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DISTRIBUTION AND FATE OF ^{3}H-OXYPERTINE IN RATS

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Abstract—The distribution and fate of tritiated oxypertine (Win 18,501-T) was studied in rats after intravenous administration.

Brain and lung became radioactive in 10 min, but the activity quickly disappeared. The highest activities at times between 30 min–72 hr after administration were found in glandular tissues. Lacrimal and salivary glands, liver and kidney showed a high activity, and even in testes, vesicula seminalis and the walls of stomach and duodenum the concentrations were higher than in blood. In adrenals the radioactivity increased during the first 3 hr and at later times the adrenals were the most active tissue of the rat.

In nonanaesthetized rats half the radioactivity was excreted in 18 hr. Most was found in urine, about 20 per cent in faeces and very small amounts in expired air. In anaesthetized rats bile contained more radioactivity than urine during the first 8 hr but later the proportion in urine increased. About 4 per cent of the dose was excreted in the gastric juice in 24 hr. Practically all the radioactivity in bile and urine was in the form of metabolites of oxypertine. Chromatographic and mass spectrometric analysis indicated that oxypertine is metabolized by *O*-demethylation and by alcoholic and phenolic hydroxylations. Dihydroxylated derivatives were found as major metabolites. The alcoholic metabolites were partly oxidized further to the corresponding acid and the demethylation product was found as a chinone imine.

OXYPERTINE (Win 18,501) is a psychosedative indole-ethyl-phenylpiperazine derivative (1-[2-(5,6-dimethoxy-2-methyl-3-indolyl)ethyl]-4-phenylpiperazine) that has been used in clinical trials since 1963. Its chlorpromazine-type tranquillizing effect seems to originate from its central sympatholytic action and the release of brain noradrenaline.[1] It may be more specific than reserpine in releasing noradrenaline although in high doses it releases brain dopamine and 5-hydroxytryptamine as well.[2–6]

Our previous paper described the distribution of tritiated oxypertine in mice and some organs of cats measured by total body autoradiography.[7] The aim of this study was to investigate the fate of oxypertine in rats.

MATERIAL AND METHODS

The unlabelled oxypertine was used as hydrochloride (Win 18,501-2). The labelled oxypertine (Win 18,501-T) was made by the Radiochemical Centre, Amersham, England by tritiating the H-atoms at the positions 4 and 7 in the indole nucleus. Its specific activity (using TRR 1 *n*-hexadecane-1,2-T as reference material) was 455 mc/mM or 1·2 mc/mg and the radiochemical purity 95 per cent.

Male Sprague–Dawley rats were used in all experiments. When *non-anaesthetized rats* (weight 130–180 g) were used they had standard rat food (Hankkija, Helsinki) before the experiment. Oxypertine was dissolved in a small volume of acetic acid, diluted with water containing some drops of alcohol and injected into a tail vein in a dose of 1 μc/g (double dose for metabolic studies) corresponding to 0·83 μg/g body weight. Then the rats were kept separately in metabolism cages for the collection of urine and faeces. They could drink water *ad lib.* but were without food if the experiment lasted 24 hr or less. During longer experiments the rats were allowed to eat standard rat food for 20 min once a day.

At the end of the experiment, the rats were killed by decapitation. Some rats, however, were anaesthetized with ether and blood samples were taken from the abdominal aorta into polyethylene tubes. The tissues were homogenized in three volumes of 10% trichloroacetic acid in a glass homogenizer of Potter–Elvehjem type, except adrenal glands, pineal, hypophysis and lacrimal glands where higher dilutions were used. After centrifugation at 2500 *g* for 15 min the activity of the clear supernatant was measured in glass-wool paper by liquid scintillation counting according to the method reported by Slot[8] (1965). For correction of the quenching effect ^{3}H-oxypertine, 1–10 μc/ml was used as an internal standard and values were converted to the 100 per cent recovery.

