ABSORPTION AND FATE OF PROPYLTHIOURACIL AND METHIMAZOLE IN THE RAT

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by

Daniel Samuel Sitar

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Dedicated

to my wife

Maureen Elizabeth

ABSTRACT

The absorption and fate of two antithyroid drugs were investigated. 6-n-Propyl-2-thiouracil-6-^{14}C and methimazole-2-^{14}C were administered orally, intraperitoneally and intravenously to Sprague-Dawley rats of either sex at a dose of 20 mg/kg. Both drugs were completely absorbed after oral administration. Plasma half-lives of propylthiouracil after intravenous, intraperitoneal and oral administration were 1.5, 9 and 12 hours respectively. The corresponding half-lives for methimazole were 4, 4 and 5 hours. Equilibrium dialysis demonstrated that propylthiouracil was bound to plasma proteins to the extent of 57% and methimazole was bound to the extent of 5%. The disappearance of propylthiouracil from the plasma after intravenous administration could be represented by a two compartment model. The corresponding data obtained after an intravenous dose of methimazole indicated a one compartment model.

Tissue distribution of the two drugs was investigated. Propylthiouracil was not concentrated in any tissue. In contrast, methimazole was concentrated in the kidney. Since the concentration of propylthiouracil was higher than methimazole in the stomach after an oral dose, this indicated that propylthiouracil was more slowly absorbed. These findings are consistent with the partition and solubility data for these drugs.

Up to 80% of both drugs and their metabolites were

excreted in the urine in 24 hours, and little or no radioactivity was excreted in the feces, regardless of the dosage route. Metabolites of both drugs were excreted into the bile. Total biliary excretion, 10 hours after an oral dose, accounted for 15% of the administered dose of propylthiouracil and 10% of the administered dose of methimazole. Thus, an enterohepatic circulation of these drugs was present.

Biliary and urinary metabolites were qualitatively similar. After doses of either drug, 15% of the radioactivity in the 24 hour urine samples was accounted for by unchanged propylthiouracil, and 20% by unchanged methimazole. The major metabolite of either drug was a glucuronide conjugate. These products accounted for 45-60% of the radioactivity in the 24 hour urine samples. An unidentified metabolite was present in the urine after the administration of either drug which represented 20-30% of the radioactivity in the 24 hour urine samples.

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INTRODUCTION

A. Historical

Although intensive research in the field now known as drug metabolism is a relatively recent event, the actual beginnings of these studies go back more than a century. The earliest observation of metabolism of a foreign compound is attributed to Gmelin, who reported in 1824 on a garlic-like odor in the viscera of animals poisoned by tellurium. It was not until 1855 that Wöhler identified the odor as dimethyl telluride. The first report of oxidative metabolism was made by Schultzen and Naunyn in 1867. They reported that phenol was excreted in the urine of dogs and men who ingested benzene.

A different type of transformation mechanism, now referred to as conjugation, was demonstrated by Keller in 1842. He showed that when mammals were fed benzoic acid, the excretory product in the urine was hippuric acid, i.e. by conjugation with glycine. This work was followed by reports of sulfate conjugation of phenol by Baumann in 1876, glucuronic acid conjugation of camphor by Schmiedeberg and Meyer in 1879, and mercapturic acid conjugation of halogenated phenols by Jaffe and independently by Baumann and Preuse in 1879. (For a more detailed review, see Williams 1959a.)

An important advance, in the more recent literature, was reported by Brodie *et al.* (1955). They demonstrated

that many of the metabolic transformations of drugs took place in the microsomal fraction of the liver. This discovery is the basis for much of the recent research concerning the mechanisms of biotransformation of foreign compounds. The role of microsomes in drug metabolism has been recently reviewed by Gillette et al. (1969).

The question arises why foreign compounds should be metabolized at all. Brodie (1964a) has suggested that the kidney is poorly equipped to excrete foreign compounds which are lipid soluble and not ionized at physiological pH. Brodie and Hogben (1957) found that nonionic, lipid soluble foreign compounds were almost completely reabsorbed from the kidney tubules. They found that almost all drug metabolites were less lipid soluble than the parent compound. These authors suggested that foreign compounds, which undergo biotransformation, are converted to more polar, less lipid soluble products that can be more readily excreted by the kidney.

B. Factors Affecting the Fate of Administered Foreign Compounds

1. Absorption

Foreign compounds and drugs may be absorbed from a great variety of sites, e.g. the mouth, stomach, small intestine, colon, rectum, skin, trachea and lungs.

Absorption of a foreign compound may occur by one or more of the following mechanisms; diffusion, filtration

and active transport. Of these mechanisms, simple diffusion is often the principle mechanism by which foreign compounds are transferred across cell membranes. Active transport may occur with certain compounds. However, the role of filtration is largely unknown in the mammal (Brodie 1964a; Parke 1968a).

The absorption of a drug by diffusion has been demonstrated to be governed by the physico-chemical properties of that drug (Brodie and Hogben 1957; Brodie 1964b; Parke 1968b). The rate of diffusion may be expressed by Fick's Law:

$$\text{Rate of diffusion} = \frac{kA(c_1 - c_2)}{d}$$

where $c_1 - c_2$ represents the concentration gradient of free drug across the membrane, A the surface area of the membrane, d the thickness of the membrane, and k the diffusion constant of the drug. The diffusion constant is characteristic of the drug, and is dependant upon a number of factors, including molecular weight, configuration, ionization constant and lipid solubility.

The work of Hogben *et al.* (1957), together with the findings of Schanker *et al.* (1957), resulted in the general conclusion that absorption from the stomach of man was the same as that observed in the rat for a wide variety of drugs which they investigated. Hogben *et al.* (1959) presented evidence to support the concept that it is the

unionized form of a drug which is preferentially absorbed. Schanker (1960) stated that two physical properties were important in determining the rate of absorption of a drug from the gastrointestinal tract; the dissociation constant, and the lipid:water partition ratio of the undissociated drug.

In applying physico-chemical criteria to drug absorption, it should be noted that many drugs are weak organic acids or bases which exist in solution in both the undissociated and dissociated forms. The proportion of each form is governed by the pH of the medium. Thus weak organic acids or bases, present in the unionized form, are readily absorbed from the gastrointestinal tract, i.e. acids from the stomach, and bases from the intestine. Completely ionized drugs, such as quaternary ammonium compounds and sulfonic acids, are absorbed slowly (Schanker 1962a). However, unionized substances, that have low lipid solubility, are also slowly absorbed, e.g. sulfaguanidine (Weinstein 1970). The above characteristics of absorption are best explained by the assumption that the barrier between the gastrointestinal tract and blood behaves as a lipid membrane. Hence, lipid soluble substances may readily diffuse through the membrane, while substances with low lipid solubility would diffuse less readily, or not at all.

2. Protein Binding

Foreign compounds may become bound to plasma and

tissue proteins by covalent, hydrogen, ionic and dipole-induced dipole bonds (Goldstein et al. 1968). When bound, foreign compounds are not freely diffusable across biological membranes. When binding is reversible, equilibrium between the bound and unbound forms determines the rate of passive diffusion across membranes. It is generally considered that protein binding is most commonly associated with plasma albumin which comprises approximately 50% of the total plasma proteins. This protein molecule contains a large number of charged sites which are capable of interaction with foreign compounds. These potential binding sites may also be occupied by endogenous substrates which may be displaced by foreign compounds in the plasma. More stable binding to plasma albumin may occur by the formation of disulfide bonds, as has been found to occur with disulfiram (Antabuse^{R}) (Strömme 1965).

Serum globulins have also been found to be involved in protein binding of foreign compounds. This has been noted with certain sulfa drugs e.g. 3-sulfamido-6-methoxypyridazine (Lederkyn^{R}) (Clausen 1966).

Genazzani and Pagnini (1963) found species differences in the binding of several sulfonamides to serum proteins. They found that serum proteins from man bound sulfa drugs more extensively than did serum proteins from mouse, rat, rabbit and other mammals used in their study. The protein binding was not found to correlate with the proportion

of albumin in the serum protein samples.

3. Excretion

The principal routes of excretion of drugs and their metabolites are via the kidneys, biliary system, intestine and sometimes the lungs. Of these organ systems, the kidneys are considered to be the most important. However, drugs and metabolites may also be excreted in sweat, milk and saliva.

a) Kidney

Excretion by the kidney may involve one or more of three processes; glomerular filtration, passive tubular transfer (diffusion), and active transport (Schanker 1962a).

Glomerular filtration yields an ultrafiltrate of blood plasma containing foreign compounds and their metabolites in approximately the same concentration as their free concentration in blood. Thus extensive protein binding could be a limiting factor in excretion by glomerular filtration alone (Kakemi et al. 1962), although few examples are known.

Passive tubular transfer is similar to the absorption process in that it varies with pH and the partition coefficient of the compound involved. Thus the rate of excretion of organic electrolytes may vary with the pH of the urine. For example, phenobarbital (pK_a = 7.3) is more rapidly excreted in an alkaline than in a neutral or acidic urine (Waddell and Butler, 1957).

Active transport of organic anions and cations is associated with separate transport mechanisms. Both processes occur in the proximal tubule, and the transported molecules are thought to be highly ionized. However, Parke (1968c) stated that it is unlikely the active transport system can distinguish between weak and strong electrolytes, and suggested that both are probably excreted by the same mechanism. Examples of organic acidic compounds that have been found to be excreted by this mechanism include ether glucuronides e.g. phenol and glucuronic acid, ester glucuronides e.g. o-aminobenzoic acid and glucuronic acid, and amino acid conjugates e.g. benzoic acid and glycine; phenylacetic acid and glutamine; aromatic hydrocarbons and glutathione. A similar transport system is also thought to exist in the liver and central nervous system (Schanker 1962a). Furthermore, relatively polar, lipid insoluble drugs and metabolites are less readily reabsorbed, and are thus subject to a higher renal clearance than less polar analogs. Rapid elimination of these polar compounds is thus observed (Parke 1968d).

b) Bile

Brauer (1959) stated criteria, which governed the excretion of foreign compounds into the bile, to be as follows: the compound should have a molecular weight of greater than 300, and be bound to plasma proteins, especially albumin. However, it is not at all clear that protein

binding is necessary for the biliary excretion of foreign substances. Brauer divided these compounds into three categories, depending on their concentration in the bile: 1) bile concentration greater than blood concentration (10 - 1000 x)
2) bile concentration equal to blood concentration
3) bile concentration much less than blood concentration. He stated that drugs could belong to any one of these three categories. Williams et al. (1965) divided foreign compounds excreted in bile of rats into two groups; less than 5% of the administered dose, and 5 - 95% of the administered dose. Although both groups included conjugates, the second group was composed primarily of conjugates.

The secretion of organic anions in bile is well established, and has been reviewed by Sperber (1959). Schanker (1962b) described the "concentrative" transfer of organic cations into bile. Procaine amide ethobromide and mepiperphenidol were transported, but decamethonium and tetraethylammonium were not. Thus, it appears that both anions and cations may be transported into bile.

Conjugates excreted into bile may be hydrolyzed by enzymes in the bile and intestine, and by the gut flora. Glucuronides are thought to be more susceptible to such hydrolyses than are ethereal sulfate conjugates. The hydrolysis products are then generally less polar, and are reabsorbed. They may subsequently recirculate to the liver

where they may be remetabolized and re-excreted into the bile. This process is referred to as enterohepatic circulation (Parke 1968e). Abou-el-Makarem et al. (1966) found species variation in the extent of biliary excretion of foreign compounds. Biliary excretion of relatively small aromatic molecules was found to be low in all species, but with compounds of higher molecular weight, considerable species variation was noted.

C. Mechanisms of Termination of Drug Action

Drug action may be terminated by one or more of the following mechanisms; redistribution, biotransformation and excretion.

An excellent example of the termination of drug action by redistribution is that of thiopental, whose anesthetic action has been found to be terminated by redistribution from the brain to other tissues of the body (Goldstein and Aranow 1960; Mark 1963).

Biotransformation is also an important factor in the termination of drug action. However, some drugs are activated via biotransformation. A classical example is the transformation of prontosil to sulfanilamide. Biotransformation may also result in metabolites with increased toxicity. Thus, parathion is desulfurated to yield the potent cholinesterase inhibitor paraoxan (Davison 1955). These examples demonstrate that the generally used term "detoxication" is somewhat of a misnomer, and the term biotransformation, or

metabolism would better describe the process. Generally speaking, biotransformation results in the formation of a more polar compound, and thus enhances the possibility that the drug will be excreted. Biotransformation reactions may be classified as follows; oxidation, reduction, hydrolysis and synthesis (conjugation). These reactions have been reviewed extensively, and details may be found in many books and reviews, some of the major works including Williams (1959b), Gillette (1963 and 1966), Dutton (1966), Parke (1968f) and McMahon (1970).

D. The Thionamide Drugs

Propylthiouracil and methimazole are the most popular of the antithyroid drugs used in North America, although other compounds, e.g. thiouracil, may enjoy wider use in other parts of the world (Greer 1964). Propylthiouracil is accepted as a safe and effective drug in the treatment of hyperthyroidism (F.D.A. 1969). Both methimazole and propylthiouracil act by inhibiting the formation of iodothyronines. Maloof and Soodak (1961) suggested that thiourea cleaved a disulfide bond in the thyroid microsomal fraction, and formed a mixed disulfide bond. Upon hydrolysis, the complex was cleaved to yield a thiosulfenic acid and urea. Maloof and Soodak (1963) presented the hypothesis that a sulfenyl iodide, formed from the cleavage of a disulfide bond by iodide, was the active iodinating species in the thyroid tissue. Cunningham (1964) showed that β-lactoglobu-

lin sulfenyl iodide reacted with thiourea and thiouracil to form the mixed disulfide bond postulated by Maloof and Soodak. Jirousek and Cunningham (1968) gave further support to this hypothesis when they demonstrated that beef thyroid microsomes bound increased amounts of thiouracil in the presence of sulfenyl iodide groups. However, these sulfenyl iodide compounds have not yet been isolated and characterized.

In the studies of the metabolism of thionamide drugs, Williams and Kay (1944) measured the urinary excretion of thiouracil in man. They found about one third of an administered dose was excreted unchanged in the urine after 24 hours. They assumed that the difference between the administered dose of this drug, and its urinary excretion, was due to metabolism. Sarcione and Sokal (1958) demonstrated that Sprague-Dawley rats methylated thiouracil and excreted 2-methylthiouracil as a urinary metabolite. Spector and Shideman (1959) demonstrated the desulfuration of thiouracil by the Holtzman rat to give uracil as a urinary metabolite. They also demonstrated the <u>in vitro</u> metabolism of thiouracil by whole liver mince to give uracil, β-alanine and ammonia.

There has been no systematic study on the absorption, distribution, metabolism and excretion of propylthiouracil and methimazole, despite the fact that they have been used for over ten years. Moreover, the drugs are recommended for use in children and pregnant women, where the risk of

surgery is usually unwarranted. The side effects, although rare, may be serious. Therefore, a comprehensive study of the absorption, distribution, metabolism and excretion of these drugs appeared to be necessary. The only study to this time is a preliminary report by Alexander et al. (1969) who investigated the serum levels of ^{35}S-labelled drugs in two patients with hyperthyroidism, one of whom had severe renal impairment. The hypothesis of Maloof and Soodak, concerning the mechanism of action of thionamide drugs would appear to cast doubt on the usefulness of using ^{35}S label in these drug studies. It might be predicted that the sulfur would be lost during metabolism, and the fate of the remainder of the molecule would not be known.

EXPERIMENTAL METHODS

A. Synthesis of 6-n-Propyl-2-thiouracil-6-^{14}C

1. Butyryl Chloride-1-^{14}C

Butyric acid (8.8 g, 0.1 mole) was added to sodium butyrate-1-^{14}C (40 mg, 0.8 mCi; Amersham-Searle, Toronto, Canada). Hydrogen chloride gas (Matheson of Canada Ltd.) was passed into this solution for ten minutes to ensure equilibration of the label. To this solution, in a 50 ml round bottom flask equipped with a short distillation column, was added benzoyl chloride (28.2 g, 0.2 mole), and butyryl chloride was distilled over as rapidly as possible. When no more distillate formed, butyric acid carrier (0.96 g, 0.01 mole) and benzoyl chloride (2.8 g, 0.02 mole) were added to the distillation flask, and distillation was recontinued until no more product could be collected. The yield of butyryl chloride was 9.0 g (76%), boiling range 100 - 104°C; reported 101 - 102.5°C (Brown 1938).

