Biochemical Pharmacology, Vol. 26, pp. 1275–1280. Pergamon Press. 1977. Printed in Great Britain.

ABSORPTION AND METABOLISM OF THE PHENOTHIAZINE DRUG PERAZINE IN THE RAT INTESTINAL LOOP

URSULA BREYER and DIETRICH WINNE

Institut für Toxikologie and Abteilung für Molekularpharmakologie, Pharmakologisches Institut, 7400 Tübingen, West Germany

(*Received* 15 *September* 1976; *accepted* 3 *January* 1977)

Abstract—Jejunal loops of male rats were instilled with [^{35}S]perazine and venous blood from the loops was collected. Plasma, erythrocytes, intestinal wall and intestinal contents were analysed for perazine and its metabolites by reverse isotope dilution; purification to constant specific radioactivity was carried out by thin-layer chromatography. Within 60 min, 57 per cent of the material appeared in blood, more than four-fifths in the form of unchanged perazine. The principal metabolites present in plasma were 3-hydroxyperazine glucuronide and perazine sulfoxide; besides the sulfoxide, red cells contained small quantities of desmethyl perazine. This metabolite was predominantly located in the intestinal wall which contained a total of 17 per cent of the administered radioactivity, mostly as unmetabolized perazine. Another 17 per cent was found in the intestinal contents and here the proportion of perazine sulfoxide was one-third. Besides perazine as the major compound small amounts of hydroxyperazine glucuronide and desmethyl perazine and traces of perazine *N*-oxide were present in the intestinal lumen.

An oral availability of distinctly less than unity has been demonstrated for phenothiazine neuroleptics in man [1–3] and rat [4–6]. However, the urinary excretion of chlorpromazine metabolites did not differ whether patients had been treated orally or intramuscularly [3]; these findings are in accordance with data obtained on tricyclic antidepressants administered *via* various routes [7–9]. Therefore, absorption was assumed to be complete and the reduction in availability was ascribed to a pronounced first-pass metabolism. Though the liver is regarded to play the major role in this process [10], the contribution of the intestinal mucosa must also be taken into consideration.

Curry and co-workers found similar chlorpromazine plasma levels in rats after i.v. and i.p. injection [5] and hence postulated that the decomposition largely took place in the intestinal wall [6]. Experiments in which a [^{35}S]chlorpromazine solution was circulated through the lumen of the rat jejunum *in vitro* were reported to verify this assumption in that a large part of the radioactivity which appeared at the serosal side was not identified as chlorpromazine [6]. This study suffered from several drawbacks since the intestinal preparation lacked a blood supply, a large part of the drug was adsorbed to the apparatus and metabolites produced were not identified.

In the present study, an intestinal loop preparation of high functional integrity [11, 12] was used for studies on the disposition of a ^{35}S-labelled phenothiazine drug with a piperazine ring in the side chain. This drug, perazine, is extensively used as a neuroleptic in some countries. Its metabolism largely proceeds *via* the pathways described for other phenothiazines [13, 14]. Specific determination of the drug and its metabolites in the experimental compartments was carried out by reverse isotope dilution analysis.

MATERIALS AND METHODS

Drug and metabolites

[^{35}S]Perazine (Per) as dimalonate was supplied by Byk Gulden (Konstanz, Germany). Its specific activity was 4 mCi/m-mole at the beginning of the experiments and 1.7 mCi/m-mole at their end. The radiochemical purity was 92%; therefore the material was purified by thin-layer chromatography (TLC) in solvent A (Table 1) with subsequent isolation from the gel by distribution between 2 N ammonia and benzene. The substance then was homogeneous upon TLC. It was dissolved in the calculated quantity of 0.1 N HCl and adjusted with 0.9% NaCl to a concentration of approximately 450 nmol/ml. The exact concentration and the specific radioactivity were determined by subjecting weighed aliquots to spectrophotometry and scintillation counting. In some control experiments C^3H_3-perazine with a specific activity of 60 mCi/m-mole was used which had been prepared by reacting desmethyl perazine with tritiated methyl iodide*. Unlabeled Per dimalonate was a gift from Chemische Fabrik Promonta (Hamburg, Germany).

