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Review Article

Metabolism of Phenothiazine Drugs

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OVER A PERIOD of years beginning in 1944, the research workers of the Rhône-Poulenc Research Laboratories in France synthesized a number of amine derivatives of phenothiazine. One of the most important of these was chlorpromazine prepared by Charpentier in 1950. This compound was found to exert a remarkable number of pharmacological effects involving the central and autonomic nervous systems. Courvoisier, *et al.* (1), have published an extensive report on the pharmacological activity of chlorpromazine in animals. The most striking aspect of this drug, however, was its therapeutic value in patients with certain emotional disturbances. This discovery provided the initial stimulus for the development and use of phenothiazine drugs in psychiatric practice.

The search for new drugs to treat specific mental disorders and the attempts to isolate a particular pharmacological effect by chemical alteration of the phenothiazine molecule has resulted in the introduction of several useful phenothiazine drugs of varying actions and potencies. These developments offer a unique opportunity, not only for the study of structure-activity relationships, but also for the examination of the effect of different chemical substituents on the biological fate of these compounds, their metabolism, distribution, and excretion.

Following oral or parenteral administration, most of the phenothiazine derivatives are extensively localized in body tissues. This property of the parent compound and/or its metabolites is probably responsible for the unusually prolonged excretion which has been observed (2, 3). The possibility that metabolites may be present in the body for long periods of time underscores the importance of the identification of these compounds and the role they play in the effectiveness of the phenothiazine drugs.

Results of studies on model compounds strongly suggest that the metabolites contribute to the action of the parent drug (4). Clinical changes in psychotic patients, the type of psychiatric disorder, or the rate of regression after drug withdrawal may be correlated with the nature and quantity of the metabolic products found in the urine. These factors have tended to focus attention on the biological fate of the phenothiazine drugs.

This review of the literature and summary of the present state of knowledge concerning the metabolism of the phenothiazine drugs deals primarily with the biological transformation of phenothiazine derivatives currently used as psychotherapeutic agents. The equally important topics of absorption, tissue distribution, and excretion will be considered in a subsequent report. A substantial part of the present review is devoted to the discussion of chlorpromazine metabolism. Chlorpromazine has been used as a representative compound for the following reasons: 1. the preponderance of the relevant literature concerns chlorpromazine; 2. chlorpromazine data have, in many cases, been independently verified; 3. chlorpromazine was the first compound of this series to be examined for metab-

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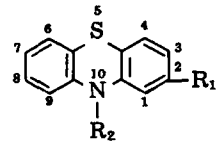
TABLE I.—CHEMICAL STRUCTURES OF SOME PHENOTHIAZINE DRUGS



Compound	R_1	R_2
Chlorpromazine	—Cl	$-CH_2CH_2CH_2N(CH_3)_2$
Methoxypromazine	—OCH_3	$-CH_2CH_2CH_2N(CH_3)_2$
Promazine	...	$-CH_2CH_2CH_2N(CH_3)_2$
Promethazine	...	$-CH_2CH(CH_3)N(CH_3)_2$
Fluphenazine	—CF_3	$-CH_2CH_2CH_2N$ piperazine $N-CH_2CH_2OH$
Perphenazine	—Cl	$-CH_2CH_2CH_2N$ piperazine $N-CH_2CH_2OH$
Thioridazine	—SCH_3	$-CH_2CH_2-$ piperidine $N-CH_3$
Mepazine	...	$-CH_2-$ piperidine $N-CH_3$
Trifluoperazine	—CF_3	$-CH_2CH_2CH_2N$ piperazine $N-CH_3$

olism. A recent review by Bousquet (5) may be consulted for current information in the general area of drug metabolism. The reader is also referred to a review of the metabolism of psychoactive drugs by Carr (6) and an excellent review of the pharmacology of the tranquilizer drugs by Domino (7). The latter paper also discusses the metabolic fate of these compounds. Harwood (8) has reviewed the metabolism of phenothiazine.