2. Ethyl 3-Oxohexanoate-3-^{14}C

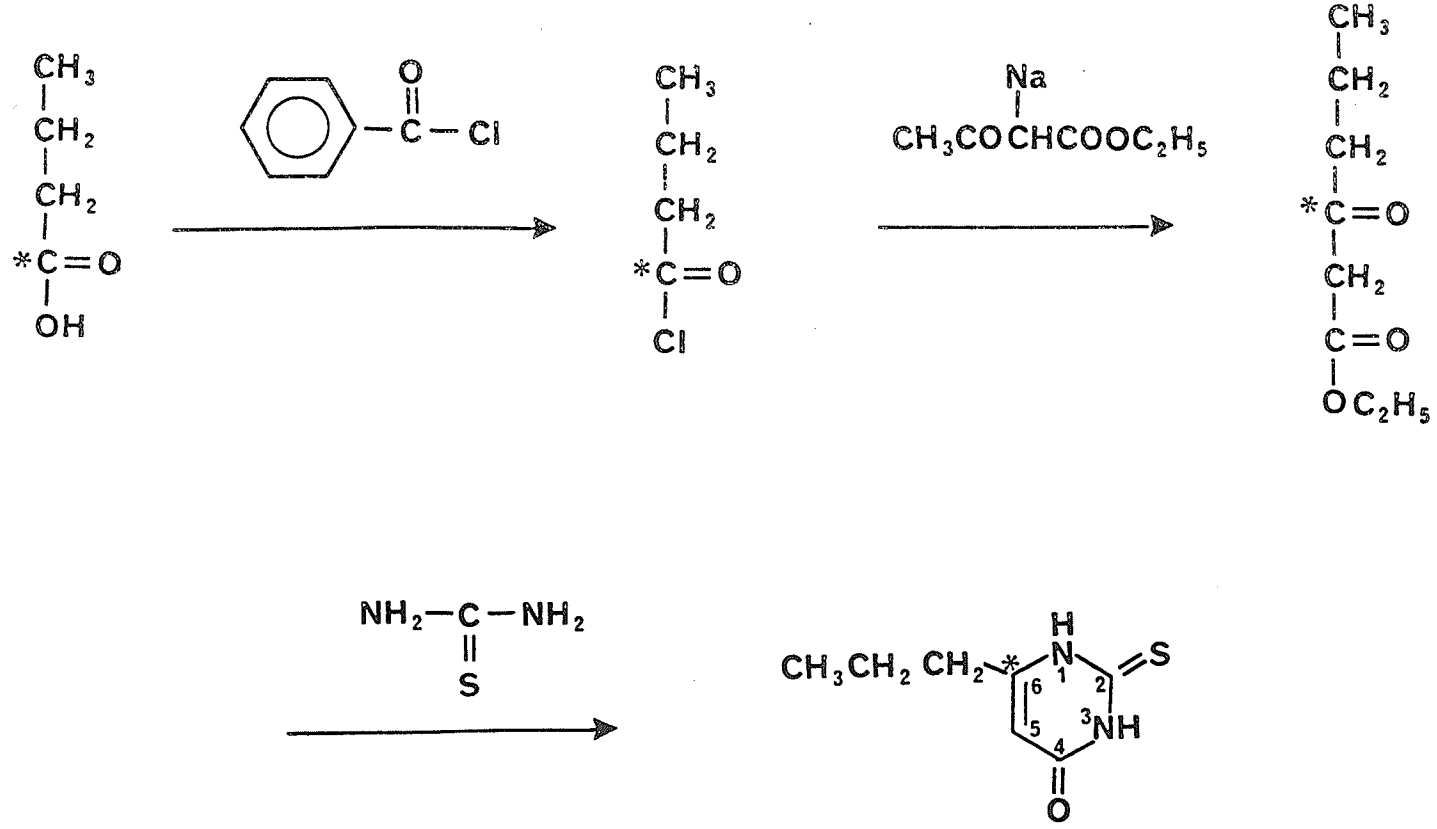
Sodio ethylacetoacetate was prepared by the method of Zaugg *et al.* (1961). Reagent grade ether was dried over Linde 3A molecular sieve until the ether no longer reacted with sodium. An ether solution of acetoacetic ester (32.75 g, 0.25 mole) was added to small chips of sodium metal (5.75 g, 0.25 mole) stirred in anhydrous ether. The reaction was completed by refluxing for two hours, and the product allowed to stand overnight. Sodio ethylacetoacetate

was filtered, washed with anhydrous ether, and stored in a desiccator. Butyryl chloride-1-^{14}C (9.0 g, 0.08 mole) was added dropwise, over a period of eighteen minutes, to a slurry of sodio ethylacetoacetate (12.8 g, 0.08 mole) which was stirred in 50 ml of anhydrous ether. The reaction mixture was allowed to stand overnight. Sodium chloride was removed by filtration, and dry ammonia gas (20 g, 1.2 moles; Matheson of Canada Ltd.) was passed for six hours into the ether solution cooled in ice. The reaction mixture was then allowed to stand overnight at room temperature. The mixture was washed with water and then stirred with 10% hydrochloric acid (30 ml) for two hours. The ether layer was then rewashed with water and 5% w/v sodium bicarbonate solution. The ether layer was evaporated and the residue was extracted with five successive portions of 5 ml saturated sodium bisulfite solution. The residue was washed with water, dissolved in ether, and dried over anhydrous sodium sulfate. The ether solution was decanted and ether was removed by distillation. The remaining oil was distilled under vacuum to yield 2.6 g (0.02 mole) ethyl-3-oxohexanoate-3-^{14}C, boiling range 94 - 105°C at 15 mm Hg; reported 93 - 94°C at 15 mm Hg (Anderson et al. 1945). Carrier ester (1.0 ml, 0.97 g) was added to the boiler flask and the distillation was recontinued to completion. The infrared spectrum of the distillate was identical to that of authentic material (Aldrich Chemical Co., Milwaukee, Wisconsin).

3. 6-n-Propyl-2-thiouracil-6-^{14}C

Ethyl 3-oxohexanoate-3-^{14}C (3.51 g, 0.02 mole), dissolved in anhydrous ethanol (5 ml), was added to a solution of sodium metal (1.1 g, 0.05 mole) and thiourea (2.35 g, 0.03 mole) in anhydrous ethanol (25 ml). The mixture was heated on a steam bath for six hours and allowed to stand overnight at room temperature. The flask was connected to a distillation assembly and most of the ethanol was distilled. The distillation of the ethanol was completed at reduced pressure. The residue was dissolved in water (20 ml) and the desired product was precipitated by acidification with concentrated hydrochloric acid (3 ml). The slurry was adjusted to pH 4.0 with glacial acetic acid, and the product was filtered, washed with cold water, and dried under vacuum to yield 2.69 g (0.016 mole) 6-n-propyl-2-thiouracil-6-^{14}C. The product was recrystallized from hot water to constant specific activity (17.5 uCi/mM). The melting point of the final product was 217 - 218°C; reported 218 - 219°C (Anderson *et al.* 1945). A summary of this synthetic method is illustrated in Fig. 1. The overall chemical yield based on butyryl chloride was 14%, and the radiochemical yield based on sodium butyrate-1-^{14}C was 34%. Radiochemical purity was confirmed by thin-layer chromatography on fluorescent silica gel (Merck, Darmstadt, Germany) in two solvent systems. The developers used were isopropanol:benzene (15:85) and benzene:methanol:acetic acid (79:14:7). The product migrated to

Fig. 1: Synthesis of 6-n-propyl-2-thiouracil-6-^{14}C from butyric acid-1-^{14}C. The asterisk denotes the position of the radioactive label.

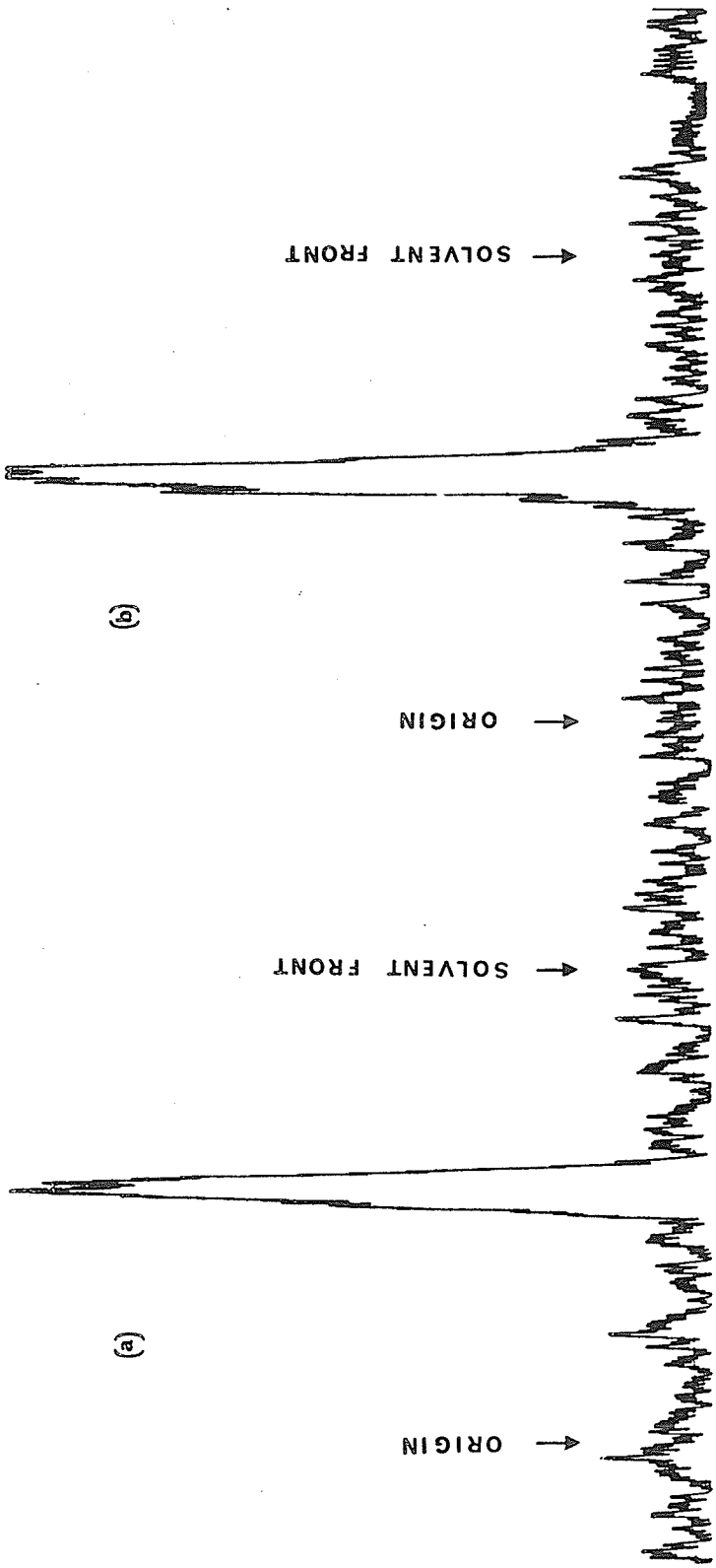


give a single spot in both solvent systems with R_f values of 0.53 and 0.54 respectively. Radioscans of the developed thin-layer plates (Fig. 2) demonstrated a single peak which corresponded to the quenching of fluorescence when the plate was viewed under ultraviolet light.

B. Methimazole-2-^{14}C

Methimazole-2-^{14}C (Fig. 3) was a gift from Eli Lilly Research Laboratories through the courtesy of Dr. R.E. McMahon. As the drug was not radiochemically pure, 0.1 mCi was separated on a preparative thin-layer plate of fluorescent silica gel (Macheray, Nagel and Co., Düren, Germany) 1 mm thick. Isopropanol:benzene (1:1) was used as the developer. The R_f of the radiochemically pure drug was 0.50. The labelled methimazole was extracted from the silica gel with ethanol. The solvent was removed, and recrystallized methimazole (1.0 g) was added to the radiochemically pure product. The resulting mixture was recrystallized from acetone:hexane to constant specific activity (22.1 uCi/mM). The melting point of the final product was 143 - 144°C; reported 146 - 148°C (Stecher 1968). Radiochemical purity was confirmed by thin-layer chromatography on fluorescent silica gel in two solvent systems. The developers used were isopropanol:benzene (1:1) and benzene:methanol:acetic acid (79:14:7). The product migrated to give a single spot in both solvent systems with R_f values of 0.50 and 0.34 respectively. Radioscans of the developed thin-layer plates

Fig. 2: Radiochromatograms of 6-n-propyl-2-thiouracil-6-^{14}C. Chromatogram (a) was developed with isopropanol:benzene (15:85), and chromatogram (b) with benzene:methanol:acetic acid (79:14:7). (a) R_f = 0.53 (b) R_f = 0.54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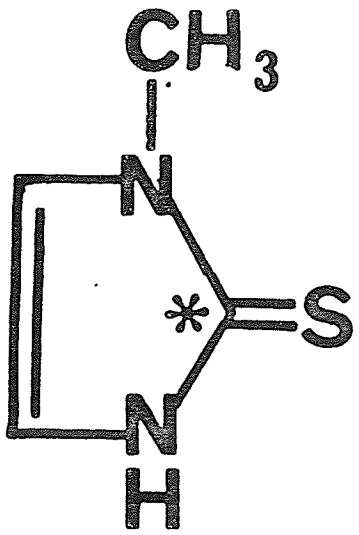


Fig. 3: Methimazole-2-^{14}C. The asterisk denotes the position of the radioactive label.

(Fig. 4) demonstrated a single peak corresponding to the quenching of fluorescence when the plate was viewed under ultraviolet lights.

C. Animals

Male and female Sprague-Dawley rats which weighed 175-225 g were obtained from a colony maintained at the Faculty of Dentistry, University of Manitoba. The animals were fasted overnight before experiments, and at least three animals were used in each experiment.

D. Drug Solutions

1. Propylthiouracil

Labelled propylthiouracil was dissolved in sufficient dilute sodium hydroxide solution to form the monosodio derivative. Distilled water was then added to give a solution of final concentration equal to 5 mg propylthiouracil/ml. Solutions for intravenous injection were prepared as above, except that the monosodio derivative was diluted with isotonic saline solution.

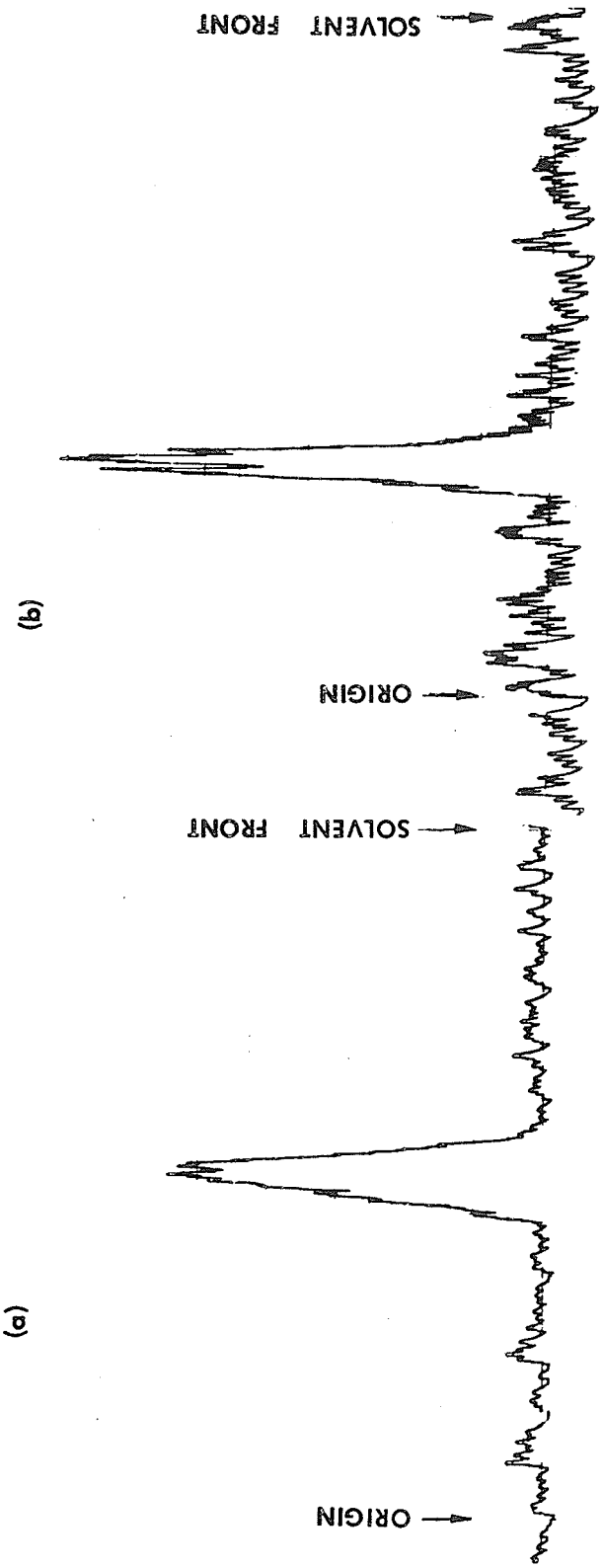
2. Methimazole

Labelled methimazole was dissolved in distilled water to give a concentration of 5 mg/ml. Solutions for intravenous injection were prepared by dissolving the drug in isotonic saline solution.

E. Administration of Drugs

Doses of labelled propylthiouracil or methimazole

Fig. 4: Radiochromatograms of purified methimazole-2-^{14}C. Chromatogram (a) was developed with isopropanol:benzene (1:1), and chromatogram (b) with benzene:methanol:acetic acid (79:14:7). (a) R_f = 0.50 (b) R_f = 0.34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(b)
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(a)

(20 mg/kg) were administered orally (by gavage needle), intraperitoneally, and intravenously (via the jugular vein) to the experimental animals.

F. Plasma Levels of Radioactivity and Administered Drugs

1. Total Radioactivity

After the drug was administered (P.O., I.P. or I.V.), animals were placed in restraining cages and allowed free access to water. The tip of the tail was severed, and blood was collected in a heparinized capillary tube at suitable time intervals (Table 2). The tubes were then centrifuged in an IEC desk top centrifuge (head IEC 275). Measured lengths of these capillary tubes, which contained known volumes of plasma, were placed in scintillation vials and broken after the addition of formic acid (0.50 ml) and 30% hydrogen peroxide (0.20 ml). The vials were sealed and heated to 70°C for one hour. The vials were cooled in ice and radioactivity was determined by liquid scintillation counting after the addition of scintillation fluid. Plasma levels of labelled drug, measured one minute after an intravenous dose, were confirmed from blood samples obtained by cardiac puncture.

2. Propylthiouracil

Aliquots of blood (200 - 400 ul), squeezed from the end of a severed rat tail onto a polyethylene sheet, were placed in 15 ml centrifuge tubes. Since the partition co-

efficient of propylthiouracil was maximal at pH 6.0 (Table 1), 1.0 ml pH 6.0 4M phosphate buffer was added to each aliquot. The samples were mixed and extracted with three successive portions of 2 ml dichloromethane. The combined extracts of each sample were evaporated to dryness in a stream of nitrogen. Scintillation fluid was added and radioactivity was determined. The resulting data were converted to plasma concentrations of propylthiouracil by using the factor 1.3, which was obtained from the plasma/blood concentration ratio, determined in distribution studies with propylthiouracil (Table 4).

Preliminary experiments demonstrated that the only radioactive component extractable from both hydrolyzed and unhydrolyzed urine samples with dichloromethane at pH 6.0 was unchanged drug. Ethyl acetate extracted small quantities of more polar derivatives as shown by examining thin-layer chromatograms for radioactivity. Control experiments were carried out to determine the extent of recovery of propylthiouracil after extraction of whole blood with dichloromethane. Propylthiouracil was added to whole blood, that had been collected in a heparinized container, to give final concentrations of 5 - 50 ug propylthiouracil/ml. Aliquots of blood (400 ul) were placed in 15 ml centrifuge tubes, and the experiment was continued as described above for the actual assay after a dose of labelled propylthiouracil. Over the concentration range stated, the amount of drug

extracted was 64 ± 2% (mean ± S.E.).

3. Methimazole

Aliquots of blood (200 - 400 ul), obtained from the rat tail in the same manner as in the propylthiouracil experiments, were placed in 15 ml centrifuge tubes. To each aliquot was added 1.0 ml pH 6.0 4M phosphate buffer. The samples were mixed and extracted with three successive portions of 2 ml ethyl acetate. The combined extracts from each sample were evaporated to dryness in a stream of nitrogen. Each sample was then extracted with ethanol (200 ml). An aliquot (150 ul) was applied to a thin-layer fluorescent silica gel plate with a Cordis^{R} applicator (Cordis Labs., Miami, Florida), and developed for 15 cm with benzene:methanol:acetic acid (79:14:7). The radioactive zone, which corresponded to unchanged methimazole, was scraped from the plate and placed in a scintillation vial. Radioactivity was determined by liquid scintillation counting after scintillation fluid was added to the sample.

