METABOLIC FATE[1,2]

By F. E. Shideman[3] and G. J. Mannering[3]

Department of Pharmacology and Toxicology, The University of Wisconsin, Madison 6, Wisconsin

In this as in many other areas of pharmacology much has been added to our knowledge within the past year. Boyland & Booth (20), in their excellent review in the previous volume of this publication, chose to emphasize mechanisms of detoxification. Maynert (123), a year earlier, considered only the excretory products of useful drugs, excluding *in vitro* studies of drug metabolism. Our emphasis lies somewhere between those of the two previous reviewers. We have elected to direct our attention again to the metabolic fate of therapeutically useful drugs but reference will be made to other chemical agents when it is believed the information may have some bearing on the compounds of primary interest. Metabolic fate will be interpreted in the strictest sense for purposes of this presentation, and other aspects of physiological disposition will be ignored except when there may be some important relationship to the biochemical alterations involved. Space limitation precludes any consideration of the chemical transformations of drugs effected by plants or microorganisms. Hormones, vitamins, biologically important catecholamines and serotonin have been excluded since these represent areas of specialization which are the subjects of periodic review. Because of a somewhat different approach from that of Boyland & Booth (20) a significant portion of the information in this review is found in publications appearing as early as May 1960. Finally, definitive data from *in vitro* studies are included since they represent biochemical transformations available to the organism even though *in vivo* they may not always be important.

General Metabolic Reactions

Considerations of the general metabolic reactions (oxidations, reductions, hydroxylations, demethylations, conjugations, etc.) which drugs and other related foreign compounds undergo have been reviewed extensively in the recent literature (15, 16, 19, 20, 46, 195). The following studies, which attracted our interest, have appeared since these reviews were written.

Hydroxylation.—A study of the substrate specificity of the hydroxylating

[1] The survey of the literature pertaining to this review was concluded in May 1962.

[2] Abbreviations used in this chapter include: ADH (alcohol dehydrogenase); AT (3-amino-1,2,4-triazole); CoA (coenzyme A); NAD (nicotinamide-adenine dinucleotide); $NADH_2$ (nicotinamide-adenine dinucleotide reduced form); NADP (nicotinamide-adenine dinucleotide phosphate); $NADPH_2$ (nicotinamide-adenine dinucleotide phosphate reduced form); TEPA (triethylenephosphoramide).

[3] Present address: Department of Pharmacology, College of Medical Sciences, University of Minnesota, Minneapolis 14, Minnesota.

system whereby phenols and dihydrodiols were formed led to the conclusion that a family of hydroxylating enzymes resides in the liver microsomes (150). Although dihydrodiols have been proposed as possible intermediates in the formation of phenols, in the case of the microsomal hydroxylation of benzene, napthalene and quinoline, they do not appear to serve such a role (149). Conversely, 1-naphthol and 3-hydroxyquinoline are not intermediates in the formation of their corresponding diols. There is a good possibility that dihydrodiols and phenols are derived from a common intermediate. Using isotopes to study the conversion of acetanilid to 4-hydroxyacetanilid it was shown that molecular oxygen rather than water participates in the hydroxylation. Further, the hydrogen of tritiated water is not incorporated into the hydroxylated product (149). A detailed study has been made of the microsomal hydroxylating system using acetanilid as a substrate (110). Riboflavin at a concentration of 10^{-4} causes a 95 per cent inhibition of the hydroxylation of pyrine *in vitro*, and *in vivo* a dose of 0.1 mg in the mouse results in almost complete failure to excrete the phenolic derivatives seen normally after administration of benzpyrine (77). N-Hydroxylation of 4-aminobiphenyl and 4-acetylaminobiphenyl in the rat has been demonstrated (134). The solubilization of an aromatic hydroxylase system from liver microsomes and the requirement of this system for a lipid-like factor have been described (87).

Dehydroxylation.—Scheline, Williams & Witt (156) have provided further support for a role of aromatic dehydroxylation as a reaction of metabolic significance with the finding that rabbits dehydroxylate 3,4-dihydroxyphenylacetic acid in both the *meta* and *para* positions. This raised speculation that catecholamines might also be dehydroxylated, e.g., the dehydroxylation of epinephrine to give *p*- or *m*-hydroxyphenylmethylaminoethanol. Indeed, at about the time this speculation was made, Pisano *et al.* (147) isolated *p*-hydroxyphenylmethylaminoethanol (Synephrine) from human urine. The finding of *m*-tyramine in the urine of humans given the monoamine oxidase inhibitor, 1-phenyl-2-hydrazinopropane (160), raises the possibility that dopamine is dehydroxylated *in vivo* (156).

Oxidative N-*dealkylation.*—McMahon (127) studied the demethylation of propoxyphene and five other arylalkyldimethylamines of varying fat solubilities in the mouse, rat, and guinea pig using whole animals and liver microsomes. Reasonably good correlation was obtained between the demethylation rates found in the three species and between the *in vitro* and *in vivo* results. Demethylation rates increased with increasing fat solubility of the amines which would tend to support the hypothesis of Gaudette & Brodie (69) that the microsomal enzymes are protected by a lipoidal barrier that is penetrated only by fat-soluble compounds. 3-Methyl-3-(N-methyl-*t*-butylamino)-butyne-1, butynamine, a tertiary aliphatic amine possessing hypotensive activity, was found to be readily demethylated *in vivo* and *in vitro* (130). Oxidative deethylation appears to be the main metabolic reaction affecting lidocaine (81).

Conjugation.—Glucuronide synthesis by way of enzymatic transfer of

glucuronic acid from uridine diphosphate glucuronic acid is not restricted to the liver, but can be performed by renal cortex, gastrointestinal mucosa and skin (47, 165). The suggestion that a single glucuronyl transferase is responsible for the synthesis of ester, ethereal and N-linked glucuronides has been dispelled by the finding that solubilized glucuronyl transferase from rabbit liver microsomes catalyzes the formation of ester and ethereal glucuronides but not of N-linked glucuronides (89). Further evidence for different glucuronide-forming enzymes was obtained by Arias (4) using homozygous Gunn rats, which suffer from nonhemolytic acholuric jaundice due to their inability to form bilirubin glucuronide, an ester glucuronide. The urinary excretion of aniline glucuronide was found to be the same in normal and Gunn rats as was the formation of aniline glucuronide by liver slices from both groups of animals. On the other hand, *O*-aminophenyl glucuronide formation was markedly decreased in hepatic slices from Gunn rats as compared with slices from normal rats.

The belief that young subjects, particularly females, are more liable to chronic benzene intoxication gains experimental support from studies employing adult male, adult castrate male, adult female, young male and young female rats, where variations of sulfurylating enzyme activity with sex and age showed a well matched correlation with susceptibility to benzene intoxication (86). The sulfate conjugation of aliphatic alcohols will be considered under the discussion of ethanol metabolism.

Miscellaneous.—Because SKF 525 A (diethylaminoethyl diphenylpropylacetate) inhibits drug metabolism in a general manner, affecting a variety of metabolic routes occurring in hepatic microsomes, it has been suggested that it acts by blocking the entrance of drugs into the microsomes. The finding that the solubilized microsomal enzyme responsible for the hydrolysis of monoethylglycinexylidide, a metabolite of lidocaine, was clearly more inhibited by SKF 525 A than the particulate enzyme from which it was derived, casts serious doubt on the theory that this inhibitor functions by interfering with the penetration of drugs into the microsomes (82, 83).

The microsome fraction of liver is composed of fragments of the endoplasmic reticulum of which there are at least two types: rough-surfaced (caused by granules rich in ribonucleic acid) and smooth-surfaced (associated with areas rich in glycogen). Drug-metabolizing enzymes appear to be localized mainly in the smooth-surfaced reticulum (61).

Factors Influencing Drug Metabolism

The activity of drug-metabolizing enzymes in liver microsomes can be modified markedly by the administration of numerous drugs and other foreign compounds, by hormones, and by the age, sex, strain and nutritional status of the animal. Conney & Burns (31), who have been major contributors in this area of biochemical pharmacology, have recently reviewed the subject and only items of interest which have been reported since their review was written are included here.

The microsome fractions of liver from adult or weanling rats pretreated with nikethamide showed an enhanced capacity to metabolize pentobarbital (22). This change was paralleled by a reduction in the duration of action of the barbiturate. A decrease in strychnine toxicity and an increase in octamethylpyrophosphoramide toxicity were observed in rats treated 48 hours previously with phenaglycodol or thiopental (95). It is well known that octamethylpyrophosphoramide itself has no anticholinesterase activity, but that it must be acted upon by the liver before exerting its toxic effects. The rate of degradation of carisoprodol by liver slices was increased when rats were pretreated with phenobarbital, phenaglycodol or glutethimide (97). This drug-induced increase in metabolic activity, which was maximal 48 hours after treatment, was inhibited by the coadministration of ethionine. Rats treated with phenobarbital, phenaglycodol, glutethimide, nikethamide, meprobamate, chlorobutanol or chlorpromazine showed both an increased pentobarbital metabolism and a diminished sleeping time after pentobarbital administration (96). Ethionine inhibited the inductive effects of both phenobarbital and phenaglycodol on the metabolism of pentobarbital and meprobamate (100, 101, 102, 107). Norleucine, *p*-fluorophenylalanine and methioninesulfoxide did not modify the inductive effect of phenobarbital (100), nor were X- or gamma-irradiation or the carcinostatic agent, 6-mercaptopurine, effective (101). A slight inhibition of induction was obtained with 8-azaguanine.