In order to facilitate the presentation of data, the literature is discussed under subject headings which relate to a particular type of metabolic reaction. For the sake of brevity and continuity, the generic names of the phenothiazine drugs have been used. Table I lists the generic names and chemical structures. The structural formulas of several known and hypothesized metabolites are given in Table II. Although some later papers are included, this review is chiefly concerned with the literature through August 1962.

SULFOXIDATION

Prior to 1950, the oxidation of organic sulfur compounds to sulfoxides was a rather novel type of biological transformation. Other than the work of Clare, *et al.* (9), on the metabolism of phenothiazine, little was known of this metabolic reaction. Phenothiazine-5-oxide was found in the blood and aqueous humor of the eye in calves which had been treated with the anthelmintic, phenothiazine. Today the accumulated evidence indicates that the formation of the sulfoxide (5-oxide) is a metabolic reaction common to all the phenothiazine derivatives in current use.

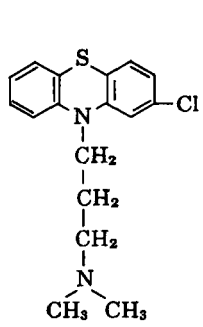
Salzman, *et al.* (10), reported in 1955 that they were able to isolate, by counter-current methods, a major metabolite of chlorpromazine and to identify this compound as the sulfoxide. In studies of urinary excretion (11), this form was found to comprise from 10–14% of the administered dose of chlorpromazine in dogs and about 5% of the dose in man. Only very small amounts of the drug were excreted unchanged by either species. In a study of the urinary excretion patterns of patients receiving daily chlorpromazine medication, Huang and Kurland (12) have shown that the average urinary excretion (*per diem*) of chlorpromazine and its sulfoxides varied from 1–20% of the daily dose. Sulfoxide excretion ranged from 1–18%, while the amount of free chlorpromazine in the urine was usually less than 1% of the dose. The average ratio of free chlorpromazine to its sulfoxides was found to be approximately 1:16.

Fishman and Goldenberg (13), by chromatographic studies, were able to show ten metabolites of chlorpromazine which were extractable at an alkaline pH from human urine. Six of these compounds gave ultraviolet spectra characteristic of sulfoxides. In addition to chlorpromazine sulfoxide, two demethylated sulfoxides were shown by chromatography. In subsequent

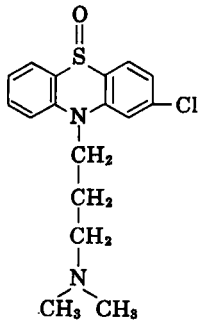
studies, these workers were able to quantify some of the urinary metabolites of chlorpromazine in man and dog (14). Values for total sulfoxide excretion were given as 5.9% of the dose in man and 11.3% in the dog. These figures agree closely with the earlier findings of Salzman and Brodie (11). It should be pointed out that excretion data for sulfoxides, as reported by Goldenberg and Fishman (14), were calculated as the sum of three different sulfoxides separated by chromatography and determined individually. Since the colorimetric assays in earlier reports gave no indication of an alteration in the side chain, the quantities given for a sulfoxide metabolite probably represented some demethylated products.

Sulfoxides have been found to be the principal metabolic products of chlorpromazine in the mouse. Upon administration of S^{35}-labeled chlorpromazine to mice, the radioactivity in the