The following perazine metabolites were prepared by previously described methods: perazine sulfoxide (Per-SO) [13], desmethyl perazine (DMP) [15] and *N*-[γ-(phenothiazinyl-10)-propyl]-ethylene-diamine (PPED) [16]. Perazine *N*-oxide (Per-NO) was isolated from the urine of patients treated with perazine [13]. 3-Hydroxyperazine (OH-Per) was obtained from the bile of rats given 50 mg/kg Per i.p. Male or female

* I. Jahns and U. Breyer, to be published.

rats were anesthetized with urethane (0.9 g/kg), a teflon tubing was inserted into the common bile duct and after administration of an isotonic neutralized Per solution (2 ml/kg) bile was collected for 4–8 hr. It was diluted with saline to 10 ml, adjusted to pH 9 with 0.25 N sodium hydroxide and extracted twice with 5 ml of chloroform. After addition of 0.1 N acetate buffer pH 4.5 (2 ml for 1 g of bile) and 0.2 ml of β-glucuronidase/arylsulfatase (Boehringer, Mannheim, Germany), the bile was incubated at 37° for 3.5 hr. Liberated phenols were extracted at pH 9 with two 5-ml-portions of chloroform and purified by TLC in solvent C (Table 1). The identity of OH-Per was proven by mass spectrometry using a MS 902 S (A.E.I., Manchester, England) with direct inlet. At 70 eV and 120° the substance gave the expected mole peak at m/e 355. The spectrum was identical with that of OH-Per isolated from the urine of Per-treated patients [13].

Preparation of the intestinal loop

In urethane-anesthetized male Wistar rats (300–350 g), a jejunal loop 13–25 cm from the *flexura duodenojejunalis* and 4.5–6 cm long was prepared as described previously [11, 12, 17]. A weighed aliquot (0.51–0.54 g) of the [^{35}S]Per solution was instilled into the closed loop and blood was sampled from the vein for three successive 20 min-periods. The blood flow amounted to 0.3–0.4 g/min corresponding to 0.8–1.2 ml/min per gram of tissue. Aliquots were taken for the determination of hematocrit and total radioactivity, then the samples were centrifuged and plasma and red cells stored at −20°. The intestinal contents were collected together with 1 ml of saline used for washing the loop.

Drug and metabolite measurements

Determination of total radioactivity. Blood (100 μl) was solubilized in 1 ml of a 1:1 mixture of isopropanol and soluene^{R}100 (Packard Instrument, Frankfurt, Germany) and decolourized with 0.4 ml of 30% hydrogen peroxide. After 15 min, 10 ml of a scintillation mixture (2 g of PPO + 25 mg of dimethyl-POPOP + 400 ml of ethanol + 600 ml of toluene) were added and the samples were kept at 37° for 3 hr. Then luminescence had declined and they could be counted in a liquid scintillation counter. According to internal standardization, counting efficiency was 78 per cent.

Weighed aliquots of plasma (about 50 mg), intestinal contents (10 mg) and intestinal wall homogenate (corresponding to about 7 mg of tissue) were dissolved in 200 μl of 1 M hyamine^{R} hydroxide, decolourized with benzoyl peroxide during 1 day at room temperature and subjected to liquid scintillation counting after addition of 10 ml of Bray's solution [18].

Determination of single compounds. To plasma and erythrocyte samples, 0.2 ml each of aqueous solutions (1 mg/ml) of perazine, Per-SO and DMP were added, to plasma also a solution containing a known quantity (150–200 μg) of OH-Per. In some experiments, plasma was spiked with Per-NO in addition and red cells with OH-Per. Per gram of original material 0.25 g of NaCl, 0.02 ml of sodium deoxycholate solution (10%, w/v) and 0.1 ml of 0.2 N NaOH were added[15] and extraction was carried out with four 2 ml-portions of benzene. From the evaporated extracts, 2% were taken for ^{35}S counting, the remainder was subjected to TLC on 20 × 20 cm plates manually coated with silica gel (Kieselgel GF_{254}, Merck, Darmstadt, Germany). With two 0.1 ml portions of chloroform the material was transferred to a band of 4 cm width. The plate was pre-run to the upper edge with chloroform–isopropanol (10:1, v/v), dried for 3 min and developed in solvent A (Table 1). After drying for 7 min and spraying with water, u.v. absorbing bands were removed, suspended in 1 ml of 2 N ammonia and extracted twice with 2 ml of chloroform. The residue of the organic phase was dissolved in a defined volume of 1.2-dichloroethane and aliquots were taken for the determination of absorbance and radioactivity. While the ratio of these two values did not change upon rechromatography of Per and Per-SO in other solvents, DMP and Per-NO needed purification in solvent B, OH-Per in solvent C and PPED in solvent D (Table 1) in order to attain a specific activity which was not altered by further chromatographic steps. In order to additionally verify the identity of Per-NO, it was reduced with titanous chloride to Per and purified in this form in solvent A.

