THE METABOLIC ALTERATION OF DRUGS

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The role of drug metabolism in the investigation of substances of therapeutic interest has undergone considerable change in the past decade. What had been considered to be quasi-academic luxury is now regarded as an essential component in any study of new drug candidates. Drug metabolism, or to use a currently popular euphemism, "biotransformation," is indeed, a two-edged sword. Judiciously applied it can be of immense value and will contribute substantially toward a meaningful interpretation, as well as a scientifically acceptable explanation, of biologic responses observed by our associates in pharmacology, pathology, and toxicology. Improperly applied, it is a panacea that will answer, glibly and superficially, questions which plagued investigators in these fields for a goodly number of decades. It is entirely too easy to assume that the biological half-life measured in the rat is also that which will be seen in the human, or that the liver of a dog will produce the same metabolites (and in the same amounts) as the liver of a woman.

Even today there is a tendency to speak of a metabolic study in a particular species as "the metabolism of X" when it is nothing more or less than the metabolism in a particular species, and often a single strain, of some member of the animal kingdom.

Papers describing the biotransformation of organic molecules have, within the past few years, undergone a marked improvement in quality. More and more frequently one finds that serious attempts were undertaken to separate, characterize, and estimate the products of these often profound alterations of the original molecule although, for a variety of reasons rigorous structure proofs, or comparison with known structures often cannot be undertaken. Authors no longer interpret radioactivity measured in tissues or during the course of an excretion study as being necessarily the same compound they introduced into the subject. In general, it is probably fair to say the investigators today will usually attempt at least to determine the extent to which metabolic alteration has taken place.

The papers selected for discussion in this review article will doubtlessly reflect the author's personal biases and as such no apology will be made. In keeping with the instructions of the editors, no attempt has been made to

present a comprehensive survey and a limited number of papers will be presented in considerable detail.

Mescaline

The well known hallucinogenic effect of mescaline, although studied for many years, is still imperfectly understood. The suggestion that (1) the biological properties of the compound may be attributed to one or more metabolites of mescaline has spurred other investigators to explore this possibility. Metabolites have been examined in a variety of species (rat, dog, human) as well as under *in vitro* conditions. Studies (1) have shown that mescaline binds on liver protein. Oxidative deamination was reported to lead to 3,4,5-trimethoxyphenylacetic acid (2), and as minor metabolites, 3,4-dihydroxy-5-methoxyphenylacetic acid (2) and 3,4-dimethoxy-5-hydroxyphenylethylamine (3) have been isolated from human urine. The rat has been reported to form 3,4,5-trimethoxyphenylethanol (4). This metabolite may be formed initially by oxidative deamination, followed by reduction to the aliphatic alcohol side chain. Under *in vitro* conditions, mescaline can be converted to 3,4-dimethoxy-5-hydroxyphenylethylamine and 3,5-dimethoxy-4-hydroxyphenylethylamine (5).

It has been reported that mescaline is a substrate for dopamine-β-hydroxylase (6) and also that biogenic amines undergo N-acetylation and O-demethylation under *in vivo* conditions (6, 7, 8). Strong evidence is presented (9) for conversion of mescaline to N-acetylmescaline, N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine, and N-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine by the rat. During the course of a twenty-four hour collection period, these metabolites represented 1.7 percent, 15.1 percent, and 14.4 percent of the radioactivity excreted in this period, respectively. The major metabolite was trimethoxyphenylacetic acid (42.3 percent). This figure may include a contribution from trimethoxyphenylethanol which was not separated by the investigators. About 20 percent of the radioactivity was in the form of unchanged mescaline. Only 6 percent of excreted radioactivity was associated with unidentified substances.

One would reasonably anticipate that the action of a monoamine oxidase inhibitor would lead to an increased excretion of amines or their N-acetyl derivatives or both. Administration of iproniazid led to increased excretion of unchanged mescaline (43.1 percent), N-acetylmescaline (5.6 percent), and N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine (27.8 percent). Interestingly, the relative amounts of N-acetyl-3,4-dimethoxy-5-hydroxyphenylethylamine and the unknown portion were not materially altered (16.9 percent and 5.3 percent respectively) upon introduction of iproniazid. Trimethoxyphenyl-acetic acid excretion dropped markedly (1.5 percent). These results are in agreement with the statement (10) that mescaline was found to be a substrate of diamine oxidase, an enzyme which is inhibited by iproniazid. N-acetyl-mescaline does not appear to cause any physiological or behavioral changes in humans (11).

As part of this study (9) rats were given carbon-14 labeled N-acetylmescaline by the intraperitoneal route. Approximately 58 percent of the radioactivity was excreted in urine during the 24-hour collection period. Only a small amount (5.4 percent) of the excreted radioactivity was in the form of unaltered N-acetylmescaline. The largest portion of the radioactivity (53 percent) was associated with N-acetyl-3,5-dimethoxy-4-hydroxyphenylethylamine. Unidentified materials accounted for 14 percent.

These observations permit one to construct a reasonable assumption as to the probable sequence of steps leading to the observed biotransformations. Musacchio & Goldstein (9) discovered that N-acetylated metabolites were demethylated in positions 4 and 5 of the benzene ring and that neither O-demethylated mescaline or O-demethylated acidic metabolites of mescaline could be detected in the urine of rats. This finding indicated to these investigators that N-acetylation took place prior to O-demethylation. Furthermore, these authors feel that additional support for this assertion is derived from the fact that N-acetylmescaline undergoes considerable conversion to O-demethylated compounds under *in vivo* conditions. This writer fails to see the justification for this statement, inasmuch as it would be necessary to administer the appropriate unacetylated O-demethylated compounds and then fail to recover the corresponding N-acetylated products in order to validate this point. Man also possesses an O-methyl transferase enzyme that leads to O-demethylation of mescaline (3) and of trimethoxyphenyl-acetic acid (12). It appears, therefore, that in man as well as in animals, N-acetylation and O-demethylation of mescaline take place. In addition to the formation of trimethoxyphenylacetic acid as a metabolite of mescaline in the urine of man and rodents (2, 13), the cat may generate this metabolite exclusively (14), although the evidence is not entirely conclusive. Figure 1 summarizes the metabolite data discussed and suggests a plausible, though speculative sequence for these steps.