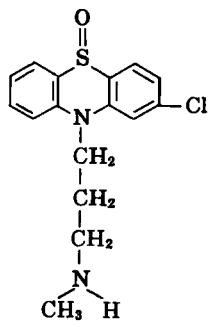
TABLE II.—SOME EXAMPLES OF REPORTED METABOLIC REACTIONS OF PHENOTHIAZINE DRUGS



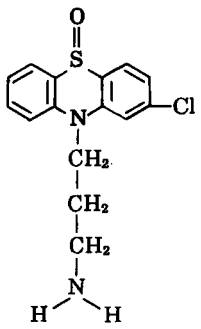
Chlorpromazine



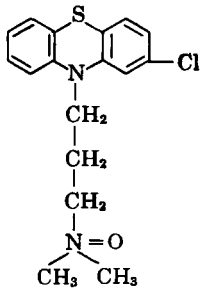
Chlorpromazine sulfoxide



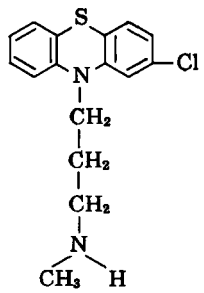
Desmethyl chlorpromazine sulfoxide



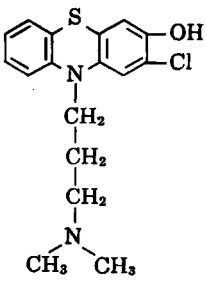
Desdimethyl chlorpromazine sulfoxide



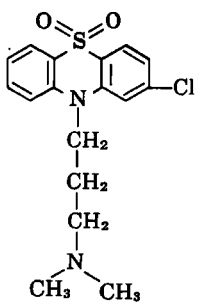
Chlorpromazine-N-oxide



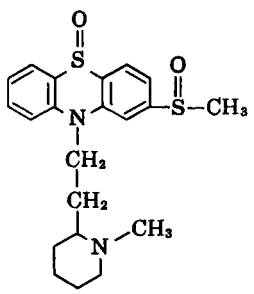
Desmethyl chlorpromazine



3-Hydroxy-chlorpromazine[a]



Chlorpromazine sulfone[a]



Thioridazine disulfoxide

[a] Hypothetical metabolites of chlorpromazine.

urine was found in the forms of free sulfate (3–5%), "combined sulfate" (23–48%), and the sulfoxide of chlorpromazine (49–73%) (15). In the rat, Ogawa, *et al.* (16), found that 27% of the dose of chlorpromazine appeared in the urine within 72 hours. The unchanged drug accounted for 8.1% of the dose while 18.9% was present as the sulfoxide. Other studies have shown that approximately 40% of the administered radioactivity is excreted by the rat in the urine when S^{35}-chlorpromazine is given (17, 18). Emmerson and Miya (18) were able to account for 12% of the dose in the urine as unmetabolized drug and a similar amount as sulfoxides of chlorpromazine.

Tissue homogenates have been used to study the oxidation of chlorpromazine *in vitro*. Chlorpromazine sulfoxide has been isolated from the incubation media. The ability to metabolize chlorpromazine is apparently centered in the liver, for other tissues have shown little activity in this respect (19, 20).

By intravenous administration of chlorpromazine sulfoxide to dogs, it has been shown that the sulfoxide itself is metabolized. Less than one-quarter of the original dose was excreted unchanged, and chromatography of the urine revealed a further metabolic product of the sulfoxide (11). Similar results have been reported in man (21). The biological half-life of the sulfoxide in human plasma has been calculated to be about 3 hours. The duration of its sedative effect was short; it usually lasted not more than 1 hour. Following an intravenous dose to patients, only 2–3% of the dose could be recovered as unchanged drug during the subsequent 24 hours (21).

In vitro studies have provided further evidence that chlorpromazine sulfoxide undergoes additional metabolism (19, 20). It has been postulated that chlorpromazine sulfone (chlorpromazine-5-dioxide) may be one of the *in vitro* products (20). Chlorpromazine sulfone has not been encountered in the investigations involving the urinary metabolites (13, 18, 22). However, Zehnder, *et al.* (23), have, by the use of S^{35}-thioridazine and inverse-isotope dilution techniques, shown that thioridazine disulfone is a metabolite which is found in the bile and urine of the rat. The metabolism of thioridazine is complicated by the presence of another oxidizable sulfur atom in the 2-methylmercapto group of the phenothiazine ring. By inverse dilution analysis, the ring sulfoxide metabolite (the 5-oxide), the side-chain sulfoxide metabolite (oxidation of the methylmercapto sulfur atom), and the disulfoxide metabolite of thioridazine were demonstrated and quantified in addition to the previously mentioned disulfone. The disulfoxide was excreted in the largest quantities. However, all of the metabolites which were determined in the urine represented only 1–2% of the administered radioactivity. These metabolites made up approximately 20% of the radioactive substances in the bile of the rat. Only traces of unchanged thioridazine were excreted in either bile or urine.