Administration of drugs that increase the activity of liver microsomal enzymes is also known to accelerate the synthesis of ascorbic acid from glucose and galactose in rats (31). Kato, Vassanelli & Frontino (102) have observed that phenobarbital and chlorobutanol induce both accelerated drug metabolism and ascorbic acid synthesis; but that, while ethionine prevents the effect on drug metabolism, it does not prevent increased ascorbic acid synthesis. This is quite surprising in view of the observation by Touster *et al.* (176, 177) that ethionine prevents the enhancement of ascorbic acid synthesis resulting from the administration of barbital and methylcholanthrene, compounds which also increase drug metabolism.

Sex differences in the pharmacological effects of pentobarbital, carisoprodol, strychnine and octamethylpyrophosphoramide in adult rats have been related to rates of drug metabolism as influenced by hormones (98, 99).

Regenerating liver is deficient in its capacity to oxidize the side-chain of hexobarbital and the ring-sulfur of chlorpromazine and to reduce the nitro-group of *p*-nitrobenzoic acid (62). Full recovery of these enzyme activities occurs about 10 days after partial hepatectomy. The rate of O-dealkylation of codeine is affected only during the first phase of regeneration and returns to normal in about four days.

Depressants of the Central Nervous System

Barbiturates.—Bush, Mazel & Chambers (25) have adequately demonstrated that exposure of thiobarbital to extraction procedures involving ether

results in conversion of some of the drug to barbital and that, by use of a technic which eliminates this pitfall, small amounts (five to seven per cent) of the thiobarbiturate are found in the urine of man as the oxygen analogue. Nevertheless, a controversy still persists in the German literature as to the existence of such a metabolic reaction *in vivo*. Block & Ebigt (12), utilizing 5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid, Inactin, labeled either in the 5-position with ^{14}C or at the 2-position with ^{35}S as well as nonlabeled compound, found no conversion to the barbiturate in the mouse. Similarly, no *in vivo* formation of pentobarbital from thiopental was observed. These workers, in another publication (13), employed thiopental and the sulfur analogue of butabarbital, labeled in the 5-position with ^{14}C, in mice and at no time after administration of the drugs could they find any pentobarbital or butabarbital in the tissues. Frey (63), also, has demonstrated the lability of thiobarbiturates in the presence of ether, but has presented evidence that such is not the case with chloroform. Employing a chloroform extraction procedure, Frey, Doenicke & Jäger (65) have identified significant but variable amounts of pentobarbital or butabarbital in the serum or plasma of the dog or man following administration of the sulfur analogues of these barbiturates. In other studies by Frey (64) it is claimed that butabarbital is detectable in dogs which received 5-ethyl-5-(1-methylpropyl)-1-methyl-2-thiobarbituric acid.

In the case of butabarbital, as well as its sulfur analogue, evidence is available that hydroxylation occurs on the methylpropyl side chain of the 5-position (12). Introduction of a hydroxyl group in the 2-position appears to predominate but other alcohols are formed by oxidation of the terminal methyl groups of the methylpropyl chain. Further oxidation to the acids appears likely. One can add N-methyl-butabarbital as well as 5-ethyl-5-(1-methylpropyl)-1-methyl-2-thiobarbituric acid to the list of compounds which undergo N-demethylation *in vivo* (64). It is also claimed that the latter compound is converted to N-methylthioureido-(1-methylpropyl)ethyl malonic acid and N-[(1-methylpropyl)-ethylacetyl]-N′-methylthiourea by the dog but the data are not particularly convincing. Work by Glasson & Benakis (73) has established that the rat, like certain other species, is capable of converting phenobarbital to 5-ethyl-5-(*p*-hydroxyphenyl)-barbituric acid and conjugating the latter with glucuronic acid. Four other metabolites of the drug were detected but not identified. That 5-ethyl-5-(3-hydroxy-3-methylbutyl)-barbituric acid is a metabolite of amobarbital in man, as it is in the dog, is indicated by the isolation of this compound from the urine of an individual who had ingested equal amounts of amobarbital and secobarbital (90).

Ethanol.—Because of its widespread use, it is natural that the metabolic fate of ethanol should have received as much attention as it has. Even so, many unanswered questions remain. The enzyme(s) involved in the second stage of the oxidation, acetaldehyde to acetic acid, has not been fully characterized. Other questions concern: (*a*) the locations within the body at

which the various steps occur, (*b*) the mechanisms, (*c*) the rates at which the reactions take place, (*d*) whether or not the rates can be influenced by other pharmacological agents, (*e*) the extent to which alternate pathways perform *in vivo*, (*f*) and whether or not such alternate pathways are more prominently operative in chronic alcoholism. Certain of these questions have been considered in a recent review (93).

Metabolic pathways other than the familiar oxidation of ethanol to acetaldehyde, the direct or indirect conversion of the latter to acetyl coenzyme A (CoA), and the ultimate oxidation of acetyl CoA through the citric acid cycle are known, but it is doubtful that they play an important role quantitatively. After intraperitoneal administration of ethanol the blood of rats was found to contain acetate and acetoacetate, and blood from perfused rat liver showed the presence of acetate, acetoacetate, hydroxybutyrate, pyruvate and an unidentified acid, all partially derived from labeled ethanol (60). The earlier work of Kendall & Ramanathan (106), which employed liver preparations to demonstrate the dismutation of aldehydes to form both acids and alcohols, and the formation of methyl formate when methanol was present, has been repeated using purified horse liver alcohol dehydrogenase, ADH (2). It is suggested that ADH may be the only enzyme involved in the dismutation of aldehydes. The stereo-specificity of the reactions has been established (1); both the ADH-mediated oxidation of aldehyde to acid and the reduction of aldehyde to alcohol utilize the same side of the nicotinamide ring of NAD. ADH can oxidize alcohol and transfer the hydrogen directly to a different aldehyde, such as to glyceraldehyde, to form the corresponding alcohol (153). Aliphatic alcohols have long been known to form glucuronides, but it has been shown only recently that they are also excreted in the urine as sulfates (14, 15, 182, 183). A metabolic pathway for ethanol other than one proceeding directly through acetate and acetyl CoA has been implicated by the report that protein, glycogen, fatty acids and cholesterol from rats given a single dose of radioactive alcohol contained two to three times the labeled carbon found in the same constituents from rats receiving ^{14}C acetate (157). This is refuted in more recent studies (161, 163) where equal amounts of ^{14}C were incorporated into tissue fatty acids, nonsaponifiable fraction, and phospholipids after radioactive alcohol or acetate administration. However, acetate-1-^{14}C is utilized more readily than ethanol-1-^{14}C by the livers of normal rats in forming citric acid cycle intermediates. The opposite is true of livers from rats chronically treated with ethanol (36). While containing only about one-fourth of the concentration of ADH, rat kidney slices metabolize ethanol more rapidly than do rat liver slices (189). The formation of acetaldehyde, which was found in the incubation medium of kidney, but not of liver slices, was prevented with 3-amino-1,2,4-triazole, a catalase inhibitor. Because of differences in rates of oxidation of the metabolites of ethanol by liver and kidney slices and because the incubation of these tissues together resulted in rates of oxidation of ethanol greater than the summation of their individual rates, it was suggested that *in vivo*,

organs may combine to improve metabolism, with the metabolites from one organ being oxidized by another organ. These considerations should be viewed with the knowledge that the total amount of ethanol oxidized to CO_2 by liver and kidney slices accounted for only about one-eighth of the oxidation found in *in vivo* experiments.

While the catalase·H_2O_2 complex undoubtedly participates in the metabolism of aliphatic alcohols, the extent of this contribution to the overall metabolism of ethanol is not known. It has been suggested by Von Wartburg & Rothlisberger (190) that the peroxidative mechanism may play a more important role in chronic alcoholism. The livers from rats treated with ethanol or methanol over long periods of time metabolized ethanol and methanol at increased rates. This was associated with an increase in the catalase rather than the ADH activity of the livers. Although erythrocytes contain abundant catalase they do not normally oxidize alcohols. This was shown to be due to the failure of erythrocytes to provide H_2O_2 for the catalase·H_2O_2 complex required for alcohol oxidation (167, 168). Oxidation of both ethanol and methanol by washed erythrocytes occurred after the addition of glucose and methylene blue, presumably as a result of H_2O_2 generation by means of the pentose shunt.

The daily administration of hexobarbital to mice and rabbits for five days increased ethanol metabolism by as much as 40 per cent (55, 56). Phenobarbital was less effective. Concomitantly, the NAD:$NADH_2$ ratio in the liver was increased (54), an alteration which would be expected to favor ethanol metabolism. The total diphosphonucleotide content of the liver was not changed as a result of barbiturate treatment nor was the catalase concentration affected. Two mechanisms for the shift in NAD:$NADH_2$ ratio are considered: (*a*) that the metabolism of hexobarbital is mediated through $NADPH_2$ and the resulting NADP is reduced through other enzymes employing $NADH_2$, and the more likely possibility, (*b*) that hexobarbital inhibits oxidative phosphorylation (3) which in turn is responsible for the maintenance of reduced NAD and NADP. In view of unsuccessful attempts in the past to accelerate ethanol metabolism by means of thyroxin, dinitrophenol or other agents which raise the general metabolic rate, the report by Goldberg, Hehir & Hurowitz (74) that triiodothyronine, given intravenously, greatly increased the disappearance of alcohol from the blood of acutely intoxicated humans, is of considerable interest. No such effect of triiodothyronine on ethanol metabolism was observed in dogs (144), and others (93) have not been able to duplicate the effect in humans. It is quite possible that the discrepancy in results may relate to the choice of subjects, as, for example, whether or not they are alcoholics.