Control experiments were performed previously to determine the extent of recovery of added methimazole to whole blood. Labelled methimazole was added to whole blood, that had been collected in a heparinized container, to give final concentrations of 10 - 50 ug methimazole/ml. Aliquots of blood (400 ul) were placed in 15 ml centrifuge tubes, and the experiment was continued as described above for the assay of plasma concentration after a dose of labelled methimazole. The transposition of the ethanol aliquot to the

thin-layer plate required several applications with the Cordis^{R} applicator, with forced drying between sample applications. Comparative chromatography experiments were carried out with urine samples which contained metabolites, to demonstrate that free drug was adequately separated from these metabolites. (For R_f values, see Table 12.) The recovery of unchanged methimazole from whole blood was measured by liquid scintillation counting prior to loading onto thin-layer plates, and after recovery from the developed chromatograms. Recovery by the solvent extraction technique was 76 ± 2%, and for the combined sequence of extraction and thin-layer chromatography 54 ± 3% (mean ± S.E.).

G. Distribution Studies

At suitable time intervals after an oral dose of drug (Tables 4 and 11), animals were killed by decapitation and allowed to exsanguinate. Tissue samples (approximately 100 mg wet weight) were taken, blotted, weighed, and added to formic acid (0.50 ml) in a scintillation vial. The vials were sealed and heated to 70°C until dissolution of the sample was complete (approximately one hour). The vials were then cooled in ice and color was bleached by the addition of 30% hydrogen peroxide (0.20 ml). Scintillation fluid was added to each sample and radioactivity was determined by liquid scintillation counting.

H. Protein Binding

Blood was obtained from rats by cardiac puncture with a heparinized syringe and was immediately centrifuged. Samples of plasma (1.0 ml) were placed inside Oxford microdialysis bags. The bags were tied and immersed in 5 ml pH 7.4 0.1M phosphate buffer. Aqueous solutions of drug (5 mg/ml) were added to the external compartment to give final drug concentrations of 5 - 50 ug propylthiouracil/ml or 5 - 30 ug methimazole/ml. The increase in volume of the external compartment, due to drug addition (5 - 50 ul), was not significant. The mixtures were incubated for periods up to 24 hours at 37°C with intermittent agitation. Each compartment was sampled for radioactivity at various time intervals to ensure that equilibration had been achieved. Experiments were performed in duplicate, and radioactivity was determined by liquid scintillation counting.

I. Excretion Studies

1. Biliary Excretion

Animals were anesthetized with an intraperitoneal injection of a solution of chloralose:urethane at a dose of 75:750 mg/kg. A 10 gauge polyethylene tube was tied into the common bile duct. The abdominal incision was closed and radioactive drug was administered orally (gavage). Bile was collected continuously for 10 hours. The animals were kept supine and warmed by illumination with a desk lamp. A furthur dose of anesthetic was required after approximately six

hours, when animals exhibited a withdrawal response to pinching of the feet. Aliquots of bile were dissolved in scintillation fluid, and radioactivity was determined by liquid scintillation counting.

2. Urinary and Fecal Excretion

After the drug was administered, animals were placed in Acme metabolism cages, and urine and feces were collected at regular intervals. Urine containers were packed in ice during the collection period. Aliquots of urine were dissolved in scintillation fluid, and radioactivity were determined. The remaining urine samples were frozen and stored in the freezer for further analysis. Feces samples were dried in air and ground with a mortar and pestle. The samples were then extracted with water and methanol in a Soxhlet tube. An aliquot of the combined extracts was dissolved in scintillation fluid, and radioactivity determined by liquid scintillation counting. Portions of feces were also weighed and counted by liquid scintillation as a gel suspension with Cab-o-sil^{R} (New England Nuclear, Montreal, Quebec) to ensure that the extraction procedure was quantitative.

J. Partition and Solubility Determination

Thiouracil (Aldrich Chemical Co., Montreal, Quebec) was recrystallized twice from hot water. Samples (250 ul) of an aqueous solution (5 mg/ml) were pipetted into 5 ml buffer solution in a centrifuge tube. Chloroform (5.0 ml)

was added and the mixture was shaken intermittently. Buffers employed were pH 1.8, 5.0, 6.0, 7.4 and 8.0. Experiments were performed in duplicate. Thiouracil was estimated in each phase by measuring the optical density at the wavelength of maximum absorption (chloroform 290 nm; aqueous 259 nm). In each experiment, the pH of the solution was measured after drug addition, to ensure that it had not changed. The same method was used to determine the partition ratio of propylthiouracil and methimazole, but the assay system involved counting the radioactivity of aliquots from each phase by liquid scintillation.

The solubility of thiouracil, propylthiouracil and methimazole were determined at pH 1.8 and 7.4. Excess non-radioactive drug was added to the buffer solution, and the optical density of an aliquot of the supernatant was measured at the wavelength of maximum absorption (thiouracil 259 nm; propylthiouracil 274 nm; methimazole 252 nm). The pH of the buffer was measured after the drug was added to ensure that it had not changed.

K. Determination of Radioactivity

A Philips Liquid Scintillation analyzer (Philips, Netherlands) was used for liquid scintillation counting. The external standard channels ratio method was used to determine the extent of quenching of radioactivity. A quench curve was derived with picric acid as the quenching agent and ^{14}C toluene as the radioactive standard. Polynomial

regression analysis of the data from the standard quench curve on a Digital PDP-8/I computer (Digital Equipment Corp., Massachusetts) demonstrated that the best fit of the data was a quadratic function. A program of this function was used with an Olivetti Programma 101 computer (Olivetti Underwood Corp., New York) to convert data from counts per minute (cpm) to disintegrations per minute (dpm).

The scintillation fluid in the propylthiouracil experiments consisted of naphthalene 80 g, 2,5-diphenyloxazole (PPO) 5 g, 1,4-Bis-[2-(5-phenyloxazolyl)]-benzene (POPOP) 0.5 g, and 333 ml each of toluene, methylcellosolve and dioxane. Aquasol^{R} (New England Nuclear, Boston, Massachusetts) was used as the scintillation fluid in the methimazole studies.

Thin-layer plates were scanned on an Actigraph III instrument (Nuclear Chicago, Des Plaines, Illinois) with an attachment for thin-layer plates.

L. Hydrolysis of Excreted Metabolites

1. Acid Hydrolysis of Urine Samples

Aliquots of urine (30 ml) were placed in a two ounce screw cap bottle. Concentrated hydrochloric acid (6 ml) was added to give a final solution of 2N. The container was sealed and hydrolyzed in a pressure cooker for one hour at 121^{o}C.

2. Enzymic Hydrolysis of Urine and Bile

Urine samples were adjusted to pH 5.3 with glacial acetic acid. Glusulase^{R} (Endo Labs. Inc., Garden City, New York), which contained 179,500 units β-glucuronidase/ml (pH optimum 4.7), and 47,000 units sulfatase/ml (pH optimum 5.8) was added to each sample (1.0 ml/100 ml urine). The urine was then incubated in a water bath at 37°C for varying periods of time (24 - 48 hours). The pH of 5.3 was chosen as it is the midpoint between the pH optimum for each of the enzymes β-glucuronidase and sulfatase.

Bile samples were diluted with three volumes of water, and then adjusted to pH 5.3 with glacial acetic acid. Glusulase^{R} was then added and hydrolysis was carried out by the same procedure used for urine samples.

M. Extraction of Urine and Bile Samples

1. Propylthiouracil

Hydrolyzed and unhydrolyzed urine samples were adjusted to pH 7.0 with dilute sodium hydroxide solution and were then extracted with dichloromethane (4 x 50 ml). The aqueous layer was then extracted with ethyl acetate (4 x 50 ml). The urine sample was adjusted to pH 2.0 with concentrated hydrochloric acid to allow the extraction of acidic substances. The urine sample was then extracted as described at pH 7.0, i.e. dichloromethane followed by ethyl acetate. The aqueous phase that remained was adjusted to pH 7.0 with dilute sodium hydroxide and freeze dried. The freeze dried residue was

then extracted with ethanol (4 x 50 ml). Radioactivity was determined in each extract by liquid scintillation counting.

Bile samples, after hydrolysis, were extracted with ethyl acetate (4 x 30 ml), and aliquots were used to determine the radioactivity present.

2. Methimazole

Hydrolyzed and unhydrolyzed urine samples were adjusted to pH 7.0 with dilute sodium hydroxide and were extracted with ethyl acetate (6 x 50 ml). The pH of the urine sample was adjusted to 2.0 with concentrated hydrochloric acid so that acidic substances might be extracted. The extraction was repeated exactly as described at pH 7.0. The remaining aqueous phase was adjusted to pH 7.0 and freeze dried. The freeze dried residue was extracted with ethanol (4 x 50 ml). Radioactivity was determined in the extracts in the same manner as in the propylthiouracil study.

Bile samples were hydrolyzed, extracted, and radioactivity determined in the same manner as in the propylthiouracil study (Section II, M, 1).

N. Microsomal Metabolic Studies

Male rats were killed by decapitation and allowed to exsanguinate. The livers were rapidly removed and placed on ice. Microsomes were prepared at 0 - 2°C. The livers were weighed and placed in 0.1M phosphate buffer. The tissue was minced with scissors and homogenized with a Dounce homo-

genizer, first with a loose fitting, and then with a tight fitting pestle. Phosphate buffer was then added to make a final suspension of 20% with respect to liver weight. The homogenate was centrifuged at 15,000 g for thirty minutes, and the pellet (nuclei, mitochondria and cellular debris) was discarded. The supernatant fluid was used in the incubation mixture to study the microsomal metabolism of propylthiouracil. The incubation mixture consisted of:

15,000 g supernatant fraction	50 ml
0.25M phosphate buffer	25 ml
distilled water	18 ml
glucose-6-phosphate (G-6-P)	2.5 ml (0.6 mM)
Triphosphopyridine Nucleotide (TPN)	20 mg (25 uM)
magnesium chloride	5 ml (2.5 mM)

All of the above, except TPN, were added to a 250 ml round bottom flask. Labelled propylthiouracil (40 mg) was added to the flask which was placed in a water bath at 37°C and stirred. The TPN was added in two portions of 10 mg, one portion at the start of the incubation, and the other portion after one and a half hours. The total incubation time was three hours. The incubation mixture was extracted in a similar fashion to that described for urine samples in the propylthiouracil study.

0. Chromatography

1. Column Chromatography

The ion exchanger DEAE-Sephadex A-25 (Pharmacia,

Montreal, Quebec), of particle size 40 -120 u, was used to separate metabolites from urine and bile. The exchanger was recycled by washing with distilled water, dilute formic acid, distilled water, dilute sodium hydroxide, and distilled water on a Buchner funnel. The exchanger was then washed with 0.05M ammonium carbonate solution. A glass column (1.2 x 25 cm) was packed by the open flow procedure, and was allowed to equilibrate overnight with 0.05M ammonium carbonate solution. The column was loaded with 5 ml of urine, or bile sample, which had been adjusted to pH 8.0 with ammonia. The column was eluted in a stepwise fashion with 50 ml 0.05M, 25 ml each of 0.20, 0.50 and 1.0M and 100 ml of 2.0M ammonium carbonate solutions. Fractions (5 ml) were collected, and aliquots (0.20 ml) of each fraction were added to scintillator fluid to determine radioactivity. The column was continuously monitored for ultraviolet absorption (methimazole 254 nm; propylthiouracil 280 nm) with a flow cell of path length 0.2 cm (Instrument Specialties Co., Nebraska).

2. Thin-Layer Chromatography

Fluorescent silica gel plates (Merck, Darmstadt, Germany) were used for preliminary work. Preparative plates (1 mm thick) were spread with fluorescent silica gel (Macheray, Nagel and Co., Düren, Germany) in this laboratory, and used to isolate metabolites from urine. Solvent systems used with this technique were:

1.	isopropanol:benzene	1:3
2.	isopropanol:benzene	1:1
3.	benzene:methanol:acetic acid	79:14:7
4.	ethanol:acetic acid	9:1

3. Gas-Liquid Chromatography

An F & M 402 High Efficiency Gas Chromatograph (Hewlett Packard, Avondale, Pennsylvania) was used in this study. Glass columns (4 ft x 6 mm OD; 3 mm ID), containing 1% OV 17 on Gas-Chrom Q 100/120 mesh (Applied Science Labs., State College, Pennsylvania) were used. Nitrogen was used as the carrier gas, and sample elution from the column was monitored with a flame ionization detector. Gas flows were as follows: hydrogen 35 ml/min, nitrogen 70 ml/min and air 350 ml/min.

P. Mass Spectrometry

Samples were introduced into a mass spectrometer indirectly via a glass reservoir or directly on a probe. This service was carried out by Morgan Schaffer Corp. (Montreal, Quebec) on an Hitachi-Perkin-Elmer RMU-6D mass spectrometer. The resulting spectra were interpreted in our own laboratory.

Q. Spray Reagent

Naphthylresorcinol reagent was used to detect glucuronide conjugates (Dawson et al. 1969).

RESULTS

A. Partition and Solubility Data

Methimazole exhibited the greatest partition into chloroform of the three drugs investigated (Table 1). Partition of propylthiouracil into the lipid phase was less than one at all pH values investigated. An accurate measure of the partition of thiouracil into the lipid phase could not be made, as the levels were below the sensitivity of the method. Methimazole also exhibited the greatest water solubility of the three antithyroid drugs. Both propylthiouracil and thiouracil exhibited similar low water solubility.

Table 1: Chloroform:water partition coefficients and aqueous solubility (g/L) of thiouracil, propylthiouracil and methimazole at different pH values.

Chloroform:water partition	1.8	5.0	6.0	7.4	8.0
Thiouracil	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Propylthiouracil	0.73	0.85	0.92	0.69	0.34
Methimazole	3.9	3.8	3.6	3.3	3.4
Aqueous solubility					
Thiouracil	0.8	--	--	1.3	--
Propylthiouracil	1.0	--	--	1.6	--
Methimazole	208	--	--	188	--

B. Propylthiouracil

1. Absorption

The plasma levels of radioactivity and of unchanged drug after intravenous, intraperitoneal and oral doses of labelled propylthiouracil are listed in Table 2. Absorption was more rapid after intraperitoneal than after oral administration. Sustained plasma levels of propylthiouracil resulted after both routes of administration. The plasma half-lives of propylthiouracil after intravenous, intraperitoneal and oral doses were 1.5 hours, 9 hours and 12 hours (by extrapolation) respectively.

A semilogarithmic plot of plasma levels of propylthiouracil, which resulted from an intravenous dose, indicated that the disappearance of the drug from the plasma could be represented by a two compartment model with two first order decay components. A digital computer analysis of the curve indicated that the model could be described by the following equation:

$$[PTU] = (19 \pm 2)e^{-(0.070 \pm 0.006)t} + (38 \pm 1)e^{-(0.003 \pm 0.001)t}$$

([PTU] = the plasma concentration of propylthiouracil at time "t")

The slope, intercept and half life of each compartment are

Table 2

Plasma levels[a] of labelled propylthiouracil, and of total radioactivity calculated as ug propylthiouracil/ml

Time (min)	Route of Administration[b]			
	Oral		Intraperitoneal	Intravenous
	Total ^{14}C	P.T.U.	Total ^{14}C	P.T.U.
1	-	-	-	58 ± 5
5	-	-	-	42 ± 7
15	9 ± 2	12 ± 2	26 ± 9	37 ± 4
30	17 ± 2	19 ± 3	30 ± 8	33 ± 3
60	24 ± 4	25 ± 3	35 ± 7	33 ± 1
90	25 ± 2	23 ± 6	34 ± 7	31 ± 2
120	26 ± 3	27 ± 3	34 ± 3	27 ± 3
180	26 ± 2	28 ± 3	31 ± 1	24 ± 1
240	26 ± 2	27 ± 5	31 ± 1	20 ± 1
300	25 ± 3	-	29 ± 2	17 ± 1
360	21 ± 2	21 ± 1	28 ± 2	14 ± 0
420	22 ± 2	-	23 ± 2	12 ± 1
480	20 ± 1	21 ± 2	22 ± 1	10 ± 1
540	18 ± 1	-	18 ± 1	9 ± 3
600	-	-	17 ± 1	8 ± 2
660	-	-	15 ± 2	7 ± 1
720	-	-	13 ± 1	5 ± 1
1440	4 ± 1	1	-	-

[a] Values quoted represent mean ± standard error. Three animals were used for each experiment.

[b] The dose was 20 mg/kg.

listed in Table 3.

Table 3: Pharmacokinetic parameters of the disappearance of labelled propylthiouracil from the plasma after an intravenous dose.

Compartment	Intercept (ug/ml)	Slope (ug/ml/min)	$t_{1/2}$ (min)
1	19 ± 2	-0.070 ± 0.006	10 ± 1
2	38 ± 1	-0.003 ± 0.001	267 ± 15

The apparent volume of distribution of labelled propylthiouracil one minute after an intravenous dose was calculated to be 34.5 ml/100 g of rat. Assuming a whole blood content of 5 ml/100 g of rat (Goldstein and Aranow 1960), the level of drug in the plasma one minute after intravenous administration represents only 9% of the administered dose.

2. Tissue Distribution and Protein Binding

The distribution of radioactivity in various tissues of female rats at the specified time intervals after an oral dose of propylthiouracil is listed in Table 4. Radioactivity was found in all tissues examined. A difference between the blood and plasma concentrations of radioactivity was noted. This difference indicated that propylthiouracil was probably bound to plasma proteins. Equilibrium dialysis demonstrated that the drug was bound to plasma proteins to

Table 4

Tissue distribution of ^{14}C after oral dosage of labelled propylthiouracil to female rats

Sample	Time (hr)		
	2	4	8
Plasma[a]	25.7 ± 2.5	25.9 ± 1.7	20.2 ± 1.1
Blood	18.1 ± 0.9	22.8 ± 0.9	14.8 ± 2.9
Brain[b]	6.3 ± 0.2	5.5 ± 0.5	3.4 ± 1.0
Heart	13.7 ± 1.4	16.5 ± 0.8	10.5 ± 2.3
Lungs	16.9 ± 0.6	17.2 ± 0.6	12.1 ± 1.6
Liver	19.1 ± 0.9	19.7 ± 1.1	12.4 ± 2.9
Kidney	19.8 ± 1.6	22.1 ± 1.0	16.1 ± 2.6
Spleen	12.0 ± 0.6	13.0 ± 0.8	7.4 ± 1.8
Abdominal Fat	9.9 ± 2.9	13.0 ± 0.8	9.0 ± 1.3
Skin	13.1 ± 1.8	13.4 ± 0.6	8.7 ± 2.4
Eye	8.3 ± 0.7	10.0 ± 0.3	6.6 ± 1.7
Red Marrow	12.7 ± 0.5	11.4 ± 0.3	10.3 ± 3.5
Yellow Marrow	8.5 ± 0.5	8.6 ± 0.3	4.5 ± 1.2
Skeletal Muscle	13.3 ± 2.9	12.7 ± 0.2	5.9 ± 1.2
Thyroid Gland	19.3 ± 3.7	16.1 ± 0.9	9.1 ± 2.3
Stomach	179.2 ±57.1	67.4 ±12.1	38.0 ±16.9
Duodenum	16.7 ± 1.7	17.1 ± 0.9	15.0 ± 2.4

Values are expressed as [a] ug drug per ml or [b] ug drug per g tissue, wet weight.

