was deduced by sequential analysis of the fragments obtained by digestion with chymotrypsin(C) and subtilisin (S) followed, as shown in the chart, by digestion with carboxypeptidase A (CP-A), aminopeptidase M (AP-M), partial acid hydrolysis (PAH) and dansylation (DNS). The dipeptide Pyr-AsnOH was identified by its electrophoretic behaviour.

It is possible that the small amounts of caerulein-like activity (guinea-pig gall bladder) found in extracts of Kassina senegalensis (1–5 μg/g fresh skin) and of Phlyctimantis verrucosus (5–7 μg/g dry skin) are due, at least in great part, to Asn[2], Leu[5]-caerulein.

Hylambates-caerulein displayed on the isolated and in situ guinea-pig gall bladder, as well as on the isolated guinea-pig ileum and rabbit large intestine, stimulant effects which were qualitatively identical with those elicited by caerulein. From a quantitative point of view, definitive conclusions are not possible because pure synthetic Asn[2], Leu[5]-caerulein was not available. However, the 2 peptides may be considered approximately equiactive.

In addition to Asn[2], Leu[5]-caerulein, methanol extracts of the skin of Hylambates maculatus contain 4 or 5 other active peptides, mainly belonging to the tachykinin family. The isolation of 2 of them is in progress.

Biliary excretion and metabolism of ^{14}C cimetidine following oral administration to male and female rats

F. J. McPherson and R. M. Lee

The Research Institute, Smith Kline and French Laboratories Ltd., Welwyn Garden City (Herts., England), 18 February 1977

Summary. In rats, the bile is not a major route of excretion of cimetidine or its metabolites, since only 10% of the ^{14}C associated with an oral dose of labelled cimetidine was excreted in the bile during 8 h after dosing.

Cimetidine is an orally active histamine H_2-receptor antagonist[1], marketed under the trade mark 'Tagamet'. When this compound was given orally to male rats[2,3] (30 mg/kg of cimetidine labelled with ^{14}C in the 2-position of the imidazole ring; figure) 58% of the radioactivity was excreted in the urine during the 24 h after dosing and approximately 50% of this was unchanged cimetidine. Similarly, 64% of the radioactivity administered orally to female rats was excreted in the urine within 24 h, but a larger proportion of the eliminated ^{14}C was associated with unchanged cimetidine. The significance in rats of biliary excretion of cimetidine and its metabolites has not been reported previously, and was the subject of the separate study described in this communication.

Materials and methods. 4 male and 4 female Wistar rats, weighing 198–201 g and 194–203 g respectively, each received by gastric intubation, 30 mg/kg of 2-^{14}C-cimetidine dissolved in 0.9% (w/w) saline. This resulted in the administration of 10 μCi of ^{14}C to each animal. After dosing, the animals were anaesthetized with 2% halothane in oxygen containing 5% CO_2, and the bile duct cannulated

1 R. W. Brimblecombe, W. A. M. Duncan, G. J. Durant, J. C. Emmett, C. R. Ganellin and M. E. Parsons, J. int. Med. Res. *3*, 86 (1975).
2 D. C. Taylor and P. R. Cresswell, Biochem. Soc. Trans. *3*, 884 (1975).
3 D. C. Taylor, Personal communication.

Biliary excretion and metabolism of ^{14}C cimetidine in male and female rats

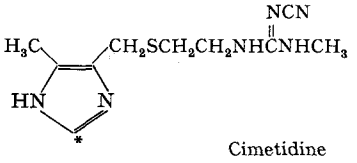
Time (min) after administration of the drug	Male rats ^{14}C Activity excreted (%)	Cimetidine excreted (%)	Sulphoxide excreted (%)	Female rats ^{14}C Activity excreted (%)	Cimetidine excreted (%)	Sulphoxide excreted (%)
60	1.22 ± 0.20	81.0 ± 13.0	19.0 ± 13.0	1.52 ± 0.47	85.0 ± 7.0	15.0 ± 7.0
90	1.95 ± 0.05	71.5 ± 14.8	28.5 ± 14.8	1.92 ± 0.22	63.5 ± 24.0	36.5 ± 24.0
120	1.57 ± 0.66	56.5 ± 12.1	43.5 ± 12.1	1.70 ± 0.41	57.5 ± 10.6	42.5 ± 10.6
180	0.90 ± 0.08	45.0 ± 8.4	55.0 ± 8.4	1.02 ± 0.17	58.5 ± 9.1	41.5 ± 9.1
240	0.90 ± 0.11	46.0 ± 8.5	54.0 ± 8.5	1.05 ± 0.26	59.0 ± 1.4	41.0 ± 1.4
300	0.80 ± 0.14	46.5 ± 6.3	53.5 ± 6.3	1.10 ± 0.21	59.5 ± 2.1	40.5 ± 2.1
360	0.90 ± 0.08	45.4 ± 4.7	54.6 ± 4.7	1.02 ± 0.12	57.6 ± 3.4	42.4 ± 3.4
420	0.92 ± 0.09	46.2 ± 6.4	53.8 ± 6.4	0.97 ± 0.09	58.3 ± 4.1	41.7 ± 4.1
Average total	9.16			10.30		

The data given are the means ± SD; n = 4 for each estimation.