Walkenstein and Seifter (24) recovered 70–80% of the radioactivity in the urine and 20–30% in the feces of the dog following a single intraperitoneal injection of S^{35}-promazine. From 3–5% was shown to be the sulfoxide, 2–3% was excreted unchanged, and 3–4% of the dose was comprised of monomethyl promazine and the monomethyl sulfoxide of promazine.

By paper chromatography of urine, Hoffmann, *et al.* (25), have isolated the sulfoxide as a metabolite of mepazine in the rat. Evidence has been given which indicates that a metabolite of perphenazine in liver tissue of the rat is the sulfoxide of this drug (26). Up to one-third of the dose of methoxypromazine is excreted in the urine by man, almost wholly in the sulfoxide form (27). After parenteral administration of promethazine-S^{35} to rats, the largest portion of the radioactive dose appeared in the urine. The sulfoxide of this phenothiazine derivative was found to be an important urinary metabolite in the rat (28). Chlorprothixene [2-chloro-10-(3-dimethylaminopropylidene)thiaxanthene HCl] is subject to biological oxidation in the same manner as the phenothiazine derivatives. A sulfoxide metabolite of this compound has been shown to be formed by man and the rat with the aid of paper chromatography and ultraviolet absorption analysis (29).

The sulfur atom of the phenothiazine ring is particularly subject to oxidation. Apparently the initial stages involve electron transfer and free radical formation. This phenomenon will be discussed later. In studies with mepazine, Block (30) found that sulfoxide formation can occur due to the presence of peroxide contamination of ethyl ether. More recently, Ross, *et al.* (31), have noted *in vitro* sulfoxidation of desmethyl chlorpromazine which occurred during ether extraction or subsequent chromatography. These findings emphasize the care which must be taken in experiments with the phenothiazine compounds in order to prevent (and recognize) the formation of artificial metabolites.

DEMETHYLATION

Ross, *et al.* (32), using chlorpromazine-(N-methyl)-C^{14}, have found that 12–16% of the label is expired as $C^{14}O_2$ by the rat during the first 6 hours. Animals maintained for 12 hours continued to expire 0.7–2.0% of the radioactive dose each hour throughout the observation period. An active system which demethylates drugs has been shown to be present in the liver (33). Upon incubation of chlorpromazine with rabbit-liver homogenates, prepared according to the method of La Du, *et al.* (33), Salzman and Brodie (11) reported that no demethylation occurred. After *in vivo* demethylation of chlorpromazine had been shown (32), Young, *et al.* (34), demonstrated that properly fortified homogenates could demethylate the drug. After a 1-hour incubation, approximately 40% of the substrate carbon-14 could be recovered as liberated CO_2.

The metabolic fate of S^{35}-promazine has been studied in dogs by Walkenstein and Seifter (24). From 70–80% of the administered radioactivity was eventually excreted in the urine. By the use of chromatography and the authentic compounds as non-labeled carriers, several metabolites of promazine were identified: promazine sulfoxide (3–5%); desmethyl promazine sulfoxide (2.5–3.5%); unchanged promazine (2–3%); and desmethyl promazine (0.75–1%). Injection of either desmethyl promazine or promazine sulfoxide gave rise to desmethyl promazine sulfoxide. Thus it appears that neither monodemethylation nor sulfoxidation (as the initial reaction) prevents the second metabolic change resulting in the desmethyl sulfoxide of promazine.