Plasma extracted with benzene was subsequently shaken twice with 2 ml of chloroform. This extract contained only traces of radioactivity and therefore was discarded. The aqueous phase was adjusted to pH 4.8 with 0.25 N acetic acid, mixed with a solution of 150 μg of OH-Per and 50 μl of β-glucuronidase/arylsulfatase and incubated at 37° for 3.5 hr. Then 0.25 N NaOH was added to bring the pH to 9 and extraction was carried out three times with 2 ml of benzene. The extract was purified by TLC in solvent C.

To the intestinal wall solutions of Per, DMP, Per-SO, OH-Per and in some cases PPED were added. After addition of 0.05 ml of 10% sodium deoxycholate solution and sufficient 10% (w/v) NaCl to make a total weight of 5 g it was homogenized with an Ultra Turrax blender (Janke & Kunkel, Staufen, Germany). An aliquot of about 100 mg was taken for total radioactivity determination, the remainder was mixed with 0.5 ml of 25% ammonia, shaken with

Table 1. R_f values of Per and metabolites in TLC on silica gel

Compound	R_f value in solvent*			
	A	B	C	D
Per	0.72	0.36	0.85	0.65
OH-Per	0.61	0.32	0.43	0.60
Per-SO	0.40	0.14	0.58	0.28
DMP	0.26	0.38	0.48	0.28
PPED	0.13	0.31	0.23	0.56
Per-NO	0.11	0.38	0.06	0.28

* A. isopropanol–chloroform–25% ammonia–water (16:8:1:1, v/v).
B. 1.2-dichloroethane–ethyl acetate–ethanol–acetic acid–water (15:26:12:8:7.5, v/v).
C. chloroform–isopropanol (20:2, v/v), atmosphere saturated with ammonia by placing into the tank a trough with 3 ml of 25% ammonia.
D. acetone–isopropanol–1 N ammonia (9:7:4, v/v).

15 ml of benzene and centrifuged. An aliquot of the organic phase was evaporated and subjected to TLC as described above.

The intestinal contents were mixed with solutions of Per, Per-SO, DMP and OH-Per and in a few cases Per-NO. The pH was adjusted to 9 with 0.25 N NaOH and extraction was carried out with three 2 ml-portions of chloroform. After removal of 2% of the extract for radioactivity measurement the rest was separated by TLC as described for the other tissues. To the aqueous phase another portion of OH-Per was added and incubation with β-glucuronidase/arylsulfatase, extraction and TLC were carried out as with plasma.

Pre-extracted plasma and intestinal contents from one experiment were divided into to equal parts and after addition of OH-Per incubated with β-glucuronidase/arylsulfatase and with the equivalent quantity of pure β-glucuronidase (Serva, Heidelberg, Germany), respectively, in parallel.

Dichloroethane solutions (0.05–0.2 ml) of the chromatographically purified compounds were shaken with 3 ml of 0.1 N H_2SO_4 and the absorbance of the aqueous solution was read in a spectrophotometer at 1 cm lightpath. A_{255}–A_{280} was used for calculating the amounts of non-phenolic sulfides. An absorbance difference of 1.000 corresponded to 123.8 nmol of Per or 124.4 nmol of DMP or 121.4 nmol of PPED in 3 ml. Per-SO was evaluated from A_{270}–A_{285}, a difference of 1.000 corresponding to 470 nmol/3 ml. OH-Per was reacted with sulfanilic acid and $NaNO_2$ to a purple dye [13] the absorbance of which was measured at 555 and 600 nm. With the aid of experiments on [^{35}S]Per without isotope dilution* it was found that 293 nmol/3 ml result in an A_{555}–A_{600} value of 1.000.