ADH continues to serve as a model in enzyme kinetic studies (8, 53, 118, 119, 148, 172, 173, 174, 175, 197, 198, 199). Adenosinediphosphate ribose was found to be a potent inhibitor of ADH and may account for the decreased activity of certain purified ADH preparations (38).

The assumption that 2-fluoroethanol is toxic by virtue of its biotrans-

formation to fluorocitrate has proven accurate, but the assumption that ADH mediates the initial step in this conversion has been shown to be incorrect (178). In fact, 2-fluoroethanol competitively inhibits the oxidation of ethanol by purified horse liver ADH. A second liver ADH of different specificity readily oxidized 2-fluoroethanol. NAD, and to a lesser extent, NADP, act as cofactors in the reaction. The role *in vivo* of this new ADH in the oxidation of ethanol and other alcohols, including methanol, awaits investigation. A study of the metabolism of fluoroacetate-2-^{14}C shows that fluorocitrate in the urine represents only three per cent of the total radioactivity and that the major radioactive metabolite in the urine did not inhibit aconitase (68). It is suggested that more than one pathway may exist for fluoroacetate metabolism.

Methanol.—Interest in methanol metabolism continues largely because of the conviction that visual disturbances and blindness, which would seem to be peculiar manifestations of methanol toxicity in man, and perhaps in monkeys, are not caused by methanol *per se* but by products of its metabolism (32, 33, 107, 108, 196). The question of whether the first step in the oxidation of methanol is accomplished by means of the peroxidative system involving catalase or through the action of a dehydrogenase also continues to stimulate interest. It was concluded by Tephly *et al.* (169) who employed liver homogenates from rats treated with either 3-amino-1,2,4-triazole (AT) to inhibit hepatic catalase activity, or with sodium tungstate, to reduce peroxide-generation, that, at least *in vitro*, a peroxidative system involving liver catalase and peroxide generating enzymes is the major mechanism for methanol metabolism. Their studies employing ethanol and other aliphatic alcohols as inhibitors support this view. In similar studies using intact rats and liver slices it was concluded that ethanol is oxidized by ADH while methanol is oxidized by catalase (162). Recent studies, employing labeled methanol, with and without other alcohols and AT as inhibitors, conclusively show that the peroxidative mechanism performs a major role in the oxidation of methanol in the intact rat (170). Contrary to much popular opinion, it has been shown recently by Kini & Cooper (108) that methanol will serve as a substrate for ADH, which is not too surprising since it has been known for some time that ADH catalyzes the reduction of formaldehyde (171, 196). Despite a very unfavorable Michaelis constant for the reaction *in vitro*, they concluded from studies where the methanol disappearance rate from the blood of the monkey was compared with the hepatic ADH concentration that ADH plays the major role in methanol oxidation. Unfortunately, these investigators based their conclusions on the assumption that all of the administered methanol was concentrated in the blood rather than equally distributed throughout the body water. This results in an eight-fold error. Re-evaluated, the data show that a major role of ADH in methanol metabolism in the monkey is highly unlikely (120).

Meprobamate.—Emmerson, Miya & Yim (49) have investigated the distribution and metabolism in the rat of meprobamate labeled with ^{14}C in the carbamate portion of the molecule. Chromatograms of extracts of

blood from animals which had received the drug indicated the presence of three radioactive compounds. Their R_f values tentatively identified them with meprobamate, hydroxymeprobamate (2-hydroxymethyl-2-*n*-propyl-1,3-propanediol dicarbamate), which had previously been identified as a metabolite by Walkenstein and co-workers (188), and a glucuronide. Only unchanged drug was detected in the brain.

Mebutamate.—Mebutamate (2-methyl-2-*sec*-butyl-1,3-propanediol dicarbamate, Capla) is stated to be a centrally acting hypotensive agent. The urine of dogs which have received this drug contains the parent compound, a hydroxylated derivative, 2-methyl-2-(β-hydroxyl-α-methylpropyl)-1,3-propanediol dicarbamate and glucuronide conjugates (43). The hydroxylated derivative accounts for approximately 60 per cent of the known metabolites, only two to four per cent of the drug being excreted in the unchanged form. Another agent of recent origin possessing hypotensive activity not necessarily of central origin is butynamine, 3-methyl-3-(N-*t*-butylmethylamino-butyne-1. ***In vitro*** studies (129) indicate that it is N-demethylated by a microsome fraction of rat liver to form the des-N-methyl compound. Data from studies on rats and man suggest that this is the major metabolic product ***in vivo***.

Narcotic analgesics.—The literature on the metabolic fate of morphine and its surrogates and the pharmacologic implications of these transformations has been reviewed up through the early part of 1960 by Way & Adler (192). Both ***in vivo*** and ***in vitro*** studies, employing liver microsomes, showed that the guinea pig forms glucuronides of morphine, nalorphine, levorphanol and codeine (7). Tritium-labeled normorphine is conjugated by the dog and monkey (136). Takemori (166) studied the effects of acute and chronic administration of morphine on the hepatic enzymes responsible for glucuronide synthesis in the rat. Uridine diphosphate glucose dehydrogenase activity increased in both treated groups, and glucuronyl transferase activity decreased as animals received repeated doses of morphine, but the data reveal a lack of relation between changes in enzyme activities and the development of tolerance. The role of demethylation in the action of narcotic analgesics (9) and the similarity between the receptors for these drugs and the N-demethylating enzyme present in liver (5) have been subjects of much recent work (192). Substitution of deuterium for the N-methyl hydrogens of morphine produced a reduction in analgesic potency and lethality in mice (48). Deuterated morphine was also N-demethylated less readily by liver microsomes. These findings are consistent with, but do not prove a relationship between N-demethylation and analgesic action. Interpretation of this work is made difficult because deuteriomorphine is more basic than morphine. Receptor affinity is known to relate to the pK_a of narcotic drugs, and the difference in potencies between morphine and deuteriomorphine could be due to differences in affinities for the receptors as well as to differences in rates of demethylation. Chronic administration of morphine to rats is known to cause a diminution in the capacity of liver microsomal preparations to N-demethylate narcotic drugs. Abrupt withdrawal of the drug is followed by

restoration of enzyme activity within 12 days. Thyroxin prevented this recovery besides having a depressant effect of its own on the N-demethylating activity of liver preparations (29). Evidence has been presented for the *in vivo* biotransformation of morphine to normorphine by the rat (137, 138). N-Methylation of narcotic drugs has also been demonstrated. An enzyme found in rabbit lung has been described by Axelrod (6) which, with S-adenosylmethionine-(^{14}C-methyl) as a methyl donor, forms morphine, meperidine, codeine and nicotine from their nor-derivatives. Independently, Clouet (28) showed that the nor-derivatives of morphine and codeine are N-methylated *in vitro* by liver and brain tissue preparations using S-adenosylmethionine as the donor, and *in vivo* in brain using methionine-(^{14}C-methyl).

Appreciable amounts of methadone are metabolized to the quaternary methyl product (155), which would explain the failure of earlier workers to account for a large part of the administered drug. The rates of transformation of heroin to 6-monoacetylmorphine and morphine have been determined *in vivo* and *in vitro* in the mouse (193).

Nonnarcotic analgesics.—The metabolism of (^{14}C-carbonyl)-salicylamide was studied in normal and hypophysectomized rats (17). Free salicylamide was identified, but gentisamide, which was found in the urine to the extent of 5 to 30 per cent, was excreted entirely in conjugated form. The products obtained in the urine of hypophysectomized animals were identical with those recovered from normal rats. Phenyramidol, 2-(β-hydroxyphenethylamino)-pyridine, a drug possessing both analgesic and muscle relaxant properties, is excreted by the dog largely as the glucuronide (135). Less than two per cent of the administered oral dose was found in the urine in the unmetabolized form.

Other depressants.—Although it has been known that the major portion of a dose of ethinamate is hydroxylated and that this metabolite is partially excreted as a glucuronide, the position of the hydroxyl group(s) was not established until recently when Murata (139) reported the isolation and identification of the glucuronide of 1-ethynyl-4-hydroxycyclohexyl carbamate from the urine of man. He (140) also identified 1-ethynyl-*trans*-1,2-cyclohexanediol as a metabolite in man. In addition to providing an excellent review of the metabolism of glutethimide, Keberle, Hoffman & Bernhard (103) have presented findings which add to our knowledge of the biochemical fate of this drug. In preliminary studies with the dog using the racemic drug they obtained evidence for the metabolism of glutethimide by two different routes and postulated that this could represent different pathways taken by the two optical antipodes. Analysis of the urine from dogs fed either the levorotatory or the dextrorotatory form of the drug established the pathways of metabolism outlined in Figure 1.