Demethylated derivatives were found to account for at least two of the sulfoxide metabolites noted by Fishman and Goldenberg (13). Additional evidence was given in a later report to substantiate the formation of the desmethyl and desdimethyl sulfoxide metabolites of chlorpromazine (14). The desdimethyl sulfoxide, a primary amine, is suggested to be the major "non-polar" metabolite of chlorpromazine in human urine. Their results may be summarized as follows: 1. Man—desdimethyl chlorpromazine sulfoxide, 3.7%; desmethyl chlorpromazine sulfoxide, 1.8%; chlorpromazine sulfoxide, 0.4%; unchanged chlorpromazine, 0.2%; 2. Dog—desdimethyl chlorpromazine sulfoxide, 1.1%; desmethyl chlorpromazine sulfoxide, 5.1%; chlorpromazine sulfoxide, 5.1%; unchanged chlorpromazine, 2.8%. Huang and Kurland (12) observed the same metabolites by two-dimensional paper chromatography. An additional metabolite of chlorpromazine, believed to be desmethyl chlorpromazine, was noted by Goldenberg and Fishman in the urine of the dog, but not of man. Desmethyl chlorpromazine has been shown to be an *in vitro* metabolite of chlorpromazine (31). Desdimethyl chlorpromazine sulfoxide is a more prominent metabolite of chlorpromazine in man than in the dog. It is possible, considering the fact that the dimethylaminopropyl side chain is common to both promazine and chlorpromazine, that a desdimethyl metabolite of promazine could be demonstrated in man.

With the exception of the unchanged drug in the urine, the excretion patterns are very similar in the rat (18) and the dog (14) after one dose of chlorpromazine. The rat excretes as much as 12% of the dose of chlorpromazine in the urine as unmetabolized drug. Fedorov (17) has reported that urinary excretion of unchanged chlorpromazine by the rat accounts for 25% of the dose given.

The N-demethylation of thioridazine was studied by using the $\diagdown N—C^{14}H_3$ form of the drug (23). Measurement of $C^{14}O_2$ excretion in the respired air indicated that the rat demethylates 30–40% of a dose of this drug. In light of the extensive demethylation of this compound, it is likely that desmethyl analogs of the sulfoxide and sulfone metabolites are also formed. The number of theoretical metabolites is formidable.

Imipramine [N-(3-dimethylaminopropyl)-imino bibenzyl HCl], a compound similar in structure to promazine, is demethylated and hydroxylated. Herrmann, *et al.* (35, 36), found both monomethyl and completely demethylated derivatives of imipramine in rabbit urine. The metabolism of this compound in rabbits is extensive, whereas man excretes much of the ingested imipramine in unmetabolized form.

N-OXIDE FORMATION

As part of their continuing studies on the metabolism of chlorpromazine by man, Fishman, *et al.* (37), have recently reported the isolation of an N-oxide metabolite from urine. The presence of an oxygen atom attached to the nitrogen of the side chain was found to cause minor but detectable differences in the chemical properties of chlorpromazine. It should be noted that the ultraviolet spectra of chlorpromazine and chlorpromazine-N-oxide were indistinguishable. Chlorpromazine-N-oxide was not a major urinary excretion product of chlorpromazine in man or in the dog. In patients receiving chlorpromazine orally, the N-oxide accounted for

0.7% of the daily dose. The corresponding figure for dogs was somewhat higher, as much as 2–3.5%. This work has been extended (38) to include imipramine, which has the same side chain as chlorpromazine. Patients on imipramine therapy excreted approximately 2% of the administered drug as imipramine-N-oxide.

HYDROXYLATION

Upon fractionation of urine from patients receiving chlorpromazine, three polar metabolites were observed by Lin, *et al.* (39). Treatment with β-glucuronidase gave rise to free glucuronic acid and three new phenothiazines. These compounds were not chlorpromazine or chlorpromazine sulfoxide. A fourth metabolite showed an ultraviolet spectrum similar to that of chlorpromazine with an additional absorption peak at 284 mμ, a property suggestive of a ring-hydroxyl function. Following enzymatic hydrolysis, the released phenothiazines became extractable with ethyl ether from an acid medium. This group believes that the products isolated were phenolic metabolites and that hydroxylation constitutes an important aspect of chlorpromazine metabolism in man. Posner (40) has drawn the same conclusion from his chromatographic studies. He had proposed, as have others (2, 12, 14), that glucuronides are the major excretory products in man rather than sulfoxides.