Values quoted represent mean ± standard error. Three animals were used for each experiment.

the extent of 56.5 ± 1.1% (n = 12; the value represents the mean ± S.E.) over the range of drug concentration from 5-50 ug/ml.

Except for the stomach wall, tissue levels of radioactivity did not exceed blood levels in any tissues examined, and in most tissues maximum levels of radioactivity were found within two hours after an oral dose of propylthiouracil. At all time intervals investigated, there was a higher concentration of radiolabel in red marrow than in yellow marrow.

3. Excretion

Cumulative urinary excretion of radioactivity following oral, intraperitoneal and intravenous doses of propylthiouracil to rats is illustrated in Fig. 5. The total radioactivity excreted in the urine varied from 75 to 90%. Mean urinary excretion was determined to be 80% of the administered dose within 24 hours. Most of the label had been excreted within approximately 15 hours. The half-life of urinary excretion of radioactivity in rats was found to be as follows: male (P.O.) 6.5 hours, female (P.O.) 4 hours, male (I.P.) 7.5 hours, and male (I.V.) 6.5 hours. Thus, it appears that female rats may excrete the drug and its metabolites more rapidly after an oral dose than do male animals, although the total urinary excretion of radioactive label was not significantly different from that of male rats. Urinary excretion of radioactivity after an

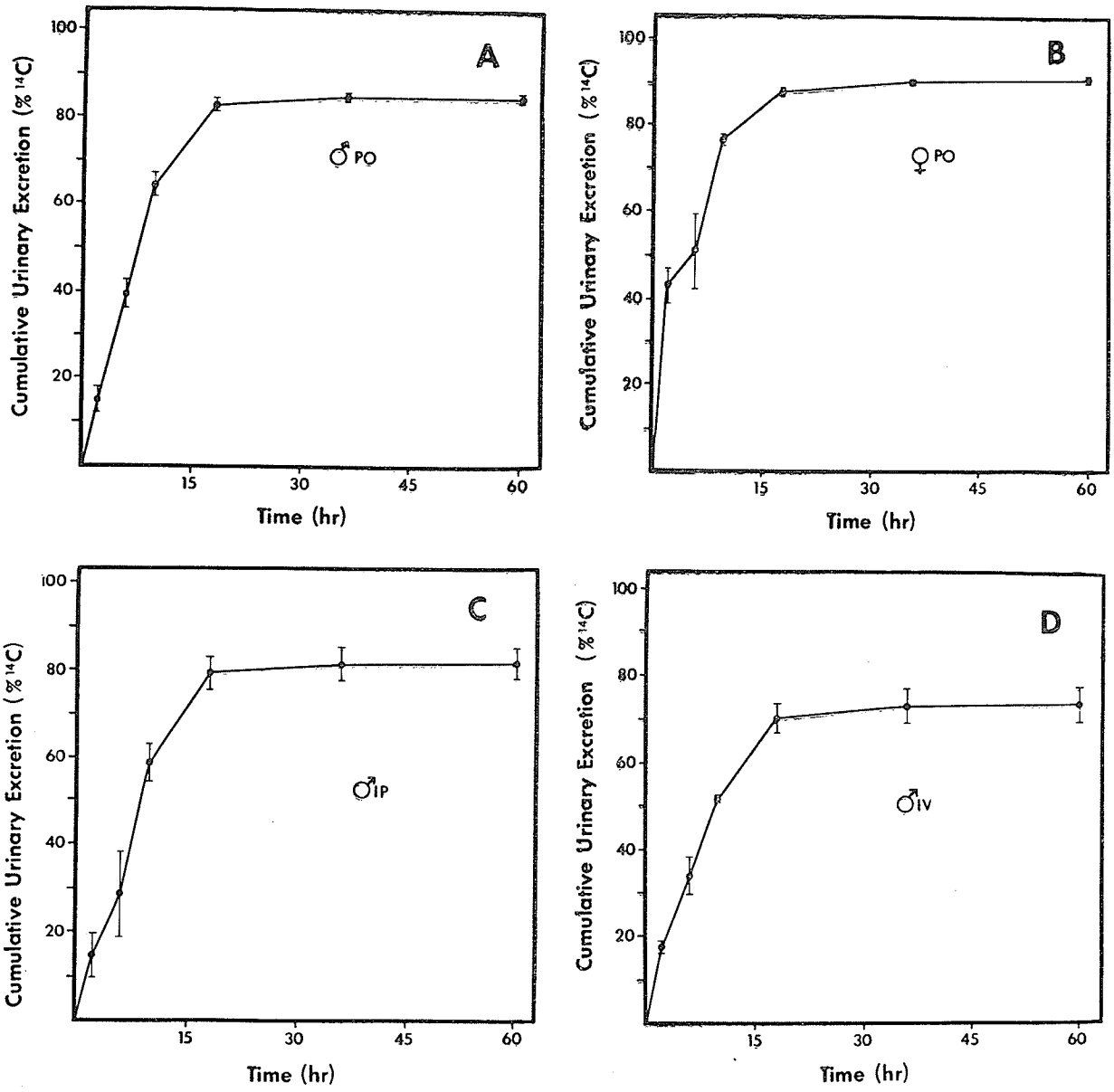


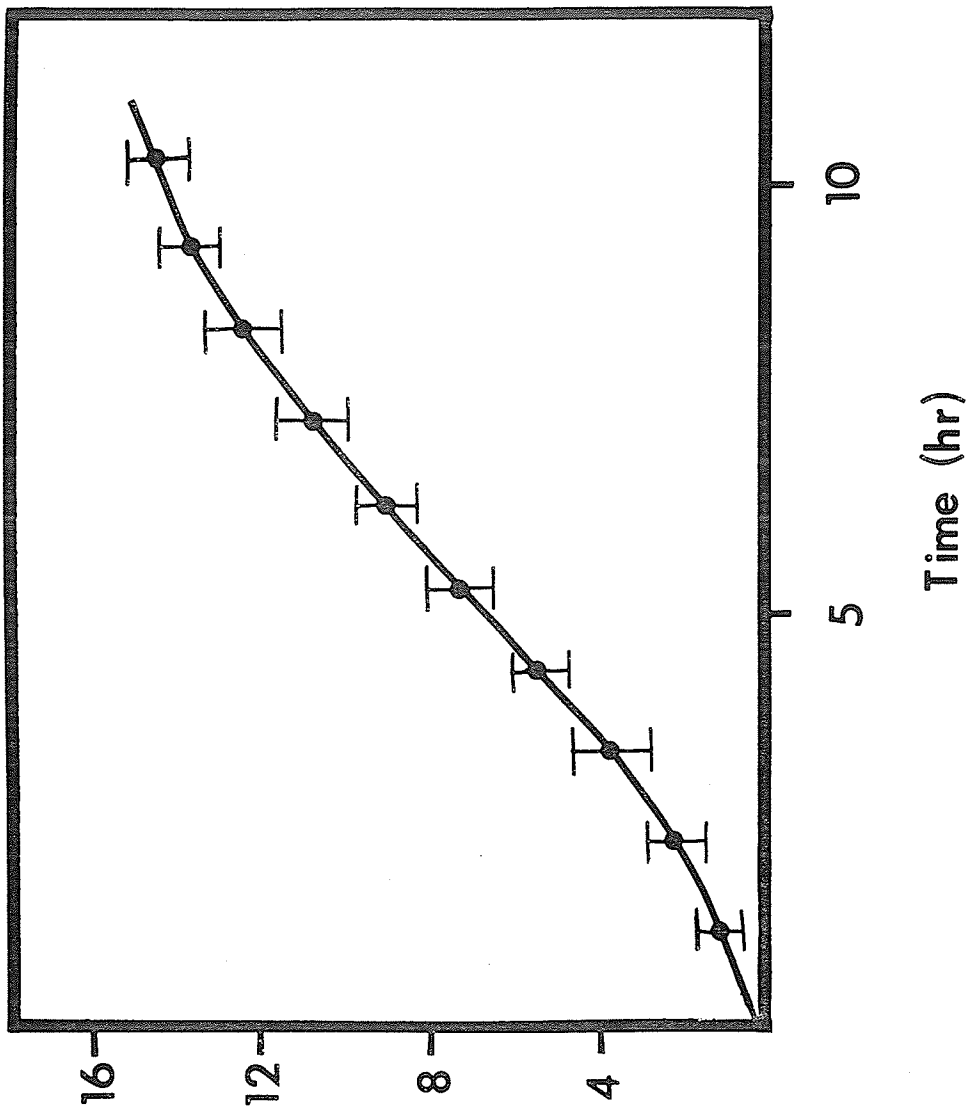
Fig. 5: Mean cumulative urinary excretion of radioactivity expressed as a percentage of the administered dose of labelled propylthiouracil. Six animals were used in each experiment, and vertical bars indicate standard errors. Dosage routes: Panel A. male rats P.O., Panel B. female rats P.O., Panel C. male rats I.P., Panel D. male rats I.V.

intravenous dose of propylthiouracil appeared to be slightly less than after an oral or intraperitoneal dose. Radioactivity was not detectable in the urine of any animals after 72 hours. The half-life of urinary excretion did not appear to differ significantly with the route of administration.

The time course of biliary excretion of radioactivity after an oral dose of propylthiouracil is illustrated in Fig. 6. After 10 hours, approximately 15% of the administered dose had been excreted into the bile. Other experiments demonstrated that there was no significant difference in the biliary excretion of radioactivity after 10 hours when compared to intraperitoneal or intravenous doses.

Fecal excretion of radioactivity over a period of 48 hours following a dose of labelled propylthiouracil was found to be; 1.6 ± 0.2% after an oral dose to male and female rats, 1.4 ± 0.3% after an intraperitoneal, and 1.6 ± 0.4% after an intravenous dose to male rats (all values are means ± standard errors). Thus, fecal excretion of radioactivity did not differ significantly with the route of administration. Identical results were obtained by counting a Cab-o-sil^{R} suspension of feces, or an aliquot of the Soxhlet extract of feces.

Fig. 6: Mean biliary excretion of radioactivity after an oral dose of labelled propyl-thiouracil. Three female rats were used, and vertical bars represent standard errors.


Cumulative Biliary Excretion (%^{14}C)
16
12
8
4
5
10
Time (hr)

4. Metabolic Studies

a) Urinary Metabolites

Elution profiles from column chromatography of urine samples, before and after hydrolysis with Glusulase^{R} (pH 5.3), are illustrated in Figs. 7 and 8. The elution profiles were similar for all other urine samples analyzed in this manner. A comparison of Figs. 7 and 8 demonstrates that there was a peak shift in the elution profile after hydrolysis of the urine sample. Thus, the major peak in Fig. 7 (peak 1) was very much reduced in size, and the radioactivity was much enhanced in peak 2 of Fig. 8. Thus, peak 1 must be a conjugate. Thin-layer chromatography of the major radioactive peak (peak 1) from Fig. 7 indicated that it corresponded to the radioactivity which remained in the urine after the sequence of extractions with organic solvents (Table 5). Treatment of this spot on the thin-layer chromatogram with naphthoresorcinol spray reagent yielded a blue color which corresponded to the radioactive zone. This is further evidence that the compound was a glucuronide conjugate. Incubation of this compound with Glusulase^{R} at pH 4.7, the pH optimum for β-glucuronidase, followed by extraction with ethyl acetate, yielded propylthiouracil, identified by its identical thin-layer chromatographic properties compared to authentic propylthiouracil (Table 5). Thin-layer chromatography of peak 2 from Fig. 8 also yielded similar thin-layer chromatographic character-

Fig. 7: Elution profile of radioactivity from the ion exchange chromatography of an unhydrolyzed 24 hour urine sample collected after administration of labelled propylthiouracil.

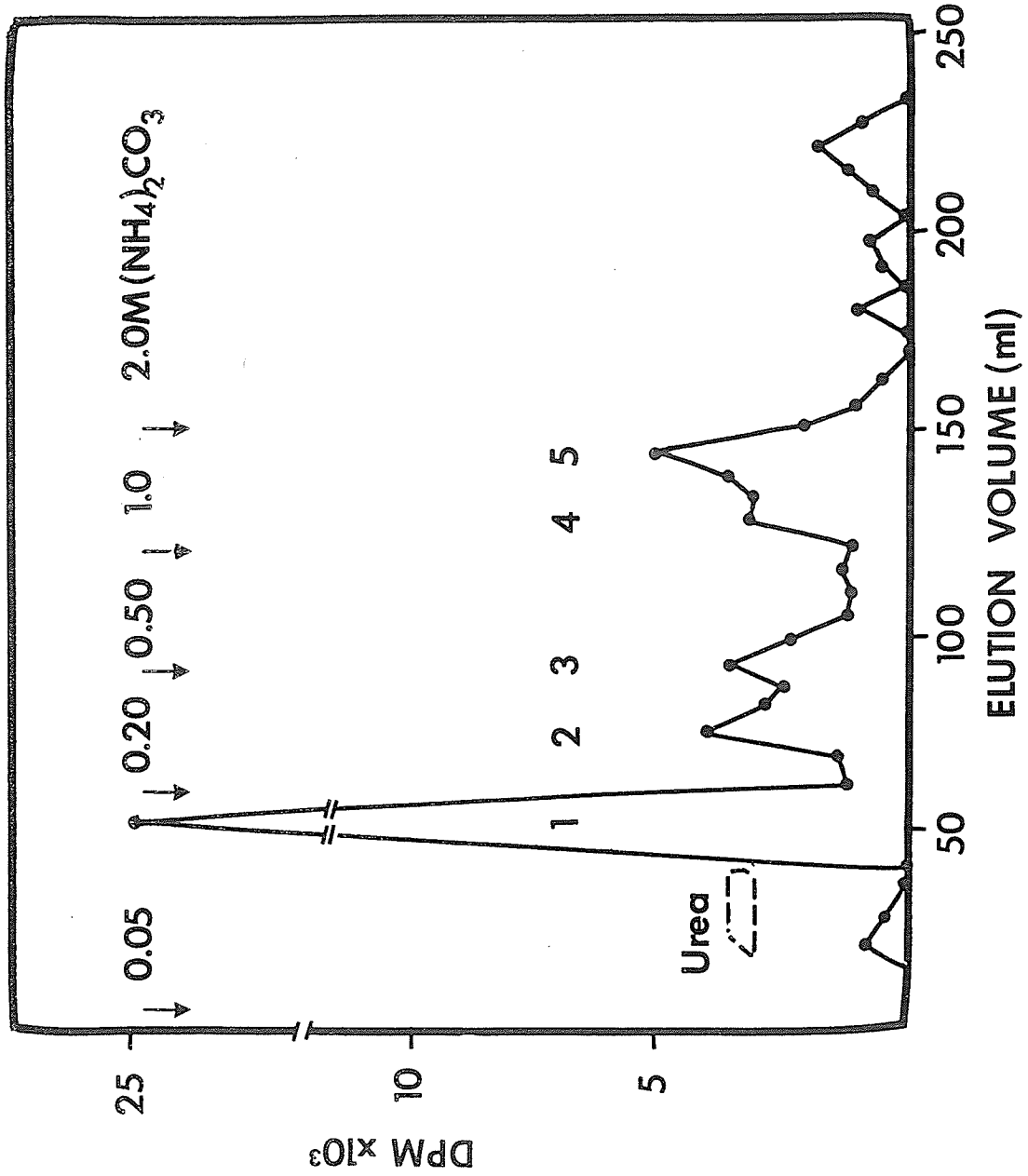
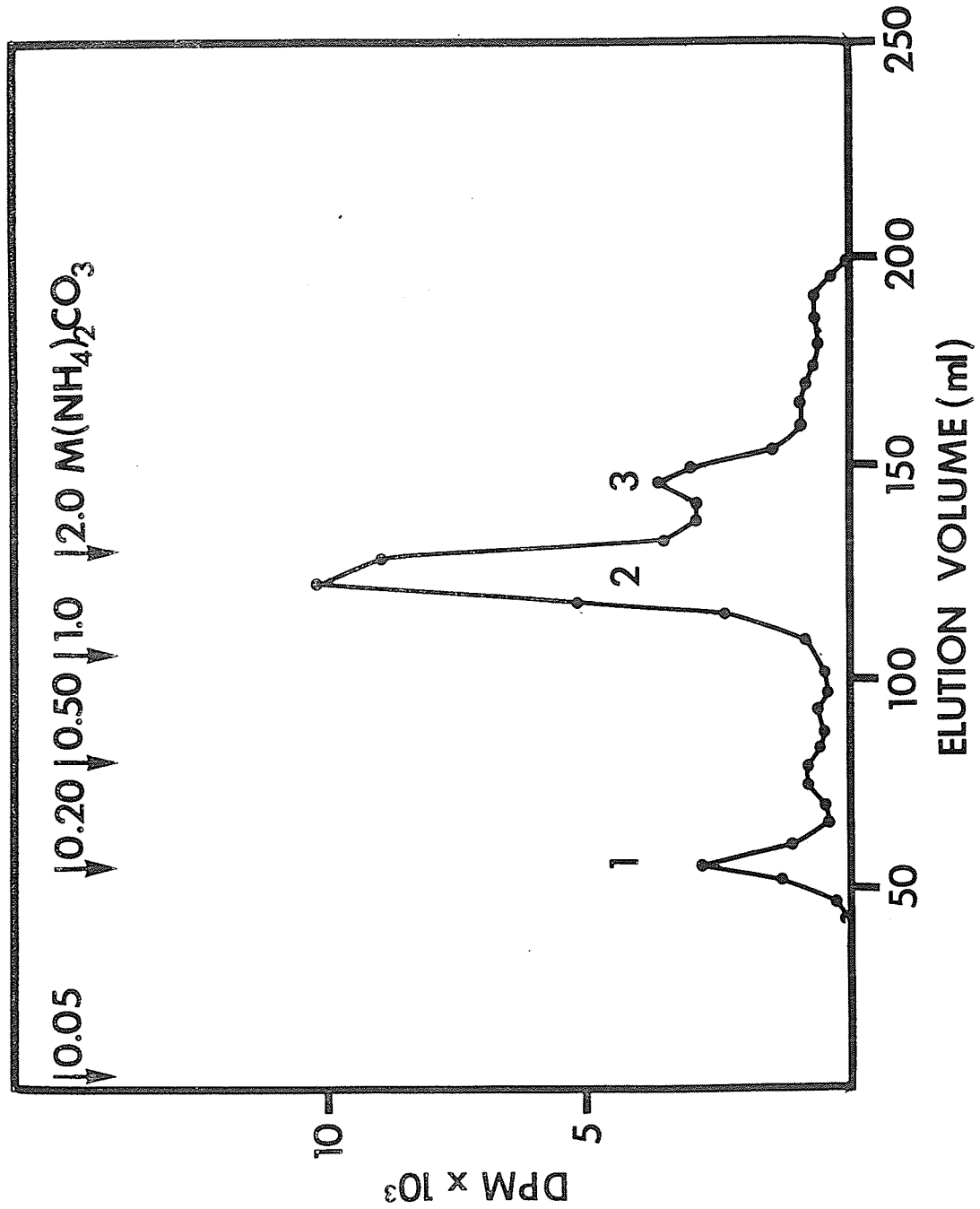

DPM x10³
25
10
5
0.05
0.20
0.50
1.0
2.0M (NH4)2CO3
1
2
3
4
5
Urea
50
100
150
200
250
ELUTION VOLUME (ml)

Fig. 8: Elution profile of radioactivity from the ion exchange chromatography of a Glusulase^{R} hydrolyzed 24 hour urine sample collected after administration of labelled propylthiouracil.


