Biochemical Pharmacology, 1962, Vol. 11, pp. 535–550. Pergamon Press Ltd., Printed in Great Britain.

THE METABOLISM OF THIORIDAZINE (MELLARIL®) AND ONE OF ITS PYRROLIDINE ANALOGUES IN THE RAT*

K. ZEHNDER, F. KALBERER, W. KREIS and J. RUTSCHMANN

Biochemical Department, Sandoz Ltd., Basle

(*Received* 24 *January* 1962; *accepted* 1 *February* 1962)

Abstract—The fate of the phenothiazine derivatives thioridazine and ET 758 in the rat organism was investigated using the compounds labelled with ^{35}S in the phenothiazine ring or with ^{14}C in the N-methyl group of the basic side chain. The following metabolic reactions were observed:

(1) oxidative demethylation at the tertiary nitrogen atom of the side chain;
(2) oxidation at both sulphur atoms to sulphoxide and sulphone; and
(3) formation of glucuronides of hydroxylated derivatives. In the case of thioridazine both monosulphoxides, disulphoxide, disulphone and northioridazine with its oxidation products were determined in the urine and bile by inverse isotope dilution analysis. The derivatives make up some 20 per cent in the bile and some 50 per cent in the urine of the excreted radioactive substances. The rest, i.e. the largest part of the administered substance, occurs as glucuronides of the hydroxylated derivatives.

INTRODUCTION

DESPITE the importance of phenothiazines containing basic substituents in psychiatry, only little is as yet known of their metabolism. Some of the material published in the literature is contradictory. It is generally based on determinations made by extraction and colorimetry, a method which in our experience—at least in some cases—is rather uncertain. Metabolism studies generally only deal with substances excreted in the urine. Hitherto the often dominant role of excretion in the bile has not been considered adequately. Salzmann and Brodie,[2, 3] Berti and Cima,[4] Young *et al.*,[5, 6] Walkenstein and Seifter,[7] Lin *et al.*,[8] Block[9] and others have established *in vivo* and partly *in vitro* the following metabolic reactions:

(1) Oxidation of the phenothiazine ring sulphur atom to sulphoxide, possibly also to the sulphone; an intermediate formation of radicals[10, 11] has been suggested for this oxidation.
(2) Oxidative demethylation[6, 12] at the tertiary amino group of the aminopropyl side chain.
(3) Formation of glucuronides[8, 13, 14] of hydroxylated phenothiazines, for which it is at present assumed that the hydroxyl group is in one of the two *p*-positions with respect to the phenothiazine ring nitrogen atom.

In the present work, we describe investigations of the metabolism of thioridazine (Mellaril® = 3-methylthio-10-[β-(1'-methyl-2'-piperidyl)-ethyl]-phenothiazine, (I)) and

* Reported in part at the 6th *Journées Biochimiques Latines*, 25–28 May 1961 in Geneva.[1]

ET 758 (3-methylthio-10-[β-(1′-methyl-2′-pyrrolidyl)-ethyl]-phenothiazine, (II)). In these compounds additional metabolic reactions caused by the presence of a second sulphur atom would be expected.

MATERIALS AND METHODS

A. Preparation and separation of thioridazine sulphoxides

1. *Oxidation of thioridazine.* A weight of 11·2 g of thioridazine hydrochloride (27·5 mM) in 300 ml of absolute ethanol was boiled under reflux for 4 hr with 3·85 ml of 40% hydrogen peroxide. After evaporation of the solution *in vacuo*, the residue was treated with sodium carbonate solution and the liberated base extracted with chloroform. Yield: 12·6 g of a mixture of bases as a viscous oil.

2. *Separation of mono- and di-sulphoxides.* The mixture of bases was transferred to a column containing 750 g of aluminium oxide (basic, activity II–III Brockmann standard) which had been prepared with benzene–chloroform (3 : 1). The following fractions were obtained with the solvents given:

Fraction no.	Solvent	Volume (ml)	Weight (g)	Colour reaction*
C 1	Benzene–chloroform 3:1	2000	—	—
C 2	Benzene–chloroform 1:1	3500	0·13	—
C 3	Benzene–chloroform 1:1	5000	9·01	violet blue
C 4	Chloroform	500	0·30	violet
C 5	Chloroform	3000	2·61	cherry red

* Colour reaction: A solution of the substance in glacial acetic acid is mixed with an equal volume of concentrated sulphuric acid.

Fraction C 3 represents a mixture of the isomeric monosulfoxides which was separated in the partition mentioned later. Fraction C 5 contained pure *disulphoxide* which crystallized from ethanol–acetone as the fumarate upon addition of 900 mg of fumaric acid. The salt was purified by liberating the base and repeating the fumarate precipitation. Yield: 2·7 g of thioridazine–disulphoxide–fumarate (m.p. 125–127 °C, decomposition). The salt gives a fairly intensive cherry red colour reaction.

3. *Separation of the monosulphoxides.* A weight of 9·0 g of the monosulphoxide mixture (fraction C 3) was dissolved in 250 ml of 60% methanol saturated with *cyclo*hexane containing 10 ml of 2 N sodium carbonate. The solution was passed through ten separating funnels, each containing 250 ml of *cyclo*hexane saturated with 60% methanol. Three 250 ml portions of methanol mixture were used for washing. Four consecutive fractions of the lower phase (D1–D4) were thus obtained. Dilution of D1 and D2 with water and extraction with chloroform yielded 4·5 g of base, from which 4·6 g of thioridazine-ring sulphoxide–fumarate were obtained by crystallization from ethanol acetone upon addition of 1·5 g fumaric acid. By combining and evaporating the *cyclo*hexane fractions 5–10, 1·8 g of the side chain sulphoxide-base was obtained as a viscous resin. The base liberated from the mother liquors, together with fractions D3 and D4, was subjected again to a similar partition, 280 mg ring sulphoxide–fumarate and 1·1 g thioridazine-side-chain–sulphoxide-base thus being obtained. The combined ring sulphoxide fumarate fractions were purified over the

base and yielded 4·5 g of pure *thioridazine-ring sulphoxide fumarate* (m.p. 140–165 °C, decomposition). This showed an intensive pure blue colour reaction. The side-chain sulphoxide fractions were decolorized by filtration of their chloroform solution over a small quantity aluminium oxide; 2·8 g of resinous, slightly yellow *thioridazine-side-chain–sulphoxide-base* were obtained. The colour reaction of this base with acid was first pinkish but quickly changed to a weak violet.

The three compounds were shown to be uniform in a thin layer chromatogram (silica gel G "Merck") with ethyl acetate–glacial acetic acid–water (5 : 2 : 2) as the mobile phase (see Table 1).