Diethylpropion

Diethylpropion (1-phenyl-2-diethylamino-1-propanone hydrochloride) is a sympathomimetic agent widely used as an anorexient in the management of obesity. When administered to humans (15) by the oral route the compound is rapidly and completely absorbed. Excretion of carbon-14 labeled metabolites takes place exclusively via the renal pathway. The elimination of diethylpropion and its metabolites is essentially completed in forty-eight hours. Using a three compartment model which assumed that absorption from the gastrointestinal tract could be treated kinetically as a simple first order diffusion process, and the excretion of drug and its metabolites was also a first order process, the absorption constant was calculated to be 88 percent per hour and that the rate of excretion was 7.7 percent per hour. A semi-logarithmic plot of plasma levels and excreted radioactivity afforded two parallel straight lines. The linearity of the urinary excretion data suggested that if metabolism of diethylpropion did occur, its biotransformation

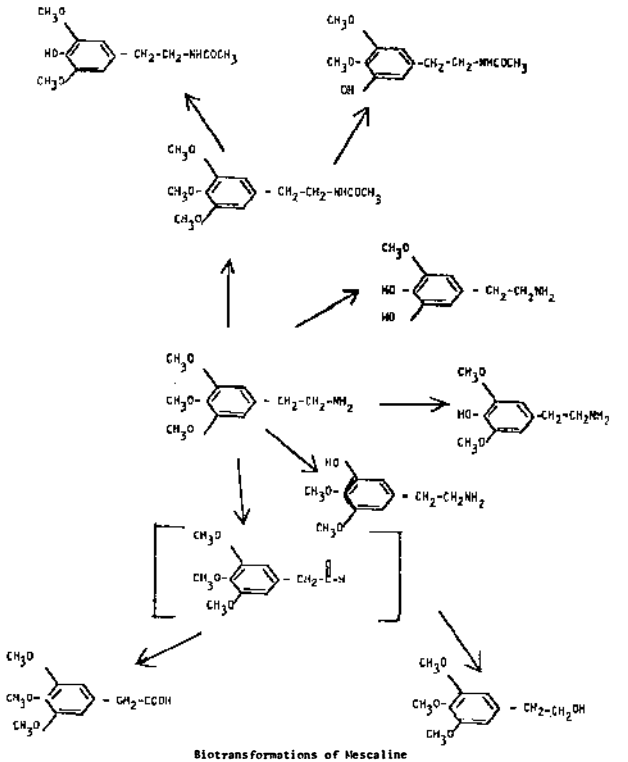


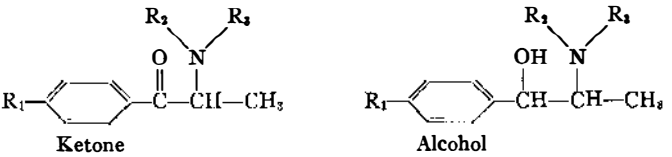
FIG. 1.

products must either have roughly equal biological half-lives or the excreted radioactivity must be largely associated with a single substance. A normalized pool consisting of urine collected from four subjects 8 to 12 hours after drug ingestion (16) was subjected to exhaustive solvent extraction under a variety of conditions. Five classes of metabolites were separated: nonacidic metabolites, acidic metabolites, conjugated nonacidic metabolites, conjugated acidic metabolites, and unextractable polar substances. These classes accounted for 51.8 percent, 32.9 percent, 4.4 percent, 1.5 percent and 12.3 percent of the carbon-14 content of the pooled urine, respectively.

Thin-layer chromatography of the nonacidic fraction revealed that 13 components constituted this group of substances. These materials accounted for the entire fraction. The following table describes these compounds, all but two of which were identified by comparison with authentic reference materials.

The largest identifiable component of this fraction was unchanged diethylpropion (7.8 percent of the total radioactivity in the original urine pool). Two unidentified metabolites, present in approximately equal quanti-

TABLE I



Reference Compound	Basic Structure	Substituent		
		R_1	R_2	R_3
I	Ketone	H	C_2H_5	C_2H_5
II	Ketone	H	H	C_2H_5
III	Ketone	H	H	H
IV	Alcohol	H	C_2H_5	C_2H_5
V	Ketone	OH	C_2H_5	C_2H_5
VI	Alcohol	H	H	C_2H_5
VII	Alcohol	OH	C_2H_5	C_2H_5
VIII	Ketone	OH	H	H
IX	Alcohol	H	H	H
X	Ketone	OH	H	C_2H_5
XI	Alcohol	OH	H	C_2H_5
XII	Alcohol	OH	H	H

ties, accounted for another 25 percent of the pool. In addition to the structures shown in Table I, Schreiber and his co-workers (16) also compared these unknown substances with *dl*-erythro-2-amino-1-phenyl-1,3-propanediol and *dl*-threo-2-amino-1-phenyl-1,3-propanediol. Unfortunately, the migration of these possible metabolites did not agree with either of these unknown substances.

The acidic fraction was found to be constituted of four substances, all of which proved to be identifiable. Hippuric acid (XIII) accounted for 26.5 percent (by integration of peak areas) or 28.6 percent (by isotope dilution) of the original urine radioactivity. Mandelic acid was found to be 0.3 percent of the total, and, interestingly, 3,4-dihydroxy-benzoic acid was shown to represent 2.9 percent. Benzoic acid, 3.4 percent of the radioactivity, was also measured. The author feels, however, that value may be an artifact stemming from the decomposition of basic, nonphenolic metabolites occurring during the extraction procedures.

Hydrolysis of the conjugated fraction with β-glucuronidase resulted in the freeing of seven basic compounds from glucuronide conjugates. The compounds released by this hydrolysis were identified as V, VII, VIII, and X to the extent of 1.8, 0.6, 0.8, and 0.3 percent, respectively. Compound V,

which was present in the nonacidic metabolite fraction as only a trace, was present in this fraction as an obviously measurable amount. Compounds XI and XII were present in readily detectable amounts but were not well enough resolved for purposes of individual quantitative estimation.

The fraction which contained acidic metabolites released by β-glucuronidase contained only a single radioactive substance which was thought to be *p*-hydroxyhippuric acid.

The metabolism of diethylpropion proceeds through an extremely complex pathway and is influenced by a number of enzymes. Sequential N-deethylation, aromatic ring hydroxylation, conjugate formation, and oxidative cleavage occur when diethylpropion is metabolized by the human.