0.05
0.20
0.50
1.0
2.0 M(NH4)2CO3
1
2
3
DPM x 10³
10
5
50
100
150
200
250
ELUTION VOLUME (ml)

istics. Thus, it is reasonable to suppose that this substance was a glucuronide conjugate of propylthiouracil. This metabolite was found to account for 50 - 61% of the radioactivity in the pooled urine samples collected over 24 hours.

Table 5: Thin-layer chromatography of radioactive compounds excreted in pooled 24 hour rat urine collected after a dose of labelled propylthiouracil. The migration of substances on thin-layer plates is expressed as their R_f values.

Substance	Developer		
	i-propanol: benzene 1:3	benzene:methanol: acetic acid 79:14:7	ethanol: acetic acid 9:1
P-1[a]	0.35	0.40	--
Propylthiouracil glucuronide[b]	0.00	0.00	0.29
Propylthiouracil[c]	0.56	0.43	--

[a] P-1 is the unidentified metabolite in the 24 hour urine.

[b] This metabolite is the component of peak 1 in the elution profile illustrated in Fig. 7.

[c] This urinary component gave identical thin-layer characteristics to authentic propylthiouracil.

The presence of unchanged drug in the 24 hour urine samples was detected by solvent extraction of unhydrolyzed urine, and by column chromatography. Thin-layer chromatography of peak 4 (Fig. 7) demonstrated that it behaved simi-

larly to authentic propylthiouracil. The radioactive compound in the dichloromethane extract of unhydrolyzed urine samples (pH 7.0) behaved similarly when subjected to thin-layer chromatography (Table 5). The identity of unchanged drug was confirmed by gas-liquid chromatography and by mass spectrometry (Table 6). The fragmentation pattern from the mass spectrum of the isolated radioactive product is consistent with the molecular structure of a pyrimidine (Budzikiewicz et al. 1967a), and was identical to the spectrum of a reference standard of propylthiouracil. The proportion of unchanged drug present in pooled 24 hour urine samples was found to be 13% in male rats and 11% in female rats after an oral dose, 16% in male rats after an intraperitoneal dose, and 19% in male rats after an intravenous dose.

The substance designated P-1 in Table 5 obtained from extracts of Glusulase^{R} hydrolyzed 24 hour urine samples, is equivalent to peak 5 in Fig. 7 and peak 3 in Fig. 8. A comparison of the two figures indicated that at least some P-1 was excreted as unconjugated product into the urine. It was not possible from the two figures to establish whether the relative amount of P-1 was increased in Fig. 8 after hydrolysis with Glusulase^{R}. The mass spectral data of P-1, purified by thin-layer chromatography, are listed in Table 7. The metabolite did not give a parent ion. This indicates that P-1 might be a conjugate which was not hydrolyzed by Glusulase^{R}, or some other polar metabolite which

Table 6

Mass spectrum of unchanged propylthiouracil obtained from the urine of rats

m/e	intensity[a]	m/e	intensity[a]	
35	8	67	5	
36	14	68	22	
38	5	69	8	
39	10	70	5	
40	10	73	9	
41	18	82	6	
42	6	83	100	
43	12	84	5	
44	6	85	65	
45	6	87	11	
47	22	96	8	
48	11	110	6	
49	7	114	11	
50	5	142	26	
54	5	147	5	
55	12	155	7	
57	6	170	60	Parent ion
60	6	171	5	

[a] Peak intensities are expressed relative to m/e 83 which has been assigned a value of 100.

Table 7

Mass spectrum of P-1 obtained from the urine of rats treated with propylthiouracil

m/e	intensity[a]	m/e	intensity[a]
14	6	39	6
15	35	42	13
17	5	43	81
18	19	44	100
26	7	45	6
27	10	58	42
28	20		

a Peak intensities are expressed relative to m/e 44 which has been assigned a value of 100.

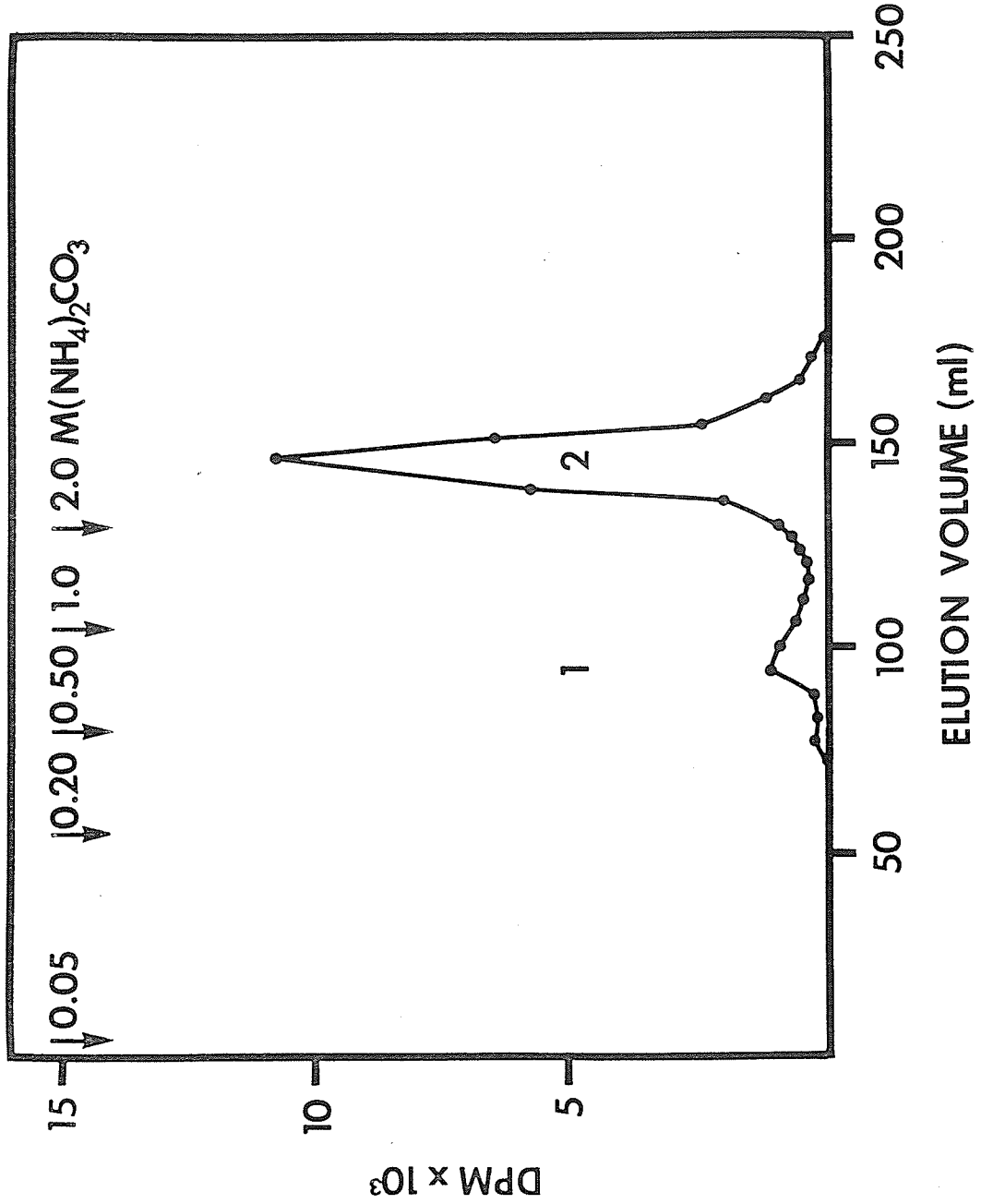
did not have a sufficient vapor pressure to give a satisfactory mass spectrum without decomposition at the sample inlet when the temperature was increased.

However, a fragmentation pattern corresponding to acetone (m/e 58) (Budzikiewicz et al. 1967b), indicates the possibility that oxidation of the 2 position of the side chain may have occurred in the metabolism of propylthiouracil to P-1. This interpretation is based on the fact that acetone was never used as a solvent in the isolation or purification of any metabolite in this study. This metabolite was found to account for 12-20% of the radioactivity found in pooled 24 hour urine samples after hydrolysis with Glusulase^{R}.

b) Biliary Metabolites

The results of column chromatography of an aliquot of pooled bile samples, collected after an oral dose of propylthiouracil, are illustrated in Fig. 9. It can be seen that only two compounds excreted into the bile were resolved, and one compound (peak 2) accounted for 90% of the radioactivity in the sample. Thin-layer chromatography of the two radioactive peaks (Table 8) indicated that neither one was unchanged drug. The failure of the two biliary metabolites to migrate in the benzene:methanol:acetic acid developer indicated that they were more polar than propylthiouracil. Thin-layer chromatography after Glusulase^{R} hydrolysis at pH 4.7 yielded only one radioactive peak

Fig. 9: Elution profile of radioactivity from the ion exchange chromatography of bile collected from rats given an oral dose of labelled propylthiouracil.


0.05
0.20
0.50
1.0
2.0 M $(NH_4)_2CO_3$
1
2
15
10
5
DPM x 10^3
50
100
150
200
250
ELUTION VOLUME (ml)

extractable into ethyl acetate. The R_f of this substance was found to correspond to that of authentic propylthiouracil (Table 5). The major biliary metabolite, before hydrolysis, gave a positive blue color with naphthoresorcinol spray reagent. Thus, the major metabolite present in the bile of rats dosed with propylthiouracil is almost certainly a glucuronide conjugate of propylthiouracil.

Table 8: Thin-layer chromatography of radioactive peaks isolated by the column chromatography of rat bile after an oral dose of propylthiouracil. The migration of substances on the thin-layer plate are expressed as their R_f values.

Substance	Developer	
	benzene:methanol: acetic acid 79:14:7	ethanol: acetic acid 9:1
Peak 1	0.00	0.52
Peak 2	0.00	0.43

c) Fecal Metabolites

Feces samples were not investigated to determine the nature of the radioactive compound(s), since only traces of radioactivity were present in them.

d) Microsomal Metabolites

Incubation of labelled propylthiouracil with a microsomal preparation (section II, N) indicated the presence

of only one metabolite. Thin-layer chromatography of this metabolite indicated that it corresponded to P-1, the unidentified metabolite from the pooled 24 hour urine samples. The formation of this metabolite was very slow, since less than 1% of the propylthiouracil was transformed to P-1 during the three hour incubation.

C. Methimazole

1. Absorption

The plasma levels of radioactivity and unchanged drug after intravenous, intraperitoneal and oral doses of labelled methimazole are listed in Table 9. Maximum plasma levels of methimazole were attained within half an hour after an oral dose, and within one hour after an intraperitoneal dose. The plasma half-lives of unchanged methimazole after intravenous, intraperitoneal and oral doses were 4 hours, 5 hours (estimated) and 5 hours respectively. The half-life of plasma radioactivity after intravenous, intraperitoneal and oral doses were 5 hours, 10 hours and 10 hours respectively.

A semilogarithmic plot of the plasma levels of methimazole, which resulted from an intravenous dose, indicated that the disappearance of the drug from the plasma could be represented by a single compartment model with one first order decay component. A digital computer analysis of the curve indicated that the model could be described by the

Table 9

Plasma levels[a] of labelled methimazole, and of total radioactivity calculated as ug methimazole/ml

Time (min)	Route of Administration[b] Oral Total ^{14}C	Oral Methim	Intraperitoneal Total ^{14}C	Intravenous Total ^{14}C	Intravenous Methim
1	-	-	-	27 ± 4	30 ± 2
5	-	-	-	24 ± 2	25 ± 4
15	8 ± 1	-	6 ± 1	22 ± 1	19 ± 2
30	16 ± 4	16 ± 1	9 ± 1	21 ± 1	20 ± 2
60	17 ± 1	15 ± 0	16 ± 2	20 ± 1	20 ± 1
90	17 ± 2	-	14 ± 2	-	-
120	16 ± 1	14 ± 2	15 ± 2	19 ± 3	16 ± 2
180	15 ± 2	11 ± 1	14 ± 1	17 ± 1	19 ± 1
240	13 ± 1	11 ± 2	13 ± 1	17 ± 3	14 ± 3
300	12 ± 1	-	12 ± 1	14 ± 1	-
360	13 ± 2	7 ± 1	11 ± 1	12 ± 1	10 ± 1
420	12 ± 1	-	10 ± 1	10 ± 1	-
480	10 ± 1	4 ± 1	10 ± 1	9 ± 1	5 ± 1
540	10 ± 1	-	9 ± 1	8 ± 1	-
600	8 ± 1	3 ± 1	8 ± 1	7 ± 0	4 ± 1
660	8 ± 1	-	7 ± 1	-	-
720	7 ± 1	-	8 ± 1	-	-
1440	1 ± 0	trace	-	-	-

[a] Values quoted represent mean ± standard error. Three animals were used for each experiment.

[b] The dose was 20 mg/kg.

following equation:

$$[\text{Methimazole}] = (25 \pm 1)e^{-(0.0031 \pm 0.0002)t}.$$

([Methimazole] = the plasma concentration of methimazole at time "t")

The slope, intercept and half life of the compartment are listed in Table 10.

Table 10: Pharmacokinetic parameters of the disappearance of methimazole from the plasma after an intravenous dose.

Compartment	Intercept (ug/min)	Slope (ug/ml/min)	$t_{1/2}$ (min)
1	25 ± 1	-0.0031 ± 0.0002	231 ± 18

The apparent volume of distribution of labelled methimazole one minute after an intravenous dose was 66.7 ml/100 g rat.

2. Tissue Distribution and Protein Binding

Distribution of radioactivity in various tissues of female rats at specified time intervals after an oral dose of methimazole is listed in Table 11. Radioactivity was found in all tissues examined. In all tissues, maximum levels of radioactivity were found within two hours. Radioactivity in the kidney, liver and duodenum exceeded plasma levels at each of the time intervals examined. The concentration of radioactivity in the stomach wall was also

Table 11

Tissue distribution of ^{14}C after oral dosage of labelled methimazole to female rats

Sample	Time (hr)		
	2	4	8
Plasma[a]	16.1 ± 1.3	12.5 ± 0.2	9.9 ± 0.7
Blood	15.6 ± 2.1	10.5 ± 1.1	9.4 ± 1.5
Brain[b]	14.6 ± 3.2	6.8 ± 0.9	6.8 ± 1.4
Heart	13.8 ± 0.8	8.0 ± 0.9	8.5 ± 1.7
Lung	15.2 ± 1.3	9.0 ± 1.0	9.3 ± 1.8
Liver	19.5 ± 0.1	13.8 ± 1.1	13.6 ± 3.0
Kidney	28.2 ± 1.5	20.7 ± 1.6	20.5 ± 3.5
Spleen	12.9 ± 1.0	7.9 ± 1.4	7.9 ± 1.7
Abdominal Fat	7.1 ± 1.3	6.7 ± 1.6	7.4 ± 4.2
Skin	12.1 ± 1.0	7.3 ± 0.9	9.5 ± 2.3
Eye	12.5 ± 1.1	8.5 ± 1.3	7.8 ± 1.6
Red Marrow	14.0 ± 0.1	6.6 ± 0.8	6.3 ± 1.3
Yellow Marrow	9.1 ± 1.1	3.3 ± 0.3	3.1 ± 0.9
Skeletal Muscle	15.7 ± 1.3	9.3 ± 1.5	7.2 ± 1.4
Thyroid Gland	13.8 ± 1.4	8.6 ± 0.5	9.5 ± 1.8
Stomach	105.0 ±31.3	68.5 ±15.8	23.3 ± 6.1
Duodenum	35.3 ±13.9	23.2 ± 3.2	14.8 ± 3.6

Values are expressed as [a] ug drug per ml or [b] ug drug per g tissue, wet weight.

Values quoted represent mean ± standard error. Three animals were used for each experiment.

higher than plasma concentration at the time intervals examined. A comparison of the blood and plasma levels of radioactivity indicated that they were not significantly different. Equilibrium dialysis experiments indicated that the drug was bound to rat plasma proteins to the extent of 5.1 ± 1.0% over the range of concentrations from 5 - 30 ug methimazole/ml (n = 8; the value represents the mean ± S.E.). The radioactive label was also found to be more concentrated in red marrow than in yellow marrow. Tissue concentrations of radioactivity present 4 hours and 8 hours after an oral dose of methimazole were essentially similar, although they were lower than the peak values observed at two hours after an oral dose.