Anaesthetized rats (weight 180–250 g, urethane 1·2 g/kg) were used for simultaneous collection of bile and urine. Gastric juice was also collected from some of them. The bile duct and urinary bladder were cannulated, the abdomen closed and tap water (3·5 per cent of rat weight) given via a stomach tube before a slow injection of tritiated oxypertine into a jugular vein. When gastric juice was also collected, the animals were kept on liquid food for 1 day and fasted another day before the experiment but received water *ad lib.* The pylorus was ligated and a collection cannula for the gastric juice was inserted through the pylorus. To prevent the rat swallowing saliva the oesophagus was ligated beneath the diaphragm leaving vagus nerves undamaged outside the ligature if possible. In order to increase the gastric juice volume, histamine (0·40–0·75 mg/kg s.c.) was administered 2–7 hr after the ^{3}H-oxypertine injection. The loss of fluid volume was corrected by giving saline and Ringer solution subcutaneously. The radioactivity of bile and gastric juice was measured in the same way as the tissue samples. Urine samples were pipetted directly into the glass-wool paper.

For measuring the *expired radioactivity*, some rats were kept in a special cage where the expired air flowed through a piece of glass-wool paper and twice through absolute alcohol. The radioactivity (^3H_2O) taken up by the glass-wool and alcohol was measured as before.

Thin layer chromatography (TLC) was performed on glass plates coated with silica gel G to 0·25 or 1·00 mm thickness. Solvent systems used were:

(I) isopropanol–ammonia–water (8:1:1),

(II) *n*-butanol–acetic acid–water (4:1:5).

In two dimensional chromatography the system (I) was carried out allowing the solvent to run to a height of 12–15 cm above the application spot. Before the second run in system (II) the plates were left to dry in air for 15 hr. One dimensional chromatography in solvent system (II) was used for preparative purpose. Urine was applied after partial desalting with ethanol in a line of 19 cm length and allowed to run to a height of 10 cm above the application line. Ten bands, each 1·0 cm in height were scraped off and

eluted by shaking with 10 ml methanol for 1 hr. The methanol eluate was centrifuged to remove the absorbent, evaporated to dryness and dissolved in *n*-hexane.

For fluororadiography the plates were coated with silica gel and antracene (50% ± 50%).[9] Solvent systems were as described above (I) and (II). For the localization of radioactivity the fluorograms were exposed to X-ray film (Kodak).

Preparation of derivatives for gas–liquid chromatography (GLC)

An ether solution of diazomethane (prepared by ether codistillation from Diazald, Aldrich Chemical Co.) was added to the methanol solution of urinary metabolites to convert all acids to methyl esters. The ether solution was added until a yellow colour persisted; the reaction time was 1–2 min (the reaction of diazomethane with organic carboxylic acids in ether–methanol solution is virtually completed when complete mixing is achieved). The ether, excess diazomethane and methanol were removed with the aid of a stream of nitrogen and the residue was dissolved in 0·15 ml of pyridine. After the addition of 0·1 ml of hexamethyldisilazane and 0·05 ml of trimethylchlorosilane the tube was stoppered tightly (Teflon-lined cap) and allowed to stand overnight. The mixture was centrifuged to remove the precipitate and the supernatant solution was used directly for GLC analysis.

The reaction with diazomethane, followed by reaction with hexamethyldisilazane-trimethyl-chlorosilane converts carboxylic acids to methyl esters, and alcohol and phenolic groups to trimethylsilyl ethers. Glucuronides are converted to methyl ester-trimethylsilyl ethers.

GLC analysis

A Perkin–Elmer F-11 gas chromatograph equipped with a hydrogen flame ionization detector was used. The column was a 6 ft × 3 mm coiled glass tube with a packing of 2·2% SE-30 on 80–100 mesh Gas Chrom Q. The coating was done according to the method of Horning *et al.*[10] For all the TLC fractions the analysis was carried out isothermally at 200° and for the conjugated fractions at R_f 10–30 also temperature programming 160–300° at the rate of 3°/min was used. Carrier gas was nitrogen, flow rate 20 ml/min.

Mass spectrometry

The instrument was a LKB 9000 combination GLC-mass spectrometer. Helium was used as carrier gas at a rate of 25 ml/min. The ionizing potential was 70 eV when the scanning was done.

RESULTS

1. *Distribution*

Oxypertine was rapidly taken up by the tissues. Ten minutes after the intravenous administration of tritiated oxypertine several tissues showed higher radioactivity than blood (Table 1). Although the drug passed rapidly into the *brain*, its concentration there was not very high in the 3-hr sample. A small residuum of radioactivity, however, remained in the brain for several days.