At least thirteen basic (I, II, IV through XII and μ_1 and μ_2) substances, four acidic metabolites (XIII through XVI) and eight glucuronides (conjugates of V, VII, VIII, X, XI, XII, μ_2, and possibly *p*-hydroxyhippuric acid) were found in pooled human urine collected 8 to 12 hours after the drug ingestion. Compound III, which was not found in this collection period, has been unambiguously identified in several urine fractions collected in early periods (17). In these earlier periods, considerable differences in the relative amounts of the reported compounds were seen and these values were found to vary systematically from fraction to fraction. These variations are doubtlessly caused by differences in the rate of generation of these metabolites and also stem from the fact that each substance may have its own unique biological half life. Not yet identified are μ_1 and μ_2; they may be structures arising from oxidation of the terminal methyl group, possibly coupled with ring hydroxylation.

The acidic metabolites of diethylpropion, hippuric, mandelic, benzoic, *p*-hydroxyhippuric, and 3,4-dihydroxy-benzoic acids are especially interesting and significant. Hippuric acid is certainly the metabolite present in the largest amount and suggests that oxidative cleavage is probably an important route in the biotransformation of this series of compounds. Mandelic acid, present in only small amounts, is another compound whose presence may be attributed to an oxidative mechanism or pathway. In light of the considerable preponderance of hippuric acid as compared to the detected mandelic acid, it seems entirely reasonable to speculate that compounds in this series that have a carbonyl group adjacent to the aromatic ring lend themselves to biologic oxidation more readily than those having a secondary alcohol group in the same position. The presence of small amounts of 3,4-dihydroxybenzoic acid leads one to think that 3,4-dihydroxylation of one or more aromatic structures can take place before scission of the side chain occurs. Compounds similar to catecholamines may be formed during the course of the various metabolic alterations. The generation of such compounds could be responsible for drug effects on the central nervous system. Conjugation of 3,4-dihydroxybenzoic acid does not appear to take place in the human to any significant extent inasmuch as hydrolysis with β-glucuronidase failed to generate detectable amounts of the parent acid.

The benzoic acid which was detected during the course of the investigation can have its origin in the instability of these compounds in an alkaline medium. It appears most unlikely, however, that brief exposure of these substances to an alkaline environment (pH 12) at room temperature is responsible for the presence of this material. A more attractive explanation is that some benzoic acid was not conjugated with glycine to form hippuric acid. One cannot exclude this possibility unless one entertains the idea that oxidative generation of benzoic acid must be followed by immediate conjugation with glycine.

The formation of glucuronide conjugates cannot be a dominant mechanism in the metabolism of diethylpropion. Where compounds were found to be excreted in the form of such conjugates, usually the unconjugated form was present in equal or substantially greater amounts than the corresponding glucuronide. Compound V is an exception; barely detectable amounts of the free compound were seen, but the glucuronide of V accounted for 1.8 percent of the radioactivity measured in the original urine pool. Glucuronide formation appears to be restricted to substances containing a phenolic (i.e., acidic) hydroxyl group. Compounds IV, VI and IX, which all possess a secondary hydroxyl function but are devoid of a phenolic aromatic ring, do not appear to be converted to the corresponding glucuronide.

The wide spectrum of biotransformation reported, and the appearance and disappearance of metabolites as a function of time should serve to caution one when speaking of the "metabolites of ——" unless they are careful to consider temporal questions that may have bearing on the situation. Qualitative and quantitative relationships that vary with time can easily explain bizarre or unpredictable responses following drug administration, especially when highly complex pathways for biotransformation exist.

Figure 2 shows the author's concept of possible pathways of formation of the nonacidic metabolites of diethylpropion.

Pronethalol

The metabolic studies reported by Bond & Howe (18) are particularly thorough and of an exceptionally high quality. Pronethalol, Figure 3, is racemic 2-isopropylamino-1-(2-naphthyl)ethanol. Black (19) reported its action as specifically blocking cardiac and other β-adrenergic receptors.

Following oral or subcutaneous administration to rat, mouse, guinea pig, and rabbit, excretion was found to be primarily via the renal pathway. In the mouse, 61 percent of the radioactivity appeared in urine in 72 hours, the vast majority of which was excreted during the first 24 hours. Only 9 percent of the ^{14}C was eliminated in feces. No $^{14}CO_2$ was detectable in expired air. Three humans dosed with pronethalol excreted an average of 100 percent (94 to 105 percent) of the ingested ^{14}C in 72 hours. Most of the radioactivity appeared during the first 24 hours.

Urine collected during the course of these studies was subjected to paper electrophoresis to achieve separation of drug and its metabolites. In each in-

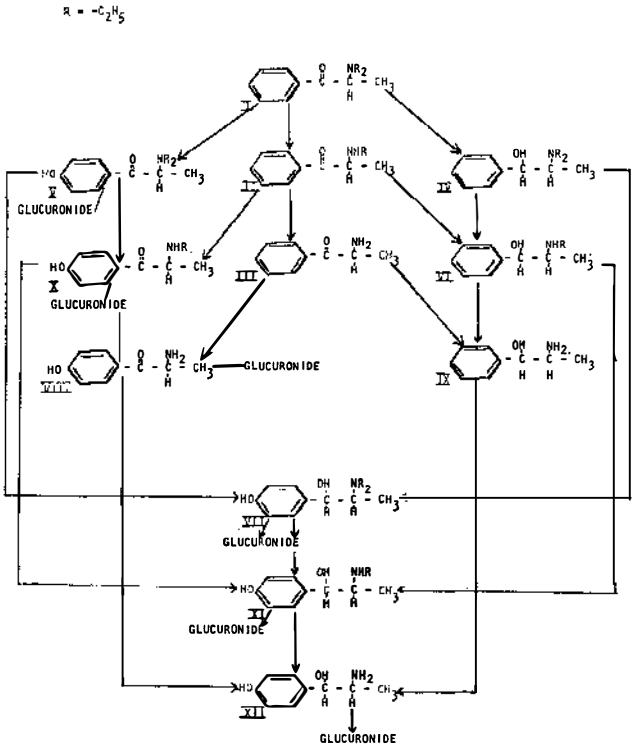


FIG. 2.

stance basic, amphoteric, and acidic metabolites were detectable. The relative proportions of these broad classes of metabolites, studied in a variety of species, varied quite markedly (Table II) from the mouse, where 85 percent of the metabolites were basic or amphoteric substances, to the guinea pig where 77 percent of the metabolites were acidic in nature. Examination of distribution of metabolites, even when confined to rodent species, shows a surprising degree of variability.