3. Excretion

Cumulative urinary excretion of radioactivity after oral, intraperitoneal and intravenous doses of labelled methimazole to rats is illustrated in Fig. 10. The total radioactivity excreted in the urine varied between 80 and 90% of the administered dose. Mean urinary excretion within 24 hours was determined to be 80% of the administered dose. These data suggest that there was no significant difference between the urinary excretion patterns of male or female rats that received an oral dose of methimazole, since the half-life for the excretion of radioactivity in the urine was seven hours in both cases. After an intraperitoneal or

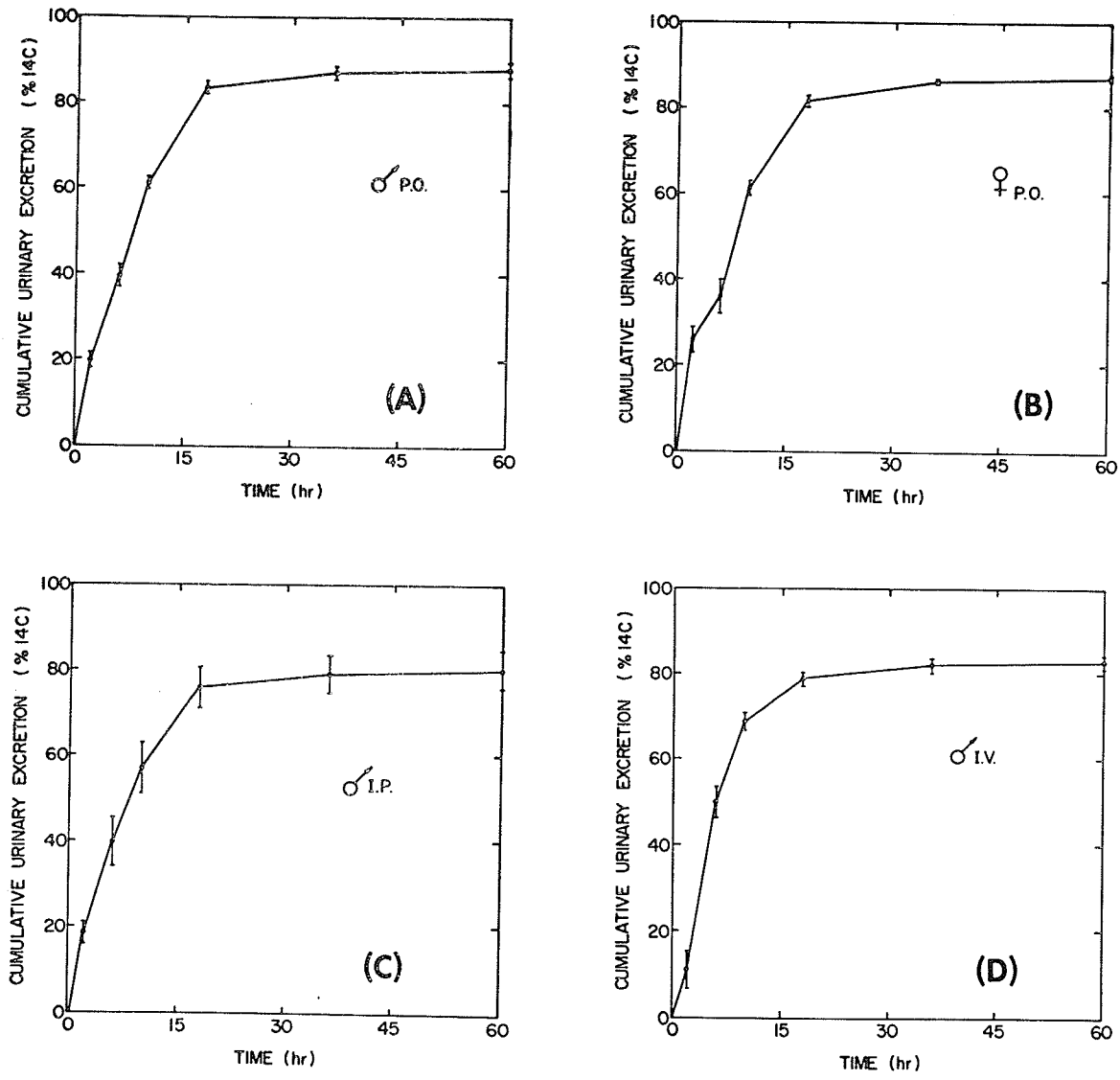


Fig. 10: Mean cumulative urinary excretion of radioactivity expressed as a percentage of the administered dose of labelled methimazole. Six animals were used in each experiment, and the vertical bars represent standard errors. Dosage routes: Panel A. male rats P.O., Panel B. female rats P.O., Panel C. male rats I.P., Panel D. male rats I.V.

intravenous dose of methimazole to male rats, the half-life of excretion of radioactivity into the urine was found to be 6 hours and 5 hours respectively. These data indicate that urinary excretion of radioactivity is similar regardless of the dosage route or sex of the animal.

The time course of biliary excretion of radioactivity after an oral dose of methimazole is illustrated in Fig. 11. The data in Fig. 11 demonstrate that 10 hours after an oral dose radioactivity was still being excreted into the bile. Biliary excretion up to this time approximated 10% of the administered dose.

Fecal excretion of radioactivity over a period of 48 hours after doses of labelled methimazole was found to account for 1.3 ± 0.2% after an oral dose to male and female rats; and 1.6 ± 0.1% after an intraperitoneal, and 1.6 ± 0.3% after an intravenous dose to male rats. These data demonstrate that fecal excretion did not differ with dosage route. Identical results were obtained from either direct counting of a fecal suspension or prior extraction.

4. Metabolic Studies

a) Urinary Metabolites

Elution profiles from the column chromatography of urine samples, before and after hydrolysis with Glusulase^{R}, are illustrated in Figs. 12 and 13. The elution profiles were similar for all other urine samples analyzed in this

Fig. 11: Mean biliary excretion of radioactivity after an oral dose of labelled methimazole. Three female rats were used and vertical bars represent standard errors.

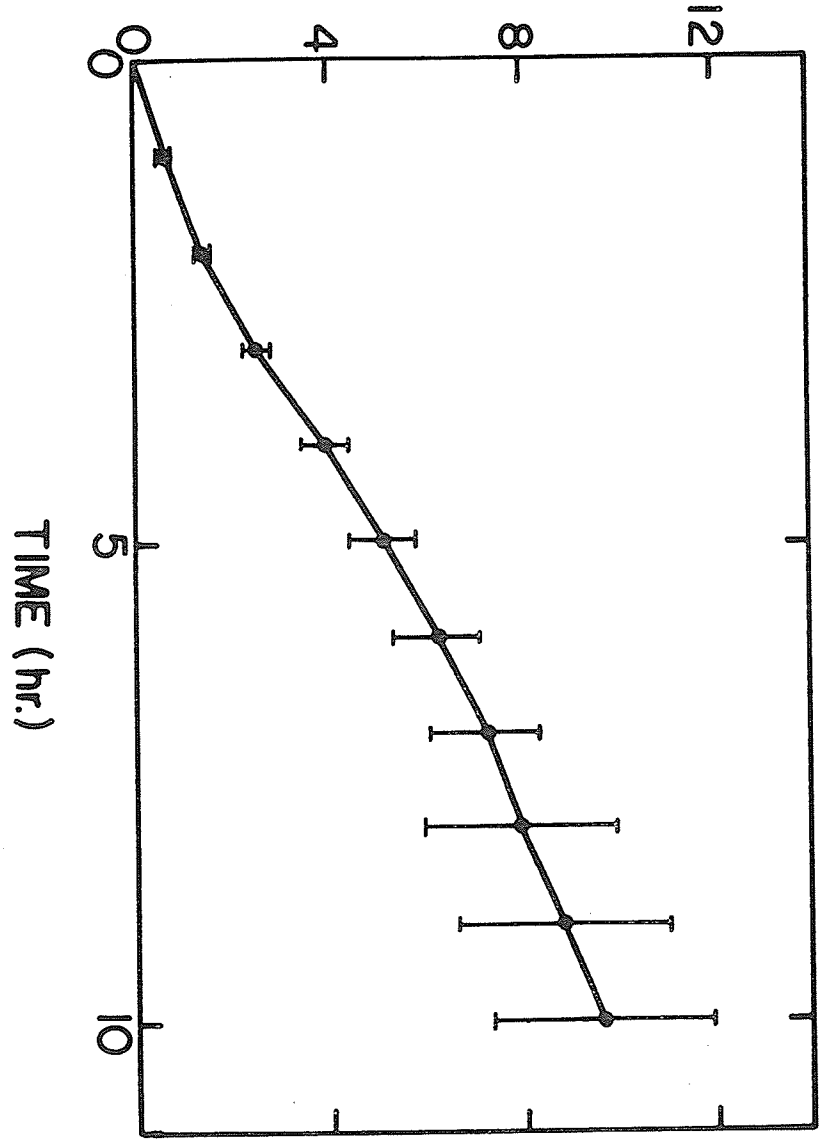

CUMULATIVE BILIARY EXCRETION (% 14 C)
0
4
8
12
TIME (hr.)
0
5
10

Fig. 12: Elution profile of radioactivity from the ion exchange chromatography of an unhydrolyzed 24 hour urine sample collected after administration of labelled methimazole.

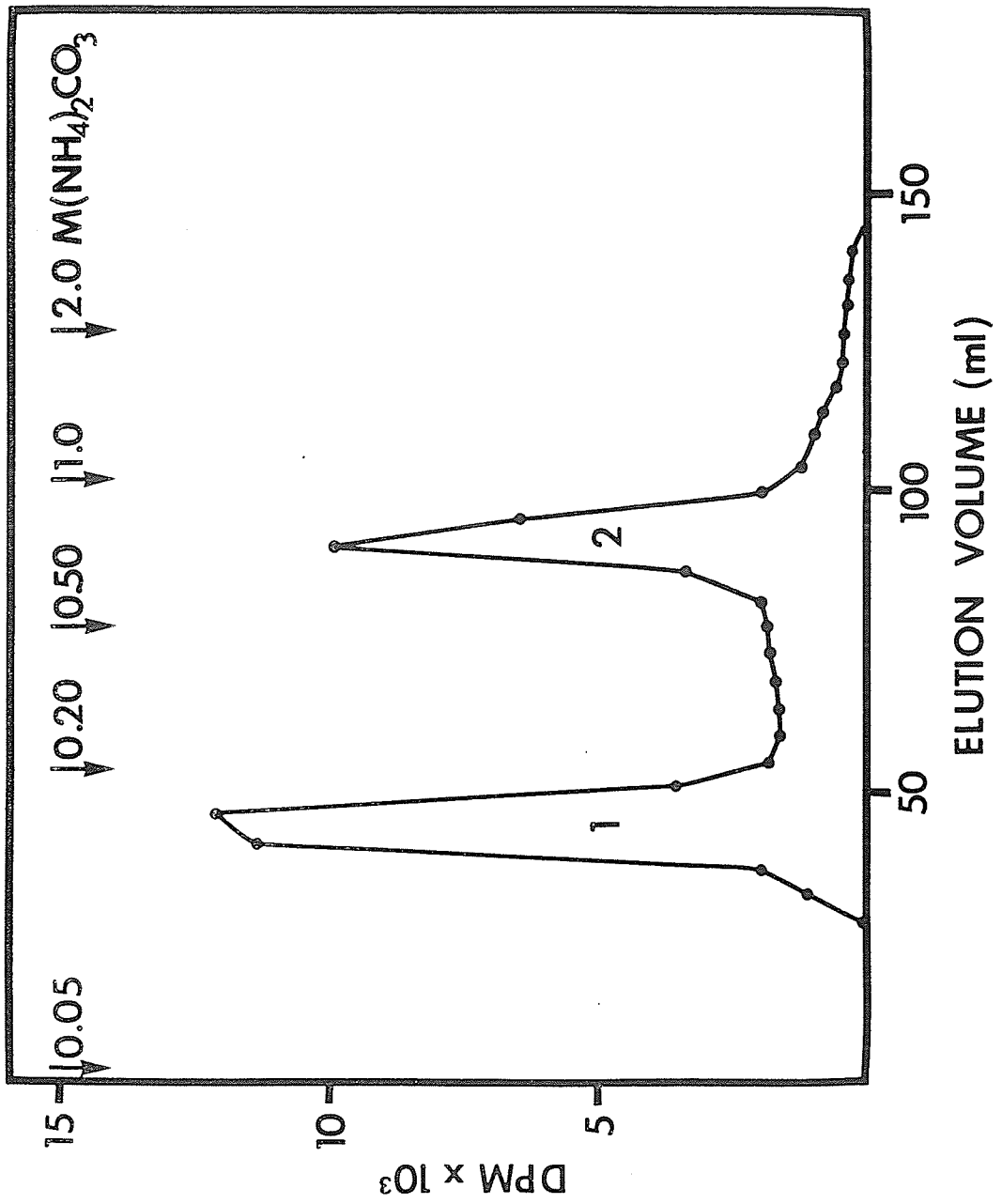
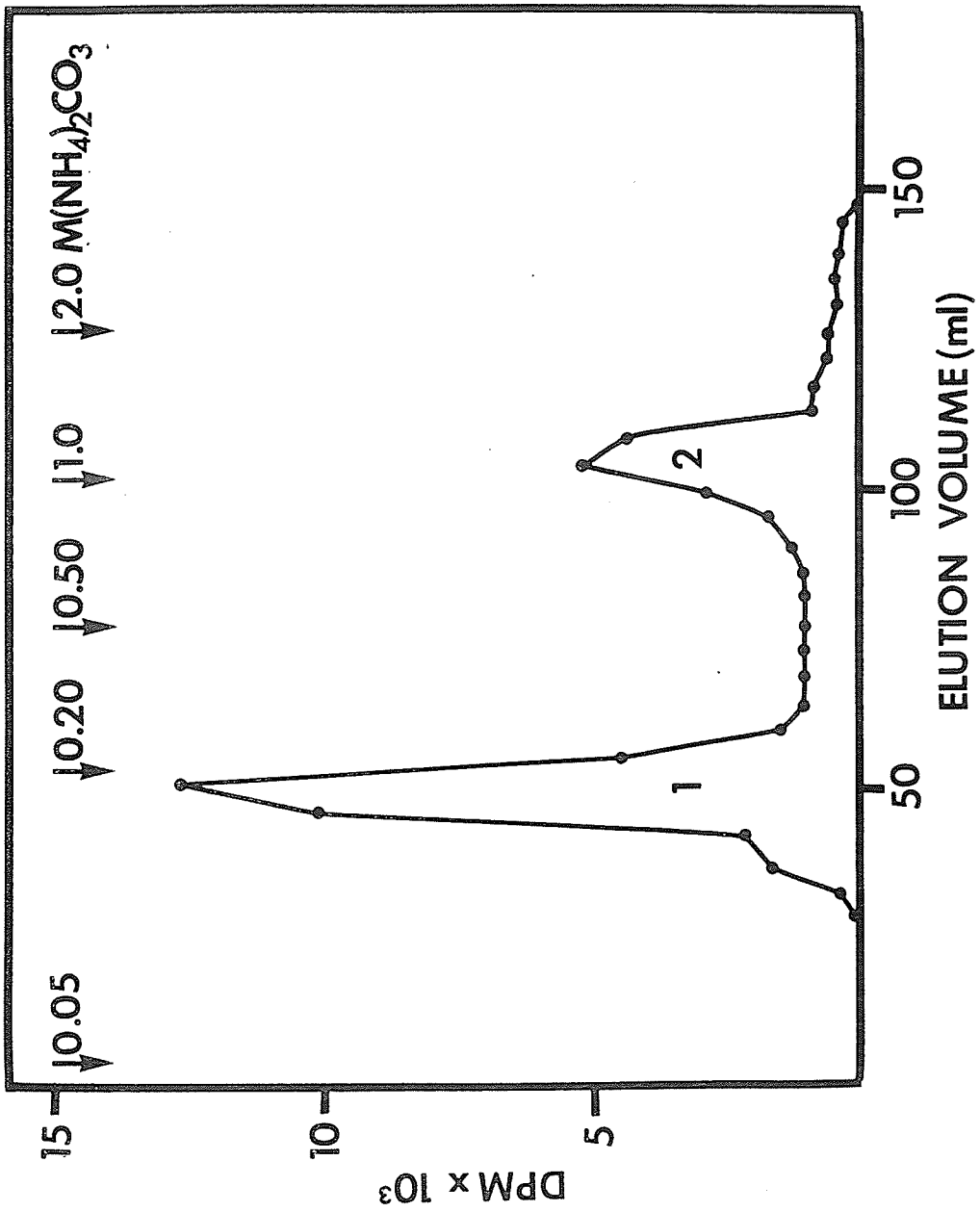

0.05
0.20
0.50
1.0
2.0 M$(NH_4)_2CO_3$
1
2
15
10
5
DPM x 10^3
50
100
150
ELUTION VOLUME (ml)

Fig. 13: Elution profile of radioactivity from the ion exchange chromatography of a Glusulase^{R} hydrolyzed 24 hour urine sample collected after administration of labelled methimazole.


DPM x 10³
15
10
5
0.05
0.20
0.50
1.0
2.0 M(NH4)2CO3
1
2
50
100
150
ELUTION VOLUME (ml)

manner. Hydrolysis of the urine sample with Glusulase^{R} at pH 5.3, prior to column chromatography, appeared to have little or no effect on the elution profile. However, acid hydrolysis of the urine sample and subsequent column chromatography yielded a different elution profile, as illustrated in Fig. 14. The new peak, which resulted from hydrolysis with acid, i.e. peak 2 in Fig. 14 corresponded with the elution volume for unchanged drug. This indicated that peak 1 in each of Figs. 12, 13 and 14 is a conjugate of methimazole.

Thin-layer chromatography of urine extracts, before and after Glusulase^{R} hydrolysis, and of the radioactive peaks collected from the column indicated the presence of three radioactive compounds in the 24 hour urine. Their thin-layer chromatographic characteristics are listed in Table 12.

Peak 1 in Figs. 12, 13 and 14 was characterized as a glucuronide conjugate of methimazole. Thin-layer chromatography indicated that this peak corresponded to the radioactivity which remained in the urine after the sequence of extractions with organic solvents (Table 12). Treatment of this spot on the thin-layer plate with naphthoresorcinol spray reagent yielded a blue color which corresponded to the radioactive zone. When the compound was incubated with Glusulase^{R} at pH 4.7, and then extracted with ethyl acetate, only one radioactive substance was found in the extract.

Fig. 14: Elution profile of radioactivity from the ion exchange chromatography of an acid hydrolyzed 24 hour urine sample collected after administration of labelled methimazole.