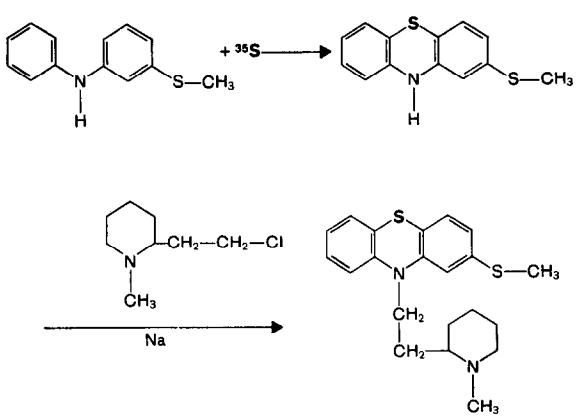
The infra-red spectrum of the side-chain sulphoxide shows, among others, a simple broad band in the sulphoxide region at 1050 cm^{-1}, while the ring sulphoxide shows a double band with centres at 1020 and 1045 cm^{-1}. The spectrum of the disulphoxide in this region corresponds to a superposition of the monosulphoxide bands.

4. *Preparation of northioridazine-disulphone.* This compound was obtained by treatment of northioridazine in acetic acid in the presence of sulphuric acid with an excess of hydrogen peroxide. The hydrochloride crystallized from methanol-ethanol (m.p. 301–303°).

Anal: Calcd. for $C_{20}H_{25}O_4N_2S_2Cl$ (m. w. 457·0): C, 52·6; H, 5·5; O, 14·0; N, 6·1; S 14·0. Found: C, 52·5; H, 5·6; O, 13·9; N, 6·0; S, 14·2.

B. Synthesis of thioridazine-9-[^{35}S]

Elementary ^{35}S was obtained by known methods[16] from KCl irradiated in the reactor "Saphir" (Schweizerisches Institut für Reaktorforschung). The synthesis of thioridazine-[9-^{35}S] (I) is shown in the following diagram.



I

1. *3-Methylmercapto-phenothiazine*[9-^{35}S]. A weight of 155 mg (4·85 mM) of radioactive sulphur and 525 mg (2·45 mM) of 3-methylmercapto-diphenylamine were dissolved together in a few millilitres of carbon disulphide and the solution evaporated to dryness *in vacuo* at 40 °C in a bulb tube. Ten milligrams of iodine were then added and the bulb tube was connected consecutively to a freezing trap cooled

in liquid air, a phosphorus pentoxide tube and a wash bottle containing cadmium acetate. The bulb tube was immersed for 20 min in an oil bath at 160 °C, and then cooled for 10 min with liquid air to freeze out the hydrogen sulphide formed. After removal of the phosphorus pentoxide tube, the apparatus was connected to a vacuum pump and the hydrogen sulphide and iodine were quantitatively distilled into the cooling trap at 0·001 mm Hg and 80 °C (oil bath). The radioactive sulphur was recovered from the distilled hydrogen sulphide by reaction with iodine solution and hydrochloric acid.

TABLE 1

Substance	R_f	Colour reaction with		Fluorescence
		Perchlorid acid	Ceric sulphate	
Thioridazine	0·77	blue-green	blue-green	weak orange
Thioridazine-ring sulphoxide	0·45	blue	—	blue
Thioridazine-side-chain sulphoxide	0·44	pink	pink	blue
Thioridazine-disulphoxide	0·19	cherry red	—	blue
Thioridazine-disulphone[15]	0·55	—	—	greenish
Northioridazine[16]	0·80	blue-green	blue-green	weak orange

Ten milligrams of copper bronze were placed in the bulb tube containing the black-green phenothiazine melt and distillation was carried out under high vacuum. Up to 130° C/0·001 mm Hg some sulphur with a little brown oil distilled. The main fraction distilled as a light green oil at 150–160 °C and 0·001 mm Hg. Towards the end of the distillation the temperature was raised to just below 180 °C. A small black residue remained. The main fraction weighed 538 mg (91 per cent).

In a second batch, 376 mg (87 per cent) of distilled phenothiazine were obtained from 113 mg (3·53 mM) of recovered and somewhat diluted sulphur and 390 mg (1·81 mM) of 3-methylmercaptodiphenylamine.

The two fractions (914 mg) were recrystallized together from ethanol. The 3-methylmercapto-phenothiazine crystallized in small light yellow plates with a melting point of 136–137 °C (uncorr.). After addition of 20 mg of inactive material, a second fraction with a melting point of 134–136 °C was obtained from the mother liquor. Yield: 547 mg of pure 3-methylmercapto phenothiazine-[9–^{35}S] free of isomers.

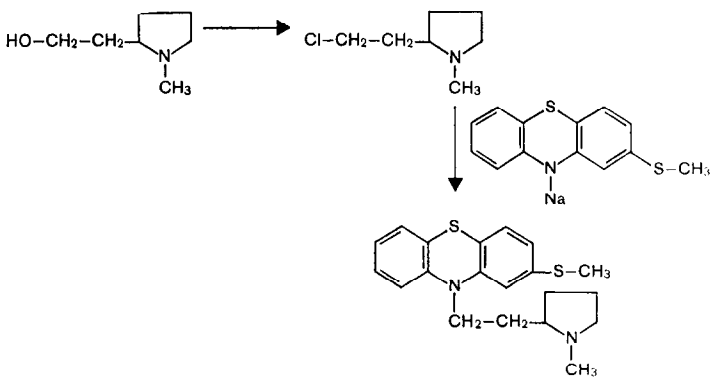
2. *Thioridazine*-[9–^{35}S]. A solution of 547 mg (2·2 mM) of 3-methylmercapto-phenothiazine- [9–^{35}S] in 15 ml of absolute xylene was added to 80 mg (3·5 mM) of sodium in 3 ml of absolute methanol and the methanol distilled off. A solution of 542 mg (3·4 mM) of freshly distilled 2-(1′-methyl-2′-piperidyl)-ethyl chloride in 2 ml of absolute methanol was then added, the methanol distilled off in an oil bath at 100 °C and the temperature raised to 150–160 °C until approximately 2 ml of xylene had distilled over. The mixture was magnetically stirred and heated under reflux at an oil bath temperature of 140 °C for 5 hr, and then cooled and diluted with ether. The solution was shaken three times with 10% tartaric acid in water, the combined tartaric acid extracts were made alkaline with potassium carbonate and extracted with ether. The residue of the extract was distilled in a bulb tube at 175–185 °C and 0·001 mm Hg. Yield: 646 mg (79 per cent) of yellow oil.

The base was neutralized in methanol with methanolic hydrogen chloride. After evaporation to dryness *in vacuo*, the residue was taken up in acetone, boiled under reflux with active carbon and filtered over Hyflo. The filtrate was reduced to 2–3 ml *in vacuo*. The hydrochloride crystallized in light yellow platelets. A second fraction was recrystallized together with the main portion from acetone, m.p. 157–158 °C (sintered from 155 °C). In a thin layer chromatogram, (silica gel G "Merck", with acetic ester–glacial acetic acid–water (5 : 2 : 2) as mobile phase) the product was shown to be radiochemically uniform and identical with pure inactive material. Yield: 408 mg, specific activity 7·5 mC/mM.