In a study comparing the metabolism of chlorpromazine in man and dog, Goldenberg and Fishman (14) noted both qualitative and quantitative differences in the urinary metabolic patterns. Man excreted predominantly phenolic metabolites of chlorpromazine, and the urine was found to contain one series of phenolic metabolites which were entirely absent in dog urine. These workers estimate that there may be as many as 24 different metabolites of chlorpromazine. Huang and Kurland (2, 12, 41) also reported that hydroxylated products and their glucuronides constitute the chief metabolites of chlorpromazine in man. The glucuronides could be detected for several weeks after discontinuation of treatment.

It was concluded from studies involving β-glucuronidase and paper chromatography that thioridazine is excreted into rat bile primarily as hydroxylated metabolites conjugated with glucuronic acid (23). Thioridazine and its products are excreted primarily in the feces. Studies by Herrmann, *et al.* (35, 36), have shown conclusively that ring hydroxylation occurs in the metabolism of imipramine. The 2-hydroxylated derivative of imipramine has been synthesized and shown to be identical with one of the metabolites in rabbit urine. This metabolite is also excreted as a conjugate with glucuronic acid.

Nadeau and Sobolewski (42, 43) have investigated the excretion products in daily urine samples from patients receiving chlorpromazine over a period of many months. More than half of the compounds excreted in the urine were shown to be present as conjugates, apparently involving glucuronic acid. It was found that the capacity for this conjugation was not altered by massive dosage or prolonged administration of the drug.

In a discussion of the absorption spectra of some metabolites of chlorpromazine, Kinbergen (44) postulated that a certain shift in absorption maxima pointed to the presence of one or two hydroxyl groups on the phenothiazine nucleus. References to conjugated, polar, or possible phenolic metabolites of the phenothiazine derivatives are common (13, 14, 23, 45–47).

Interest in possible hydroxylated metabolites of the amine substituted phenothiazines has been spurred by the knowledge that mono- and di-hydroxylated derivatives of phenothiazine are formed by a number of animals (48–50). The hydroxylated metabolites of phenothiazine are of two basic types—leucophenothiazone (3-hydroxy phenothiazine) and leucothionol (3,7-dihydroxyphenothiazine)—each of which also occurs in an enolic form, phenothiazone (phenothiaz-3-one) and thionol (7-hydroxyphenothiaz-3-one), respectively. Berti and Cima (51) have stated that chlorpromazine compounds of the phenothiazone type are rather improbable. They feel that the substituted nitrogen in chlorpromazine would prevent the shift in double bonds necessary for phenothiazone formation. They also refer to the absence of color in the urine of animals after chlorpromazine treatment. The phenothiazone metabolites impart a red color to the urine of animals treated with phenothiazine. This does not exclude, however, the possibility that certain metabolites of the substituted phenothiazines may be in the leuco or colorless forms analogous to those found with phenothiazine.

OTHER METABOLIC CHANGES

A ferric chloride-sulfuric acid reagent has been developed to yield a color with the metabolites in the urine of a patient which is proportional in intensity to the amount of chlorpromazine ingested daily (52). It was hypothesized that the violet color observed was due to the reaction of