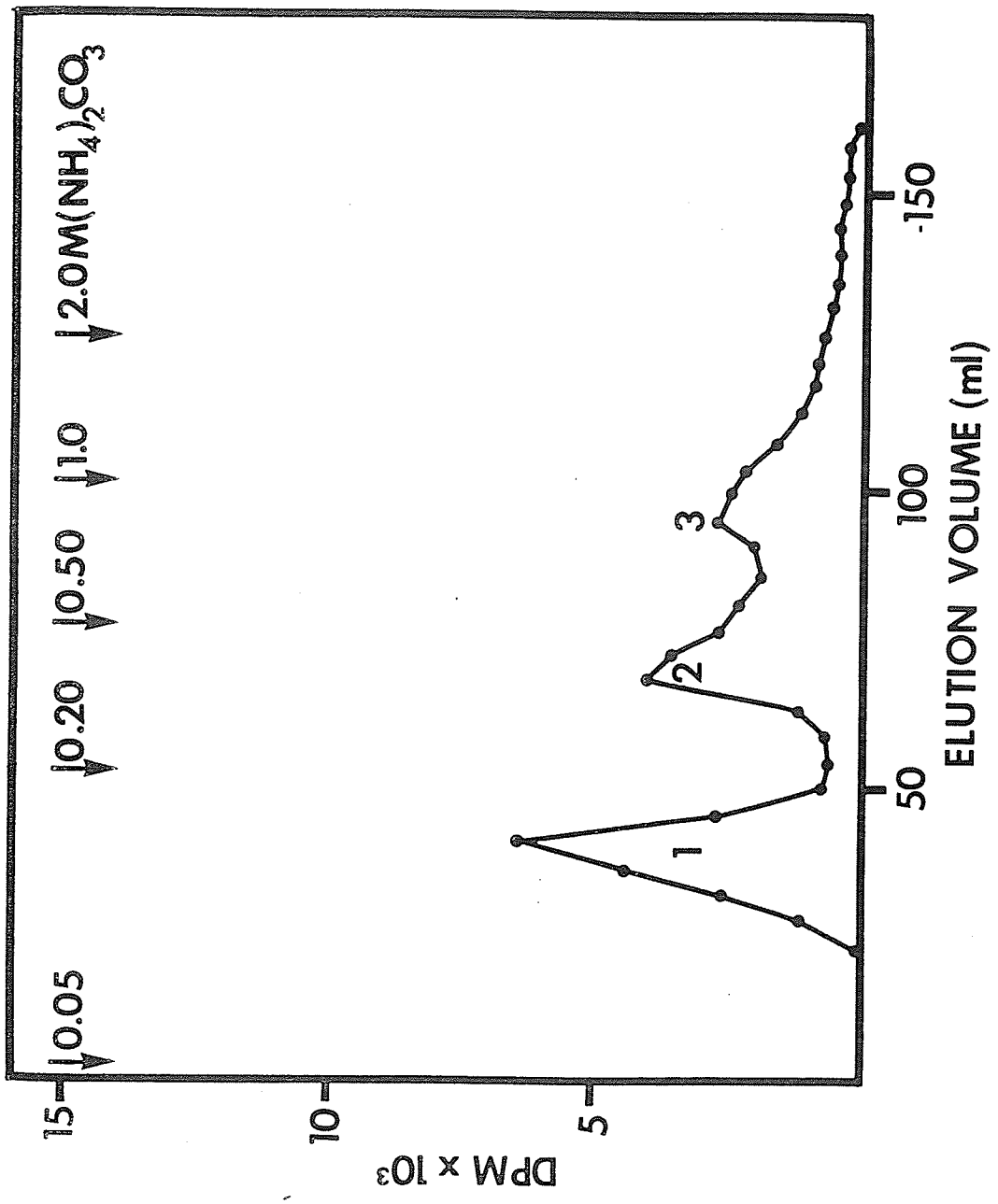

0.05
0.20
0.50
1.0
2.0M $(NH_4)_2CO_3$
1
2
3
15
10
5
DPM x 10^3
50
100
150
ELUTION VOLUME (ml)

Table 12: Thin-layer chromatography of radioactive compounds excreted in rat urine after a dose of labelled methimazole. The migration of substances on thin-layer plates is expressed as their R_f values.

Substance	Developer		
	ethanol: acetic acid 9:1	i-propanol: benzene 1:1	benzene:methanol: acetic acid 79:14:7
Methimazole	--	0.50	0.34
M-1[a]	0.61	0.00	0.11
Methimazole glucuronide[b]	0.47	0.00	0.00

[a] M-1 is the unidentified metabolite in the 24 hour urine sample.

[b] This metabolite corresponds to peak 1 in Fig. 12.

This substance was found to have identical thin-layer chromatographic properties to authentic methimazole (Table 12). Thus, it is reasonable to conclude that this metabolite is a glucuronide conjugate of methimazole. This metabolite was found to account for 45 - 60% of the excreted radioactivity in the 24 hour urine sample.

Thin-layer chromatography of ethyl acetate extracts of urine samples before hydrolysis, indicated that unchanged drug was excreted in the urine. It was found that 19% was excreted by male rats and 26% by female rats in the urine

over a period of 24 hours after an oral dose. Male rats were found to excrete 18% after an intraperitoneal dose, and 23% after an intravenous dose in the form of unchanged drug in pooled urine samples collected over 24 hours. This unchanged drug is believed to correspond to the area between the first and second peaks in Figs. 12 and 13, and becomes more prominent after acid hydrolysis of urine samples prior to column chromatography. The characterization of methimazole was confirmed by thin-layer chromatography in two solvent systems and compared with authentic material (Table 12), by gas-liquid chromatography, and by mass spectrometry (Table 13). The fragmentation pattern is consistent with the structure of methimazole (Budzikiewicz et al. 1967c), and was identical to the spectrum of a reference standard of methimazole.

The second peak in Figs. 12 and 13, and the third peak in Fig. 14 are attributed to M-1, the unidentified metabolite isolated from the urine of rats treated with methimazole. Its thin-layer chromatographic properties are listed in Table 12. Mass spectral analysis of this metabolite is listed in Table 14. The data did not allow us to determine the structure of this metabolite. The sample was contaminated with acetic acid. However, there was no significant peak at m/e 114. This indicated that the substance designated M-1 is not unchanged methimazole, and must have undergone some metabolic transformation. Strong absorption at

Table 13

Mass spectrum of unchanged methimazole from the urine of rats

m/e	intensity[a]	m/e	intensity[a]	
40	5	59	6	
41	5	69	16	
42	22	72	28	
43	7	81	15	
44	5	86	6	
45	8	99	5	
54	11	114	100	Parent ion
55	7	115	12	
56	8	116	6	
57	7			

[a] Peak intensities are expressed relative to m/e 114 which has been assigned a value of 100.

Table 14

Mass spectrum of M-1 obtained from the urine of rats treated with methimazole

m/e	intensity$_a$	m/e	intensity$_a$
50	10	79	20
51	18	80	10
52	28	81	35
53	19	82	57
54	30	83	42
55	72	84	18
56	33	85	28
57	63	91	18
58	100	92	10
59	100	93	10
60	*	94	15
61	28	95	40
67	24	96	16
68	22	97	34
69	38	98	20
70	23	99	10
71	28	105	13
72	14	107	12
73	44	109	36
74	28	110	10
77	22	111	20
78	10	133	20

a Peak intensities are expressed relative to m/e 58 which are assigned a value of 100.

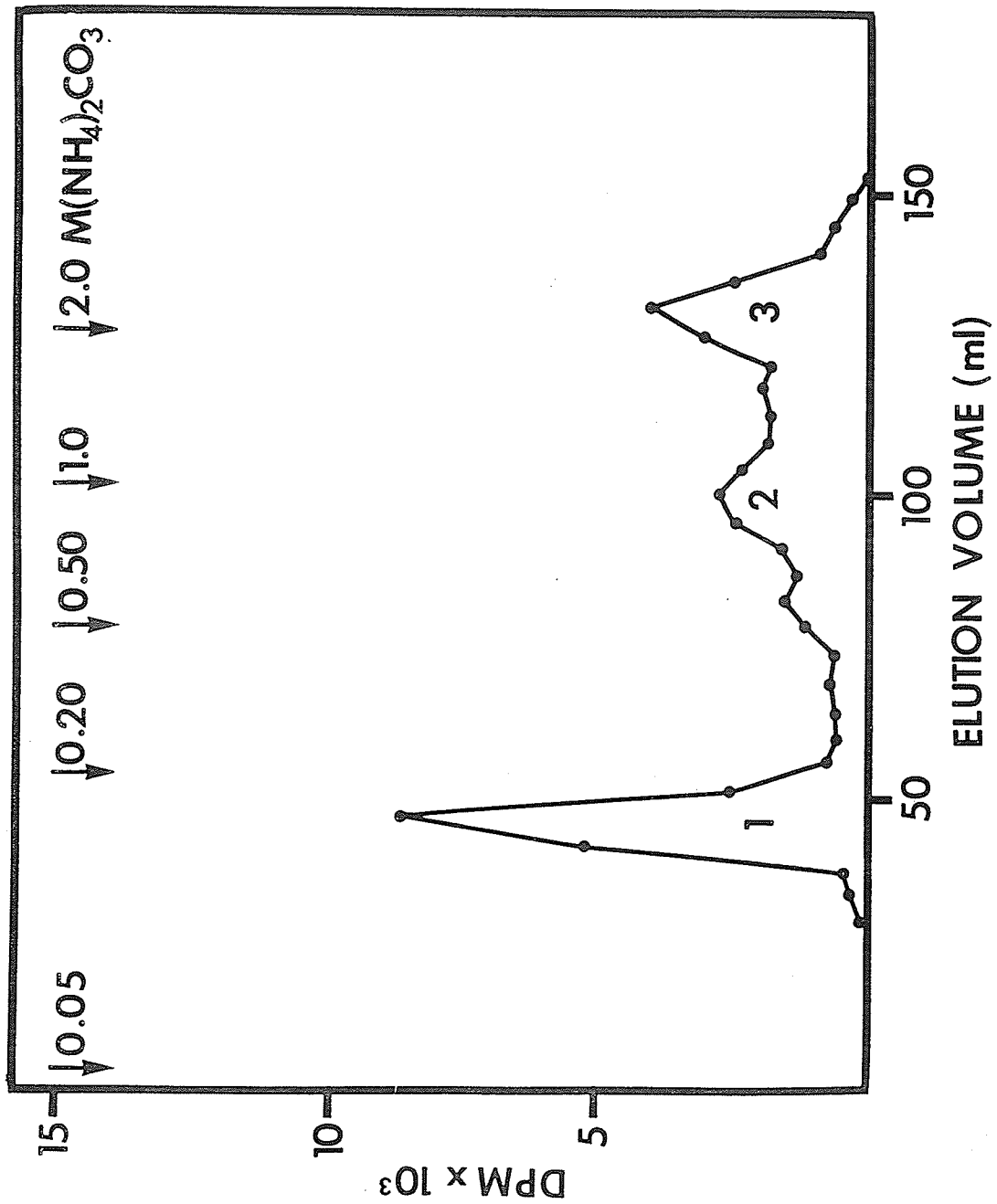
* acetic acid contamination.

254 nm by this compound indicated that the ring structure must still have been intact. The only similarity between the mass spectrum of methimazole and that of M-1 is the similar intensities of m/e 81 and 69 relative to each other. This metabolite was found to account for 13 - 25% of the radioactivity in samples of pooled 24 hour urine.

b) Biliary Metabolites

The results of column chromatography of an aliquot of pooled bile samples collected after an oral dose of methimazole are illustrated in Fig. 15. The elution profile indicates the presence of three radioactive compounds. The thin-layer chromatographic properties of these compounds are listed in Table 15. It can be seen from the R_f values of these compounds that no detectable quantities of methimazole were excreted into the bile, i.e. all of the isolated compounds were metabolites. Peak 1 in Fig. 15 exhibited the same chromatographic properties as peak 1 in Figs. 12, 13 and 14. This indicates that they are the same compound, i.e. a glucuronide conjugate of methimazole. This conclusion is supported by the thin-layer chromatographic characteristics of peak 1 from Fig. 15 (Tables 12 and 15). The compound was hydrolyzed with Glusulase^R at pH 4.7 and the ethyl acetate extract of the hydrolysate was subjected to thin-layer chromatography. Radiochromatograms of the developed plates revealed the presence of methimazole and

Fig. 15: Elution profile of radioactivity from the ion exchange chromatography of bile collected from rats given an oral dose of labelled methimazole.


0.05
0.20
0.50
1.0
2.0 M(NH4)2CO3
1
2
3
15
10
5
DPM x 10³
50
100
150
ELUTION VOLUME (ml)

M-1 in the ethyl acetate extract. The biliary metabolites were not further characterized. Methimazole glucuronide (peak 1, Fig. 15) accounted for 40% of the biliary radioactivity.

Table 15: Thin-layer chromatography of radioactive peaks from bile after an oral dose of labelled methimazole. The migration of substances on thin-layer plates is expressed as their R_f values.

Substance	Developer	
	benzene:methanol: acetic acid 79:14:7	ethanol: acetic acid 9:1
Peak 1	0.00	0.49
Peak 2	0.00	0.61
Peak 3	0.00	0.27

c) Fecal Metabolites

Feces samples were not investigated to determine the nature of the radioactive compound(s), since only traces of radioactivity were present.

DISCUSSION

A. Position of the Radioactive Label in the Thionamide Drugs

The initial problem was to introduce the label at a position where it would not be cleaved during metabolic degradation of the drugs, and thus result in the inability to trace the major products. Earlier reports on the metabolism of thiouracil indicated that it was metabolized by desulfuration and ring fragmentation (Spector and Shideman 1959) to yield carbon dioxide, ammonia, inorganic sulfate and a β-amino acid. This indicated that labelling with ^{35}S or ^{14}C at the position of the C=S bond, i.e. at the 2 position of the ring (Figs. 1 and 3) would be inappropriate, because the label might be lost during metabolism of both propylthiouracil and methimazole. It was therefore decided that labelling within the ring with ^{14}C would yield the most useful information. A review of the methods available for the synthesis of propylthiouracil (Anderson *et al.* 1945) indicated that the 6 position of the ring would be a feasible position to insert the label. The synthesis of labelled propylthiouracil was therefore carried out with this in view (Section II, A). A basic principle of radiolabel synthesis is to attempt to insert the label into the desired position as the final step in the reaction sequence to conserve expensive label. The method used in this synthesis required the insertion of the label in the first of a three

step procedure. The use of carriers gave a radiochemical yield of 34% despite the overall chemical yield of 14%.

Labelled methimazole was a gift from the Eli Lilly Research Laboratories through the courtesy of Dr. R.E. McMahon. The ^{14}C label was present at the 2 position of the ring (Fig. 3). The label was incorporated into this position rather than the N-methyl position because N-demethylation of foreign compounds is a common metabolic route in the mammal. It was considered that this label would remain in most of the possible metabolites.

B. Absorption

Propylthiouracil was found to be more rapidly absorbed after an intraperitoneal dose than after an oral dose (Table 2). With methimazole, the reverse was true (Table 9). This difference may be explained by the solubility and partition characteristics of each substance (Table 1). At the pH of the stomach, propylthiouracil is not completely soluble in the volume of the vehicle administered to rats, i.e. 5 mg/ml. Thus, it may be predicted that this drug precipitated in the stomach. This precipitate might then serve as a reservoir to give constant absorption over a long period of time. This concept is compatible with the finding that radiolabel concentration in the stomach exceeded plasma concentration at least for four hours after an oral dose of propylthiouracil

(Table 4). The surface area of the peritoneum is much larger than that of the stomach. Also, the solubility of propylthiouracil is greater at the pH of the peritoneum than that of the stomach (Table 1). Thus, it would be expected from these data that propylthiouracil would be more readily absorbed from the peritoneum, and hence result in higher plasma levels than after an oral dose. This is compatible with the data in Table 2.

On the other hand, the partition coefficients and water solubility of methimazole (Table 1) are relatively constant over the pH range measured. This would indicate that the rate of absorption of methimazole after an oral dose should be similar to the rate of absorption after an intraperitoneal dose, especially since the solubility data indicate that methimazole should not precipitate in the stomach. There is, however, a difference in the initial rates of absorption. Methimazole was initially absorbed more rapidly after an oral than after an intraperitoneal dose. This difference was not detectable one hour after administration of this drug by either route.

The results of these studies demonstrate that methimazole is more rapidly absorbed following an oral dose than is propylthiouracil. This confirmed by the time to achieve peak plasma levels after an oral dose (Tables 2 and 9) and by the amount of radioactivity in the stomach after an oral dose (Tables 4 and 11). This finding is in marked

contrast to those of Alexander et al. (1969). These workers stated that propylthiouracil was absorbed more rapidly than methimazole in one human because they observed that peak plasma levels of radioactivity were obtained more rapidly after an oral dose of propylthiouracil than after methimazole. However, these workers did not measure the rate of disappearance of the drug from the stomach. They monitored only plasma concentration of ^{35}C radioactivity after an oral dose of each drug to one patient with normal renal clearance.

Plasma levels of a drug, measured after an oral dose, do not produce evidence to allow definitive conclusions to be drawn concerning the rate of absorption. Peak plasma levels indicate only that the rate of distribution into the tissues, metabolism, and excretion have reached the same rate as the absorption of the drug from the gastrointestinal tract.

The half-lives of unchanged drug present in the plasma after an intravenous dose of propylthiouracil or methimazole were 1.5 hours and 4 hours respectively. Pharmacokinetic analysis of plasma concentrations of unchanged drug, after an intravenous dose, demonstrated a basic difference in their pattern of disposition. The disappearance of propylthiouracil from the plasma could be represented by a two compartment model (Table 3), while the disappearance of methimazole from the plasma could be represented by a single

compartment model (Table 10). The single compartment that was demonstrated for methimazole very closely resembled the second compartment for propylthiouracil. The slopes of these compartments were the same, i.e. -0.003, and their half-lives were similar, i.e. 267 minutes for propylthiouracil, and 237 minutes for methimazole. These data suggest that the rate of metabolism and/or excretion of methimazole was similar to that of propylthiouracil. The failure to demonstrate a rapid first compartment for methimazole, comparable to that found for propylthiouracil, can be explained by its more rapid distribution into the tissues. This interpretation is compatible with the partition and solubility data of both drugs (Table 1).

Another interesting finding was the relative amounts of free drug concentrations present in the plasma after oral and intravenous doses of each drug. If the amount of protein binding is taken into account, the concentration of free drug in the plasma is very similar for both drugs. This observation might lead to the assumption that the absorption of the two drugs was similar. However, the concentration of the radiolabel present in the stomach wall after an oral dose tends to rule out this interpretation (Tables 4 and 11). This finding is exactly opposite to that reported by Alexander et al. (1969), who obtained results from only one experiment. No reasonable explanation

can be offered for this difference other than to make the comment that the single experiment of Alexander et al. can be considered to be a preliminary study. However, the differences in half-lives found in this study are compatible with differences in the absorption of methimazole and propylthiouracil. Unchanged drug present in the plasma following an intraperitoneal dose of either drug was not determined in this study. Since no detectable amounts of metabolites were demonstrated to be present in the plasma of rats after intravenous or oral administration of propylthiouracil, it would be reasonable to suppose that a similar condition would exist after intraperitoneal administration of this drug. Thus, the half-life of unchanged propylthiouracil in the plasma after an intraperitoneal dose to male rats would therefore be 9 hours. In the case of methimazole, circulating metabolite(s) were demonstrated after intraperitoneal and intravenous doses. However, the plasma concentrations of radioactivity were similar after intraperitoneal and oral administration of this drug. Thus, it could be assumed that the proportion of circulating metabolite(s) would also be similar after a dose of this drug administered by either of the two routes mentioned above. Thus, the plasma half-life of methimazole is approximately 5 hours after an intraperitoneal dose given to male rats.