After addition of 150 mg of inactive thioridazine, an additional portion of 320 mg of labelled product (specific activity 3·5 mC/mM) was obtained from the mother liquors.

C. Synthesis of ^{35}S-ET 758

This synthesis is shown in the following diagram:



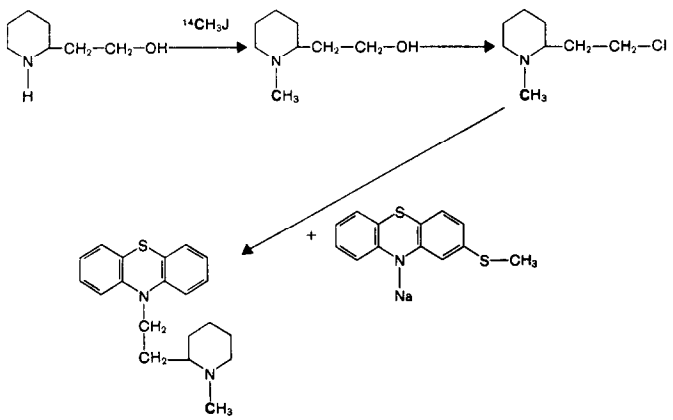
II

1. 1-*Methyl*-2-(2′-*chloroethyl*)-*pyrrolodine*. A solution of 260 mg (2 mM) 1-methyl-2-(2′-hydroxy-ethyl)-pyrrolidine in 5 ml of chloroform was saturated with dry hydrogen chloride and, after 2 ml of distilled thionyl chloride had been added, heated under reflux for 2 hr. After evaporating to dryness *in vacuo*, the residue was taken up three times in a little methanol, the solvent being evaporated each time, and finally dried at 50 °C under high vacuum for 1 hr.

2. *ET* 758-*fumarate*. A weight of 293 mg (82 per cent) of ET 758 base were obtained as yellow oil at 180–185 °C and 0·001 mm Hg as described under ^{35}S-thioridazine from a mixture of 100 mg of sodium in 5 ml of absolute methanol, 246 mg (1 mM) of 3-methylmercapto-phenothiazine-[9-^{35}S] in 15 ml xylene and the 1-methyl-2-(2′ chlorethyl)-pyrrolidine dissolved in 3 ml of methanol. A yield of 273 mg of ^{35}S—ET 758 fumarate was obtained from the base by treatment with fumaric acid and crystallization from ethanol–acetone.

D. Synthesis of thioridazine-[N-^{14}C]

Thioridazine-[N-^{14}C] was synthesized from ^{14}C-methyliodide which was obtained by Zeisel cleavage of 3-nitrobenzoic acid methyl ester. To prevent quaternization as much as possible, a large excess of 2-(2′-piperidyl)-ethanol was treated with the ^{14}C-methyl-iodide in methanolic solution at room temperature. The methylated product was separated from the starting material by acetylation, five partitions between water and ether (the methylated product remaining in the ether phase), distillation in a bulb tube and alkaline saponification. 2-(N-Methyl-2′-piperidyl)-ethyl chloride was obtained by reaction with thionyl chloride as the hydrochloride and was condensed with an excess of 3-methylthio-phenothiazine and sodium alcoholate in xylene, according to the following diagram:



II

1. 3-*Nitrobenzoic acid methyl ester-*^{14}C. Radioactive diazomethane from 570 mg ^{14}C-nitroso-tosyl methylamine[17] was collected in a receiver at 0 °C containing 670 mg of 3-nitrobenzoic acid dissolved in 40 ml of ether. The ether solution was washed with sodium bicarbonate solution, the ether distilled off and the residue sublimed at 70–75 °C and 0·05 mm Hg. Yield: 392 mg (80·5 per cent), m.p. 75–76 °C.

2. ^{14}C-*methyl iodide.*The 392 mg of labelled 3-nitrobenzoic acid methyl ester were dissolved in 2·5 ml of acetic anhydride and 10 ml of hydriodic acid ($d \doteq 1{\cdot}70$). A volume of 0·6 ml of 50% hypophosphorous acid was added with ice-cooling. The solution was boiled for 1½ hr while a weak stream of nitrogen was bubbled through it. The methyl iodide was passed through three wash bottles, the first containing 5% ascorbic acid solution and the second and third, 5% copper sulphate solution. It was then dried over phosphorus pentoxide and condensed in a spiral condenser cooled with liquid air. The methyl iodide was ready for further use after this treatment.

3. N-*methyl-*[^{14}C]-*pipecolyl carbinol.* The radioactive methyl iodide, 1·5 g of freshly distilled α-pipecolyl carbinol and 12 ml of absolute methanol were sealed into an

ampoule and left to stand for 14 days at 20 °C. Four millilitre of concentrated hydrochloric acid were then added and the mixture was evaporated to dryness *in vacuo*. The oily residue was taken up in water, made alkaline with potassium carbonate and extracted with ether.

After drying, the ether extract was reduced to a few millilitres, 5 ml of acetic anhydride were added, the ether was distilled off completely and heating was carried out for 1 hr on a water bath. The excess anhydride was then hydrolysed by adding 2 ml of water and heating for a further 15 min. The acetic acid was distilled off *in vacuo* the residue taken up in ether, made alkaline with potassium carbonate and the mixture of acetyl derivatives was partitioned between water and ether. The combined ether solutions were dried, the solvent evaporated off and the residue distilled at 110–120 °C and 0·010 mm Hg.

A yield of 383 mg (96 per cent) of O-acetyl-*tert*-amine was obtained as a colorless oil. This product, 3 ml of ethanol and 2 ml of 2 N sodium hydroxide were heated under reflux on a water bath for 1 hr. The mixture was then diluted with ether and the aqueous phase saturated with solid potassium carbonate. After decantation and washing several times with ether, the ethereal solution of N-methyl-[^{14}C]-pipecolyl carbinol was ready for further use.

4. 2-(1′-*Methyl*-[^{14}C]-2′-*piperidyl*)-*ethyl chloride hydrochloride*. The ethereal carbinol solution was made acid with chloroform, saturated with hydrochloric acid gas, and then evaporated *in vacuo*. The residue was taken up in 8 ml of chloroform–hydrochloric acid, heated with 2·5 ml of thionyl chloride under reflux for 2 hr and the solution evaporated.

The oily residue was taken up three times in chloroform and three times in methanol, the solvent being distilled off *in vacuo* each time. The remaining oil crystallized completely on standing. Yield: 406 mg.