Two major acidic metabolites, 2-naphthyl-glycolic and 2-naphthoic acids, were isolated from guinea pig, rat, and rabbit urine, and identified by thin layer chromatography in the human urine.

Two amphoteric metabolites appear to be glucuronides. The major

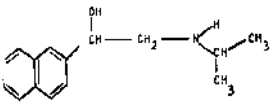


FIG. 3.

TABLE II

PRONETHALOL METABOLISM

Species	% of Drug Metabolized to Basic and Amphoteric Metabolites	% of Drug Metabolized to Acidic Metabolites
Rabbit	65	35
Dog	54	46
Cat	69	31
Rat	71, 67	29, 33
Monkey	70	30
Mouse	85, 86	15, 14
Guinea Pig	23	77
Human		
Patient 1	53	47
Patient 2	56	44
Patient 3	76	24

glucuronide is that formed from the 7-hydroxy analogue of pronethalol, and this substance was recovered from rat, mouse, and human urine. The amount of 7-hydroxy-pronethalol isolated after treatment with β-glucuronidase (*Helix pomatia*) leads to the conclusion that the sample contained at least 91 percent of the glucuronide of this compound. A minor metabolite present in this fraction was not identified.

The basic metabolites found in the urine of the guinea pig were identified as unchanged pronethalol and the unconjugated form of its 7-hydroxy metabolite. Less than 1.5 percent of the administered pronethalol, as estimated in a fluorimetric procedure, was present in the urine of mouse, rat, rabbit, and guinea pig. Only trace amounts of pronethalol were liberated upon treatment of mouse, rat, and rabbit urine with β-glucuronidase. Serum levels of pronethalol and certain metabolites following oral administration to humans were measured; the data from one such experiment is reported in Table III. These data support this writer's contention that the time course of metabolite patterns cannot be neglected in studies of this nature. In the case being discussed, the 7-hydroxy analogue of pronethalol is about five times more active than pronethalol in its ability to antagonize isophenaline induced tachycardia in the cat.

In addition to the intact animal studies, Bond & Howe (18) have also reported on their findings when this compound was introduced into liver homogenate preparations. Whole homogenates and 10,000 g supernatants from rat, mouse, and guinea pig livers differed strongly in their ability to oxidize the alkylamine side chain of pronethalol. Guinea pig liver was found to be much more active than rat liver. Mouse liver had virtually no activity. In these species, in all instances, the 10,000 g supernatant was more active than the liver homogenate. The rate of disappearance of pronethalol and the rate

TABLE III

PATIENT 1

Time	Total Metabolites	Pronethalol	7-hydroxy-Pronethalol	Acids
hr.				
1/2	4.4	0.1	0.4	1.0
1	9.2	0.3	0.3	3.2
2	8.6	0.15	0.9	1.7
4	7.3	0.1	1.2	0.9

All levels expressed in μg per ml of serum.

of formation of acidic metabolites were almost identical in a rabbit liver preparation. Bond & Howe (18) suggest that *in vitro* pronethalol disappears largely by side chain oxidation. Rabbit liver preparations do not seem to be capable of aromatic ring hydroxylation of pronethalol, a naphthalene ring compound. Such preparations have been shown to hydroxylate a benzene nucleus (14).

The earliest metabolite of the side chain oxidative pathway is the primary amine which has undergone the loss of its two methyl groups. This compound could only be detected in liver preparations and was not seen in intact animal studies. The primary amine is probably acted upon by monoamine oxidase and an aldehyde dehydrogenase to form 2-naphthyl-glycolic acid, the simplest acidic metabolite found in urine. Addition of a monoamine oxidase inhibitor completely blocked oxidation of the pronethalol side chain. Subsequent dehydrogenation of 2-naphthyl-glycolic acid would result in the formation of the next metabolite, 2-naphthyl-glyoxylic acid. Oxidative decarboxylation then results in 2-naphthoic acid.

The 7-hydroxy analogue is the only biotransformation product on the ring hydroxylation pathway. This substance, whose identity was confirmed by comparison with authentic material, is excreted chiefly in the form of the glucuronide conjugate. Conjugation probably takes place on the aromatic hydroxyl group.

In most of the species studied, ring hydroxylation and conjugation may be considered the major pathways. Only in the guinea pig is the acidic fraction of metabolites greater than the amphoteric and basic substances. In the woman these fractions are nearly equal. In most other species the basic and amphoteric fraction is from two to six times larger than the acidic biotransformation products fraction.

These results can be interpreted as reflecting the relative activities of the drug-metabolizing enzymes of these species. Such differences in activity can result in ring hydroxylation or N-dealkylation. The initial enzyme reaction then determines the remainder of the sequence. Apparently, the enzymes in-

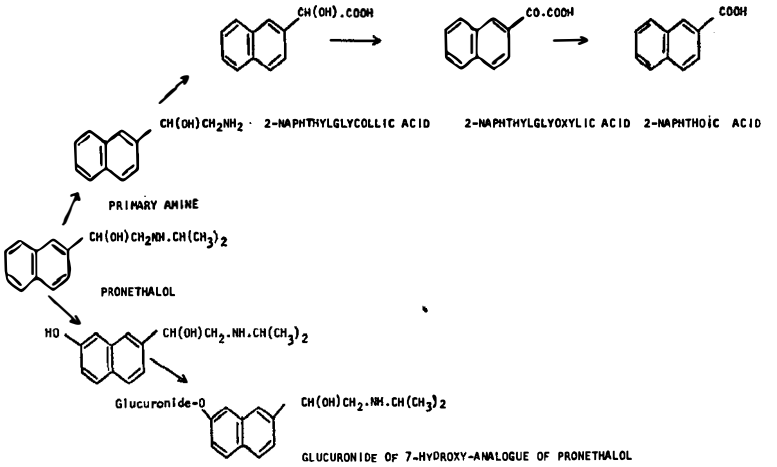


FIG. 4.

volved along these metabolic pathways have a significant degree of selectivity, inasmuch as the authors did not report the presence of metabolites which one might guess to be the 7-hydroxy analogues of 2-naphthyl-glycolic, 2-naphthyl-glyoxylic, or 2-naphthoic acids. Such materials, if present at all, probably exist only as trace amounts.

Evidence exists to support this point of view: the guinea pig has been shown to dealkylate N-methyl compounds more readily than the rat (20), whereas the rat is more efficient than the guinea pig in the hydroxylation of aromatic compounds (21, 22). The cat excreted the greatest proportion of unconjugated base following subcutaneous administration of pronethalol, which is in agreement with earlier work (23) that reported that this species does not form glucuronides with great facility. The main metabolites formed in the woman are the same as those detected in various animal species.