Based on parallel changes in activity with purification, cofactor requirements and susceptibility to inhibition by various agents, Friedman & Cooper (67) have provided convincing evidence that liver alcohol dehydrogenase and the enzyme responsible for the conversion of chloral hydrate to trichloro-

ethanol are identical. Curry (35) has identified diethylacetylurea in the intestinal contents of an individual who had been taking carbromal and bromisovalum.

Butler (26) has presented evidence for the reduction of small amounts of chloroform and carbon tetrachloride to methylene chloride and chloroform, respectively, *in vitro* by mouse liver. These reductions also can be effected by certain agents of biochemical significance, e.g., ascorbic acid, cysteine, reduced glutathione, and cytochrome C. In the case of carbon tetrachloride, reduction *in vivo* also took place to a limited degree as was evidenced by the pulmonary excretion of chloroform by dogs following administration of carbon tetrachloride. Although the compound is not of therapeutic impor-

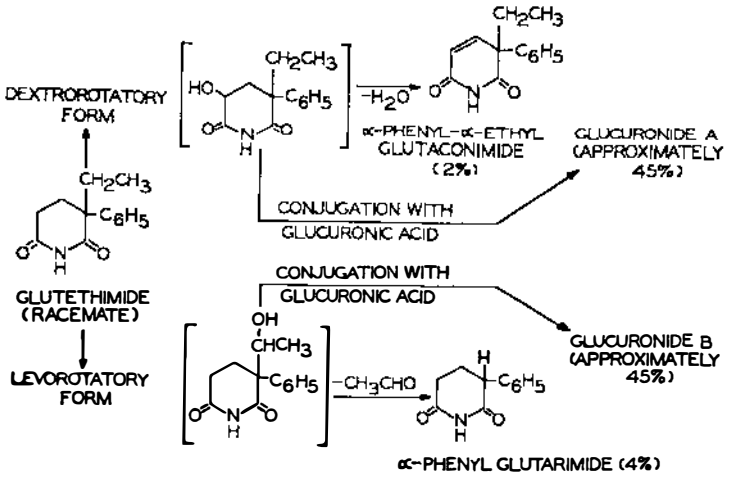

FIG. 1. Metabolism of the optical antipodes of glutethimide.

tance, it is of interest that the methyl derivative of chloroform, 1,1,1-trichloroethane, is almost entirely (99 per cent) excreted by the rat via the respiratory route in unchanged form (76). One-half of one per cent appears in the expired air as carbon dioxide and much of the remainder is found in the urine as the glucoronide of the parent compound.

ANTIEPILEPTICS

The studies described by Maynert (123) with respect to the fate of diphenylhydantoin in the dog, rat and man have been published in detail (122). Following administration of 1,3-dimethyl-5-ethyl-5-phenylhydantoin to dogs 5-ethyl-5-phenylhydantoin (Nirvanol) was identified in the plasma and was the major metabolite found in the urine (186). The double demethylation apparently consists of initial rapid removal of the methyl group in the 1-position followed by slower demethylation in the 3-position. Broadhead & Kilpatrick (23) have studied the fate of ^{35}S-labeled 2-thiohydantoin and 5-

carboxymethyl-2-thiohydantoin in the rat and found that the major portion of both compounds is excreted unchanged in the urine.

Agents of Psychopharmacologic Interest

Tranquilizing Agents.—In addition to being susceptible to sulfoxidation, demethylation and hydroxylation, chloropromazine is metabolized to its N-oxide. Fishman, Heaton & Goldenberg (59) have identified chlorpromazine-N-oxide in the urine of patients receiving the drug. It is not a major metabolic route, accounting for less than one per cent of the dose. Somewhat greater amounts (2–3.5 per cent) are excreted by the dog as the N-oxide. The mechanism involved in the sulfoxidation of the phenothiazines by a liver microsome preparation has been studied in detail by Gillette & Kamm (79). Oxidation of chlorpromazine or 4,4-diaminodiphenyl sulfide to sulfoxides

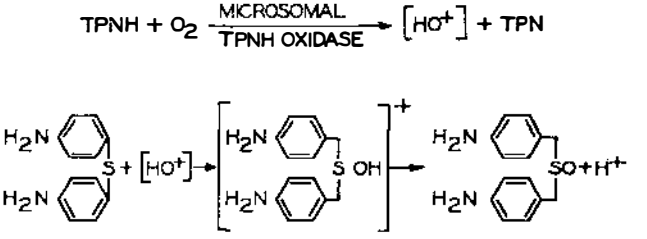

Fig. 2. Proposed mechanism for sulfoxide formation.

requires $NADPH_2$ and oxygen in addition to the enzyme preparation. Of the various tissues of the guinea pig examined, only liver showed activity. The proposed mechanism for sulfoxide formation is depicted in Figure 2. Haynes (78) has reported that 5.5 to 7 per cent of chlorpromazine is eliminated as free and bound chlorpromazine sulfoxide and bound chlorpromazine by patients receiving low daily doses (200 mg) of the drug. At higher levels of dosage (800 mg) somewhat greater amounts (7 to 9 per cent) are eliminated in these forms. Fishman & Goldenberg (57) have obtained tentative evidence for the presence of at least six sulfoxide derivatives of chlorpromazine in the urine of patients receiving the drug.

The metabolism of ethoxybutamoxane (2-*n*-butylaminomethyl-8-ethoxy-1,4-benzodioxane) and its chlorine derivative, chlorethoxybutamoxane (2-*n*-butylaminomethyl-5-chloro-8-ethoxy-1,4-benzodioxane), has been the subject of recent publications. McMahon, Welles & Lee (131) have employed ethoxybutamoxane with ^{14}C in the *alpha* carbon of the ethoxy group or in the *alpha* carbon of the *n*-butyl group in metabolic studies in rats and dogs. The major pathway for metabolism in the rat appears to be cleavage of the ether group followed by oxidation of the 2-carbon fragment to carbon dioxide. In addition to the parent compound, two metabolites were found in the urine. One of these, amounting to approximately 10 per cent of the dose, was identified

as 2-*n*-butylaminomethyl-8-hydroxy-1,4-benzodioxane. In the dog, ether cleavage plays only a minor metabolic role, whereas side chain oxidation proceeds to the extent of about 42 per cent. Another metabolite identified in dog urine was 2-carboxy-8-ethoxy-1,4-benzodioxane. After administration of chlorethoxybutamoxane (^{14}C-ethoxy) to rats, guinea pigs or mice, over 50 per cent of the radioactivity appeared as expired carbon dioxide (128). Significantly less ether cleavage occurred in the rabbit. In the dog, side chain oxidation was the major metabolic pathway, with 2-carboxy-5-chloro-8-ethoxy-1,4-benzodioxane appearing in the urine. Thus, the *in vivo* pattern of metabolism of this compound appears to be very much like that of ethoxybutamoxane.

Antidepressants.—Isocarboxazid is readily metabolized by rat liver homogenates under either aerobic or anaerobic conditions (159), benzylhydrazine being one of the metabolic products, and presumably 5-methylisoxazolylcarboxylic acid, the other. When a low dose of benzyl labeled isocarboxazid was administered to rats (one mg/kg) practically all of the drug was accounted for as radioactive hippurate (158). After large doses appreciably less drug (approximately 27 per cent) appeared in the urine as hippurate during the first 24 hr.

Demethylation of imipramine proceeds quite rapidly in the rat (11) and desmethylimipramine, (N-γ-monomethylaminopropyl)-iminodibenzyl, has been isolated and identified in the tissues of rabbits by Herrman & Pulver (79). This same metabolite has been found in the brain of the rat and evidence that it is responsible for the antidepressant activity of imipramine has been presented (71). Two other produts of imipramine metabolism have been identified. One of these, N-(γ-dimethylaminopropyl)-2-hydroxyiminodibenzyl, was found in the urine of rabbits as the glucuronide (79) and the other, imipramine-N-oxide, was isolated from human urine, (58). The latter is excreted to the extent of approximately two per cent of the daily dose.

Hallucinogenic agents.—Daly, Axelrod & Witkop (37) have conducted a rather extensive study of methylation and demethylation of mescaline *in vitro* (Fig. 3). They have noted the formation of various methoxy derivatives of 3-, 4- and 5-trihydroxyphenethylamine when the latter was incubated with a catechol-O-methyl transferase preparation. When mescaline was incubated with a rabbit liver preparation consisting of microsomes and soluble enzymes, various O-demethylated derivatives were formed, as well as 3,4,5-trimethoxyphenylacetic acid. Friedhoff & Goldstein (66) identified the latter compound as the major metabolic end product of mescaline in the urine of the rat. Pretreatment of animals with iproniazid markedly inhibited its formation and prior administration of calcium carbimide resulted in a large increase in the excretion of 3,4,5-trimethoxyphenylethanol. These data suggest that mescaline is first metabolized to an aldehyde which is then oxidized to the acid or reduced to the alcohol.

