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METABOLISM OF TRIFLUOPERAZINE, FLUPHENAZINE, PROCHLORPERAZINE AND PERPHENAZINE IN RATS: *IN VITRO* AND URINARY METABOLITES

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Abstract—*In vitro* and *in vivo* metabolites of trifluoperazine, fluphenazine, prochlorperazine and perphenazine were isolated by solvent extraction and thin layer chromatography and quantified by u.v. spectroscopy. In liver microsomes from male rats all four drugs underwent *N*-dealkylation, *N*-oxidation, sulfoxidation and aromatic hydroxylation. The relative rates of these reactions depended on the substrate concentration, *N*-oxidation being favoured at higher concentrations. *N*-Demethylation of trifluoperazine proceeded faster than removal of the hydroxyethyl group from fluphenazine which led to the same metabolite *N*[γ-(2-trifluoromethyl-phenothiazinyl-10)-propyl] piperazine. The same applied to the dealkylation of prochlorperazine and perphenazine. Following oral administration of 10 mg/kg of the drugs, male rats excreted 1·8–4 per cent of the dose within the first 12 hr in urine in the form of the sulfoxide and the *N*-dealkylated sulfoxide. *In vivo*, too, the *N*-hydroxyethyl group was removed to a smaller extent than the *N*-methyl group. *N*-Oxides were not detected in urine at this dose level, but when 25 or 50 mg/kg prochlorperazine were administered, rats excreted small amounts of the *N*-oxide.

BIOTRANSFORMATION products of the neuroleptic drug perazine found in rat tissues after repeated dosage[1] partially differ from those formed upon *in vitro* incubation[2] and detected in urine following a single dose. The present study was intended to provide data on *in vitro* and urinary metabolites of ring-substituted phenothiazine drugs that have a piperazine ring in the side chain in common with perazine. These data can be compared with those on the biodegradation products of the same drugs found in tissues and excreted in urine under the condition of chronic treatment.[3,4]*

The major *in vitro* pathways of phenothiazine metabolism are *N*-dealkylation, *N*-oxidation, sulfoxidation and hydroxylation of the aromatic ring system. Most of these reactions have already been detected when rat liver microsomal preparations were incubated with trifluoperazine (CF_3-Per) or prochlorperazine (Cl-Per), but positive identification of the demethylated products was either lacking[5,6] or unsatisfactory,[7] and on the other metabolites quantitative data were not available. Using fluphenazine (Flu) as substrate, Robinson[7] was unable to demonstrate *N*-dealkylation *in vitro*. Nor has to our knowledge the *N*-dealkylation product of Flu been shown to occur *in vivo*, though the corresponding metabolites of the closely related drugs flupenthixol[8] and clopenthixol[9] were isolated.

Studies on urinary metabolites have revealed the formation of the sulfoxides of CF_3-Per and perphenazine (Pph) in rats,[10,11] of Cl-Per in humans,[12] and of Flu in

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dogs and monkeys.[13] 7-Hydroxy-fluphenazine could be identified as the major Flu metabolite in dog and monkey faeces.[14]

The data to be presented here will show that the *N*-dealkylation products of the drugs are also consistently found in rat urine in the form of their sulfoxides and that certain correlations can be established between *in vitro* and *in vivo* metabolism.

MATERIALS AND METHODS

Drugs and drug metabolites. The drugs were donated by the following companies: trifluoperazine dihydrochloride (Jatroneural®, Röhm und Haas, Darmstadt, Germany), fluphenazine dihydrochloride (Lyogen®, Byk Gulden Lomberg, Konstanz, Germany), prochlorperazine free base (Compazine®, Smith, Kline and French, Philadelphia, U.S.A.), and perphenazine free base (Decentan®, Merck, Darmstadt, Germany). 7-Hydroxy-chlorpromazine was a gift from Dr. A. Manian (National Institute of Mental Health, Bethesda, U.S.A.).