Skeletal muscles and *the heart* showed rather low radioactivity, but because of the large muscular mass involved it accounted for one third of the body radioactivity for half an hour after oxypertine administration. The concentration in *blood* was 2·4 μc/ml in 10-min samples, half this value at 30 min and about the same in 3-hr

TABLE 1. THE TISSUE CONTENTS OF RADIOACTIVITY (μc/g $\pm$ SD) AFTER AN INTRAVENOUS ADMINISTRATION OF TRITIATED OXYPERTINE (1μc/g) IN MALE RATS

Tissue	10 min	30 min	3 hr	24 hr	72 hr
Skeletal muscle	0·89 ± 0·16 (5)	0·96 ± 0·11 (3)	0·29 ± 0·14 (4)	0·24 ± 0·09 (6)	0·04 ± 0·03 (4)
Heart	0·92 ± 0·37 (5)	0·59 ± 0·24 (3)	0·45 ± 0·12 (4)	0·24 ± 0·10 (4)	
Brain	2·39 ± 0·87 (5)	1·63 ± 0·38 (3)	0·66 ± 0·18 (4)	0·21 ± 0·11 (6)	0·15 ± 0·04 (4)
Spleen	1·68 ± 0·45 (5)	1·16 ± 0·18 (3)	0·64 ± 0·21 (4)	0·13 ± 0·06 (5)	
Lung	2·52 ± 0·80 (5)	2·10 ± 0·12 (3)	1·25 ± 0·24 (4)	0·08 ± 0·04 (5)	
Stomach wall	1·23 ± 0·38 (4)	2·01 ± 0·34 (3)	1·96 ± 0·24 (2)	0·27 ± 0·16 (3)	0·14 ± 0·02 (2)
Duodenum	2·01 ± 0·55 (4)	1·45 ± 1·00 (3)	3·76 ± 1·06 (2)	0·30 ± 0·11 (3)	
Liver	3·90 ± 1·19 (5)	3·82 ± 0·87 (3)	5·02 ± 1·30 (4)	0·48 ± 0·45 (6)	0·13 ± 0·11 (3)
Kidney	5·10 ± 2·75 (5)	3·50 ± 1·50 (3)	3·40 ± 1·88 (4)	0·84 ± 0·52 (5)	0·31 ± 0·08 (3)
Extraorbital lacrimal gland	3·30 ± 0·80 (4)	3·60 ± 0·53 (2)	2·01 ± 0·61 (4)	0·13 ± 0·09 (3)	0·02 ± 0·03 (2)
Sublingual and submandibular glands	2·45 ± 1·56 (4)	1·06 ± 0·32 (2)	1·14 ± 0·89 (4)	0·05 ± 0·05 (3)	0·02 ± 0·02 (2)
Adrenal glands	2·23 ± 1·96 (4)	2·72 ± 0·50 (2)	2·48 ± 3·03 (3)	1·74 ± 0·80 (4)	0·32 (1)
Thymus	0·77 ± 0·38 (4)	0.68 ± 0·02 (2)	0·30 ± 0·00 (2)	0·07 ± 0·03 (5)	0·04 ± 0·05 (2)
Testis	0·96 ± 0·25 (4)	1·28 ± 0·37 (2)	0·85 ± 0·50 (4)	0·37 ± 0·38 (6)	0·22 ± 0·06 (4)
Fat	0·79 ± 0·23 (5)	1·95 ± 0·96 (2)	0·90 ± 0·68 (4)	0·11 ± 0·06 (5)	0·13 ± 0·00 (2)
Gut with contents	1·63 ± 0·18 (2)	1·30 (1)	10·12 ± 0·24 (2)	1·13 (3)	0·03 ± 0·02 (2)
Blood	2·4 ± 0·8 (3)	1·2 ± 0·7 (2)	0·98 ± 0·03 (2)		0·1 ± 0·08 (3)
Hypophysis			0·26 (2)		

The number of animals in parentheses.

samples. Some activity was demonstrated in the blood for three days. The *spleen* usually contained less activity than the blood. The *lungs* showed considerable activity at first, but it soon decreased.