Incubation of psilocybin (Fig. 3) with a rat kidney homogenate resulted in the formation of psilocin (84). Various tissues of different species have been

examined for activity of the phosphatase responsible for this conversion. Small amounts of radioactivity (seven per cent of the dose) were found in the expired carbon dioxide after administration of N-methyl-^{14}C-psilocin to rats (94). Approximately 40 per cent of the activity was accounted for in the urine as parent compound and 4-hydroxyindoleacetic acid. Gessner, Khairallah, McIsaac & Page (70) administered serotonin, bufotenine or psilocybin to rats and estimated the extent of their urinary excretion as well as that of the respective hydroxyindoleacetic acids. Small amounts (seven per cent) of serotonin were excreted in the first 24 hr as unchanged drug while approximately 40 per cent was recovered as the hydroxyindoleacetic acid. The percentages of parent compound and hydroxyindoleacetic acids in the case of bufotenine and psilocybin were 22 and 7 per cent and 11 per cent

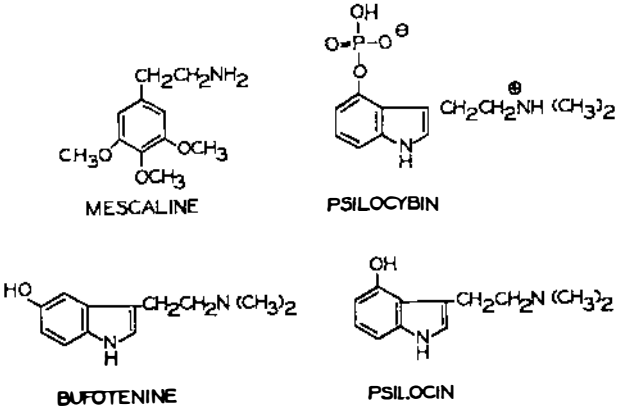

FIG. 3. Structures of hallucinogenic drugs.

and traces, respectively. The three drugs also were compared as substrates for monoamine oxidase of liver. Serotonin underwent rapid degradation to 5-hydroxyindoleacetic acid in a period of 10 minutes. Some 40 minutes were required before any trace of hydroxyindoleacetic acid could be detected when bufotenine was used, and psilocybin was unaltered after two hr of incubation.

AUTONOMIC DRUGS

Space limitation precludes adequate presentation of the more recently accumulated information on the metabolism of biologically important sympathomimetic amines and closely related compounds. Rather than slight this extremely important field of endeavor by a cursory review readers are referred to excellent articles which have been published recently in book form (181).

Several publications appearing during the past two years have contributed significantly to our detailed knowledge of the metabolism of nicotine.

Hucker *et al.* (85) have demonstrated the conversion of nicotine to cotinine by liver preparations from various species, activity being localized in the microsome fraction of the cell. The soluble fraction converted nicotine to hydroxynicotine. After administration of (−)-cotinine to dogs γ-(3-pyridyl)-β-oxo-N-methyl-butyramide was identified in the urine (124). When nicotine-(methyl-^{14}C) was administered to rats approximately nine per cent of the activity was recovered from the expired air as labeled carbon dioxdie and somewhat less than one per cent was liberated from the urine upon treatment with urease (126). From the urine of dogs which received (−)-nornicotine Wada *et al.* (185) isolated two metabolites, (−)-norcotinine and γ-3-pyridyl-γ-aminobutyric acid. They have interpreted this as indicating that the conversion of nornicotine to norcotinine proceeds via the aminobutyric

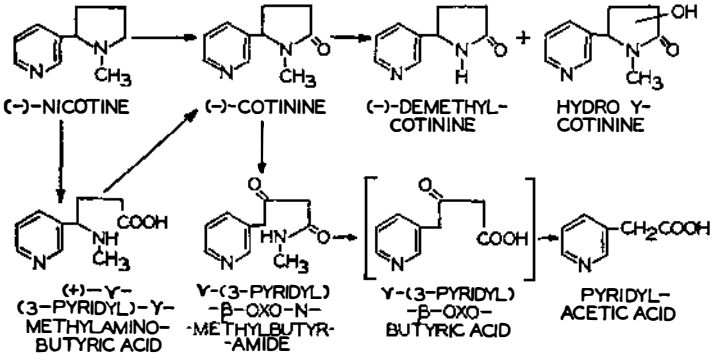

FIG. 4. Metabolism of nicotine.

acid derivative. In addition to providing evidence for the demethylation of nicotine by the rat and dog, McKennis and co-workers (125) have formulated a metabolic scheme for nicotine in the dog which takes into account findings of their own as well as findings of other laboratories (Fig. 4). The more recent studies of Bowman & McKennis (18) which demonstrate the presence of hydroxycotinine and γ-(3-pyridyl)-β-oxo-N-methylbutyramide in the urine of man after administration of (−)-cotinine indicate certain similarities between the dog and the human in the metabolism of this compound. On the other hand, no desmethylcotinine was detected in man.

Domer & Schueler (42) have shown hemicholinium number 3 to be resistant to metabolic alteration by the rat. No labeled respiratory carbon dioxide could be detected following the administration of drug labeled with ^{14}C in the N-methyl groups. Approximately 73 per cent of the administered dose was found in the urine and feces as unchanged drug during the first 24 hr. A rather unconvincing claim has been made for the excretion of two per cent of the dose of atropine by man as tropic acid (75). Little, if any, metabolism of hexamethonium, or its sulfonium or monoquaternary analogues occurs in

the mouse (114). Bretylium appears to be similarly stable since no metabolic products could be detected in the urine of cats or humans which had received the ^{14}C-labeled compound (45). Mayer (121) has investigated the fate of dichloroisoproterenol (β-hydroxy-N-isopropyl-3,4-dichlorophenethylamine), labeled in the β-position with ^{3}H, in dogs and mice. Metabolites were present in large amounts in urine but not in tissues, and were identified as 3,4-dichloromandelic acid and the glucuronide and probably sulfate conjugates of the drug.

Histamine

Lindahl (116) has described the characteristics of an enzyme system in the liver which catalyzes the transfer of the methyl group from S-adenosylmethionine to histamine at the imidazole nitrogen most remote from the side chain. In the heart-lung-liver preparation of the cat a major portion (70 per cent) of injected histamine was metabolized to 1-methylimidzole-4-ylacetic acid (115). In the heart-lung preparation the prominent metabolite was 4-(2-aminoethyl)-1-methylimidzole (methylhistamine), less than four per cent of the injected drug being converted to imidzole-4 (or 5)-ylacetic acid. Both heart and lung tissue *in vitro* methylate histamine. Inoue (88) has provided suggestive evidence for the formation *in vivo* of the N-glucuronides of histamine by man and rabbits, and Johansson *et al.* (91) have indicated that large doses of chlorpromazine have no effect on the pattern of urinary excretion of histamine and its metabolites by man. The greater excretion of endogenous histamine by female than male rats is the result of a difference in the rate of catabolism of the amine and possibly is attributable to the existence in the male of a minor pathway of metabolism which is not present in the female (143).

Oral Hypoglycemics

Nelson (142) has administered tolbutamide to man in combination with alcohol and followed the rate of urinary excretion of its oxidation product, carboxytolbutamide. He presents data which are interpreted as indicating zero order oxidation of the hypoglycemic agent. Lemieux *et al.* (113) have prepared tolbutamide-D^3 with the label in the methyl group of the toluene residue and studied its rate of oxidation to N-*p*-carboxybenzenesulfonyl-N′-*n*-butylurea in man. Since no difference in rate of conversion could be detected between this compound and its nondeuterated analogue it can be concluded that deuteration has no effect on oxidation at this site. In a study of the metabolism of 1-(*p*-acetylbenzenesulfonyl)-3-cyclohexylurea (acetohexamide) in dogs, rabbits, rats and man, Welles and co-workers (194) found only trace amounts of unaltered drug excreted in the urine. All species excreted the compound in the reduced form, 1-(*p*-α-hydroxyethylbenzenesulfonyl)-3-cyclohexylurea.

Chemotherapeutic Agents

Sulfonamides.—Nelson (141) has described the kinetics of excretion and acetylation of sulfathizole in man. Piccinini (146) has found that admin-

istration of sodium bromide or sodium iodide decreased blood concentrations of acetylated sulfonamide in rabbits which had received sulfamethoxypyridazine. These findings may be explained by the observation that sodium bromide also inhibits sulfonamide acetylation by pigeon breast muscle via the CoA pathway. Excretion studies (21) with 5-methyl-3-sulfanilamido-isoxazole indicate the major portion of the drug is eliminated in the urine during the first 24 hr, more than 60 per cent being in the acetylated form.

Antibiotics.—Kelly & Buyske (104) have identified only unaltered drug in the urine of rats and dogs and the feces of rats following intraperitoneal or oral administration of 7-tritio-tetracycline or randomly labeled ^{14}C-tetracycline. Demethylchlortetracycline is similarly resistant to chemical change in the dog since 93 per cent of the activity found in the urine after intravenous administration of drug randomly labeled with ^{14}C was in the unaltered form (105). Administration of equal doses of griseofulvin to male and female rats resulted in higher blood levels of drug in the latter (24). This apparently is due to a greater capacity of the liver of male animals to metabolize the drug. Such sex differences are not observed in the rabbit or the guinea pig.

Anticancer drugs.—Roberts & Warwick (154) have identified the major metabolite of busulfan (Myleran). More than 60 per cent of the radioactivity of the labeled compound injected into rats, mice, and rabbits appeared in the urine in the form of 3-hydroxy-tetrahydrothiophene-1,1-dioxide. The proposed route of metabolism is shown in Figure 5. Myleran (I) reacts with cysteine or a cysteinyl moiety (II) to form a cyclic sulfonium ion (III) which undergoes decomposition to tetrahydrothiophene (IV). This in turn is converted to tetrahydrothiophene-1,1-dioxide (V) and thence to 3-hydroxy-tetrahydrothiophene-1,1-dioxide (VI). While III, IV and V were not isolated, it was shown that S-β-L-alanyl-tetrahydrothiophenium mesylate (VII) and IV and V were metabolized almost entirely to VI.