5. *Thioridazine*-[N-^{14}C]. A yield of 435 mg (57 per cent) of thioridazine-[^{14}C] base was obtained as a yellow, viscous oil by reacting together 127 mg of sodium in 5 ml of methanol, 615 mg of methylthio-phenothiazine in 15 ml of xylene and 406 mg of 2-(1′-methyl-[^{14}C]-2′-piperidyl)-ethyl chloride hydrochloride in 5 ml of methanol at 195–205 °C and 0·020 mm Hg. Treatment of the base with chloroform–hydrochloric acid followed by recrystallization from acetone gave 289 mg of thioridazine-[^{14}C] hydrochloride (m.p. 158–160 °C) with a specific radioactivity of 0·438 mc/mM. After addition of 210 mg of inactive thioridazine hydrochloride and recrystallizing three times 280 mg of thioridazine-[^{14}C] hydrochloride with a specific activity of 0·171 mc/mM were obtained from the mother liquors.

The activity yield, with reference to tosyl methylamide, was 27 per cent over ten steps.

All radioactive products were compared with the authentic inactive compounds and shown to be radiochemically pure by thin layer chromatography on silica gel G "Merck", with ethyl acetate–acetic acid–water (5 : 2 : 2) as mobile phase.

E. Determination of radioactivity

The ^{35}S content was determined by oxidation of the samples with a mixture of concentrated hydrochloric acid, 70% perchloric acid and copper nitrate with the

addition of 1 N sulphuric acid as carrier, precipitation of the sulphur as barium sulphate,[18, 19] followed by counting of an infinitely thick layer in a flow counter. The value found was corrected for radioactive decay. The ^{14}C content of dried tissues and excreted materials was determined by wet oxidation as described earlier[20, 21] and measurement of the $^{14}CO_2$-activity in proportional gas counters.

F. Metabolism tests on the rat

1. *Resorption.* A weight of 20 mg/kg of ^{35}S-thioridazine or ^{35}S-ET 758 was administered by stomach tube to four male and four female rats (approx. 200 g). Two animals were killed with ether after $\frac{1}{2}$, 1, 2 and 4 hr, respectively, the abdominal wall was opened and as much blood as possible withdrawn with a syringe from the vena cava. The entire gastrointestinal tract (GIT) was clamped off at the upper end of the œsophagus and at the colon and carefully prepared. The GIT was divided into five parts which were rinsed several times in 5% tartaric acid solution. The contents and wall were then homogenized separately in a Potter homogenizer and the radioactivity was determined. The liver and lung from the same animals were also examined.

2. *Excretion in the bile.* Laparotomy was carried out on rats under ether narcosis. A slit was made in the wall of the bile duct approximately 1 cm from the junction with the duodenum, a thin polyethylene catheter approximately 20 cm long was introduced towards the liver into the bile duct and the abdominal wall was stitched together again. The rat was mounted in a specially constructed frame so that it could move only slightly. The bile was collected fractionally in volumetric flasks cooled with dry ice. Twenty milligrams of ^{35}S-thioridazine or ^{35}S-ET 758 per kg were administered either intravenously or orally to each of three approximately 200 g female rats per experiment.

3. *Excretion in the faeces and urine.* The faeces and urine of each of three approximately 200 g rats to which 20 mg ^{35}S-thioridazine or ^{35}S-ET 758 per kg had been administered either intravenously or orally, were collected separately, the experiment being carried out in a modified metabolism cage as described by Brittain.[22]

4. *Distribution in the organism.* Approximately 200 g rats were injected intravenously with 20 mg ^{35}S-thioridazine or ^{35}S-ET 758 per kg and two animals were killed and dissected after periods of 1, 2, 4, 8, 16 and 24 hr. The radioactivity of the heart, lung, liver, spleen, kidney, suprarenal gland, ovaries or testicles, stomach, small and large intestines, pancreas, thigh muscles, skin of the paws, brain, blood, lymph gland, femur, bone marrow, thyroid gland and salivary gland was determined, and expressed by the factor

$$F = \frac{\text{specific activity} \times \text{body weight}}{\text{total activity}}$$

G. Determination of the thioridazine metabolites in the urine and bile by inverse isotope dilution

1. *Determination of separate metabolites.* Bile and urine were collected in a 50- or 100-ml volumetric flask and when collection was complete, made up to the mark with water. Five millilitres were withdrawn for determination of the total activity and to the

remainder were added exactly weighed amounts (each of 400–500 mg) of inactive thioridazine, thioridazine side-chain sulphoxide, thioridazine ring sulphoxide and thioridazine disulphoxide (all as bases). Freeze-drying was carried out and the residue extracted with absolute methanol. After distillation of the solvent *in vacuo*, the residue from the methanol extract was shaken with chloroform and half-saturated potassium carbonate solution.

The residue left after evaporation of the chloroform solution (approx. 2 g) was chromatographed on a column of 150 g of basic aluminium oxide (Merck, activity II–III (Brockmann)) (see A, 2) and the mixture of the two monosulphoxides subjected to countercurrent distribution (see A, 3). The thioridazine, disulphoxide and ring sulphoxide fractions thus obtained were crystallized with somewhat more than the molar amount of fumaric acid, and purified to constant activity by liberation of the bases and crystallization of the fumarates. The side-chain sulphoxide was obtained as pure amorphous base as described.

The original urine or bile solution was divided in two for additional determination of disulphone. The first half was treated as above while 500 mg of inactive disulphone were added to the second. The diluted disulphone was isolated by freeze-drying, extraction with absolute methanol, distillation of the solvent and shaking with chloroform and potassium carbonate solution. The preparation was purified over the crystallized hydrochloride.

The original amounts of the individual metabolites present were calculated from the specific activities of the products.

2. *Determination of the total metabolites from thioridazine to thioridazine disulphone.* A weight of 500 mg of inactive thioridazine hydrochloride were added to the bile and to the urine, the mixtures made up to the mark and 5 ml removed for the total activity determination. The rest of the solution was freeze-dried. The residue was taken up in 10 ml of glacial acetic acid, 2 ml of concentrated sulphuric acid were added and the solution treated for about 2 hr at 60 °C with a total of 8 ml of 40% hydrogen peroxide in 1 ml portions. The solution, which no longer gave a phenothiazine colour reaction, was evaporated *in vacuo*, distilled three times *in vacuo* with water and the residue was shaken with chloroform and half-saturated potassium carbonate solution. The thioridazine disulphone was obtained pure from the chloroform extract by crystallization from ethanol. The total amount of N-methylated compounds originally present (thioridazine, monosulphoxides, disulphoxide, sulphone and possibly other oxidation intermediates) was calculated from the specific activity of the product.

3. *Determination of the total metabolites from northioridazine to northioridazine disulphone.* The same procedure as described under G, 2 was adopted except that 500 mg of northioridazine hydrochloride was added as carrier. The northioridazine disulphone was obtained pure from the chloroform extract in the form of the hydrochloride crystallized from a mixture of methanol and ethanol. The total amount of northioridazine and its oxidation products up to the disulphone was calculated from the specific activity.

RESULTS AND DISCUSSION

F-values for various organs and tissues are given in Table 2 and plotted for the lung, liver, brain and blood in Figs. 1 and 2. The activity in the blood falls off rapidly.