The highest radioactivity was found in organs that may be excretory paths for oxypertine metabolites. After 10 min, the *kidney* was the most radioactive tissue and always contained at least twice the activity of blood and brain. The *liver* was about as active and 3 hr after the administration, contained more of the total radioactivity than the whole musculature. The *duodenum* and *stomach wall* showed considerable continuous activity, but a part of this may have been caused by small residues of

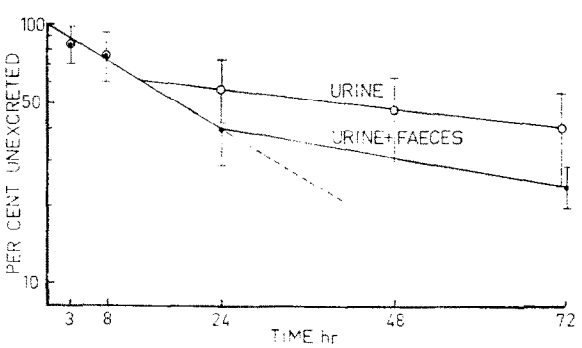

FIG. 1. Excretion of radioactivity in urine and faeces of nonanaesthetized rats after i.v. administration of tritiated oxypertine plotted on half logarithmic paper for the calculation of the initial rate half time.

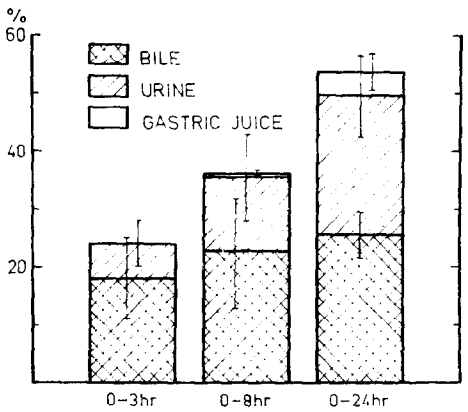

FIG. 2. Excretion of radioactivity in bile, urine and gastric juice of five anaesthetized rats after i.v. administration of tritiated oxypertine.

gastrointestinal contents in the folds of mucosa, because *gastric juice* and *bile* were more active (Fig. 2).

Exocrine and endocrine glands accumulated oxypertine and/or its metabolites. The *lacrimal glands* showed a remarkable radioactivity during the first 3 hr. Slightly lower values were found in *salivary glands*. In the *adrenals* the amount of radioactivity was at first similar, but the activity was retained for longer so that this later became the most active tissue of the rat. The *testes* collected only a moderate concentration of radioactivity over a long period. Thus, the testes later contained slightly more activity than the brain. Two *epididymis* samples measured had slightly higher activity than the testes of the same rats. The same was true for 10 and 30 min samples of *seminal vesicles*, but after 1 day they contained less activity. The *thymus* did not concentrate oxypertine.

To evaluate the amount of radioactivity in the *enterohepatic circle* the gut from the pylorus to the anus was measured with its contents. The values were high especially in 3-hr samples corresponding to the strong excretion in the bile within this time.

2. *Excretion*

Urinary excretion of the radioactivity during the first 3 hr corresponded to about 5 per cent of the dose per hour, and it decreased gradually (Fig. 1).

Within 24 hr 43·2 $\pm$ 11·6 (SD) per cent of the dose was excreted in urine and 16·3 $\pm$ 10·8 per cent in faeces. The total excretion of radioactivity from the body showed a faster initial rate with a half life of 18 hr and a slower rate later. The excretion in 72 hr was 55·5 $\pm$ 13·8 and 19·7 $\pm$ 4·7 per cent in urine and faeces respectively, thus resulting in a total of 75·2 per cent of the dose.

Expired air contained a just-detectable amount of radioactivity, when collected for 6 hr after the ^{3}H-oxypertine administration. When urine and faeces were kept in a current of air and the air measured in the same way, small traces of activity were found. Neither of these values were a significant part of the total excretion values.

In anaesthetized rats (Fig. 2) the main excretion of radioactivity occurred via *bile* during the first 8 hr, but later the activity in bile was low, and within 24 hr about the

same level was found in bile and urine. The *gastric juice* also contained some radioactivity and 4 per cent of the dose was excreted via this route in 24 hr. Because bile and gastric juice were taken out the elimination of radioactivity from the body was faster than in the nonanaesthetized rats; the anaesthetized rats excreted 50 per cent of the dose within 11 hr.