Eight to 15 per cent of thioTEPA (N,N′,N″-triethylenethiophosphoramide) administered intravenously to dogs was recovered as TEPA in the urine (132). The urinary recovery of TEPA was 24 to 34 per cent of the administered dose. After the administration of labeled sulfur mustard gas (*bis*-β-chloroethyl sulfide-^{35} S) to mice, rats and humans, large amounts of thiodiglycol and *bis*-chloroethyl sulfone were detected in the urine, largely as conjugates (39). It was concluded that the majority of the radioactivity excreted in the urine represented compounds formed from alkylation of the drug rather than metabolites formed by enzymatic action.

2-Chloropurine is the first purine for which simultaneous enzymatic oxidation (xanthine oxidase) along two different pathways has been established. Bergmann, Unger & Kalmus (10) have shown that attack at either carbon atom 6 or 8 proceeds at the same rate (Fig. 6). This phenomenon is ascribed to the mesomeric (+M) effect of the halogen in the 2-position, which exhibits pronounced aromatic properties. Labeled 6-chloropurine, administered parenterally to rats, is excreted in the urine largely as 6-chlorouric acid, an unidentified dehalogenated metabolite and the normal products of purine

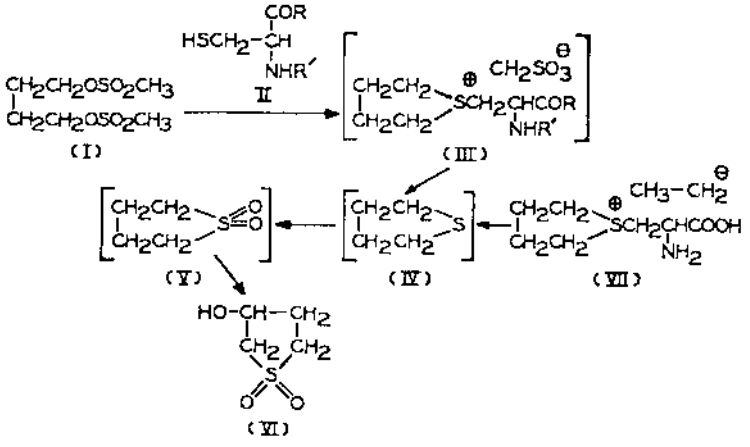

FIG. 5. Metabolism of busulfan.

catabolism (44). When 5-iodooxyuridine was administered to normal and tumor-bearing mice, the main pathway for degradation involved cleavage to 5-iodouracil and subsequent dehalogenation with the formation of uracil and inorganic iodide (152). A similar result was obtained with 5-bromo-2′-deoxycytidine (151). It is also deaminated by murine neoplastic cells and incorporated into NAD in the form of 5-bromo-2′-deoxyuridine-5′-phosphate in place of thymidylic acid (34). Studies of the distribution, excretion, and metabolism of the carcinostatic amino acid derivative, 1-aminocyclopentane-1-carboxylic acid-(^{14}C-carbonyl), in normal and tumor-bearing rats indicated that all of the radioactivity present was in the form of the original compound (164). Methotrexate (4-amino-10-methylfolic acid) is inactivated by the acetylating enzyme system of pigeon liver (111).

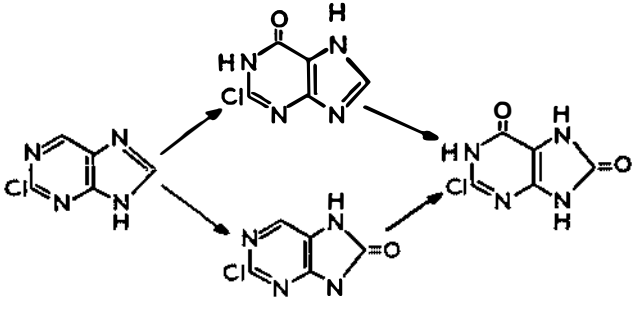

FIG. 6. Oxidation of 2-chloropurine.

Other chemotherapeutic agents.—The work of Yard & McKennis (200), along with previously established findings, indicates the following metabolic pathway for isoniazid (isonicotinylhydrazine) in man and certain other species, such as the rabbit: isonicotinylhydrazine→1-acetyl-2-isonicotinylhydrazine→acetylhydrazine→1,2-diacetylhydrazine. The dog cannot convert hydrazine or acetylhydrazine to 1,2-diacetylhydrazine. In man 75 to 95 per cent of a dose of isoniazid is eliminated via the kidneys within 24 hr (41). The major portion is present as the acetyl derivative of isoniazid (25 to 70 per cent) and isonicotinic acid (20 to 40 per cent). Administration of either pyridoxine or *p*-aminosalicylic acid did not affect this pattern or excretion.

The mono-N-glucuronides of 4,4′-diaminodiphenylsulfone and 4,4′-diaminodiphenyl sulfoxide have been identified in the urine of both the rabbit and man after administration of these drugs (179). There also is evidence that the rabbit converts 4,4′-diaminodiphenylsulfone-N,N′-diglucose sulfonate (Promin) to the N-glucoside of 4,4′-diaminodiphenylsulfone (180). Paul *et al.* (145) showed that nitrofurazone and a number of nitrofurans are metabolized by liver slices from the rat but identified none of the metabolites. In studies employing rabbits and rats Villela (184) has utilized N′-methylnicotinamide as a substrate for an enzyme referred to as quinine oxidase. The enzyme is contained in rabbit plasma but not in rat plasma. Enzyme activity increased in the rabbit after treatment with carbon tetrachloride, presumably due to liberation of the enzyme from the liver into the blood.

Miscellaneous Drugs

N-Methylpyridinium-2-aldoxime is excreted in the urine of rats or humans almost completely unchanged. However, small amounts are metabolized with the release of cyanide, as was evidenced by the increased excretion of thiocyanate after administration of the drug (50). N-Methylpyridinium-2-nitrile was tentatively identified by chromatographic and chemical means and the suggestion was made that this metabolite is the precursor of both thiocyanate and an unidentified metabolite (51). In a more recent study employing rats methyl-labeled N-Methylpyridinium-2-aldoxime iodide (2-PAM) yielded metabolites which were identified chromatographically as N-methylpyridinium-2-nitrile, N-methylpyridinium-2-carboxylic acid and N-methyl-2-pyridone (52). The chemical and physical properties of a metabolite of 2-PAM isolated from human urine suggest that it is a derivative of N-methyl picolinic acid (109).

Coumarin, although not employed as a drug, is of interest because it is the structural nucleus of a number of anticoagulants. Kaighen & Williams (92) have thoroughly investigated the metabolism of [3-^{14}C] coumarin in the rabbit. In Figure 7 are presented Kaighen & Williams findings with respect to the metabolic pathway for this compound along with the relative amounts of each metabolite found in the urine.

Other papers of interest dealing with drug metabolism but which are not

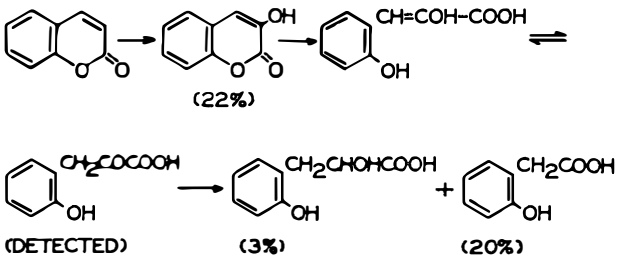

FIG. 7. Metabolism of coumarin.

discussed because of limitations in space include studies on cardiac glycosides (112), sulfobromophthalein (30), castor oil (191), α-methyl dopa and α-methyl metatyrosine (27), diuretically active methylated disulfonamides (117), thioanalogs of phenylbutazone (40), disulfiram (133), colchicine (187) and lidocaine (80).