A sequence (Figure 4) of biotransformation is proposed by Howe & Bond (18).

PERAZINE

Metabolism studies on the phenothiazines are being continued at an unabated pace. This interesting class of compounds has been investigated further by Breyer & Kanig (25). These collaborators have examined urinary metabolites of perazine (10-[3′-(4″-methylpiperazinyl)-propyl]-phenothiazine) excreted by psychiatric patients.

Approximately 15 to 30 percent (estimated spectrophotometrically) of the administered dose of the orally administered drug is excreted in the urine of these subjects, chiefly in the form of metabolites. This class of compounds is largely excreted in feces, rather than in urine.

The biotransformations detected in the metabolism of perazine are qualitatively the same as those seen during the course of investigations of chlorpromazine (26–32). The quantitative relationship, however, is quite different. The N-oxide metabolites are relatively minor in chlorpromazine (26, 28, 29, 33) but in perazine are the chief constituents of the metabolites which did not undergo aromatic hydroxylation. Breyer (24) believes this difference may be due to the relatively polar character of perazine, in contrast to chlorpromazine, stemming from the presence of three nitrogen atoms. Introduction of a N-oxide function causes perazine to become so hydrophilic that it is no longer reabsorbed in the kidney tubules and for this reason it appears in the urine in fairly large amounts. A similar argument can be made, perhaps more forcefully, for the N-oxide-sulfoxide of perazine. This suggestion, that reduced tubular reabsorption may be the reason the N-oxides of perazine are the dominant urinary metabolites, receives support from *in vitro* studies of chlorpromazine metabolism. It has been demonstrated (34) that under such conditions, chlorpromazine is converted mainly to the N-oxide. Also chlorpromazine N-oxide is readily reduced in the presence of liver enzymes (35).

Kanig (25) has reported the following metabolites in the urine of eight psychiatric patients who were treated from 3½ to 6 weeks with oral doses of perazine administered at levels of 300 to 600 mg per day: desmethyl perazine, perazine sulfoxide, desmethyl perazine sulfoxide, 3-hydroxyperazine (glucuronide), desmethyl 3-hydroxy perazine (glucuronide), perazine N-oxide, perazine N-oxide sulfoxide, and a sulfoxide with an altered piperazine ring.

Figure 5 outlines a possible sequence of the metabolite formation from perazine.

The recovery of urinary metabolites, reported by Kanig (25), condensed to average values and expressed as percent of administered dose, shows 0.7 percent excreted as perazine plus desmethyl perazine, 2.2 percent as perazine sulfoxide plus desmethyl perazine sulfoxide, 6.1 percent as 3-hydroxy perazine and desmethyl-3-hydroxy perazine (as glucuronides), 1.7 percent perazine N-oxide, 2 percent perazine N-oxide sulfoxide, and 0.6 percent as a sulfoxide with an altered piperazine ring. Both inter-individual, as well as intra-individual, variations were usually quite small.

An interesting observation was made during the course of these experiments. A distinct increase in the ratio of demethylated compounds compared to the tertiary amines was noted. This demethylation ratio varied independently of the other metabolites whose excretion was relatively constant.

A parallel behavior was seen with 3-hydroxy perazine and its demethylation product. These increases in ratio continued for three or four weeks and then remained fairly constant. Reduction of the dose by 30 percent led to a distinct increase in the demethylation rate in three of four subjects.

The increasing demethylation ratio may be due to drug-related alteration

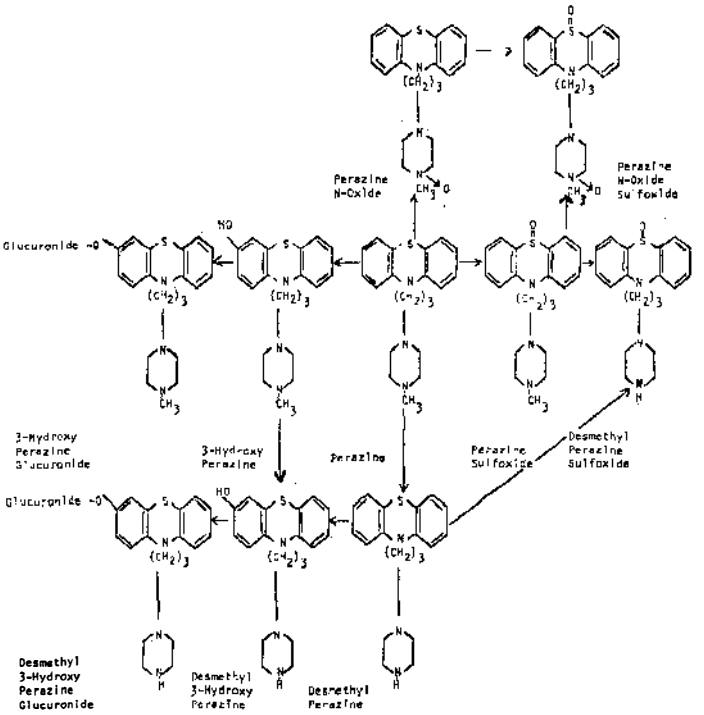


FIG. 5.