Reference compounds for the dealkylation products, *N*[γ-(2-trifluoromethyl-phenothiazinyl-10)-propyl]piperazine (CF_3-PPP) and the 2-chloro analogue Cl-PPP were prepared from the corresponding γ-(phenothiazinyl-10)-propylchlorides[15] by heating with a 5-fold excess of piperazine in dioxane (60 min at 100°). The synthetic products were also converted to the sulfoxides.

7-Hydroxy-fluphenazine (OH-Flu) was obtained from the feces of a female Beagle dog given daily 20 mg/kg fluphenazine *per os*. The feces were mixed with double the volume of 2 N ammonia and extracted several times with chloroform. From the residue of the organic phase OH-Flu was obtained by thin layer chromatography in solvent A_2 (Table 2). It exhibited the chromatographic properties described by Dreyfuss *et al.*[13]

Chemical interconversions of compounds were performed by the methods used previously.[16] The reduction of *N*-oxides to the tertiary amines was simplified by adding 0·2 ml 1 M $Na_2S_2O_5$ and 0·15 ml 2 N HCl to a solution of the *N*-oxide in 1 ml water instead of working with gaseous SO_2.

Animals. Male Wister rats weighing 240-350 g were used. They had free access to tap water and a standard laboratory chow except during the 12 hr period of urine collection. Pretreatment with phenobarbital was carried out by intraperitoneal injection of 100 mg/kg phenobarbital-Na on the 3 days preceding sacrifice.

Studies in vitro. Livers of two rats were pooled and microsomes were prepared in sucrose–EDTA–Tris buffer[17] by centrifugation of the homogenate at 100 *g* (10 min), 5000 *g* (10 min), 10,000 *g* (10 min) and sedimentation of the microsomal fraction from the supernatant at 100,000 *g* (60 min). The microsomes were washed once and resuspended in the same buffer. Protein was determined according to Lowry *et al.*[18] using bovine serum albumin as standard. The microsomes were either used at once or stored at −20° for several days.

Incubations were carried out with 2 ml samples in two different media. Per ml the samples contained: 2·5 mg microsomal protein; 1 μmole NADP; 8 μmoles sodium D, L-isocitrate; 50 μg isocitrate dehydrogenase (EC 1.1.1.42); 0·5 μmoles substrate (unless stated otherwise); 125 μmoles sucrose; 2·7 μmoles Na_2EDTA; and in addition in medium A: 7·5 μmoles $MgCl_2$; 37 μmoles KCl; 25 μmoles Tris–HCl pH 7·5; in medium B: 5 μmoles $MgCl_2$; 25 μmoles KCl; 57 μmoles Tris–HCl pH 7·5. The reaction was started by addition of the substrate in 0·1 ml water after the samples had

been pre-incubated for 5 min at 37° with shaking under air. After further shaking for 10 min the mixtures were processed as described by Breyer.[2]

In some instances, the microsomal suspensions were shaken at 37° for 10 min without addition of the NADPH-generating system. Subsequently the cofactors were added, and the pre-incubation and incubation proceeded as above.

Thin layer chromatographic separation of the metabolites on silica gel GF_{254} (Merck, Darmstadt, Germany) was carried out as described previously,[2] except that the solvent system was slightly modified (solvent A_1, Table 2). Isolation of the separated compounds from the gel was performed by partitioning between 2 N ammonia and 1,2-dichloroethane or chloroform[2,16] using two 2 ml portions of the organic solvent.

Studies in vivo. Rats were placed into individual metabolic cages at 8 a.m. and were fed 10–50 mg/kg of the drugs (calculated as free base) *per os* as aqueous solutions. Additional water was given to a total volume of 5 ml per animal. Further 3 ml portions of water were fed by oesophageal tube at 11 a.m., 2 p.m. and 5 p.m. Urine collection was terminated at 8 p.m., and the samples were stored at −20°.