the reagent with a relatively unstable metabolite which is intermediary between chlorpromazine and chlorpromazine sulfoxide. A compound that gives the same violet color can be prepared by irradiation of an aqueous solution of chlorpromazine with ultraviolet light (53). Forrest, *et al.* (53), using electron spin resonance and absorption spectra, tentatively characterized the intermediate as a thionium hydroxide with one or two hydroxyl groups attached to the sulfur atom. The first products in the oxidation of phenothiazine drugs have been shown to be ion radicals (54). There is good evidence that these radicals are responsible for the color reactions observed. The intermediate form of chlorpromazine produced by ultraviolet irradiation is apparently the same as that of the metabolite(s) occurring in the urine of treated patients. It is of interest that small amounts of these compounds are excreted for many weeks following discontinuation of treatment with a phenothiazine drug (3). A free-radical intermediary form of the phenothiazines has also been postulated by Laborit, *et al.* (55). There are several other papers demonstrating the lability of chlorpromazine in the presence of acids, bases, or light (56–59). One analytical method, designed for phenothiazine derivatives, has an end point governed by color changes during titration. A red free-radical is formed immediately upon the loss of one electron. Decoloration occurs at the equivalence point when a second electron is lost (60).

Both the chlorine atom and the side chain of chlorpromazine remain firmly attached to the phenothiazine nucleus during the passage of the drug through the animal body (51). The stability of the halogen atoms of trifluoperazine has also been demonstrated (61).

There is evidence that the thiazine ring can be degraded by the mouse. Christensen and Wase (15) recovered small quantities of the administered radioactivity as labeled sulfate after injection of S^{35}-chlorpromazine. Walkenstein and Seifter (24) have reported that 10% of the sulfur from S^{35}-phenothiazine is excreted by the dog as inorganic sulfate. This type of breakdown did not occur with promazine, however. This provides further evidence that the side chain is not metabolically removed.

Acetylfluphenazine can be enzymatically hydrolyzed *in vitro* to fluphenazine. Smith (62) has hypothesized that the same reaction may take place *in vivo* and that fluphenazine is the active agent.

The biological fate of two quaternary phenothiazine compounds has been studied in rats by the use of sulfur-35 and tracer techniques (28, 63). After oral administration, these compounds are excreted mainly in the feces. One was largely excreted as the unchanged drug with only traces of possible metabolites. Although the sulfoxide was observed with the other compound, only a small portion of the dose appeared in this form. Apparently neither compound undergoes extensive metabolism. Access to the metabolizing enzymes may be prevented by the chemical influence of the quaternary nitrogen of the side chain in these compounds.

PHARMACOLOGICAL EFFECTS OF METABOLITES

Although chlorpromazine sulfoxide exhibits pharmacological effects which are qualitatively similar to those of chlorpromazine, the latter compound has a much greater potency and a more rapid onset of action (64). Chlorpromazine sulfoxide has been shown to have a weak sedative action of short duration in man (21). For these reasons it is considered unlikely that the effects of chlorpromazine are mediated through the sulfoxide. Moran and Butler (64) have shown that chlorpromazine sulfoxide is not equally active in all species. The sulfoxide was found to be about one-tenth as active as chlorpromazine in potentiating hexobarbital anesthesia and one-eighth as active as chlorpromazine in producing sedation in the dog. Cats exhibited similar effects. In mice and rabbits, however, chlorpromazine sulfoxide was almost devoid of sedative action and had very little potentiating effect on hexobarbital sleeping time in mice. This is in marked contrast to chlorpromazine which is about equally potent, in these respects, in all of the forenamed species. The pharmacological activity of mepazine shows a decrease corresponding to that seen with chlorpromazine upon sulfoxidation (25).

Posner, *et al.* (4), have compared, in a series of behavioral and pharmacological tests, several known and model metabolites of chlorpromazine and promazine. Their results confirmed those of previous reports concerning the decreased activity of chlorpromazine sulfoxide. Of greatest interest, however, was the remarkable degree of activity shown by demethylated and hydroxylated compounds. Desmethyl chlorpromazine was only slightly less active than chlorpromazine. While the activity of chlorpromazine-N-oxide was less than desmethyl chlorpromazine, all of these chlorpromazine analogs were far more active than chlorpromazine sulfoxide. The ac-

tivity of 4-hydroxypromazine approached that of promazine, but the 2-hydroxy derivative was generally much less active. Although these phenolic derivatives of promazine are only model compounds and are definitely not metabolites of the drug (40), other phenolic metabolites are known to be formed. The studies on model compounds clearly demonstrate that activity could reside in a metabolite resulting from ring hydroxylation. The possibility that demethylated or N-oxide metabolites contribute to the action of chlorpromazine or promazine is equally intriguing.