3. *Metabolic studies*

TLC and paper chromatography. Most of the radioactivity in urine and practically all in the bile was in the form of metabolites. A very weak spot corresponding to oxypertine, however, was sometimes found in the urinary chromatograms. The presence of metabolites was also studied in extracts of brain and liver by paper chromatography and measuring its radioactivity. In chromatograms of both tissues a weak additional radioactive spot about R_f 0·50 in isopropanol–ammonium–water was seen 30 min after the oxypertine administration. The same spot was also developed from brain extracts 3 hr after the administration, though a major amount was still found in an area corresponding to oxypertine (about R_f 0·90). A considerable starting point activity, however, was evident at both times.

There were several metabolites to be found in urine and bile. Two-dimensional fluororadiography (Fig. 3) showed, especially in urine, two groups of metabolites, one with low (0–0·2) and another with higher (0·4–0·6) R_f-values both in acid and alkaline solvent systems. Most of the metabolites seemed to be present in both urine and bile, but spots 11 and 12 (Fig. 3) were among the major metabolites in bile though weak in urine, and some of the high R_f value spots were not found in bile fluororadiograms. After one dimensional layer chromatography of urine in butanol–acetic acid–water the radioactive R_f 0–0·2 zone was scratched from a silica plate and hydrolyzed with sulphate free β-glucuronidase and rechromatographed with the same system after deproteinization with methanol. The activity of the hydrolysate was measured using zonal scratching of the silica layer into the counting vials. The major radioactivity was now in R_f 0·4–0·6 zone, thus indicating that the radioactivity in the lower zone had been in the form of glucuronides, which released similar hydroxylated metabolites that were also found in free form. This radioactivity could be extracted into ethyl acetate, as well as the compounds that in the chromatograms of non-hydrolyzed urine run the same distance. A smaller amount of ethyl acetate extractable material running to higher R_f zones (maximum at R_f 0·80) was found after hydrolysis. In the latter area without hydrolysis weak spots were seen which in all the solvent systems used corresponded to two monohydroxylated reference compounds (the hydroxyls in indole-2-methyl and in the para-position of the terminal phenyl groups, respectively), but these were not major metabolites.

GLC and mass spectrometry. The layer chromatogram zones after the acid solvent run were scratched away, methylated and turned to trimethylsilyl derivatives and analyzed by gas chromatography. Table 2 shows the peaks that were additional to or much bigger than those in control samples made from the urine collected before oxypertine administration.

The radioactivity of different peaks were counted by taking in a separate run the gas directly into the counting vials through Teflon-glass tubes that were washed with the scintillation liquid. The gas chromatogram from the most radioactive zone (R_f 0·50–0·60) showed two major radioactive peaks. The larger (peak A in Table 2) of the

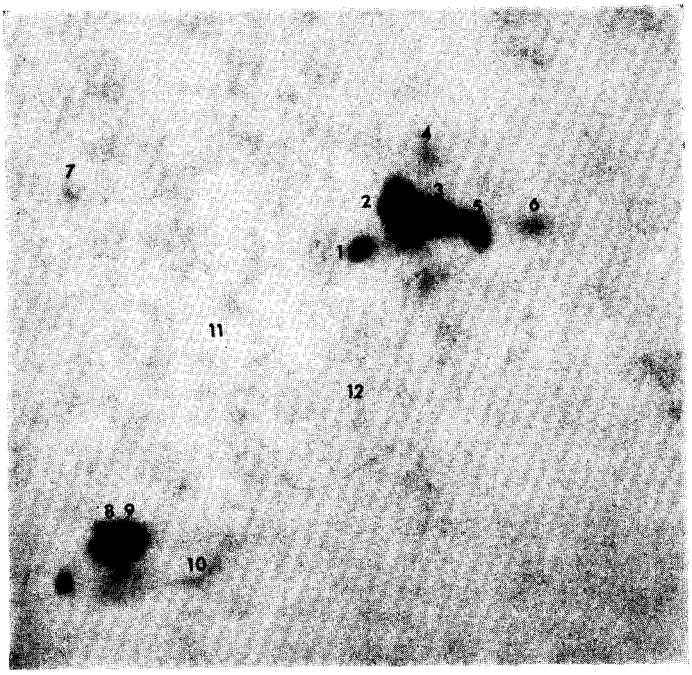

FIG. 3. Two dimensional fluorogram from urine (0–3 hr after an i.v. injection of ^{3}H-oxypertine made on thin layer composed of silica gel–anthracene (1:1). Solvent I (vertical): isopropanol–ammonia–water (8:1:1) and solvent II (horizontal): butanol–acetic acid–water (4:1:5). Most of the same spots were present also in bile fluorograms, but the major spots were 2, 8, 10, 11 and 12.