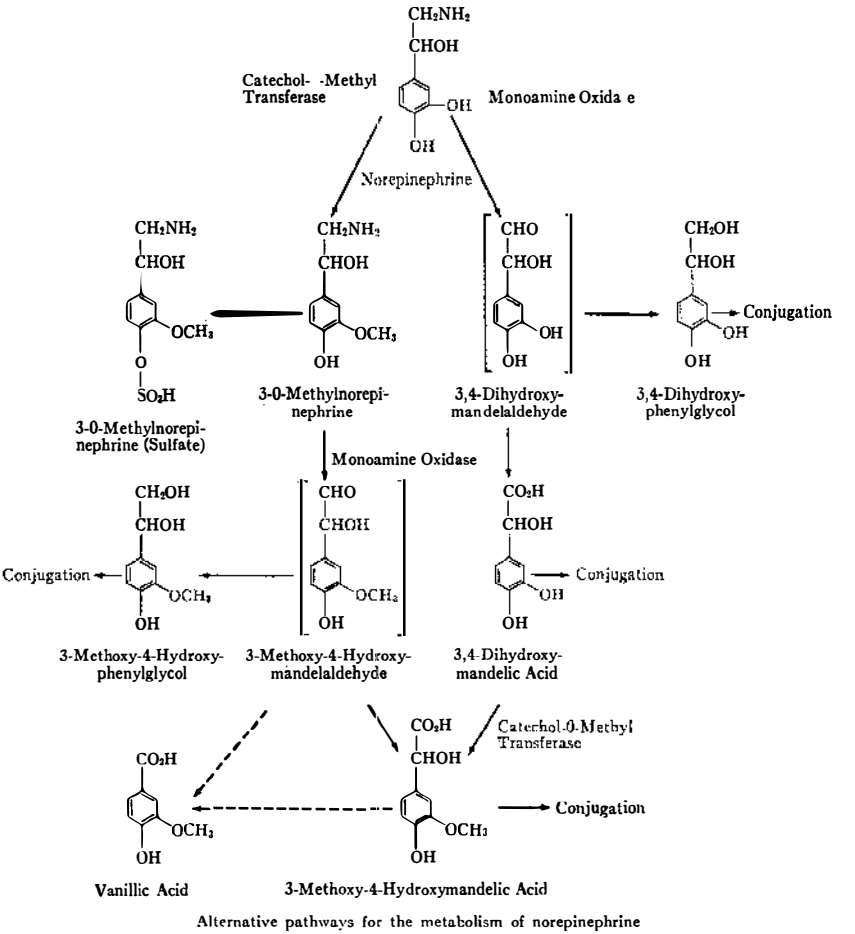
of enzyme levels, saturation of an enzyme system, or a slowly attained steady-state saturation. The suggestion that levels of drug metabolizing enzymes are altered during the course of therapy is the most readily acceptable explanation.

NOREPINEPHRINE AND EPINEPHRINE METABOLITES

Norepinephrine and epinephrine have been favorite compounds in the study of intermediary metabolism for a number of years. The following scheme, taken from a paper by Goodall & Alton (36) presents a plausible sequence (Figure 6).

Goodall and his collaborators (37–40) have shown that 3,4-dihydroxymandelic acid (DOMA) is a deamination product of both norepinephrine and epinephrine. Approximately 100 μg of this substance is found in the urine of normal human subjects in the course of a day (41–44). This increases to almost 1000 μg when pheochromocytoma is present (44, 45).

The biotransformation of DOMA has led to reports of 3-methoxy-4-hydroxymandelic acid (MOMA) as a metabolite of DOMA, but little else was



Alternative pathways for the metabolism of norepinephrine

FIG. 6.

known with regard to other metabolites of this substance. MOMA is probably the principal metabolite of norepinephrine and epinephrine.

Goodall & Alton (36) undertook to measure the extent of conversion of DOMA to MOMA by the human, and, further, to identify other, as yet unreported, metabolites of DOMA. In order to do this, these researchers administered 3,4-dihydroxy mandelic acid-2-^{14}C by rapid (one minute) intravenous injection and also by slow (1 hour) infusion. Two subjects received the principal metabolite of norepinephrine and epinephrine.

In either case, DOMA was converted to at least ten other substances, as separated by means of DOWEX 1-X2 columns. Within 24 hours after a 1 hour infusion of DOMA, 17.7 ± 2.5 percent of circulating DOMA is reported as having been converted to MOMA. The metabolite next in abun-

dance is 3,4-dihydroxybenzoic acid (DOBA) (7.7 ± 1.4 percent). Unchanged DOMA was excreted in abundant amounts (47.7 ± 2.5 percent). Small amounts of vanillic (VA) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) were identified. At least seven unidentified metabolites were excreted in small quantities (0.3 to 2.4 percent).

Previous workers (40, 46–57) had reported MOMA, DOPAC, (3,4-hydroxyphenylacetic acid), HVA, and VA to be metabolic products of both epinephrine, norepinephrine, and dopamine; however, DOMA was not. Conjugation of the phenolic acid was reported to be a relatively minor pathway of DOMA metabolite elimination. The combined conjugate fraction did not contain sufficient amounts of any particular substance to permit ready identification. The combined conjugates accounted for about 6 to 11 percent of the administered radioactivity.

Several aspects of the findings of Goodall & Alton (36) are of considerable interest and are extremely difficult to explain. Following a 1 hour infusion of DOMA-2-^{14}C, 95.3 ± 1.2 percent of the total radioactivity is recovered in 24 hours, however, only 72.3 ± 0.6 percent was recovered when the DOMA was injected during a 1 minute interval. Correspondingly reduced excretion of each of the metabolic products was also observed. Goodall & Alton (36) suggest that this may be due to a larger percentage of the DOMA being bound when DOMA is present in greater concentration in the circulation. These authors volunteer that the observed phenomenon cannot be adequately explained and this reviewer concurs with their statement. The excretion of unchanged DOMA after 1 hour infusion was 47.7 ± 4.6 percent of the dose. Following rapid injection, 40.4 ± 6 percent was excreted. Obviously, a considerable statistical overlap is present. On the other hand, the excretion of both MOMA and DOMA was statistically different after the two rates of administration.

Delvigs & Taborsky (58) have continued their program of investigation of the metabolism of indole and its related structures. Because esterification confers hormonal activity on 5-methoxytryptamine and alters its metabolism, these authors undertook the study of the acetate ester of 5-methoxytryptophol, which is the oxygen isostere of melantoin, in rats.

The indole series of compounds are metabolized, in the main, by two metabolic routes, or a combination of both. These reactions have been studied in considerable detail by the Cleveland Clinic group. An oxidative sequence can take place through a group on the side chain with amines or aliphatic hydroxyl functions being altered to acids (59–63). When this pathway is blocked by N,N-dialkylation, on N-acetylation, or an oxidizable group is not present, hydroxylation will then take place. Examples of this can be seen in the case of skatole, 3-methylindole which is converted to a mixture of monohydroxylated metabolites by the rat (64) and indole, which is converted to the corresponding 3-hydroxyindole sulfate ester (65). Metabolic introduction of a hydroxyl function in the 6-position (66) takes place to some extent with melantoin (3-(2-acetylaminoethyl)-5-methoxyindole)

(66), the pineal constituent and frog skin lightening hormone (59, 61, 67, 69) and N,N-dimethyltryptamine (68).

Administration of 3-(2-acetoxyethyl)-5-methoxyindole (58) intraperitoneally to the rat, resulted in the formation of a single metabolite. Approximately 86 percent of the administered radioactivity appeared in urine and 3 percent in feces during the course of 24 hours.