In chemical studies chlorpromazine was found to be a powerful electron donor. This property was attributed to the phenothiazine portion of the molecule. Karreman, *et al.* (65), have suggested that the therapeutic effect of the drug is related to this phenomenon.

Investigators have shown that desmethylimipramine, a known metabolite of imipramine is more potent in some respects than its parent compound (66). There is good evidence to indicate that the antidepressant action of imipramine is actually due to its demethylated metabolite (67, 68). It is more remarkable, however, that imipramine appears to antagonize the effectiveness and delay the onset of action of desmethylimipramine (68). These results serve to emphasize the problems which may also be encountered in studies on the phenothiazine derivatives concerning the evaluation of the pharmacological actions and interactions of parent compound and metabolites.

SUMMARY

Consideration has been given to the literature associated with the metabolism of modern phenothiazine drugs. Several important pathways have been elucidated. A particularly characteristic reaction of this class of compounds is sulfoxidation. Further oxidation of the sulfur atom to a sulfone has been observed only with thioridazine. A number of other metabolic reactions have been demonstrated with the phenothiazine drugs. Among these are: demethylation, N-oxide formation, and conjugation with glucuronic acid. Although the isolation and identification of an actual phenolic metabolite of a N-substituted phenothiazine has not yet been accomplished, the analysis of glucuronic acid conjugates leaves little doubt that the phenothiazine components are phenolic in nature. Evidence indicates that aromatic hydroxylation followed by conjugation with glucuronic acid is the dominant metabolic pathway for the phenothiazine drugs in man. Studies have shown that it is possible for a phenolic metabolite to contribute to the action of its parent drug, *e.g.*, aromatic hydroxylation does not necessarily result in loss of pharmacological activity. Thus, the identification of the phenolic metabolites of the phenothiazine derivatives should be considered a task of prime importance.

In view of the number of known and theoretical metabolites of the phenothiazine drugs, the species differences in the metabolism of these compounds, and the inherent lability of the phenothiazine nucleus, one must conclude, in retrospect, that the progress in elucidating the metabolic fate of these compounds has been quite remarkable. However, much work remains to be done in this area, since with the metabolites identified to date it is not possible to account for more than 8–10% of the dose of a phenothiazine drug in man.

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Research Articles

Quantitative Determination of Serotonin in *Panaeolus* Species

By J. K. WIER† and V. E. TYLER, JR.

A method was developed for the quantitative estimation of serotonin (5-hydroxytryptamine) in mushrooms, especially species of the genus *Panaeolus*. The procedure involves extraction of the dried, finely powdered carpophores with acidified alcohol, application of the extract to filter paper strips, formation of these chromatograms with *n*-propanol : 1 *N* ammonium hydroxide (5:1), development of colored serotonin spots with a modified Pauly's reagent, determination of the total color of these spots with an automatic recording electronic densitometer, and calculation of the serotonin content from a standard curve. Each step in the procedure was evaluated to determine the influence of certain variables, and the results obtained by the final method were subjected to statistical evaluation.

SEROTONIN (5-hydroxytryptamine) has received considerable attention recently due to its interesting physiological properties (1). In addition to its occurrence in certain animal tissues, the compound has been found in a number of plants (2), including carpophores of *Panaeolus* species. Several cases of cerebral mycetism reputedly have been caused by ingestion of certain species of this genus (3). Although *P. campanulatus* (Fr.) Quél. has been reported (4) to be devoid of psychotomimetic activity in the rat and in man, Tyler and Smith (5) have reported the presence of serotonin in it and in several other species of *Panaeolus*. It was of interest, therefore, to determine the concentration of serotonin in these mushrooms.

Serotonin and related indole derivatives have been quantitated by determination of their physiological effects on certain animal tissues,

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