TABLE 2. GLC PEAKS FOUND IN URINE FRACTIONS OF OXYPERTINE TREATED RATS BUT NOT CLEARLY PRESENT IN THE CORRESPONDING FRACTIONS OF CONTROL URINE

R_f in TLC	Retention time (in GLC)	Size of peak
00–10	6 min 10 sec	moderate
,,	22 min	great
10–20	22 min	great
20–30		
30–40	7 min 30 sec	great
,,	11 min 30 sec	moderate
,,	20 min 30 sec	small
40–50	6 min	great
,,	13 min 30 sec B*	moderate
,,	22 min 20 sec	moderate
50–60	6 min	great
,,	13 min 30 sec B*	small
	16 min 30 sec	moderate
,,	25 min 30 sec A*	great

* Mass spectra of the metabolites A and B are given in Figs. 4 and 5.

Fractions obtained from TLC running in butanol–acetic acid–water solvent were methylated and silylated for GLC.

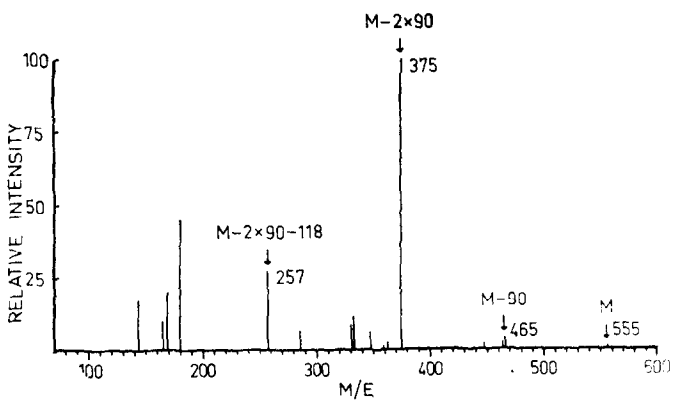

FIG. 4. Mass spectrum of the trimethylsilyl derivative of one of the main metabolites (A in Table 2) of oxypertine. The peaks corresponding the loss of one (M/E 465) and two (375) trimethylsilyl groups and the break between the carbons in piperidine ring (257) are marked with arrows.

radioactive peaks was analyzed by taking the corresponding peak in a separate run directly into mass spectrometry. The mass spectrum of this probable main metabolite (Fig. 4) indicates a molecular weight 555. This corresponds very well to the theoretical value 554·5 of a dihydroxylated oxypertine metabolite containing trimethylsilyl groups in both hydroxyls. The presence of the peaks M-90 and M-180 (465 and 375) gives additional proof of the presence of two trimethylsilyl groups. The most probable sites of the hydroxyl groups seem to be in the methyl of indole-2 position and in the para-position of the phenyl group, and these would seem likely according to the general rules of hydroxylation. The other main peak (B in Table 2) was also present in the next lowest R_f zone. Its mass spectrum indicated a lower molecular weight (407) and the presence of carboxymethyl groups (fractions M–CH_3,

M–OCH_3 and M–$COOCH_3$). Thus the indole-2-methyl group has been oxidized to carboxyl *in vivo* and this is found as a methyl ester. The compound also seems to be demethylated but not conjugated, probably because of chinone imine formation.

The radioactive low R_f area in acid layer chromatograms showed with GLC several peaks not occurring in the control urine chromatograms. The mass spectra of two major peaks contained mass peaks typical of glucosiduronic acids.[12] The structure of the glucuronides remained without exact analysis, but the mass spectrum of the major peak indicates a monohydroxylation without demethylation. The third peak of the same fraction gave a different mass spectrum (Fig. 4) which seems to indicate the demethylation of oxypertine. The molecular peak and several fraction peaks match with a compound demethylated and then oxidized to chinone imine form and containing one trimethylsiloxy group, e.g. in the methyl of indole-2-carbon. Without a reference compound we were unable to decide which of the methoxy groups was demethylated. The oxidation to form a chinone imine may have occurred *in vivo*, but could also be an artefact resulting *in vitro* during the prepurification.

