formation of the TFMA-oxanilic acid via *N*-acetyl-TFMA can occur. However, it was observed that whilst the *N*-acetyl-TFMA dosed to the animals was over 90% ^{13}C-enriched, the *N*-oxanilic acid isolated was only 67% enriched (as determined by isotope ratios on GC–MS of the esterified metabolite). This indicates that deacetylation of a portion of the *N*-acetyl-TFMA occurred before metabolism to the oxanilic acid (presumably via re-acetylation).

The requirement for *N*-acetylation prior to formation of the oxanilic acid of TFMA probably explains why this metabolite is not formed by the dog, given the well known metabolic deficiency of *N*-acetylation in this species [5]. When dosed with ^{13}C, ^{14}C-N-acetyl-TFMA (16 mg/kg) the dog also produced the expected TFMA oxanilic acid. In this case the stable isotope ratio of the metabolite was the same as that of the *N*-acetyl TFMA dosed to the animal.

As referred to in the text, the formation of oxanilic acids as minor metabolites of certain aromatic amines and their derivatives has been reported [3, 4]. Thus up to 21% of the administered 4-chloroacetanilide was recovered from the urine of rabbits as the 4-chloro-oxanilic acid metabolite within 24 hr after dosing [3]. Rabbits have also been shown to convert up to 14% of a dose of [^{35}S] sulphanilamide to *N*-(4-sulphamoylphenyl)-oxanilic acid [6]. However, the metabolism by rat of virtually the whole of an oral dose of TFMA to an oxanilic acid is unprecedented.

The species difference observed between rat and dog in the metabolism of ^{14}C-TFMA is most easily explained if formation of the oxanilic acid metabolite requires *N*-acetylation, a process at which the dog is deficient. Although we have not demonstrated that *N*-acetylation of TFMA occurs *in vivo*, the retention of the stable isotope label in the TFMA-*N*-oxanilic acid formed from [^{13}C, ^{14}C]-*N*-acetyl-TFMA provides additional support for the involvement of acetylation in this transformation.

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Influence of species and drug pretreatment on the metabolic oxidation of cimetidine and metiamide

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Cimetidine and its forerunners, buramamide and metiamide, are specific H_2-receptor antagonists but only cimetidine is currently used in the treatment and prophylaxis of gastro-intestinal ulcer disease and reduction of gastric hyperacidity states in man [1, 2]. Cimetidine and metiamide are mainly metabolized by oxidation to the sulfoxide and 5-hydroxymethyl derivatives [3, 4], but recently the *N*-glucuronide conjugate has been reported as the major metabolite of cimetidine [5]. Drug interactions with cimetidine have been well documented [6], and this paper reports the influence of species and drug pretreatment on the metabolic oxidation of cimetidine and metiamide.

Materials and methods

Cimetidine, metiamide and their derivatives were supplied by Smith Kline & French Research Limited, U.K. Phenobarbitone, anthracene and ethanol were obtained from the British Drug House, while -pregnen-3-OL-20-one and methimazole were from Sigma. Metyrapone was a gift from Ciba-Geigy, Lagos, Nigeria.

Four non-mammalian and three mammalian species (male) were dosed with 50 mg/kg of cimetidine or metiamide, and housed in appropriate metabolic cages. The excreta and urine were collected daily. Sets of rats were also pretreated with chemicals before the administration of cimetidine or metiamide.

Assay procedure. Urine samples and the excreta were made alkaline with 1.25 N NaOH and then extracted with 2 × 5 ml isopropanol–dichloromethane (1:1, v/v). The pooled extracts were evaporated to dryness on a water bath at 45° and reconstituted in ethanol (40 μl). Portions (20 μl) were examined by TLC (silica gel $60F_{254}$ 0.25 mm thick 5 × 10 cm, E. Merck, Darmstadt, West Germany) and developed in the appropriate solvents (Table 1). The compounds and their metabolites were located under u.v. light and by exposure for 15 sec in iodine vapor. The spots were scraped off and eluted into 1 N HCl (6 ml) and then quantified using a Varian 634 u.v.-visible scanning spectrophotometer.

Results and discussion

Sulfoxidation of xenobiotics has been shown to be both species and substrate dependent [7, 8]. The results (Table 2) clearly show that the rates of appearance of the sulfoxide and 5-hydroxymethyl metabolites and unchanged drug were species dependent for both cimetidine and metiamide, although by 72 hr the total amounts of dose excreted were similar except for the rat.