C. Tissue Distribution

Both propylthiouracil and methimazole were found in all tissues examined (Tables 4 and 11). After an oral dose of propylthiouracil, the stomach was the only tissue which contained a higher concentration of radioactivity than the plasma. Thus, it appears that there are no active transport systems present for propylthiouracil. Also these data indicate that there is no transport system available for propylthiouracil in the thyroid gland as has been suggested by Alexander et al. (1969). After oral administration of methimazole, tissue concentration of radioactivity exceeded plasma concentration in the kidney, liver and duodenum, as well as the stomach. The drug was not concentrated in the thyroid gland. This is analagous to the results obtained with propylthiouracil. The concentration of radioactivity in the kidney suggests the presence of a transport system for methimazole and/or its metabolites. The concentration of radioactivity in the liver, only slightly higher than that in the plasma, might reflect binding of the drug to the drug metabolizing enzyme system. The higher concentration of radioactivity present in the duodenum may be attributed either to radioactivity excreted into the bile, or to unchanged drug from the stomach which passed into the duodenum before it was absorbed.

Propylthiouracil did not readily penetrate the brain

(plasma concentration 26 ug/ml; brain concentration 6 ug/ml). In contrast, the concentration of radioactivity present in the brain two hours after an oral dose of methimazole was the same as that in plasma (plasma concentration 16 ug/ml; brain concentration 15 ug/ml). This indicated that there was a rapid equilibration of methimazole with brain tissue. The concentration of radioactivity in the abdominal fat was higher after a dose of propylthiouracil than after methimazole. Also, the ratio of concentration of radioactivity in the abdominal fat compared to the free drug concentration in the plasma was greater for propylthiouracil than methimazole two hours after an oral dose, i.e. 0.85 for propylthiouracil, and 0.48 for methimazole. From the partition data, one may have expected a higher concentration of methimazole in abdominal fat, but this was not observed. Although the concentration of radioactivity in the tissues appeared fairly constant up to eight hours after an oral dose of propylthiouracil (Table 4), this was not so after an oral dose of methimazole. The tissue concentration of radioactivity after an oral dose of methimazole was maximal within two hours after an oral dose, and then started to decline. However, the tissue concentration of radiolabel was fairly constant between the four and eight hour intervals examined in the methimazole study (Table 11). The difference in the tissue concentration of these two drugs

can be explained by the difference in absorption (Section IV, B).

On examination of the plasma levels of unchanged propylthiouracil after an oral dose (Table 2), it might reasonably be concluded that the tissue concentrations of radioactivity represent unchanged drug, since no measurable concentration of circulating metabolite(s) could be demonstrated. This may not be true in the liver and kidney, which are well known sites for the metabolism of foreign compounds. The same reasoning is also applicable to the results obtained in the methimazole study, at least to four hours after an oral dose, in that no circulating metabolite(s) could be demonstrated (Table 9). In other words, tissue concentrations of radioactivity may be said to represent unchanged methimazole for at least four hours after an oral dose of this drug.

It is interesting to speculate that higher concentrations of radioactivity in red than in yellow marrow might be a contributing factor in the blood dyscrasias observed after doses of these two drugs. This might possibly arise where renal impairment is present, since it has been demonstrated that the half-life of radioactivity in the plasma is considerably prolonged in this case (Alexander *et al.* 1969).

The concentration of radioactivity by the kidney,

after a dose of methimazole, but not after propylthiouracil, suggests that the excretion mechanisms of these drugs and their metabolites are different, i.e. secretion and/or re-absorption from the kidney tubules could account for the difference in the concentration of methimazole and propyl-thiouracil in the kidney. This last finding is of possible therapeutic significance where renal impairment is present. This concept is in agreement with the observations of Alexander et al. (1969) who found that the relative pro-longation of the half-lives of serum radioactivity was longer after a dose of propylthiouracil than methimazole when renal impairment was present.

Williams (1959c) suggested that there is no correla-tion between the amount of thionamide drug in the thyroid gland and antithyroid activity. It is obvious, however, that there must be at least a minimal concentration of these drugs at which they can exert their effect. This study demonstrated that neither of the two drugs were con-centrated in the thyroid gland. This finding is exactly opposite to that of Alexander et al. (1969) who investi-gated these two drugs. They used ^{35}S labelled drugs, but did not give any experimental details. These workers examined only total tissue radioactivity. This discrepancy can be explained on the basis of the findings of Maloof and Soodak (1957), who also found that radioactivity was

concentrated in the thyroid glands of rats that had been given ^{35}S labelled thiourea or thiouracil. However, they also demonstrated that most of the radioactivity in the thyroid gland was accounted for by $^{35}SO_4$. The thyroid/serum concentration of these drugs was approximately one. Thus, only the label, and not the drug, was concentrated in the thyroid gland. The results in this study confirm the findings of Maloof and Soodak (1957) that the thyroid does not concentrate drugs of the thionamide type. Thus, the discrepancy between the findings of Alexander et al. (1969) and the results in this study is resolved, i.e. the concentration of radioactivity in the thyroid gland which they observed can be explained by the presence of $^{35}SO_4$ which could be trapped after desulfuration of propylthiouracil.

D. Excretion

The kidney was found to be the organ responsible for the excretion of most of the radioactivity after administration of both drugs, although, as mentioned previously in the discussion of tissue distribution of these drugs (Section IV, C), the mechanism of excretion may be different. Very little or no radioactivity was excreted in the feces after doses of either drug. This excretion pattern is in agreement with other studies of the elimination of thionamide drugs (Williams and Kay 1944; Williams et al. 1944; Schulman Jr. and Keating 1950; Spector and Shideman 1959; Alexander

et al. 1969). These data indicate that the drugs were completely absorbed (Section IV, B).

Male rats, given an oral dose of either propylthiouracil, or methimazole, exhibited urinary excretion half-lives of drug and metabolites which were very similar, i.e. 6.5 and 7 hours respectively. Female rats excreted these drugs differently after an oral dose. The urinary half-lives for propylthiouracil and methimazole in the latter instance were 4 hours and 7 hours respectively. The excretion data from female rats agree with the findings of Alexander et al. (1969) who found that the two human subjects examined in their study excreted propylthiouracil and its metabolites more rapidly than methimazole and its metabolites. These data indicate that there is a sex difference in the urinary excretion of these drugs and their metabolites in rats. It is not stated in the study by Alexander et al. (1969) whether the human subjects were males or females. Thus, it is not possible to say at this time if a sex difference in urinary excretion might occur in humans given these drugs. Such a finding would have therapeutic implications.

One of the more important findings of these studies is the significant excretion of metabolites into the bile after administration of propylthiouracil and methimazole. Since little or no label was excreted in the feces, an

enterohepatic circulation must have been present. Such an enterohepatic circulation of thionamide drugs has not previously been reported. None of the radioactivity in the bile was due to unchanged drug. The characterization of these excretory products as conjugates in the bile means that the molecular weight of the biliary metabolites approaches the postulated molecular weight requirement of 300 stated by Brauer (1959). The molecular weight of propylthiouracil glucuronide is 336, while that of methimazole glucuronide is 290. However, metabolites of lower molecular weight in the bile may result if the metabolite M-1, a biliary metabolite of methimazole, has been demethylated or desulfurated.

E. Metabolism

Prior to this study, the only available data from studies of the metabolism of thionamide drugs was that reported by Schulman Jr. (1950), Maloof and Soodak (1957), Sarcione and Sokal (1958), Maloof and Spector (1959), and Spector and Shideman (1959), on the metabolism of thiourea and thiouracil. The study of propylthiouracil and methimazole metabolism reported here may be compared to those of thiourea and thiouracil. They may also be compared to the metabolism of barbiturates, since the ring structure of barbiturates is very similar. It might therefore be expected that there would be some similarity in the metabolism

of these two classes of compounds.

This study represents the first demonstration of the conjugation of a thionamide drug with glucuronic acid. The position of attachment to glucuronic acid was not determined. Glucuronic acid may combine with any one of four potential sites on the propylthiouracil molecule, and two potential sites on methimazole. These positions represent conjugation with an amide nitrogen, with sulfur, and with oxygen. Glucuronide conjugation with these functional groups has been previously described with other compounds. Examples of these reactions include the conjugation of the sulfur atom of 2-mercaptobenzathiazole in the metabolism of benzothiazolesulfonamide in the dog (Clapp 1956), and the sulfur atom of thiophenol in the metabolism of thiophenol in rabbits (Parke 1952). Conjugation of glucuronic acid with an amide nitrogen include 2-methyl-2-propyl-1,3-propanediol dicarbamate-N-glucuronide (meprobamate-N-glucuronide) in rabbits (Tsukamoto *et al*. 1963(, and sulfadimethoxine-N^1-glucuronide in the rat and man (Bridges *et al*. 1965). Conjugation of glucuronic acid with oxygen is the most commonly described glucuronide conjugate. An example of this reaction is the ether glucuronide conjugate of the pyrimidine ring in 5-hydroxy-2-methyl-6-methoxy-4-sulfanilamidopyrimidine, an oxidative metabolite from the metabolism of sulfamethomidine in man (DiCarlo *et al*. 1963).

The formation of more than one glucuronide conjugate was confirmed, at least in the metabolism of propylthiouracil. The urinary and biliary glucuronide conjugates were not the same in this study, i.e. their elution volumes were different (Figs. 7 and 9). The number of biliary metabolites in the metabolism of methimazole also suggests this possibility. Although three metabolites were demonstrated in the unhydrolyzed bile (Fig. 15; Table 15), only two radioactive compounds were demonstrated in the hydrolyzed sample. M-1 was the minor component in the hydrolyzate. This suggests that there were possibly two glucuronide conjugates of methimazole in the bile, one of which had the same elution volume as the glucuronide found in urine (Figs. 12 and 15). The data obtained in this study indicate that the oxidative metabolism of thionamide drugs plays only a minor role in their metabolism and excretion. Thus, approximately 70% of the radioactivity in 24 hour urine samples has been identified as unchanged drug, and a glucuronide conjugate of the original drug in both the propylthiouracil and methimazole studies.

Desulfuration of the thionamide drugs during hydrolysis with Glusulase^{R} does not appear to be likely. Marsh (1966) has stated that free 1-thio-D-glucuronic acid cannot be obtained from 1-thioalkyl or aryl conjugates, since hydrolysis of these occurs by cleavage of the D-glucuronosyl-S bond.

All compounds isolated in this laboratory absorbed strongly in the ultraviolet region at a similar wavelength to the parent drugs. Thus, it is most unlikely that ring fragmentation occurred to any significant extent in the metabolism of these drugs. This finding is similar to the metabolism of barbiturate drugs, where ring fragmentation has not been described as a major metabolic pathway in the metabolism of these drugs in mammals.

The inability to obtain unequivocal mass spectra of the unidentified metabolites P-1, from the propylthiouracil study, and M-1, from the methimazole study, did not allow us to make a complete identification of these metabolites. However, from the data obtained so far, some conclusions are possible concerning the structure of these compounds. Ultraviolet absorption at a similar wavelength to the parent drugs indicated that the ring structure of these metabolites was intact. However, no conclusions can be made concerning the role of desulfuration of these drugs. One would expect that this reaction must occur to some extent, since it has been demonstrated for thiourea and thiouracil (Schulman Jr. 1950; Maloof and Soodak 1957; Maloof and Spector 1959; Spector and Shideman 1959). However, Schönbaum *et al.* (1971) have not yet been able to demonstrate this metabolic reaction in the metabolism of propylthiouracil by the guinea pig.

This leaves only one remaining possibility in the

metabolism of propylthiouracil, i.e. side chain oxidation of the 6-propyl group. This would be compatible with routes of metabolism described for barbiturates. Thus, Waddell (1965) demonstrated that secobarbital was metabolized to 5-(2,3-dihydroxypropyl)-5-(1-methylbutyl) barbituric acid, and two stereoisomers of 5-allyl-5-(3-hydroxy-1-methylbutyl) barbituric acid in man. Maynert and Dawson (1952) and Maynert (1965) demonstrated that pentobarbital was metabolized to the two stereoisomers of 5-ethyl-5-(3-hydroxy-1-methylbutyl) barbituric acid in man and dog. Maynert (1965) also showed that amobarbital was excreted in the urine of man as 5-ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid. This was the major metabolite. Maynert and VanDyke (1950a and b) had previously demonstrated that ring hydrolysis in the metabolism of pentobarbital and amobarbital accounted for only trace metabolites, and thus was not the major route of metabolism of these compounds. The mass spectrum of P-1 indicated the presence of an acetone fragment. This finding is compatible with the postulate that side chain oxidation may play a role in the metabolism of propylthiouracil. This would be analagous to the metabolism of barbiturates, and suggests that ω-1 oxidation is one of the routes of metabolism of propylthiouracil.

The mass spectrum of M-1, obtained in the methimazole study, could not be interpreted. Thus, it can only be speculated that desulfuration, or N-demethylation are

possible oxidative metabolic reactions.

The excretion of unchanged drug accounted for 10 - 25% of the radioactivity in the 24 hour urine samples after a dose of either of the two drugs. This finding, along with the finding that a glucuronide conjugate of the parent drug was the major metabolite in both studies (45 - 60%), leaves approximately 20 - 30% of the urinary radioactivity to be identified. The identification of P-1 and M-1 in future studies should result in the complete identification of the oxidative metabolites which occur in the urine and bile of albino rats. Summaries of the metabolism of propylthiouracil and methimazole are presented in Figs. 16 and 17.

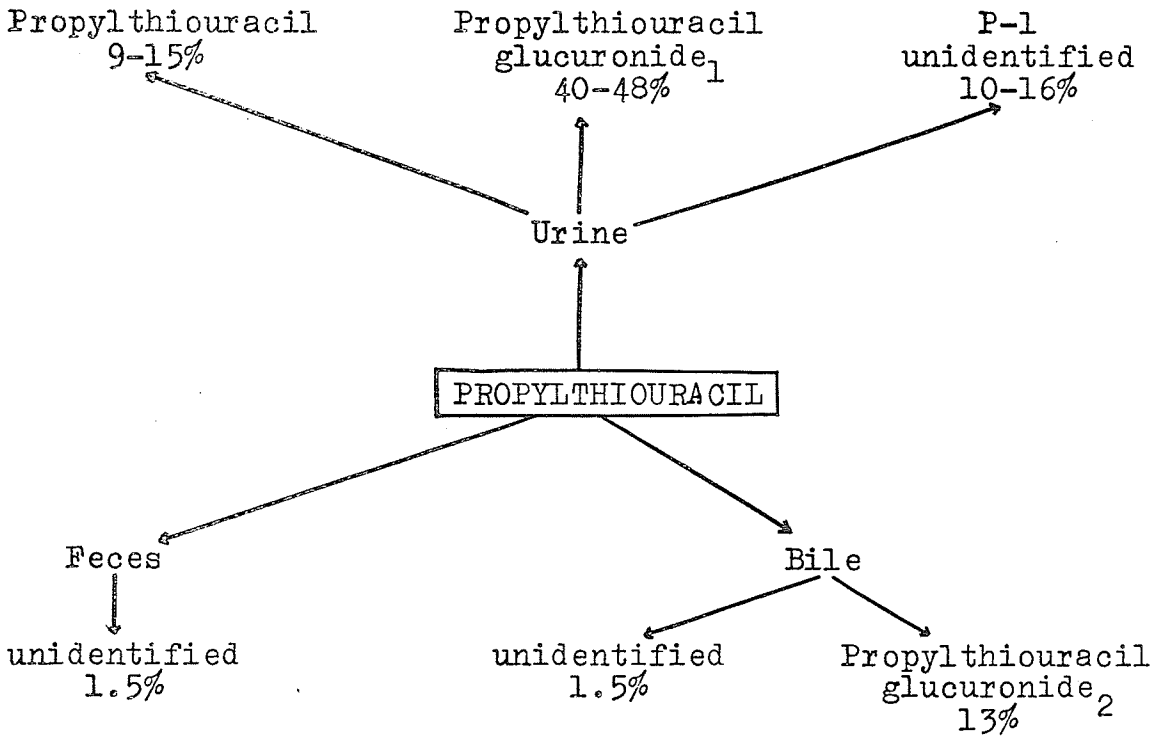


Fig. 16: Summary of the excretory products which were present after a dose of labelled propylthiouracil. Values are expressed as a percentage of the administered dose; urinary metabolites after 24 hours, fecal metabolites after 48 hours and biliary metabolites after 10 hours.

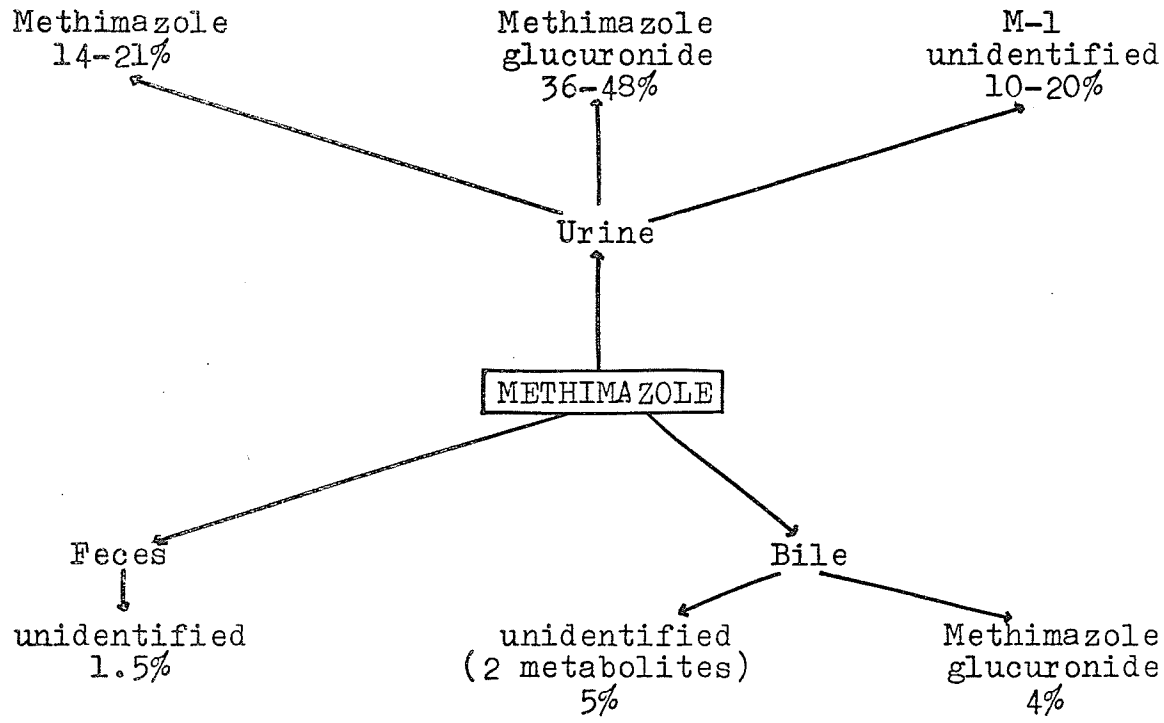


Fig. 17: Summary of the excretory products which were present after a dose of labelled methimazole. Values are expressed as a percentage of the administered dose; urinary metabolites after 24 hours, fecal metabolites after 48 hours and biliary metabolites after 10 hours.

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