Urine collected from two animals (average volume 14 ml per rat) was mixed with 1 g NaCl and 0·1 ml of 10% sodium deoxycholate solution, adjusted to pH 9–9·5 with 5 N NaOH and extracted three times with 20 ml chloroform. The filtered extracts were combined, the solvent evaporated under reduced pressure and the residue subjected to thin layer chromatography, each sample being transferred to a band 5 cm wide. The first separation was achieved in solvent A_2 (Table 2), and isolation of substances was carried out as above with chloroform as extractant. Bands containing the dealkylated sulfoxides (and, if present, the *N*-oxides) were rechromatographed in solvent C; perphenazine sulfoxide had to be purified in solvent B.

Recoveries of metabolites were measured by adding the substances to a suspension of microsomes or to rat urine, respectively, and carrying them through the procedures described.

Quantitation of metabolites. This was performed by dissolving the purified compounds in 3 ml 0·1 N HCl and—if necessary following dilution—measuring the u.v.

TABLE 1. EXTINCTION DIFFERENCES OF PHENOTHIAZINE DRUGS AND OF THEIR SULFOXIDES (CONCENTRATION 5×10^{-5} M) USED FOR QUANTITATIVE DETERMINATIONS

Compound	Extinction differences	
	$E_{256}-E_{280}$	$E_{273\cdot5}-E_{290}$
Trifluoperazine (CF_3-Per)	1·388	0·004
CF_3-Per sulfoxide (CF_3-Per-SO)	−0·015	0·315
	$E_{260}-E_{280\cdot5}$	$E_{276}-E_{285}$
Fluphenazine (Flu)	1·251	−0·001
Flu sulfoxide (Flu-SO)	0·005	0·332
	$E_{256\cdot5}-E_{270}$	$E_{275}-E_{285}$
Prochlorperazine (Cl-Per)	1·414	−0·011
Cl-Per sulfoxide (Cl-Per-SO)	0·013	0·305
	$E_{257}-E_{277}$	$E_{275}-E_{281\cdot5}$
Perphenazine (Pph)	1·442	0·000
Pph sulfoxide (Pph-SO)	0·000	0·250

Solvent 0·1 N HCl, light path 1 cm

absorption at two wavelengths. These were chosen in such a way[17] that the sulfoxidic metabolites of a drug would minimally interfere with the measurement of the non-sulfoxidic ones and vice versa (Table 1).

RESULTS AND DISCUSSION

Identification of metabolites. The sulfoxides of the drugs CF_3-Per-SO, Flu-SO, Cl-Per-SO and Pph-SO (Fig. 1) exhibited u.v. spectra with 4 maxima between 230 and 350 nm which are characteristic of phenothiazine drug sulfoxides.[19] The metabolites obtained from *in vitro* incubations and from rat urine cochromatographed with each other and with reference compounds prepared by H_2O_2 oxidation of the parent drugs. They were reduced to the original drugs by zinc–HCl.