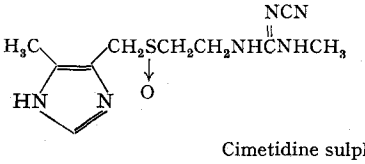
for total bile collection. Bile was collected continuously for 7 h and fractionated at 30 min or hourly intervals, with replacement of fluid loss by 0.9% saline administered i.p. The rats were kept anaesthetized throughout the experiment by placing cotton-wool pads soaked in ether over their mouth and nostrils.

The bile samples were assayed for radioactivity by liquid scintillation counting, using 20 µl aliquots of bile mixed directly into the scintillant. The number of ^{14}C-labelled compounds present in the bile, and their identity, was investigated by spotting samples onto silica TLC plates, which were developed in a solvent system containing ethyl acetate, methanol and ammonia in the ratios 5:1:1 by volume, respectively. After drying, the plates were sprayed with diazotized sulphanilic acid reagent[2], or were exposed to X-ray film for visualization of cimetidine, the known metabolites, and any other ^{14}C-labelled material. The radioactive areas shown by radioautography were scaped from the plate and counted after eluting the silica gel with 1 ml methanol. For identification of the parent compound and its major metabolites, samples of bile were streaked onto PLC plates, which were developed as previously described. The relevant areas were scraped off, eluted with methanol, and compared, by TLC and nuclear magnetic resonance (NMR) spectroscopy, with authentic cimetidine or cimetidine sulphoxide (figure) synthesized in these laboratories by Dr G. R. White.

The possibility that rat bile could contain glucuronides derived from cimetidine was investigated. Bile samples (200 µl) were incubated with 150 units of β-glucuronidase (Sigma Chemical Co. Ltd) for 20 min at 37 °C. Aliquots of these treated samples were then examined by TLC as described for untreated bile. The efficacy of the β-glucuronidase was assayed in the presence of bile by measuring the phenolphthalein released from phenolphthalein glucuronide.



Cimetidine



Cimetidine sulphoxide

* = Position of ^{14}C label

Results and discussion. The results in the table show that after 7 h an average 9.2% of the total radioactivity administered to rats was recovered in the bile collected from male animals, and 10.3% in that from females. Clearly, this indicated that the bile does not represent a major route of excretion of 2-^{14}C-cimetidine in rats, which is consistent with the finding of Taylor et al.[2] who showed that 60% of an oral dose of cimetidine was eliminated via the urine in 24 h.

In rats of both sexes, the highest rate of excretion of ^{14}C into the bile occurred between 60 and 90 min after the oral administration of cimetidine. After 2 h the rate of excretion declined to about 50% of maximum, and this rate was maintained until the experiment was terminated at 7 h after dosing. It should not be forgotten, however, that, under the experimental conditions outlined above, losses of bile would occur, which might affect the biliary excretion of drugs[4]. The TLC analysis of bile samples indicated the presence of unchanged cimetidine and one major metabolite – the sulphoxide (figure). Preparative chromatography, followed by comparative TLC and NMR spectroscopy, confirmed the identity of parent compound and metabolite. When quantitative investigations were undertaken, a similar metabolic pattern was apparent in both male and female animals (table). Coincidental with the maximal rate of ^{14}C excretion, was the maximal rate of cimetidine excretion (approximately 70% of ^{14}C activity at 90 min being unchanged cimetidine, 30% being the sulphoxide). Higher levels of unchanged drug were detected in the bile of female relative to male animals and this was in agreement with sex differences previously reported for the urinary excretion of cimetidine[2]. Treatment of bile samples from either sex with β-glucuronidase produced no significant variations in metabolic patterns, suggesting that glucuronidation does not have a significant role to play in the biliary excretion of this compound.

In summary, the bile does not represent a major route of excretion of this orally active histamine H_2-receptor antagonist, approximately 10% of the orally administered ^{14}C activity being excreted in 7 h. Examination of bile samples indicated the presence of cimetidine, and one metabolite – the sulphoxide; male animals appeared to metabolize the parent compound to a greater extent than female animals.

4 R. L. Smith, in: The Excretory Function of Bile, p. 100. Chapman and Hall, London 1973.