Control experiments were carried out in order to account for sulfoxide formation during the work-up. Known quantities of [^{35}S]Per and cold Per-SO were added to blood or plasma, intestinal wall and intestinal contents of untreated rats and the samples were processed in the way described.

Calculations. Since complete separation of plasma from erythrocytes was not possible, the amount of substances that was due to plasma entrapped in the erythrocyte fraction was calculated with the aid of the hematocrit. The calculated plasma and erythrocyte weights were compared with the experimental values and quantities of Per and metabolites found in the fractions were corrected accordingly. Besides, corrections were introduced for quantities of material taken off before addition of unlabeled substances.

RESULTS

Structural formulas of perazine and its metabolites formed in intestine are shown in Fig. 1.

Substances appearing in intestinal venous blood. The proportion of the administered radioactivity drained by the blood was 22.6 ± 1.7% during the first 20 min-period, 20.3 ± 1.4% during the second and 14.0 ± 1.1% during the third (mean ± S.E.M., n = 7). Separate analysis of single compounds in red blood

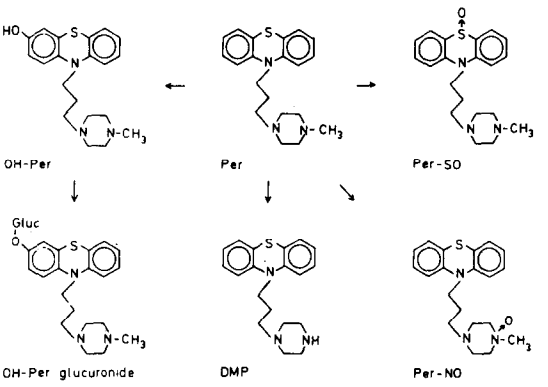

Fig. 1. Structures of perazine and its metabolites formed in the rat intestine *in vivo*.

cells and plasma gave the results presented in Fig. 2. Unchanged Per accounted for 77% of the substances occurring in plasma and for 90% of those in red cells. The major metabolite found in erythrocytes was Per-SO, while in plasma the concentration of OH-Per conjugates usually exceeded that of the sulfoxide during the first two 20 min-periods. The phenol was nearly exclusively conjugated with glucuronic acid since the OH-Per quantity liberated by pure β-glucuronidase in three plasma samples was 90.5, 94 and 100.5% of the quantity liberated by β-glucuronidase + arylsulfatase. Red cells were not analyzed for the conjugate, since phenolic glucuronides of similarly lipophilic substrates were found to be excluded from these cells [17, 19]. DMP was a minor metabolite in both blood compartments.

Since the mean hematocrit was 50%, the average Per concentration in erythrocytes was 1.8-fold (range 0.75–2.44-fold) that in plasma and the Per-SO concentration 1.35-fold. The preferential localization in red cells was even more marked with DMP which attained 7-fold (range 4–11-fold) higher levels in erythrocytes than in plasma.

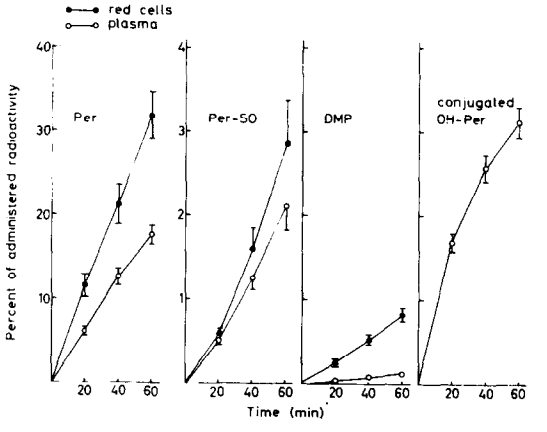

Fig. 2. Cumulative appearance of Per and metabolites in venous blood of rat jejunal loops. [^{35}S]Per (246 ± 2 nmol) was instilled into closed loops weighing 0.37 ± 0.07 g. The average amount of venous blood collected was 6.7, 7.2 and 7.1 g for the first, second and third 20 min-period, respectively. Values are means ± S.E.M., n = 7 except for Per-SO in red cells where n = 5.