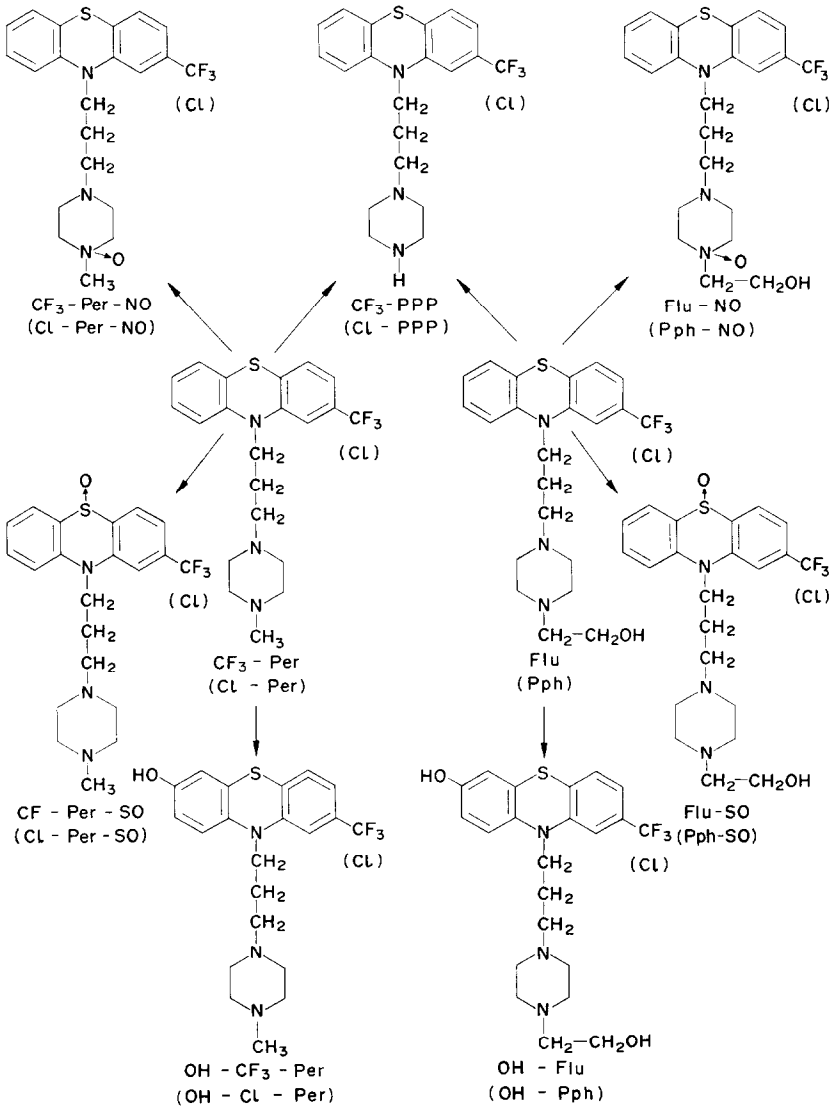

FIG. 1. Structures of the phenothiazine drugs investigated and of their *in vitro* metabolites.

TABLE 2. R_f VALUES OF TRIFLUOPERAZINE (CF_3-Per), FLUPHENAZINE (Flu), PROCHLORPERAZINE (Cl-Per) AND PERPHENAZINE (Pph) AND OF THEIR MAJOR METABOLITES IN THIN LAYER CHROMATOGRAPHY ON SILICA GEL GF_{254}

Compound	R_f value in solvent*			
	A_1	A_2	B	C
CF_3-Per	0·83	0·86	0·81	0·32
OH-CF_3-Per	0·66	0·66	0·84	0·30
CF_3-Per-SO	0·60	0·63	0·58	0·18
CF_3-Per-NO	0·18	0·19	0·42	0·32
CF_3-PPP	0·40	0·44	0·50	0·32
CF_3-PPP-SO		0·30	0·23	0·20
Flu	0·76	0·76	0·90	0·33
OH-Flu	0·58	0·59	0·90	0·31
Flu-SO	0·49	0·55	0·70	0·19
Flu-NO	0·21	0·27	0·63	0·52
Cl-Per	0·78	0·86	0·81	0·30
OH-Cl-Per	0·60	0·70	0·76	0·27
Cl-Per-SO	0·52	0·66	0·51	0·16
Cl-Per-NO	0·16	0·21	0·40	0·35
Cl-PPP	0·35	0·42	0·43	0·34
Cl-PPP-SO		0·28	0·19	0·15
Pph	0·75	0·76	0·90	0·34
OH-Pph	0·58	0·64	0·87	
Pph-SO	0·47	0·55	0·62	0·16
Pph-NO	0·21	0·25	0·59	0·44

* Solvents A_1: isopropanol–chloroform–25% ammonia–water, 32:16:1·5:1 by vol. A_2: 32:16:2:1 by vol. B: acetone–isopropanol–1 N ammonia, 27:21:12. C: 1,2-dichloroethane–ethyl acetate–ethanol–acetic acid–water, 15:26:12:8:7·5.