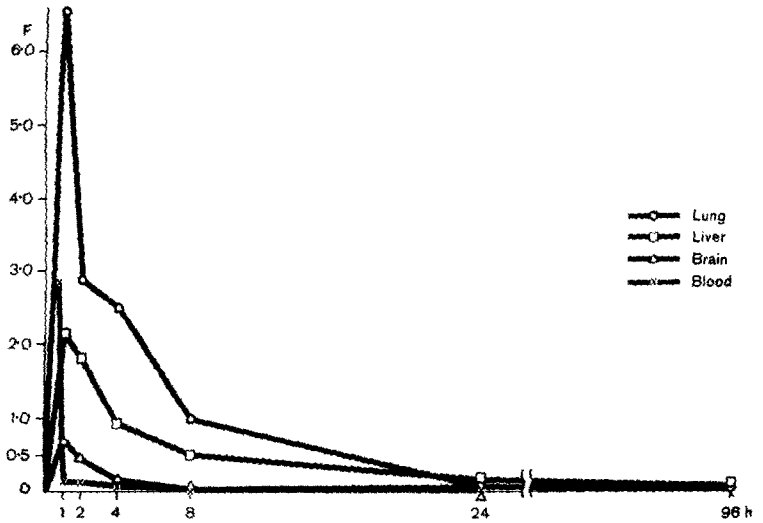


FIG. 1. Distribution of ^{35}S-thioridazine in rat after intravenous administration

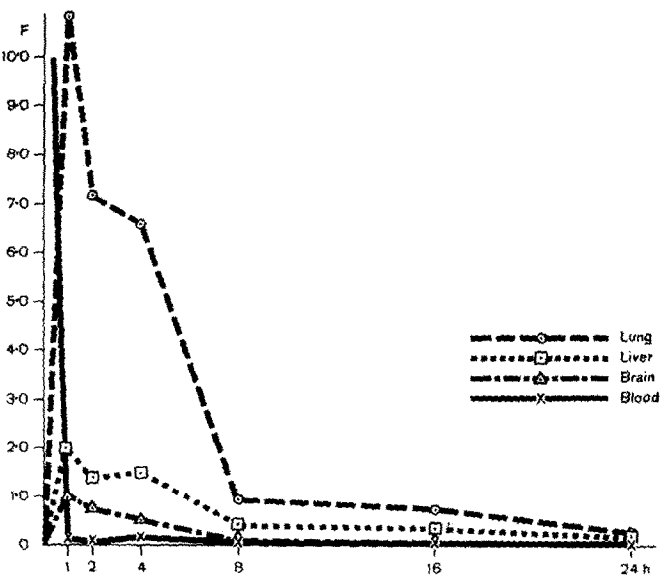


FIG. 2. Distribution of ^{35}S-ET 758 in rat after intravenous administration.

During the first 2–4 hr the *F*-value lies between 0·2 and 0·1, and after 8 hr *F* is no longer measurable with certainty. The radioactivity in the brain reaches its maximum ($F = 0{\cdot}7$) for thioridazine and 1·0 for ET 758 after 1 hr. With the exception of the intestines in which all the material excreted through the bile collects, the internal organs and glands reached their maximum *F*-values of 1–2 after approximately 1 hr. The highest *F*-value, 6·5 for thioridazine and 10·8 for ET 758 are found in the lung. This fact, previously noted for chlorpromazine, and other phenothiazines,[23] could be interpreted by assuming, that the material injected into the venous bloodstream

passes the first capillary system in the lung and is partly retained there. There is no doubt that the phenothiazines are generally absorbed intensely by tissues in general as is also apparent in the resorption experiments described below. But from all the evidence presented here and by others it must be concluded that these substances have a special affinity for lung tissue.

TABLE 2. MEAN *F* VALUES OF TWO ANIMALS AFTER INTRAVENOUS INJECTION OF ^{35}S-THIORIDAZINE AND ^{35}S-ET 758

	^{35}S-Thioridazine					^{35}S-ET 758				
	1	2	4	8	24	1	2	4	8	24 hr
Heart	0·88	0·57	0·25	0·06	0·00	1·17	0·52	0·75	0·08	0·02
Spleen	2·00	0·89	0·81	0·22	0·06	2·83	1·85	1·69	0·25	0·08
Kidney	1·65	0·87	0·62	0·15	0·07	2·03	1·31	1·43	0·19	0·04
Adrenals	2·72	2·52	0·88	0·16	0·04	3·05	2·07	1·91	0·29	0·08
Pancreas	1·12	0·72	0·60	0·12	0·02	0·94	0·81	0·66	0·11	0·02
Bone	0·23	0·22	0·11	0·04	0·00	0·32	0·14	0·26	0·04	0·01
Bone marrow	1·60	0·93	0·70	0·15	0·02	2·80	2·12	1·26	0·28	0·05
Lymph nodes	1·06	0·78	0·40	0·15	0·00	0·90	0·71	0·70	0·10	0·01
Salivary glands	1·70	1·01	0·83	0·15	0·02	1·88	1·26	1·15	0·14	0·04
Tyreoidea	1·63	1·60	0·88	0·32	0·06	1·59	1·40	1·48	0·19	0·05
Skin	0·35	0·36	0·25	0·08	0·01	0·37	0·36	0·40	0·23	0·08
Muscle	0·32	0·15	0·13	0·02	0·00	0·61	0·29	0·33	0·03	0·05
Stomach	0·60	—	0·80	0·04	0·02	2·25	1·75	1·36	0·13	0·02
Small intestine	2·20	2·32	0·52	0·35	0·03	3·10	1·40	1·67	0·43	0·05
Colon	0·75	0·47	0·32	0·12	0·05	0·99	0·62	0·84	0·84	0·31
Ovaries	—	—	—	—	—	0·83	0·79	0·67	0·10	0·01
Epididymis	0·35	—	0·35	0·30	0·20	—	—	—	—	—
Testicles	0·38	0·57	0·39	0·30	0·07	—	—	—	—	—

TABLE 3. DISTRIBUTION OF ^{35}S-THIORIDAZINE AFTER ADMINISTRATION *per os*

Time (hr)	^{35}S-activity as per cent of the dose						
	Gastrointestinal tract			Liver		Lung	
	Content	Wall	Content plus wall	F	%	F	%
½	66	9	75	0·70	2·6	0·22	0·1
1	53	15	68	1·24	5·0	0·99	0·5
2	55	8	63	0·83	3·4	0·83	0·4
4	59	12	71	1·27	4·7	1·69	0·7

TABLE 4. DISTRIBUTION OF ET 758 AFTER ADMINISTRATION *per os*

Time (hr)	^{35}S-activity as per cent of the dose						
	Gastrointestinal tract			Liver		Lung	
	Content	Wall	Content plus wall	F	%	F	%
½	61	28	89	1·45	6·6	0·99	0·5
1	54	26	80	1·96	8·5	1·18	0·7
2	69	19	88	1·63	6·0	1·84	0·9
4	50	17	67	1·73	6·8	3·47	1·7

The results of the resorption experiments contained in Tables 3 and 4 show, that about half of the thioridazine and ET 758 administered *per os* is taken up from the gastrointestinal tract. A considerable part of this portion is retained by the intestinal wall and given off to the circulation but slowly. This effect is specially pronounced in the case of ET 758. The *F*-values reached in the liver and lung after some hours are nevertheless considerable. Thioridazine and ET 758 are excreted by the rat preponderantly through the bile. In rats with a bile fistula, the upper curve in Fig. 3 which reaches an average of 75 per cent in 24 hr, is obtained after intravenous injection of ^{35}S-thioridazine. The radioactive material excreted in the urine by the same animals during the same period amounts to only a few per cent of the total quantity. ET 758 produces similar curves (Fig. 4).