The induction inhibition experiment in the rat (Table 3) also demonstrated selective effects with regard to compound and specific metabolites. The induction of cimetidine sulfoxidation by alcohol, phenobarbitone, and pregnenolone, and its inhibition by methimazole rather than metyrapone tend to suggest the involvement of a flavoprotein-mediated monooxygenase in this oxidation reaction.* Metiamide sulfoxidation appears different since it was only

* R. A. Prough and R. N. Hines, "Eighth European Workshop on Drug Metabolism", Abstr. No. 20, p. 64 (1982).

Table 1. Chromatographic properties of cimetidine, metiamide and their oxidation products

Compounds	R_f values in solvent		
	A*	B†	C‡
Cimetidine	0.64	0.63	0.51
5-Hydroxymethylcimetidine	0.39	0.49	0.42
Cimetidine sulfoxide	0.22	0.29	0.35
Metiamide	0.73	0.72	0.55
5-Hydroxymethylmetiamide	0.47	0.54	0.44
Metiamide sulfoxide	0.38	0.44	0.38

* A = chloroform–ethanol–aq. NH_3 (sp.gr. 0.88), (6:1:1, by vol.).
† B = chloroform–ethanol–aq. NH_3 (sp.gr. 0.88), (4:1:1, by vol.).
‡ C = ethylacetate–methanol–aq. NH_3 (sp. gr. 0.88), (5:1:1, by vol.).

Table 2. Species differences in the metabolic oxidation of cimetidine and metiamide (in parentheses)*

Species	Route of administration†	% Dose excreted in		% Excretion in 24 hr as		
		24 hr	72 hr	Unchanged	Sulfoxide	5-Hydroxymethyl derivative
Toad	i.m.	25 (44)	53 (60)	79 (70)	21 (20)	0 (10)
Tortoise	i.m.	27 (12)	50 (48)	80 (92)	12 (5)	8 (3)
Lizard	i.m.	18 (18)	42 (49)	99 (84)	1 (16)	0 (0)
Pigeon	s.c.	22 (28)	51 (51)	33 (63)	44 (22)	23 (8)
Rat	i.p.	55 (88)	95 (95)	85 (72)	10 (20)	5 (8)
Rabbit	i.p.	18 (16)	58 (50)	78 (70)	12 (15)	10 (15)
Cat	s.c.	25 (27)	51 (52)	47 (70)	35 (20)	17 (6)

* Results are means of five animals.
† Abbreviations: i.m., intramuscular; i.p., intraperitoneal; and s.c., subcutaneous.

Table 3. Effect of drug pretreatment on the metabolic oxidation pattern of cimetidine and metiamide (in parentheses) in the rat*

Drug	Dose	% Dose excreted in 24 hr as		
		Unchanged	Sulfoxide	5-Hydroxymethyl derivative
Control†	Normal saline (0.2 ml)	56 ± 5 (44 ± 2)	13 ± 1 (12 ± 1)	5 ± 1 (5 ± 1)
Alcohol†	25%, v/v (0.2 ml)	47 ± 6 (34 ± 3)	22 ± 4 (21 ± 3)	5 ± 1 (6 ± 1)
Phenobarbitone†	30 mg/kg	51 ± 4 (33 ± 2)	20 ± 2 (17 ± 2)	6 ± 1 (13 ± 3)
Anthracene†	50 mg/kg	54 ± 2 (50 ± 2)	10 ± 1 (15 ± 1)	3 ± 1 (13 ± 1)
Pregnenolone†	50 mg/kg	45 ± 6 (51 ± 8)	24 ± 5 (15 ± 1)	5 ± 1 (9 ± 2)
Methimazole‡	100 mg/kg	68 ± 1 (46 ± 4)	12 ± 1 (10 ± 3)	0 (trace)
Metyrapone‡	100 mg/kg	66 ± 3 (57 ± 6)	13 ± 1 (11 ± 3)	Trace (5 ± 1)

* Values are means ± S.E., N = 6.
† Pretreated for 5 days before drug administration.
‡ Coadministered with cimetidine or metiamide.

markedly induced by alcohol and phenobarbitone but was not inhibited by methimazole or metyrapone.

Sulfoxidation of chlorpromazine [9], methimazole and other thioureylenes [7] is via the flavoprotein-mediated monooxygenase system, while the sulfoxidation of thiacetamide [10], on the other hand, is catalyzed by a cytochrome P-450 containing monooxygenase system. Ethanol induces a particular form(s) of cytochrome P-450 [11] and a subpopulation of this which has a poor affinity for metyrapone [12] could be involved in the sulfoxidation of metiamide. Alternatively, a different type of *S*-oxidase might be involved which is not induced by phenobarbitone or methylcholanthrene and not drastically inhibited by classical inhibitors.*

In summary, the metabolic oxidation of cimetidine and metiamide to their sulfoxide and 5-hydroxymethyl derivatives was species dependent, and their formation in rat showed different sensitivities to the effects of enzyme inducers and inhibitors.