LITERATURE CITED

1. Abeles, R. H., and Brownstein, A. M., *Biochim. Biophys. Acta*, **44**, 598 (1960)
2. Abeles, R. H., and Lee, H. A., Jr., *J. Biol. Chem.*, **235**, 1499–1503 (1960)
3. Aldridge, W. N., and Parker, V. H., *Biochem. J. (London)*, **76**, 47–56 (1960)
4. Arias, I. M., *Biochem. Biophys. Res. Commun.*, **6**, 81–84 (1961)
5. Axelrod, J., *Science*, **124**, 263–64 (1956)
6. Axelrod, J., *Life Sciences*, **1**, 29–30 (1962)
7. Axelrod, J., and Inscoe J. K., *Proc. Soc. Exptl. Biol. Med.*, **103**, 675–76 (1960)
8. Baker, R. H., Jr., *Biochemistry*, **1**, 41–47 (1962)
9. Beckett, A. H., Casy, A. F., and Harper, N. J., *J. Pharm. Pharmacol.*, **8**, 874–84 (1956)
10. Bergmann, F., Unger, H., and Kalmus, A., *Biochim. Biophys. Acta*, **45**, 49–56 (1960)
11. Bernhard, K., and Beer, H., *Helv. Physiol. Pharmacol. Acta*, **20**, 114–21 (1962)
12. Block, W., and Ebigt, I., *Arzneimittel-Forsch.*, **10**, 709–19 (1960)
13. Block, W., and Ebigt, I., *Arzneimittel-Forsch.*, **10**, 949–52 (1960)
14. Boström, H., and Vestermark, A., *Acta Physiol. Scand.*, **48**, 88–95 (1960)
15. Boström, H., and Vestermark, A., *Biochem. Pharmacol.*, **6**, 72–81 (1961)
16. Bousquet, W. F., *J. Pharm. Sci.*, **51**, 297–309 (1962)
17. Bousquet, W. F., and Christian, J. E., *J. Am. Pharm. Assoc. (Sci. Ed.)*, **49**, 389–93 (1960)
18. Bowman, E. R., and McKennis, H., Jr., *J. Pharmacol. Exptl. Therap.*, **135**, 306–11 (1962)
19. Boyland, E., *Biochem. Pharmacol.*, **6**, 61–64 (1961)
20. Boyland, E., and Booth, J., *Ann. Rev. Pharmacol.*, **2**, 129–42 (1962)
21. Brandman, O., and Engelberg, R., *Current Therap. Res.*, **2**, 364–69 (1960)
22. Brazda, F. G., and Baucum, R., *J. Pharmacol. Exptl. Therap.*, **132**, 295–98 (191)
23. Broadhead, G. D., and Kilpatrick, R., *Biochem. J. (London)*, **76**, 63P (1960)
24. Busfield, D., Child, K. J., Basil, B., and Tomich, E. G., *J. Pharm. Pharmacol.*, **12**, 539–43 (1960)
25. Bush, M. T., Mazel, P., and Chambers, J., *J. Pharmacol. Exptl. Therap.*, **134**, 110–16 (1961)
26. Butler, T. C., *J. Pharmacol. Exptl. Therap.*, **134**, 311–19 (1961)
27. Carlsson, A., and Lindqvist, M., *Acta Physiol. Scand.*, **54**, 87–94 (1962)
28. Clouet, D. H., *Life Sciences*, **1**, 31–34 (1962)
29. Cochin, J., and Sokoloff, L., *Proc.*

Soc. Exptl. Biol. Med., **104**, 504–6 (1960)
30. Combes, B., and Stakelum, G. S., *J. Clin. Invest.*, **39**, 1214–22 (1960)
31. Conney, A. H., and Burns, J. J., *Advan. Pharmacol.*, **1**, 31–58 (1962)
32. Cooper J. R., and Felig, P., *Toxicol. Appl. Pharmacol.*, **3**, 202–9 (1961)
33. Cooper, J. R. and Kini, M. M., *Biochem. Pharmacol.*, **11**, 405–16 (1962)
34. Cramer, J. W., Prusoff, W. H. and Welch, A. D., *Biochem. Pharmacol.*, **8**, 331–35 (1961)
35. Curry, A. S., *Nature*, **188**, 58 (1960)
36. Dajani, R. M., and Orten, J. M., *J. Nutrition*, **76**, 135–42 (1962)
37. Daly, J., Axelrod, J., and Witkop, B., *Ann. N. Y. Acad. Sci.*, **96**, 37–43 (1962)
38. Dalziel, K., *Nature*, **191**, 1098–99 (1961)
39. Davison, C., Rozman, R. S., and Smith, P. K., *Biochem. Pharmacol.*, **7**, 65–74 1961)
40. Dayton, P. G., Sicam, L. E., Landrau, M., and Burns, J. J., *J. Pharmacol. Exptl. Therap.*, **132**, 287–90 (1961)
41. Des Prez, R., and Boone, I. U., *Am. Rev. Respirat. Diseases*, **84**, 42–51 (1961)
42. Domer, F. R., and Schueler, F. W., *J. Am. Pharm. Assoc.*, **49**, 553–56 (1960)
43. Douglas, J. F., Ludwig, B. J., Ginsberg, T., and Berger, F. M., *J. Pharmacol. Exptl. Therap.*, **136**, 5–9 (1962)
44. Duggan, D. E., and Titus, E. *J. Pharmacol. Exptl. Therap.*, **130**, 375–82 (1960)
45. Duncombe, W. G., and McCoubrey, A., *Brit. J. Pharmacol.*, **15**, 260–64 (1960)
46. Dutton, G. J., *Biochem. Pharmacol.*, **6**, 65–71 (1961)
47. Dutton, G. J., and Stevenson, I. H., *Biochim. Biophys. Acta*, **58**, 633 (1962)
48. Elison, C., Rapoport, H., Laursen, R., and Elliott, H. W., *Science*, **134**, 1078–79 (1961)
49. Emmerson, J. L., Miya, T. S., and Yim, G. K. W., *J. Pharmacol. Exptl. Therap.*, **129**, 89–93 (1960)
50. Enander, I., Sundwall, A., and Sörbo, B., *Biochem. Pharmacol.*, **7**, 226–31 (1961)
51. Enander, I., Sundwall, A., and Sörbo, B., *Biochem. Pharmacol.*, **7**, 232–36 (1961)
52. Enander, I., Sundwall, A., and Sörbo, B., *Biochem. Pharmacol.*, **11**, 377–82 (1962)
53. Fernandez, V. P., Mahler H. R., and Shiner, V. J., Jr., *Biochemistry*, **1**, 259–62 (1962)
54. Fischer, H. D., *Biochem. Pharmacol.*, **11**, 307–14 (1962)
55. Fischer, H. D., and Oelssner, W., *Med. Exptl.*, **3**, 213–18 (1960)
56. Fischer, H.-D., and Oelssner, W., *Klin. Wochschr.*, **39**, 1265 (1961)
57. Fishman, V., and Goldenberg, H., *Proc. Soc. Exptl. Biol. Med.*, **104**, 99–103 (1960)
58. Fishman, V., and Goldenberg, H., *Proc. Soc. Exptl. Biol. Med.*, **110**, 187–90 (1962)
59. Fishman, V., Heaton, A., and Goldenberg, H., *Proc. Soc. Exptl. Biol. Med.*, **109**, 548–52 (1962)
60. Forsander, O. A., and Räihä, N. C. R., *J. Biol. Chem.*, **235**, 34–36 (1960)
61. Fouts, J. R., *Biochem. Biophys. Res. Commun.*, **6**, 373–78 (1961)
62. Fouts, J. R., Dixon, R. L., and Shultice, R. W., *Biochem. Pharmacol.*, **7**, 265–70 (1961)
63. Frey, H. H., *Naturwissenschaften*, **47**, 471–72 (1960)
64. Frey, H. H., *Arch. Intern. Pharmacodyn.*, **134**, 175–88 (1961)
65. Frey, H. H., Doenicke, A., and Jäger, G., *Med. Exptl.*, **4**, 243–50 (1961)
66. Friedhoff, A. J., and Goldstein, M., *Ann. N. Y. Acad. Sci.*, **96**, 5–13 (1962)
67. Friedman, P. J., and Cooper, J. R., *J. Pharmacol. Exptl. Therap.*, **129**, 373–76 (1960)
68. Gal, E. M., Drewes, P. A., and Taylor, N. F., *Arch. Biochem. Biophys.*, **93**, 1–14 (1961)
69. Gaudette, L. E., and Brodie, B. B., *Biochem. Pharmacol.*, **2**, 89–96 (1959)
70. Gessner, P. K., Khairallah, P. A., McIsaac, W. M., and Page, I. H., *J. Pharmacol. Exptl. Therap.*, **130**, 126–33 (1960)
71. Gillette, J. R., Dingell, J. V., Sulser, F., Kuntzman, R., and Brodie B. B., *Experientia*, **17**, 417–18 (1961)
72. Gillette, J. R., and Kamm, J. J., *J. Pharmacol. Exptl. Therap.*, **130**, 262–67 (1960)
73. Glasson, B., and Benakis, A., *Helv. Physiol. Pharmacol. Acta*, **19**, 323–34 (1961)
74. Goldberg, M., Hehir, R., and Hurowitz, M., *New Engl. J. Med.*, **263**, 1336–39 (1961)