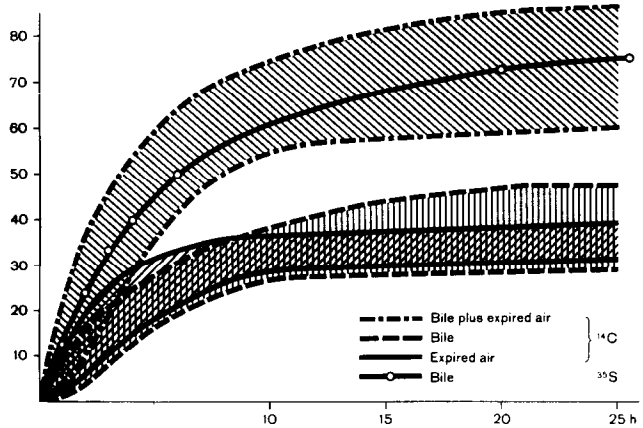


FIG. 3. Excretion of ^{14}C- and ^{35}S-thioridazine by rat after intravenous administration. Vertical axis: excretion in % of the doses.

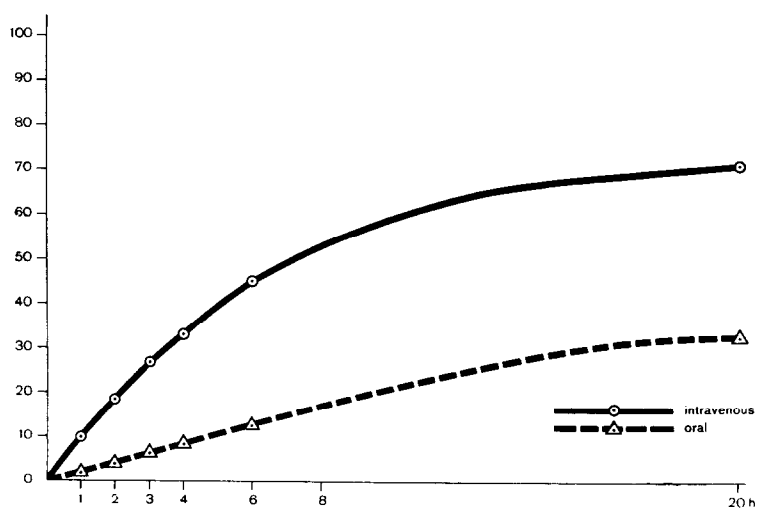


FIG. 4. Biliary excretion of ^{35}S-ET 758 by rat. Vertical axis: excretion in % of the doses.

In intact animals, approximately 80 per cent of the radioactivity of ^{35}S-thioridazine appears in the faeces within 72 hr and only 9 per cent appears in the urine (most of this during the first 24 hr) (Table 5). In comparison with animals with a bile fistula, only a small increase of the excretion in the urine is seen. This leads to the conclusion that only slight reabsorption of metabolites from the intestine takes place.

From data recently presented by Eiduson *et al*[24] it must be concluded that in the excretion of thioridazine in humans the role of the bile relative to the urine is somewhat less pronounced, but still prevalent.

TABLE 5. EXCRETION OF ^{35}S-THIORIDAZINE AND ^{35}S-ET 758 IN THE FAECES AND URINE OF INTACT RATS (SIX ANIMALS) AFTER INTRAVENOUS OR *per os* APPLICATION

Time interval (hr)	Excretion expressed as per cent of the administered dose					
	^{35}S-thioridazine		^{35}S-ET 758			
	Urine	Faeces	Urine		Faeces	
	i.v. application	i.v. application	*p.o.* application	i.v. application	*p.o.* application	i.v. application
0– 2	—	—	0·5	1·5	—	—
2– 4	—	—	4·2		—	—
4– 6	—	—	5·4	5·4	—	—
6– 8	—	—	6·4	5·6	—	—
8–20	—	—	9·6	8·9	67	55
24	7	41	—		—	—
48	9	74	—		—	—
72	9	81	—		—	—

From a comparison (Table 6) with the data published by Fyodorov[23] which were obtained by similar methods interesting conclusions regarding the resorption and excretion of the various phenothiazines, at least in the rat, can be drawn. Derivatives with aliphatic side chains such as promazine and chlorpromazine are excreted mainly in the urine[29] and less in the bile, the speeds of excretion for parenteral (s.c.) and *per os* administration being basically very similar. The picture is different for compounds with heterocycles in the side-chain. Here there is a preponderance of excretion in the

TABLE 6. COMPARISON OF THE EXCRETION OF VARIOUS PHENOTHIAZINE TRANQUILLIZERS

	Promazine		Chlor-promazine		Chlormepazine		Thioridazine		ET 758*	
	s.c	*p.o.*	s.c.	*p.o.*	s.c.	*p.o.*	i.v.	i.v.	*p.o.*	i.v.	*p.o.*
Urine											
0–24 hr	47	36	32	28	4	1	5	7	—	9	10
0–48 hr	47	40	42	34	5	2	5	9	—	—	—
0–72 hr	55	41	44	35	5	2	6	9	—	—	—
Faeces											
0–24 hr	10	24	0	1	8	61	30	41	—	55	67
0–48 hr	27	37	13	17	12	75	38	74	—	—	—
0–72 hr	30	39	21	18	14	78	45	81	—	—	—

* 20-hr values.

bile and an only very slight occurrence of metabolites in the urine. Chlormepazine is obviously not readily resorbed after subcutaneous or *per os* administration. On oral administration, a considerable part of this substance appears to pass through the intestine without ever being resorbed, as we have also established in the case of thioridazine. From this it is concluded that phenothiazine derivatives with a piperidine side-chain are more slowly resorbed and are mainly excreted in the bile whilst compounds with the classic dialkylaminopropyl side-chain are resorbed relatively quickly and favour excretion via the kidneys. The results obtained by Weikel *et al.*[25] with the pyrrolidine derivative methdilazine (= Tacaryl), which seems to be preponderantly excreted in the urine, are surprising in view of the fact established by us that ET 758, which is very similar to methdilazine, is mainly excreted in the bile.