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Effects of combined administration of thiol compounds and methylmercury chloride on mercury distribution in rats

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It was reported previously that the brain uptake of methylmercury was accelerated by combined administration of L-cysteine and depressed by the neutral amino acid, phenylalanine, but not by basic and acidic amino acids [1]. From these results, one can speculate that the blood–brain barrier transport system of neutral amino acid participates in some way in methylmercury penetration through the blood–brain barrier. Cysteine seems to be an important factor in the methylmercury uptake in the brain. On the other hand, it has been suggested that a methylmercury–glutathione complex is of importance for the methylmercury uptake in the kidney [2]. From studies on mercury accumulation at short times after the administration of inorganic mercury, Thomas and O'Tuama [3] suggested that uptake processes differ in different tissues. In order to clarify the methylmercury uptake process in the brain in comparison with other tissues, the effect of combined administration of L-, D-cysteine, glutathione or *N*-acetyl-L-cysteine and methylmercuric chloride on mercury distribution was investigated in rats.

Materials and methods

Methylmercuric chloride (purity: 98%), L-cysteine HCl, glutathione and *N*-acetyl-L-cysteine were purchased from Wako Pure Chemical Industries. D-Cysteine HCl was purchased from Nakarai Chemical Company. Each reagent was dissolved in phosphate buffered saline (pH 7.4). Kud: Wistar male rats (age 7 weeks) were used in this study. Either methylmercuric chloride (5 μmole/kg) alone, or methylmercury premixed with L-cysteine, D-cysteine, *N*-acetyl-L-cysteine or glutathione at the dose of 10 μmole/kg was injected i.v. into the rats. After 5 min, 3 and 24 hr, blood was collected from the abdominal vein of the rats under pentobarbital anesthesia. Then, the rats were perfused with saline, and the organs were removed for mercury determination. An aliquot of blood was centrifuged at 3000 rpm for 10 min for separation of plasma. Total mercury contents in the brain, kidney, liver, whole blood and plasma were determined by an oxygen combustion flameless atomic absorption system using Sugiyamagen Mercury analyzer MV250 R.

Statistical significance of mean values was calculated by Student's *t* tests and P values less than 0.05 were considered to be significant.

Results and discussion

All thiol compounds used in this experiment caused increased mercury contents in the brain, kidney and liver (Fig. 1), and lower mercury levels in the blood at 5 min (Fig. 2). The combined i.v. administration of methylmercury and thiol compounds significantly decreased whole blood: plasma ratio of mercury at 5 min (control, 169; L-cysteine, 77; D-cysteine, 92; *N*-acetyl-L-cysteine, 67; glutathione, 110). It has been reported that in the presence of glutathione or cysteine, the methylmercury uptake in red blood cells is depressed [3, 4].

The L-cysteine caused an additional increase in the mercury contents in the brain, while D-cysteine treatment showed the same mercury levels as the other thiol compounds. Such results mean that there was a stereospecificity of the blood–brain permeability to the methylmercury cysteine complex. Since it is known that barrier transport of amino acids is stereospecific [5], these results support our previous findings [1] that the blood–brain barrier transport system of amino acids might be involved in the methylmercury uptake in the brain. It has been reported that mercury–amino acid complexes might be involved in the transport of mercury into kidney cells via the same mechanism as amino acid themselves are transported [6]. In the present work, however, no stereospecific difference was observed in the renal uptake of methylmercury–cysteine complexes. The liver, blood and plasma contents also were not significantly different after simultaneous treatment with methylmercury and either D-, or L-cysteine. Alexander and Aaseth [2] reported that methylmercury glutathione complex in blood is rapidly extracted by the kidney. Our results showed that *N*-acetyl-L-cysteine and glutathione caused significantly higher mercury levels in the kidney at 5 min as compared with L- or D-cysteine treatment. The accumulation of methylmercury in the kidney may be a more complicated process and coupled with the metabolism of glutathione and cysteine in the kidney. Treatment with all of the thiol compounds increased the methylmercury uptake by the liver approximately equally. Alexander and Aaseth [2] reported that the mercury content in the liver was depressed at 1 hr in rats given methylmercury mercaptides. They suggested that liver cells are unable to take up glutathione and can export large amounts of the methylmercury glutathione complex rapidly into the