The *N*-dealkylation product of CF_3-Per and Flu, *N* [γ-(2-trifluoromethyl-phenothiazinyl-10)-propyl] piperazine (CF_3-PPP, Fig. 1) was compared to the synthetic compound in various solvent systems (Table 2) and found to have identical R_f values. The same was true for the sulfoxides of CF_3-PPP from all three sources. The secondary amine obtained from CF_3-Per as well as from Flu was converted to CF_3-Per by reacting it with methyl iodide. The identity of the dealkylated sulfoxide CF_3-PPP-SO obtained from urine after dosing rats with CF_3-Per or Flu was confirmed by chromatography of the compound itself and of its reduced analogue CF_3-PPP along with the synthetic reference compounds in solvents A_2, B and C. In addition, it could be methylated to CF_3-Per-SO. The same investigations were carried out with the corresponding metabolites of Cl-Per and Pph, namely Cl-PPP and Cl-PPP-SO.

The *N*-oxides of all drugs, CF_3-Per-NO, Flu-NO, Cl-Per-NO and Pph-NO, were reduced to the parent compounds by sulfur dioxide and by zinc–HCl. Their *in vitro* formation was impaired by incubating the microsomes for 10 min at 37° without addition of a NADPH-generating system, a procedure that is known to reduce tertiary amine oxidation.[20,21] The *N*-oxides as well as the dealkylated metabolites did not differ from the respective parent drugs in their u.v. spectra.

The phenolic metabolites OH-CF_3-Per, OH-Flu, OH-Cl-Per and OH-Pph could only be tentatively identified as the 7-hydroxy derivatives of the drugs. The criteria for their identification were: (a) colour reactions on chromatograms[22] were identical for OH-Cl-Per, OH-Pph and for authentic 7-hydroxy-chlorpromazine. The major fluphenazine metabolite extracted from dog feces which according to its chromatographic behaviour could be assumed to be 7-hydroxy-fluphenazine,[13,14] cochromatographed with OH-Flu and exhibited the same colours as OH-Flu and OH-CF_3-Per upon spraying. (b) The phenolic metabolites produced a purple dye upon reaction with sulfanilic acid and sodium nitrite. The absorption curve of the dye obtained from OH-Cl-Per was identical to that produced from 7-hydroxy-chlorpromazine.

Further minor metabolites occurred in urine. Among these, one was consistently detected when 25 or 50 mg/kg Cl-Per had been administered. The identification of this compound as γ-(2-chloro-phenothiazinyl-10) propylamine sulfoxide will be described in the accompanying paper.[4] Intermediates in the piperazine ring degradation which have been observed as tissue metabolites[3] were not formed in *in vitro* incubations nor could they be detected in rat urine.

Recoveries of metabolites. The recoveries from microsomal suspensions were measured for the metabolites of CF_3-Per. They were very similar to those found with the corresponding perazine metabolites[2] and were therefore assumed to be valid for the remaining substances, too. The quantities recovered were 86 ± 1·5% for CF_3-PPP, 89 ± 2·5% for CF_3-Per-NO and 91 ± 3% for CF_3-Per-SO ($n = 5$, mean ± S.D.).

Recoveries of urinary metabolites were measured using the Cl-Per metabolites as representatives. The fraction recovered was 87 ± 6% for Cl-Per-SO, 74 ± 5% for Cl-PPP-SO and 31 − 52% for Cl-Per-NO, depending upon the quantity added (20–80 nmoles, $n = 5$).