DISCUSSION

The fast uptake of oxypertine by nervous tissue corresponds to a rapid onset of action (less than 30 min in this study) seen in several experimental and clinical studies. The release of amines seems to be slower[5] indicating that it may be less essential for the behavioral effects of oxypertine than other effects, e.g. the blocking of central sympathetic receptors.[1] In the blood oxypertine seems to be concentrated in platelets (unpublished results) like other amine releasers, e.g. chlorpromazine,[13] reserpine[14] and guanethidine.[15]

The general distribution suggests that the partial specificity of noradrenaline release by oxypertine may be due to its concentration at the storage sites of noradrenaline, but this requires additional studies at microscopical and submicroscopical level. If this suggestion were true, the high concentrations in adrenergic organs may be connected with a central and peripheral sympatholytic action. At least in some cases oxypertine given to hypertonic patients has caused a fall in blood pressure and not decreased

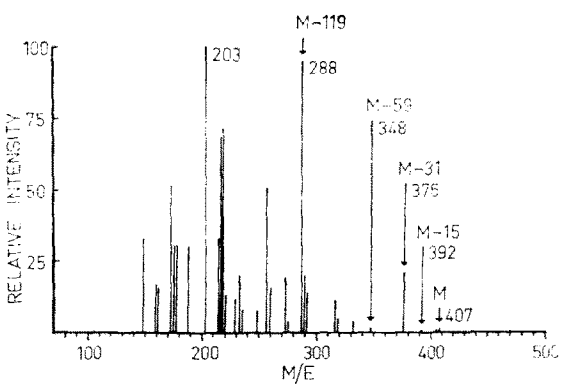

FIG. 5. Mass spectrum of the trimethylsilyl derivative of one of the main metabolites (B in Table 2) of oxypertine. The peaks corresponding the loss of CH_3O and CH_3OOCH_2 as well as the break between the carbons in piperidine ring are indicated with arrows.

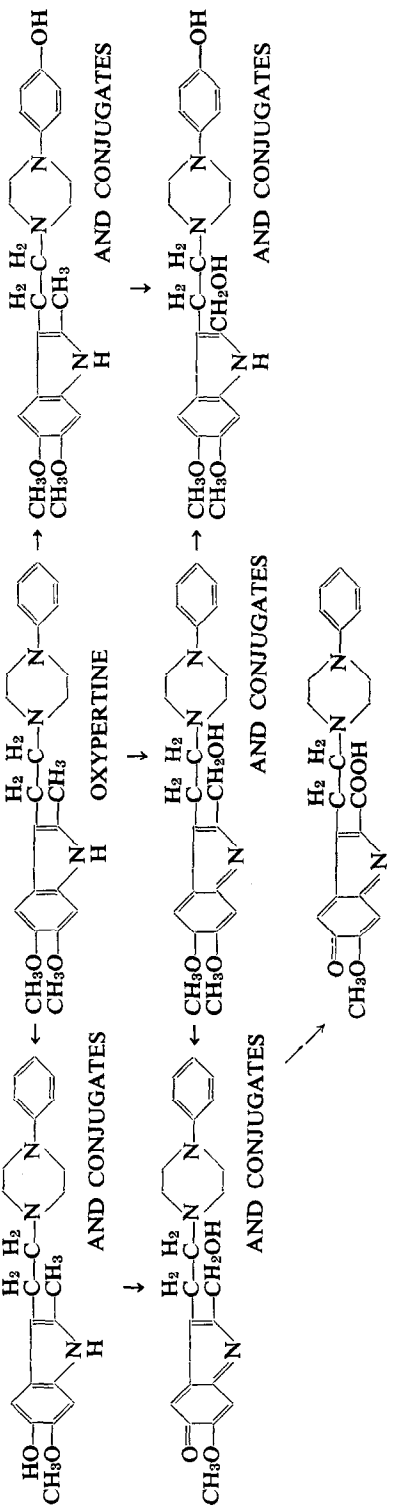

FIG. 6. Metabolic pathways of oxypertine in rat.

normal blood pressure.[16] The value of oxypertine in the treatment of hypertension may be worth studying because, unlike reserpine it does not cause mental depression.