When 5-methoxytryptophol is administered, it is quickly metabolized to the anticipated 5-methoxyindole-3-acetic acid (63). 5-Methoxy-tryptamine, and other tryptamines are readily converted to the corresponding carboxylic acid (61). When the amine function is converted to the amide, ring hydroxylation can take place (61, 67, 69).

Delvigs & Taborsky (58) considered four possible metabolic sequences, based upon existing literature. The first possibility was that only 6-hydroxylation took place as in the case of melantoin, to afford, in this instance, 3-(2-acetoxyethyl)-6-hydroxy-5-methoxyindole. Hydrolysis of the ester could take place under the influence of an esterase and metabolism identical with 5-methoxytryptophol should occur and the metabolite would be 5-methoxyindole-3-acetic acid. Another possibility was that 6-hydroxylation would be the initial step and subsequent hydrolysis of the ester linkage to result in 6-hydroxy-5-methoxytryptophol. The fourth possibility was that ester hydrolysis might be followed by an oxidative reaction pathway to give 6-hydroxy-5-methoxyindole-3-acetic acid.

Known compounds having the structures of the postulated metabolites were synthesized. None was identical with the metabolite isolated (58). 4-Hydroxy-5-methoxyindole-3-acetic acid was also found not to be the correct structure.

The authors made an unsuccessful effort to synthesize 7-hydroxy-5-methoxyindole-3-acetic acid, the structure they feel is identical with the newly discovered metabolite. They have used the following indirect, but sound, evidence. The only remaining position is the 7-position. All previous studies of indoles have shown that hydroxylation occurs only in the benzene ring and never on the 2-position or on the chain. Position 3 does not possess a replaceable hydrogen. 7-Hydroxylation had been reported for skatole.

It is suggested (58) that 6-hydroxylation is not a general biotransformation of the exogenous indoles but that 7-hydroxylation can be the exclusive hydroxylation step. The question of why 6-hydroxylation occurs in some cases, and 7-hydroxylation in others, remains to be answered.

The *In Vivo* and *In Vitro* Metabolism of Diazepam and Chlordiazepoxide

The metabolic transformations undergone by diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) and chlordiazepoxide (7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-oxide) are most remarkable (Figure 7). Of particular interest is that these compounds have been shown to undergo a series of complex biotransformations lead-

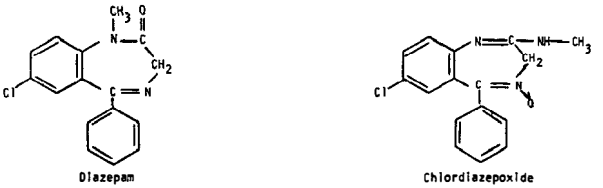


FIG. 7.

ing to the same metabolites (70-74).
which has been identified as 7-chloro-1,3-dihydro-5-(4-hydroxphenyl-2H-1, 4-benzodiazepin-2-one) as a terminal metabolic product following administration of chlordiazepoxide (74) or diazepam (70). In the dog another metabolite, oxazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4,benzodiazepin-2-one), has been identified as being a metabolite of diazepam (71, 72) and also chlordiazepoxide (73). (Figure 8).

In man and the dog the predominant route of elimination of diazepam and its metabolites is through the kidney. Man excretes approximately 71 percent in urine and 10 percent in the feces, and the dog 61 percent in the urine and 34 percent in the feces (71). The rat, on the other hand, excretes 22 percent in the urine and 57 percent in the feces following intraperitoneal administration. Following intravenous administration of diazepam, diazepam levels decline very rapidly and the formation of the N-desmethyl derivative is quite rapid. High levels of this substance persisted suggesting that further biotransformations were slower than the N-demethylation step. The N-desmethyl diazepam metabolite was present only in trace amounts in urine as either the free or conjugated form (71, 72). The primary urinary metabolite was found to be the N_1-desmethyl-C_3-hydroxy derivative of diazepam which was excreted as a glucuronide conjugate (72). Simultaneously, the C_3-hydroxy derivative of diazepam was excreted as a minor metabolite, in the conjugate form.

Four metabolites have been identified in the gastrointestinal tract of rats which had been given diazepam intraperitoneally (70). These were shown, by the use of high resolution mass spectrometry and nuclear magnetic resonance, to be the glucuronide or sulfate conjugates of 3-hydroxydiazepam,

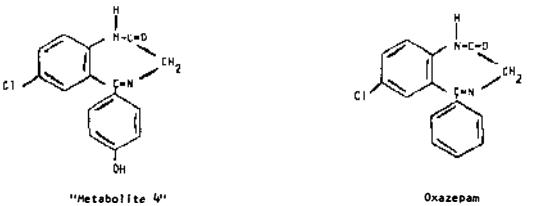


FIG. 8.

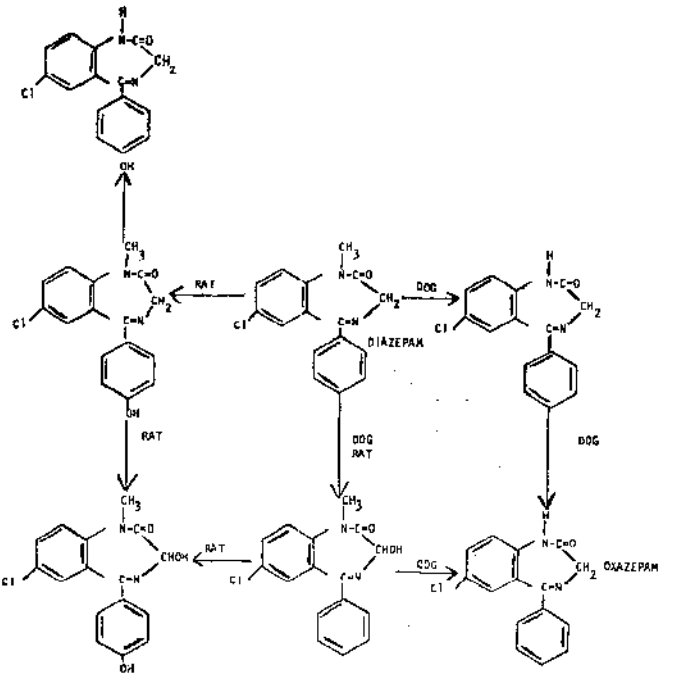


FIG. 9.

and the conjugates of 5-hydroxyphenyl analogues of diazepam, 3-hydroxy diazepam, and N-desmethyl diazepam, respectively.