* Breyer *et al.*, to be published.

Table 2. Chemical oxidation of Per to Per-SO upon tissue analysis

Material	Fraction of [^{35}S]Per converted to [^{35}S]Per-SO	n
Plasma	1.0 ± 0.3%*	5
	1.1 ± 0.06%†	3
Erythrocytes	5.3 ± 1.3%†	8
Intestinal wall	2.8 ± 0.2%	4
Intestinal contents	3.2 ± 0.3%	3
	3.7 ± 1.6%‡	6

Means ± S.D.
* Per was added to plasma.
† Per was added to whole blood and after 90 min at 0° plasma and erythrocytes were separated by centrifugation.
‡ After addition of Per, samples were incubated for 60 min at 37°.

Whereas the quantity of Per absorbed within 20 min slightly decreased towards the end of the experiment and OH-Per glucuronide exhibited a clear-cut decrease, more Per-SO was present in blood collected at later times. This must be due to the accumulation of Per-SO in the intestinal contents (see below).

Traces of unconjugated OH-Per were detected in plasma especially from the first sampling period. The quantities were, however, too small for a reliable determination. Some plasma samples were analyzed for Per-NO, but the presence of the metabolite could not be proven unequivocally.

The *in vitro* oxidation of Per to Per-SO during the extraction procedure was found to occur to an extent which could not explain the Per-SO concentrations present in the experimental samples. According to the results of control experiments (Table 2), the contribution of Per-SO resulting from chemical oxidation to the total Per-SO measured in red cells was about one-third. There was no correlation between Per and Per-SO concentrations in erythrocytes having passed the loop. A substantially larger quantity of Per (around 15%) was converted to Per-SO when the cells were extracted with chloroform instead of benzene. Much less sulfoxidation occurred upon extraction of plasma, and the percentage of Per recovered as Per-SO did not differ whether the drug had been added to whole blood or to plasma. Per-SO values contained in Figs 2 and 3 are corrected for the artificial oxidation process.

The sum of the specifically determined substances in plasma and red cells accounted for 100.0 ± 2.9% (mean ± S.E.M.) of the total radioactivity in blood. This means that no quantitatively important metabolite could have been overlooked. No appreciable quantities of polar nonhydrolyzable metabolites occurred in plasma, since after enzymatic hydrolysis and extraction the radioactivity remaining in the water phase was only 3% of the activity originally present in plasma.

Substances present in intestine. After 60 min of blood perfusion, total radioactivity in the intestinal wall and intestinal contents accounted for 15.9 ± 1.2% and 15.0 ± 2.4% (mean ± S.E.M., n = 7), respectively, of the administered dose. The sum of the specifically measured compounds was higher by one-sixteenth and one-twelfth, respectively (Fig. 3). In the latter case this must have been due to the adsorption of lipophilic compounds to mucus which caused them to escape the radioactivity determination.

The proportion of the single compounds in the intestinal wall was similar to that in red cells since unchanged Per predominated by far and the major metabolite was Per-SO. The contribution of DMP to total ^{35}S was higher than in any other compartment and the amount present in about 0.37 g of tissue came close to the quantity found in around 21 g of blood. Unconjugated OH-Per was consistently present though at a low concentration. Attempts to find PPED gave a negative result.

Little more than half of the radioactivity remaining in the intestinal lumen was due to Per while 38% consisted of Per-SO (Fig. 3). This high concentration cannot be attributed to Per-SO production at this site, since only little more Per was converted to the sulfoxide upon incubation for 1 hr with intestinal contents and subsequent extraction than during the extraction alone (Table 2). OH-Per was mainly present in the conjugated form with glucuronic acid as the predominant conjugation partner. After hydrolysis with pure β-glucuronidase, the OH-Per quantity measured was 95% of the quantity liberated with additional arylsulfatase. A small fraction of the luminal contents was represented by DMP, a metabolite not formed *in vitro* by intestinal contents. The occurrence