These results and total body autoradiographic studies in mice[7] show that a great concentration of oxypertine metabolites circulate in the enterohepatic cycle and, thus, go through the liver several times. This can perhaps be connected with the slight increase of serum transaminase values sometimes found.[17, 18] Usually the liver function tests remain normal and we have seen no reports of icterus during oxypertine treatment. A considerable amount of oxypertine is excreted into the gastrointestinal lumen. Only bile and gastric juice were analyzed in this study, but salivary glands also concentrated radioactivity. Preliminary microscopic autoradiograms from salivary glands after administration of tritiated oxypertine showed activity in the ductal lumen of thesubmandibular and parotid glands (P. Pohto, personal communication).

The study indicates that oxypertine is metabolized in rats by demethylation and by alcoholic and phenolic hydroxylation. These can be followed by glucuronidation and possibly also by sulphate conjugation (Fig. 6). Alternatively the alcoholic metabolite can be further oxidized to the corresponding acid. Both the monohydroxylated metabolites, the alcoholic and the phenolic ones still have good lipid solubility and a poorer water solubility. This might explain why they seem to be very prone to further hydroxylation and two-hydroxyl derivatives were found to be the major metabolites. A considerable formation of two-hydroxyl-metabolites has been found also from tetrabenazine,[19] a drug having some common characteristics with oxypertine in both stucture and pharmacological actions. The site of demethylation may be either or both of the methoxy groups of oxypertine. The predominant metabolite of brucine, another dimethoxy indole derivative, has been shown to be 2-methoxy-3-hydroxy-strychnine corresponding the demethylation of the methoxy groups in indole-6 position[20] although the indole-5-methoxy groups also were demethylated to a minor extent.

The presence of metabolites in brain tissue at the time of the pharmacological effects arouses the question of possible biological activity of the metabolites. At least the indole-2-methyl derivative has been administered to experimental animals without significant effects as a result (S. Archer, personal communication).

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REFERENCES

1. D. W. WYLIE and S. ARCHER, *J. med: pharm. Chem.* **5**, 932 (1962).
2. S. SPECTOR, K. MELMON and S. SJOERDSMA, *Proc. Soc. exp. Biol. Med.* **3**, 79 (1962).
3. M. MATSUOKA, *Jap. J. Pharmac.* **14**, 181 (1964).
4. M. GOLDSTEIN and K. NAKAJIMA, *Life Sci.* **5**, 1133 (1966).
5. K. FUXE, H. GROBECKER, T. HÖKFELT, J. JONSSON and T. MALMFORS, *Arch. exp. Path. Pharmak.* **256**, 450 (1967).
6. I. J. BAK and R. HASSLER, *Agressologie* **9**, 341 (1968).
7. M. M. AIRAKSINEN and J. E. IDÄNPÄÄN-HEIKKILÄ, *Psychopharmacologia* **10**, 400 (1967).
8. C. SLOTT, *Scand. J. clin. Lab. Invest.* **17**, 182 (1965).
9. U. LÜTHI and P. G. WASER, *Nature, Lond.* **205**, 1190 (1965).
10. E. C. HORNING, W. A. J. VANDENHEUVEL and B. G. CREECH, in *Methods of Biochemical Analysis*, Vol. XI, (Ed. D. GLICK) Interscience, New York (1963).

11. H. Budzikiewicz, C. Djerassi and D. H. Williams, in *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco (1967).
12. P. I. Jaakonmäki, K. L. Knox, E. C. Horning and M.'G. Horning, *Eur. J. Pharmac.* **1**, 63 (1967).
13. Liisa Ahtee and M. K. Paasonen, *J. Pharmac.* **18**, 126 (1966).
14. H. M. Solomon and P. D. Zieve, *J. Pharmac. exp. Ther.* **155**, 112 (1967).
15. D. J. Boullin and R. A. O'Brien, *Br. J. Pharmac.* **35**, 90 (1969).
16. C. D. Neal, M. P. Collins and I. W. Imlha, *Curr. ther. Res.* **11**, 357 (1969).
17. L. E. Hollister, J. E. Overall, J. Kimbell, J. L. Bennet, F. Meyer and E. Caffey, *J. new Drugs* **3**, 26 (1963).
18. W. P. K. Calwel, M. Jacobsen and A. Skarbek, *Br. J. Psychiatr.* **110**, 520 (1964).
19. D. E. Schwartz, H. Bruderer, J. Rieder and A. Brossi, *Biochem. Pharmac.* **15**, 645 (1966).
20. H. Tsukamoto, H. Yashimura, T. Watabe and K. Oguri, *Biochem. Pharmac.* **13**, 1577 (1964).