The *in vivo* metabolism of diazepam is shown in Figure 9.

Schwartz (75) has also studied the *in vitro* metabolism of diazepam using the classical 9000 g liver supernatant system. He studied both the dog and the rat. The dog liver preparation converted diazepam to the 3-hydroxy diazepam and N_1-desmethyl diazepam analogues in about equal amounts.

Oxazepam, the N_1-desmethyl-3-hydroxy derivative, which is the dominant metabolite under *in vivo* conditions, was formed in relatively small quantities. Pretreatment of dogs with phenobarbital led to a more active liver preparation which formed greater amounts of the N_1-desmethyl analogue and oxazepam but not of the 3-hydroxy metabolite. Interestingly, the rat liver preparation formed the same metabolites as did that of the dog. However, only one of these compounds, 3-hydroxy diazepam, is formed by the intact rat (70). The most prominent route of diazepam metabolism *in vivo* in the rat is the introduction of a hydroxyl function in the 5-phenyl position. These metabolites were found only in trace amounts under *in vitro* conditions. Schwartz (75) concludes that if in the rat the liver is the major site for the formation of the observed phenolic metabolites, then the super-

natant system used *in vitro* must have favored N-demethylation and hydroxylation at the C-3 position over the hydroxylation, of the phenyl ring. Phenobarbital stimulation of the rat liver system led to a marked increase in oxazepam but also resulted in the production of novel, unidentified polar metabolites.

The excretion of radioactive chlordiazepoxide and its metabolites from man, dog, and rats (76) closely parallels that which has been reported for diazepam. Koechlin (76) identified two metabolities synthesized by both man and dogs. These were the "lactam" derivative (7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one-4 oxide) which resulted from hydrolytic cleavage of the methylamino substituent at the C-2 position. This metabolite was excreted as such and also underwent cleavage to the "open lactam" or amino acid metabolite [N-(2-amino-5-chloro-α-phenyl-benzylidine) glycine-N-oxide]. This compound was excreted in the free form or as alkali labile conjugates. These metabolites were not found in the excreta of the rats.

When diazepam was introduced into man and the dog considerable hydroxylation occurred at the C-3 position. In the chlordiazepoxide study just reported (76), no evidence of a similar reaction had been seen. A subsequent study (73), wherein the lactam was orally introduced to the dog, led to the recovery of small but significant conversion to diazepam. These authors also detected oxazepam upon administration of chlordiazepoxide.

The loss of the methylamino function to form the lactam would cause one to suspect that two steps were involved in this conversion. First one would expect to see N-demethylation, and then to see this followed by deamination. Schwartz (77) incubated chlordiazepoxide in dog and rat liver preparations. The principal metabolite was found to be the N-demethylated compound (7-chloro-2-amino-5-phenyl-3H-1,4-benzodiazepin-4-oxide). This metabolite was also found to be formed in small quantities under *in vivo* conditions. It was detected in the blood of chlorazepoxide treated rats, squirrel monkeys, and humans. Lactam formation could not be demonstrated with *in vitro* experiments employing dog liver.

The principal metabolites found in the urine of rats which had received chlordiazepoxide intraperitoneally have been identified with the aid of high resolution mass spectrometry. These metabolites represent approximately one third of the administered dose of chlordiazepoxide (74). Four new urinary metabolites, all having undergone introduction of a hydroxyl function in the C-5 phenyl ring, have been identified. The introduction of the hydroxyl function is thought to occur in the *para* position in each case. These new metabolites are 7-chloro-1,3-dihydro-5-(4-hydroxyphenyl)-2H-1,4-benzodiazepine-2-one-4 oxide; 2-amino-7-chloro-5-(4-hydroxyphenyl)-3H-1,4-benzodiazepine-4-oxide; 7-chloro-5-(4-hydroxy-phenyl)-2-methylamino-3H-1,4-benzodiazepine-4-oxide; and 7-chloro-1,3-dihydro-5-(4-hydroxyphenyl)-2H-1,4-benzodiazepine-2-one. This latter metabolite differs from the others in that the N-oxide function has been removed. All metabolites ap-

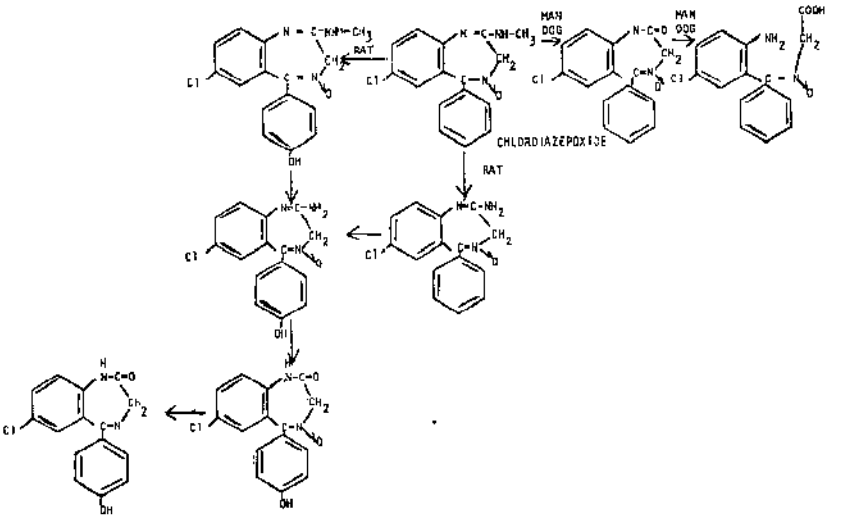


FIG. 10.

pear to be present in nearly equal quantities. The N-demethylated metabolite, although not found in this study, has been reported.

Figure 10 illustrates the known metabolic conversion of chlordiazepoxide.

The scope of this review has been confined to complex examples of drug metabolism. Too frequently biotransformations have been treated as single isolated steps and little consideration has been given to the sequential nature of these changes. The metabolism of many drugs is a highly complex series of events. Metabolites are synthesized, and then may be excreted unchanged, or in the form of conjugates, or may undergo further transformation to other substances which may be more or less polar in nature. The metabolite which is excreted may have undergone several biotransformations prior to elimination. In the case of molecules which have a marked ability to affect the biologic response of an animal, the observed therapeutic effect may arise from metabolites having a transient existence. For this reason it is desirable to determine the metabolic pathway as specifically as possible.

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