Thin layer chromatography. From the R_f values summarized in Table 2, it is obvious that separation of the four major *in vitro* metabolites of the individual drugs from each other and from the parent material can be achieved in solvent A_1. The urinary metabolites were first run in solvent A_2. Since this is not able to separate the *N*-dealkylated sulfoxides from the *N*-oxides, the corresponding bands had to be rechromatographed in solvent C. Pph-SO from urine was still contaminated with endogenous material after chromatography in solvent A_2 and had to be purified in solvent B.

Rate of in vitro *transformations.* The data contained in Table 3 show that the reaction rates were influenced by small variations in the composition of the incubation mixture. Medium B differed from medium A in containing less Mg^{2+}, which according to separate experiments was of no great influence. More important were probably the larger quantity of Tris buffer and the higher osmolarity.

In microsomes from unpretreated animals *N*-oxidation was the fastest reaction in accordance with observations on chlorpromazine[23,24] and perazine.[2] However, with Cl-Per as substrate it could be shown that increasing the drug concentration above 0·5 mM greatly enhanced *N*-oxidation while retarding demethylation and sulfoxidation (Fig. 2). Extrapolation from this observation to lower substrate concentrations (which are unfavorable for *in vitro* investigations due to rapid consumption of the substrate) leads to the conclusion that at low drug concentrations in the endoplasmic reticulum *N*-oxidation will not play a dominant part. A nearly identical dependence

TABLE 3. RATE OF METABOLITE FORMATION FROM PHENOTHIAZINE DRUGS (0·5 mM) IN RAT LIVER MICROSOMES AS INFLUENCED BY A VARIATION IN THE REACTION MEDIUM AND BY PHENOBARBITAL (PB) PRETREATMENT (3 × 100 mg/kg i.p.).

	Rate (nmoles mg protein^{-1} min^{-1})			
	Medium A		Medium B	
Reaction	Control	PB	Control	PB
CF_3-Per				
Sulfoxidation	0·5	0·9		
Demethylation	1·1 ± 0·3	1·6	1·8 ± 0·3	3·9
N-oxidation	1·6 ± 0·4	1·3	2·6 ± 0·3	1·5
Flu				
Sulfoxidation	0·4	0·6	0·4	
Dealkylation	0·2	0·5	0·4	1·1
N-Oxidation	0·9	0·6	1·3 ± 0·1	0·6
Cl-Per				
Sulfoxidation	0·5 ± 0·1	0·7 ± 0·1		
Demethylation	1·1 ± 0·2	1·5 ± 0·3	1·6 ± 0·6	
N-Oxidation	2·0 ± 0·5	1·2 ± 0·2	2·3 ± 0·2	
Pph				
Sulfoxidation	0·5	0·7	0·6	
Dealkylation	0·3	0·6	0·4 ± 0·05	
N-Oxidation	1·4	0·8	2·05 ± 0·35	

$n = 4 - 6$, where mean ± S.D. is given

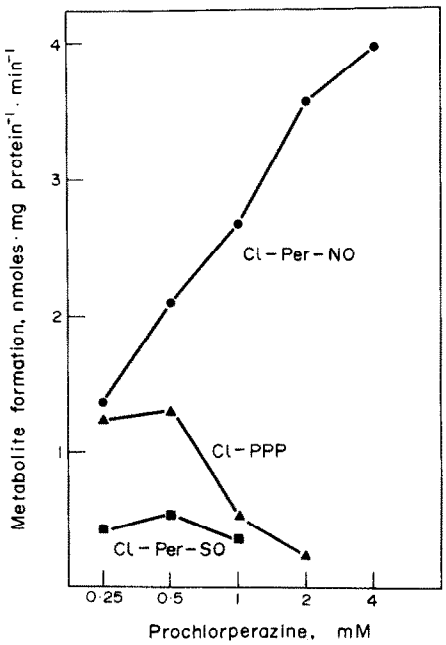

FIG. 2. Formation of prochlorperazine metabolites in rat liver microsomes as a function of substrate concentration.

of reaction rates on the substrate concentration had been demonstrated for perazine.[2]

Demethylation of CF_3-Per and Cl-Per was considerably faster than removal of the hydroxyethyl group from Flu and Pph. Formation of the sulfoxides proceeded at a similarly low rate with all four drugs. The aromatic hydroxylation, too, proved to be a slow reaction *in vitro*. The phenols were measured in a few instances only, and the rate of their formation was found to be around 0·2–0·4 nmoles mg protein^{-1} min^{-1}.