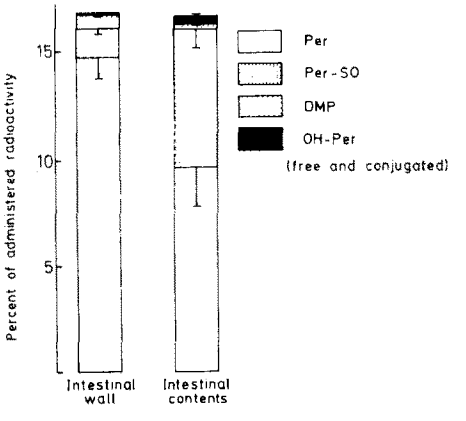

Fig. 3. Quantities of Per and metabolites contained in rat jejunal loops 60 min after instillation of 246 nmol of [^{35}S]Per. Vertical bars represent S.E.M., n = 7.

of Per-NO could definitely be proven though its quantity was only 0.02% of the dose.

Balance. Taking the data on all four compartments together, the sum of the specifically determined compounds corresponded to 92.4 ± 1.8 (S.E.M.) % of the administered Per. This quantity was composed of 74.2% of unchanged Per, 12.5% of Per-SO, 3.5% of conjugated and 0.2% of free OH-Per and 2% of DMP.

DISCUSSION

Oxidative biotransformation of foreign compounds by intestinal mucosa or microsomes prepared from it has been demonstrated in several species including the rat [20–22, see here for further references]. Intestinal microsomes from this species exhibited a relatively high activity in hydroxylating aromatic hydrocarbons, while aniline hydroxylase and ethylmorphine demethylase were not detected [21]. There was, however, measurable *O*-dealkylation activity towards *p*-nitroanisole [22].

The present investigation showed that aromatic hydroxylation of perazine was one of the major metabolic pathways in the rat intestine *in situ*. Terminal *N*-dealkylation also took place and the ratio of *N*-demethylation *vs.* aromatic hydroxylation was similar to the estimated ratio in the bile fistula rat* where metabolism proceeds primarily in the liver. DMP as a lipophilic metabolite was mainly localized in intestinal tissue and erythrocytes. Piperazine ring degradation by *N*-dealkylation[23] could not be detected.

A conspicuously large quantity of perazine was recovered as the sulfoxide. This finding is at variance with negative results obtained upon incubation of [^{35}S]perazine with rat intestinal microsomes [24]. Therefore Per-SO formation by systems other than the mixed-function oxidase of the mucosa had to be taken into consideration. Data obtained on chlorpromazine sulfoxidation by blood and hemoglobin [25] leave doubt as to the occurrence of this reaction during incubation. A lack of correlation between Per and Per-SO concentrations in erythrocytes from the perfusion experiments argues against predominant formation of the sulfoxide in blood. Besides, in control experiments no Per was oxidized to Per-SO upon storage of whole blood at 0° since the proportion of sulfoxide obtained from plasma was the same as when Per had been added to plasma. Thus it must be concluded that the artificial oxidation took place during extraction, particularly because its extent depended on the solvent used. When Per-SO quantities formed *in vitro* were subtracted from the total amount, there remained a considerable difference which indicated sulfoxidation during the perfusion. Intestinal contents could be excluded as the site of Per-SO production, therefore the mucosa is the tissue which most probably carried out the conversion. About half of the total Per-SO was found in the lumen from which it seemed to be absorbed very slowly due to its hydrophilicity.

A high conjugating activity of intestinal mucosa towards phenols has repeatedly been described [17, 20, 26]. Therefore it was not surprising that most of the OH-Per formed appeared as the glucuronide. The majority was recovered from plasma while a small fraction was secreted into the lumen. OH-Per glucuronide was the only metabolite not accumulated in the intestinal wall or contents, such that its appearance rate in plasma can be assumed to reflect its rate of formation. The decrease in the rate with time indicated that under the conditions used the enzymes producing it were not saturated at least at later times. The administered quantity of 246 nmol or 83.5 μg for an intestinal loop of 5 cm length when extrapolated to the entire duodenum and jejunum (60 cm) would correspond to 1 mg or about 3 mg/kg body weight.

It can be concluded that even small doses of this phenothiazine which in relation to body weight do not exceed low therapeutic doses are largely absorbed unchanged from the rat intestine. Intraperitoneal injection instead of oral dosage of phenothiazines and chemically related drugs with the intention to circumvent intestinal first-pass elimination does not seem justified. This applies particularly to experiments of longer duration or with repeated administration since the drugs are known to cause intestinal adhesions and megacolon [27]. From the comparison of the present results with data on trifluoperazine kinetics in the rat† it is clear that a much larger proportion of these drugs is removed by hepatic first-pass elimination than during passage through the gut wall.