METABOLISM

Like many other tertiary methylamines thioridazine is demethylated in the organism to a considerable extent. This phenomenon was followed by using the N-$^{14}CH_3$-derivative.

Fig. 5 shows a semi-logarithmic representation of the course of $^{14}CO_2$ excretion in the respiration after intravenous administration. The excretion takes place in two

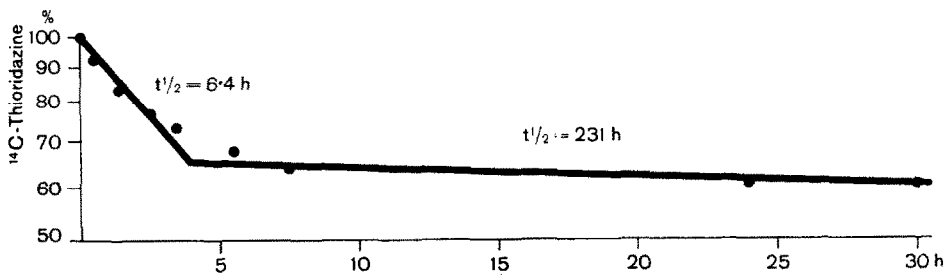


FIG. 5. Radioactivity of expired air after intravenous administration of ^{14}C-thioridazine to rats

phases. The first process has a half-life of about 6 hr and corresponds to the direct formation of CO_2 from the methyl group, while the second, with a very long half-life, can be interpreted as the normal turnover of carbon got into the general metabolism in the form of formaldehyde, formate or carbon dioxide. From 30 to 40 per cent of the thioridazine is demethylated to northioridazine.

The amounts of metabolites arising by oxidation at the two sulphur atoms of thioridazine and its demethylated derivative (see Fig. 6) viz: thioridazine, the three sulphoxides III, IV and V, the disulphone VI, the total of thioridazine and its oxidation products, as well as the total of northioridazine (VII) and its oxidation products up to the sulphones have been determined by inverse dilution analysis (Table 7).

All these metabolites together represent only about 20 per cent of the active material found in the bile and consequently 10–15 per cent of the active substance administered. The largest part of these metabolites consists of the disulphoxide. Of the active material excreted in the urine, about 60 per cent can be assigned to the known products. This, however, corresponds at most to 1–2 per cent of the total active material administered. Only traces of unchanged thioridazine are detectable.

The fate of the remaining 80 per cent of the substances administered is a matter for conjecture. Of the active material remaining in alkaline solutions of the bile after extraction with chloroform which takes up the simple metabolites mentioned (corresponding to about 25 per cent of the activity present), the greater part (50–60 per cent of the total) can be extracted with butanol. The metabolites in this extract can

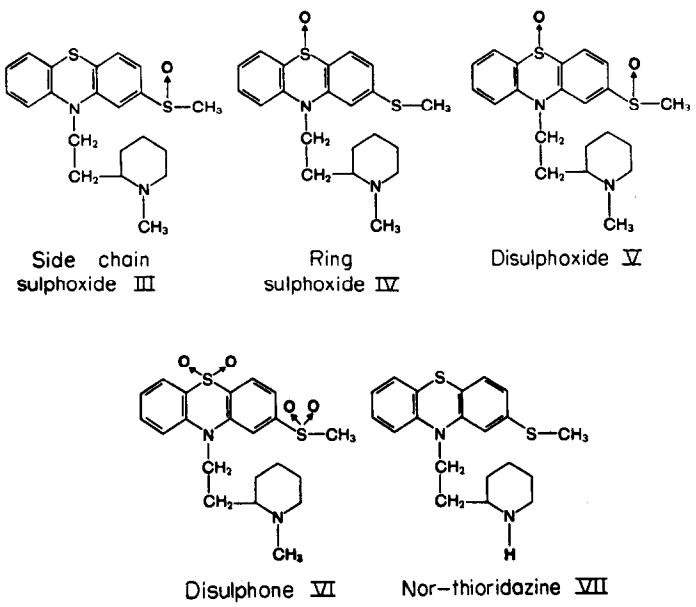


FIG. 6. Metabolites of thioridazine.

TABLE 7. IDENTIFIED METABOLITES OF THIORIDAZINE IN BILE AND URINE OF RAT (Determination by inversed isotope-dilution)

	Bile				Urine			
	^{14}C 48 hr	^{14}C 40 hr	^{35}S 72 hr	^{35}S 40 hr	^{14}C 48 hr	^{14}C 40 hr	^{35}S 45 hr	^{35}S 40 hr
Excretion in % of injected total activity	36·5	18·0	78·2	64·3	3·5	2·6	1·5	4·2
thereof: %								
Disulphoxide	6·1	—	8·3	—	12·7	—	7·2	—
Side-chain sulphoxide	1·0	—	0·8	—	4·1	—	4·7	—
Ring sulphoxide	0·1	—	0·3	—	2·1	—	2·2	—
Thioridazine	0·2	—	0·1	—	1·7	—	1·7	—
Disulphone	1·9	—	—	—	0·9	—	—	—
Total to disulphone	—	9·0	—	—	—	30·6	—	—
Total to northioridazine-disulphone	—	—	—	11·3	—	—	—	30?
% of excreted activity	9·3	9·0	9·5	11·3	21·5	30·6	15·8	30?
% of administered total activity	3·4	1·6	7·4	7·3	0·8	0·8	0·2	1·3?

be cleaved by β-glucuronidase. In Fig. 7 an autoradiogram of a two-dimensional paper chromatogram of an ethanol extract of freeze dried bile, containing all the ^{35}S-metabolites is shown. After treatment with β-glucuronidase of the extract the autoradiogram Fig. 8 results.

The main radioactive spots now are seen to lie in the region, where unconjugated thioridazine derivatives are usually found, and the radioactive spots of the more hydrophilic compounds in Fig. 7 have disappeared.

From this it is concluded that a large proportion of the material excreted in the bile consists of a series of glucuronides of hydroxylated derivatives of thioridazine and northioridazine and probably also of their oxidation products. Similar findings were recently also obtained for chlorpromazine.[8, 13, 14] For the present we assume, as do other authors, that the hydroxyl radicals have been introduced into positions 3 and/or 7 of the phenothiazine ring.

Our attempts to synthesize phenolic derivatives of thioridazine, either by Brodie's[26] method with Fe^{2+} ions and ascorbic acid in the presence of EDTA, or by enzymic methods, have as yet remained without success. On the other hand, sulphoxidation[27, 28] and demethylation[6, 12] could be detected *in vitro* with rat liver microsomes.