Table 4. Quantities of metabolites excreted in rat urine within 12 hr following oral administration of phenothiazine drugs

Drug	Dose	Metabolite	Quantity (% of the dose)
CF_3-Per	10 mg/kg	CF_3-Per-SO	0·8
		CF_3-PPP-SO	2·6
Flu	10 mg/kg	Flu-SO	1·45
		CF_3-PPP-SO	0·5
Cl-Per	10 mg/kg	Cl-Per-SO	0·95
		Cl-PPP-SO	2·2
		Cl-Per-NO	ND
	25 mg/kg	Cl-Per-SO	0·8
		Cl-PPP-SO	2·8
		Cl-Per-NO	0·05
	50 mg/kg	Cl-Per-SO	0·8
		Cl-PPP-SO	2·2
		Cl-Per-NO	0·07
Pph	10 mg/kg	Pph-SO	3·3
		Cl-PPP-SO	0·6

The values are means of 2–5 experiments on two animals each.
ND—Not detectable.

Pretreatment of animals with phenobarbital led to an increase in the dealkylation rate and to a slight enhancement of the sulfoxidation. *N*-Oxides, however, were produced at a lower rate, a feature already described for dimethylaniline[25] and perazine[26] as substrates.

Quantities of urinary metabolites. The results are presented in Table 4. A small percentage of the dose only was excreted in urine during the first 12 hr, so that not many conclusions can be drawn concerning the fate of the drugs in the organism. There is, however, a good agreement between *in vitro* and *in vivo* findings with regard to the dealkylated fraction. Following administration of CF_3-Per or Cl-Per, the majority of the urinary sulfoxides was in the form of the secondary amines, while rats treated with Flu or Pph excreted primarily the sulfoxides of the undegraded drugs. This indicates that also *in vivo* the *N*-methyl group was split off faster than the *N*-hydroxyethyl group.

A further agreement between *in vitro* and *in vivo* results can be seen in the observation that increasing the Cl-Per dose led to a slight increase in the percentage excreted in the form of the *N*-oxide. This fraction, however, was still very small following a dose of 50 mg/kg. A possible explanation for this fact may be sought in the ability

of *N*-oxides to be reduced *in vivo* to the parent amines.[27,28] An alternative explanation can be inferred from the results in Fig. 2: The actual drug concentration at the site of the enzyme may have been so low *in vivo* that *N*-oxidation was in fact a minor biotransformation pathway. An argument in favour of low substrate concentrations in the endoplasmic reticulum can be derived from the observation that increasing the Cl-Per dose from 10 to 50 mg/kg did not result in a reduction of the Cl-PPP-SO fraction in urine, i.e. there was no indication of a substrate inhibition of demethylation.

Attempts to find phenolic metabolites free or as glucuronides in the urine of rats treated with Cl-Per or Flu proved unsuccessful.

The recovery of a small fraction of the drug dose only in the form of urinary metabolites is in accordance with observations by Melikian and Forrest.[29] Following administration of ^{3}H-Cl-Per rabbits excreted 9·6 per cent of the radioactivity in urine within the first 24 hr, and guinea-pigs excreted 4·9 per cent, part of the activity being in the form of phenols. Since in rats the threshold for biliary excretion of organic anions is lower than in rabbits and guinea-pigs,[30] phenolic glucuronides of Cl-Per can be expected to occur in the urine of the latter two species but not in that of the rat.

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