Acknowledgements—The authors wish to thank Chemische Fabrik Promonta, Hamburg, and Byk Gulden, Konstanz, Germany, for the gift of [^{35}S]perazine and Dr. I. Jahns for the preparation of [^{3}H]perazine. They are grateful to Prof. Dr. A. Prox, Thomae GmbH, Biberach, for measuring the mass spectra. Thanks are due to Miss S. Rehmer for excellent technical assistance. The work has been financially supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

1. S. H. Curry, J. M. Davis, D. S. Janowsky and J. H. L. Marshall, *Archs Gen. Psychiat.* **22,** 209 (1970).
2. S. H. Curry, J. H. L. Marshall, J. M. Davis and D. S. Janowsky, *Archs Gen. Psychiat.* **22,** 289 (1970).
3. L. E. Hollister, S. H. Curry, J. E. Derr and S. L. Kanter, *Clin. Pharmac. Ther.* **11,** 49 (1970).
4. K. Zehnder, F. Kalberer and J. Rutschmann, *Biochem. Pharmac.* **11,** 551 (1962).
5. S. H. Curry, J. E. Derr and H. M. Maling, *Proc. Soc. exp. Biol. Med.* **134,** 314 (1970).
6. S. H. Curry, A. D'Mello and G. P. Mould, *Br. J. Pharmac.* **42,** 403 (1971).
7. B. Alexanderson, O. Borgå and G. Alvan, *Eur. J. Clin. Pharmac.* **5,** 181 (1973).
8. L. F. Gram and J. Christiansen, *Clin. Pharmac. Ther.* **17,** 555 (1975).
9. L. F. Gram and K. Fredericson-Overø, *Clin. Pharmac. Ther.* **18,** 305 (1975).
10. M. Gibaldi and S. Feldman, *Eur. J. Pharmac.* **19,** 323 (1972).
11. D. Winne, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmac.* **254,** 199 (1966).
12. H. Ochsenfahrt and D. Winne, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmac.* **264,** 55 (1969).
13. U. Breyer, *Biochem. Pharmac.* **18,** 777 (1969).
14. U. Breyer, *Biochem. Pharmac.* **20,** 3341 (1971).
15. U. Breyer and K. Villumsen, *Eur. J. Clin. Pharmac.* **9,** 457 (1976).

* Breyer, Jahns *et al.*, to be published.

† G. Schmalzing, *Drug Metab. Dispos.*, in press.

16. U. Breyer, W. Junginger and D. Villumsen, *Biochem. Pharmac.* **25,** 2623 (1976).
17. K. W. Bock and D. Winne, *Biochem. Pharmac.* **24,** 859 (1975).
18. G. A. Bray, *Analyt. Biochem.* **1,** 279 (1960).
19. C. von Bahr and O. Borgå, *Acta pharmac. tox.* **29,** 359 (1971).
20. K. Hartiala, *Physiol. Rev.* **53,** 496 (1973).
21. R. S. Chhabra, R. J. Pohl and J. R. Fouts, *Drug Metab. Dispos.* **2,** 443 (1974).
22. H. Hoensch, C. H. Woo, S. B. Raffin and R. Schmid, *Gastroenterology* **70,** 1063 (1976).
23. U. Breyer, D. Krauss and J. C. Jochims, *Experientia* **28,** 312 (1972).
24. R. Knoll, W. Christ and B. Müller-Oerlinghausen, *Naunyn-Schmiedeberg's Arch. Pharmac.* **293,** Suppl. R 54 (1976).
25. R. Minder, F. Schnetzer and M. H. Bickel, *Naunyn-Schmiedeberg's Arch. Pharmac.* **268,** 334 (1971).
26. D. Josting, D. Winne and K. W. Bock, *Biochem. Pharmac.* **25,** 613 (1976).
27. H. I. Vapaatalo, J. E. Idänpään-Heikkilä, P. J. Neuvonen and M. K. Paasonen, *Acta pharmac. tox.* **27,** 262 (1969).