REFERENCES

1. J. RUTSCHMANN, F. KALBERER, W. KREIS and K. ZEHNDER, *Chimia* **15**, 406 (1961).
2. N. P. SALZMANN and B. B. BRODIE, *Nature, Lond.* **176**, 1122 (1955).
3. N. P. SALZMANN and B. B. BRODIE, *J. Pharm., Lond.* **118**, 46 (1956).
4. T. BERTI and L. CIMA, *Farmaco, Ed. sci.* **12**, 159 (1957).
5. R. L. YOUNG, J. J. ROSS, JR. and A. R. MAASS, *Nature, Lond.* **183**, 1396 (1959).
6. J. J. ROSS, JR., R. L. YOUNG and A. R. MAASS, *Science* **128**, 1279 (1958).
7. S. S. WALKENSTEIN and J. SEIFTER, *J. Pharm., Lond.* **125**, 283 (1959).
8. T. H. LIN, L. W. REYNOLDS, I. M. RONDISH and E. J. VAN LOON, *Proc. Soc. Exp. Biol., N.Y.* **102**, 602 (1960).
9. W. BLOCK, *Arzneimittelforschg.* **11**, 266 (1961).
10. I. S. FORREST, F. M. FORREST and M. BERGER, *Biochim. et Biophys. Acta* **29**, 441 (1958).
11. I. G. FELS and M. KAUFMANN, *Nature, Lond.* **183**, 1392 (1959).
12. B. N. LA DU, L. GAUDETTE, N. TROUSOF and B. B. BRODIE, *J. Biol. Chem.* **214**, 741 (1955).
13. V. FISHMAN and H. GOLDENBERG, *Proc. Soc. Exp. Biol., N.Y.* **104**, 99 (1960).
14. H. S. POSNER, Abstract paper presented to the American Chemical Society, 135th Meeting, 81 C (1959).
15. J. P. BOURQUIN *et al.* Unpublished.
16. J. L. WOOD, J. R. RACHELE, C. M. STEVENS, F. H. CARPENTER and V. DU VIGNEAUD, *J. Amer. Chem. Soc.* **70**, 2547 (1948).
17. A. STOLL, J. RUTSCHMANN, A. VON WARTBURG and J. RENZ, *Helv. Chim. Acta* **39**, 993 (1956).
18. S. S. WALKENSTEIN and C. M. KNEBEL, *Analyt. Chem.* **29**, 1516 (1957).
19. J. KATZ and S. B. GOLDEN, *J. Lab. Clin. Med.* **53**, 658 (1959).
20. J. RUTSCHMANN and W. SCHÖNIGER, *Helv. Chim. Acta* **40**, 428 (1957).
21. J. RUTSCHMANN, *Helv. Chim. Acta* **40**, 433 (1957).
22. R. T. BRITTAIN, *Labor Practice* **8**, 279 (1959).
23. N. A. FYODOROV, *Proceedings of the Second International Conference on the Peaceful Uses of Atomic Energy, Geneva* 1958 Vol. 24, p. 205. United Nations, New York (1958).
24. S. EIDUSON, E. GELLER, S. COHEN, Paper 18.69.766 *V International Congress of Biochemistry, Moskow* (1961).
25. J. H. WEIKEL, A. G. WHEELER and P. D. JOINER, *Tox. Appl. Pharmacol.* **2**, 68 (1960).
26. S. UDENFRIEND, C. T. CLARK, J. AXELROD and B. B. BRODIE, *J. Biol. Chem.* **208**, 731 (1954).
27. J. J. KAMM, J. R. GILLETTE and B. B. BRODIE, *Fed. Proc.* **17**, 382 (1958).
28. J. R. GILLETTE and J. J. KAMM, *J. Pharm., Lond.* **130**, 262 (1960).
29. E. HANSSON and C. G. SCHMITERLÖW, *Arch. int. pharmacodyn.* **131**, 309 (1961).

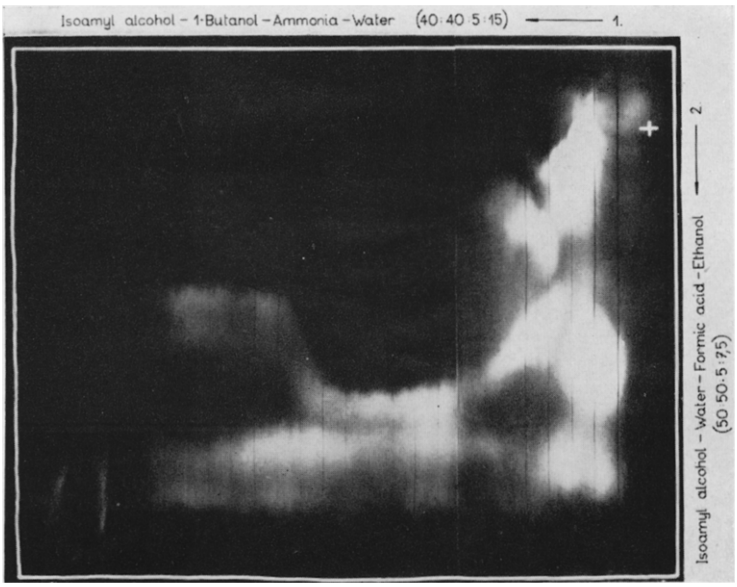


FIG. 7. Autoradiogram of ^{35}S-thioridazine-metabolites in rat.
(Absolute ethanol extract of lyophilized bile.)

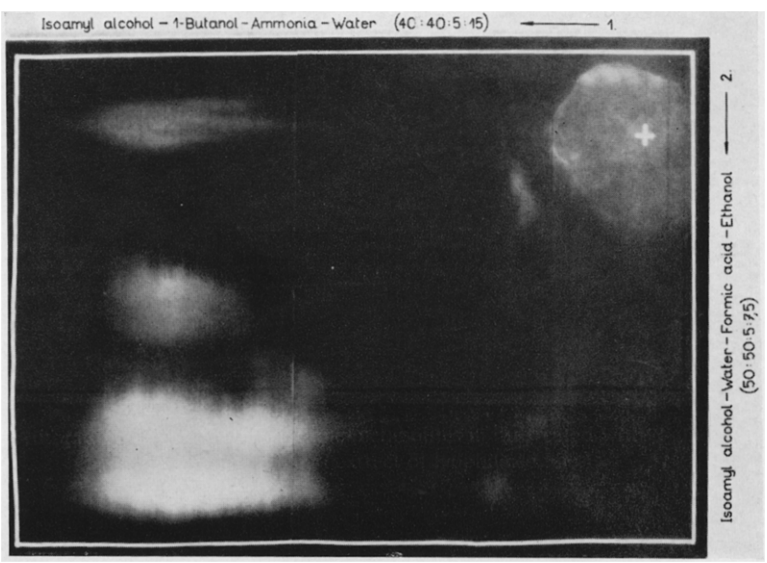


FIG. 8. Autoradiogram of ^{35}S-thioridazine-metabolites in rat treated with β-glucuronidase.
(Absolute ethanol extract of lyophilized bile.)

facing page 550