75. Gosselin, R. E., Gabourel, J. D., and Wills, J. H., *Clin. Pharmacol. Therap.*, **1**, 597–603 (1960)
76. Hake, C. L., Waggoner, T. B., Robertson, D. N., and Rowe, V. K., *Archives Environ. Health*, **1**, 101–5 (1960)
77. Harper, K. H., and Calcutt, G., *Nature*, **192**, 165–66 (1961)
78. Haynes, E. E., *J. Lab. Clin. Med.*, **56**, 570–75 (1960)
79. Herrmann, B., and Pulver, R., *Arch. Intern. Pharmacodyn.*, **126**, 454–69 (1960)
80. Hollunger, G., *Acta Pharmacol. Toxicol.*, **17**, 356–64 (1960)
81. Hollunger, G., *Acta Pharmacol. Toxicol.*, **17**, 365–73 (1960)
82. Hollunger, G., *Acta Pharmacol. Toxicol.*, **17**, 374–83 (1960)
83. Hollunger, G., *Acta Pharmacol. Toxicol.*, **17**, 384–89 (1960)
84. Horita, A., and Weber, L. J., *Biochem. Pharmacol.*, **7**, 47–54 (1961)
85. Hucker, H. B., Gillette, J. R., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **129**, 94–100 (1960)
86. Ikeda, M., Murakami, H., and Nishio, M., *Arch. Biochem. Biophys.*, **93**, 670–71 (1961)
87. Imai, Y., and Sato, R., *Biochim. Biophys. Acta*, **42**, 164–65 (1960)
88. Inoue, T., *J. the Pharm. Soc. Japan*, **80**, 831–37 (1960)
89. Isselbacher, K. J., *Biochem. Biophys. Res. Commun.*, **5**, 243–46 (1961)
90. Jackson J. V., and Moss, M. S., *Nature*, **192**, 553–54 (1961)
91. Johansson, M. B., Lindell, S. E., Roos, B. E., and Westling, H., *Med. Exptl.*, **4**, 239–42 (1961)
92. Kaighen, M., and Williams, R. T., *J. Med. Pharm. Chem.*, **3**, 25–43 (1961)
93. Kalant, H., *Quart. J. Studies Alc.*, **23**, 52–86 (1962)
94. Kalberer, F., Kreis, W., and Rutschmann, J., *Biochem. Pharmacol.*, **2**, 261–69 (1962)
95. Kato, R., *Arzneimittel-Forschung*, **11**, 797–98 (1961)
96. Kato, R., and Chiesara, E., *Brit. J. Pharmacol.*, **18**, 29–38 (1962)
97. Kato, R., Chiesara, E., and Frontino, G., *Jap. J. Pharmacol.*, **11**, 31–36 (1961)
98. Kato, R., Chiesara, E., and Frontino, G., *Biochem. Pharmacol.*, **11**, 221–27 (1962)
99. Kato, R., Chiesara, E., and Vassanelli, P., *Med. Exptl.* **4**, 387–92 (1961)
100. Kato, R., Chiesara, E., and Vassanelli, P., *Biochem. Pharmacol.*, **11**, 211–20 (1962)
101. Kato, R., Frontino, G., and Vassanelli, P., *Jap. J. Pharmacol.*, **11**, 25–30 (1961)
102. Kato, R., Vassanelli, P., and Frontino, G., *Experientia*, **18**, 9–10 (1962)
103. Keberle, H., Hoffmann, K., and Bernhard, K., *Experientia*, **18**, 1–18 (1962)
104. Kelly, R. G., and Buyske, D. A., *J. Pharmacol. Exptl. Therap.*, **130**, 144–49 (1960)
105. Kelly, R. G., Kanegis, L. A., and Buyske, D. A., *J. Pharmacol. Exptl. Therap.*, **134**, 320–24 (1961)
106. Kendal, L. P., and Ramanathan, A. N., *Biochem. J.* (*London*), **52**, 430–38 (1952)
107. Kini, M. M., and Cooper, J. R., *Biochim. Biophys. Acta*, **44**, 599–601 (1960)
108. Kini, M. M., and Cooper, J. R., *Biochem. Pharmacol.*, **8**, 207–15 (1961)
109. Kramer, B., *Biochem. Pharmacol.*, **11**, 299–305 (1962)
110. Krisch K., and Staudinger, H., *Biochem. Z.*, **334**, 312–27 (1961)
111. Krishnamurthy, V., and Braganca, B. M., *Nature*, **192**, 68–69 (1961)
112. Lauterbach, F., and Repke, K., *Arch. Exptl. Pathol. Pharmakol.*, **239**, 196–218 (1960)
113. Lemieux, R. U., Sporek, K. F., O'Reilly, I., and Nelson, E., *Biochem. Pharmacol.*, **7**, 31–34 (1961)
114. Levine, R. R., *J. Pharmacol. Exptl. Therap.*, **129**, 296–304 (1960)
115. Lilja, B., and Lindell, S.-E., *Brit. J. Pharmacol.*, **16**, 203–8 (1961)
116. Lindahl, K. M., *Acta Physiol. Scand.*, **49**, 114–38 (1960)
117. Logemann, W., and Giraldi, P. N., *Brit. J. Pharmacol.*, **18**, 61–64 (1962)
118. MacLeod, L. D., *Biochem. J.* (*London*), **77**, 10P–11P (1960)
119. Mahler, H. R., Baker, R. H., Jr., and Shiner, V. J., Jr., *Biochemistry*, **1**, 47–52 (1962)
120. Mannering, G. J., Parks, R. E., Jr., and Tephly, T. R., *Biochem. Pharmacol.*, **11**, 677–78 (1962)
121. Mayer, S. E., *J. Pharmacol. Exptl. Therap.*, **135**, 204–12 (1962)
122. Maynert, E. W., *J. Pharmacol. Exptl. Therap.* **130**, 275–84 (1960)
123. Maynert, E. W., *Ann. Rev. Pharmacol.*, **1**, 45–64 (1961)
124. McKennis, H., Jr., Bowman, E. R.,

and Turnbull, L. B., *J. Am. Chem. Soc.*, **82**, 3974–76 (1960)
125. McKennis, H., Jr., Turnbull, L. B., Schwartz, S. L., Tamaki, E., and Bowman, E. R., *J. Biol. Chem.*, **237**, 541–46 (1962)
126. McKennis, H., Jr., Wada, E., Bowman, E. R., and Turnbull, L. B., *Nature*, **190**, 910–11 (1961)
127. McMahon, R. E., *J. Med. Pharm. Chem.*, **4**, 67–78 (1961)
128. McMahon, R. E., *J. Pharmacol. Exptl. Therap.*, **130**, 383–88 (1960)
129. McMahon, R. E., and Easton, N. R., *J. Pharmacol. Exptl. Therap.*, **135**, 128–33 (1962)
130. McMahon, R. E., and Mills, J., *J. Med. Pharm. Chem.*, **4**, 211–13 (1961)
131. McMahon, R. E., Welles, J. S., and Lee, H. M., *J. Am. Chem. Soc.*, **82**, 2864–66 (1960)
132. Mellett, L. B., and Woods, L. A., *Cancer Res.*, **20**, 524–32 (1960)
133. Merlevede, E., and Casier, H., *Arch. Intern. Pharmacodyn.*, **132**, 427–53 (1961)
134. Miller, J. A., Wyatt, C. S., Miller, E. C., and Hartman, H. A., *Cancer Res.*, **21**, 1465–73 (1961)
135. Miller, L. D., *Toxicol. Appl. Pharmacol.*, **4**, 190–99 (1962)
136. Misra, A. L., Jacoby, H. I., and Woods, L. A., *J. Pharmacol. Exptl. Therap.*, **132**, 311–16 (1961)
137. Misra, A. L., Mulé, S. J., and Woods, L. A., *Nature*, **190**, 82–83 (1961)
138. Misra, A. L., Mulé, S. J., and Woods, L. A., *J. Pharmacol. Exptl. Therap.*, **132**, 317–28 (1961)
139. Murata, T., *Chem. Pharm. Bull.*, **9**, 334 (1961)
140. Murata, T., *Chem. Pharm. Bull.*, **9**, 335 (1961)
141. Nelson, E., *J. Pharm. Sci.*, **50**, 912–15 (1961)
142. Nelson, E., *Nature*, **193**, 76–77 (1962)
143. Netter, K. J., Cohn, V. H., Jr., and Shore, P. A., *Am. J. Physiol.*, **201**, 224–26 (1961)
144. Newman, H., and Smith, M. E., *Nature*, **183**, 689–90 (1959)
145. Paul, H. E., Ells, V. R., Kopko, F., and Bender, R. C., *J. Med. Pharm. Chem.*, **2**, 563–84 (1960)
146. Piccinini, F., *Arch. Intern. Pharmacodyn.*, **125**, 251–64 (1960)
147. Pisano, J. J., Oates, J. A., Jr., Karmen, A., Sjoerdsma, A., and Udenfriend, S., *J. Biol. Chem.*, **236**, 898–901 (1961)
148. Plane, R. A., and Theorell, H., *Acta Chem. Scand.*, **15**, 1866–74 (1961)
149. Posner, H. S., Mitoma, C., Rothberg, S., and Udenfriend, S., *Arch. Biochem. Biophys.*, **94**, 280–90 (1961)
150. Posner, H. S., Mitoma, C., and Udenfriend, S., *Arch. Biochem. Biophys.*, **94**, 269–79 (1961)
151. Prusoff, W. H., Cramer, J. W., Chu, M. Y., and Welch, A. D., *Biochem. Pharm.*, **8**, 324–26 (1961)
152. Prusoff, W. H., Jaffe, J. J., and Günther, H., *Biochem. Pharm.*, **3**, 110–12 (1960)
153. Redetzki, H. M., *Texas Rept. Biol. Med.*, **18**, 83–92 (1960)
154. Roberts, J. J., and Warwick, G. P., *Biochem. Pharmacol.*, **6**, 217–27 (1961)
155. Schaumann, O., *Arch. Exptl. Pathol. Pharmakol.*, **239**, 311–20 (1960)
156. Scheline, R. R., Williams, R. T., and Witt, J. G., *Nature*, **188**, 849–50 (1960)
157. Schulman, M. P., Zurek, R., and Westerfeld, W. W., *Alcoholism, Basic Aspects and Treatment*, 29–38 (Am. Assoc. Advan. Sci., Washington, D. C., 212 pp., 1957)
158. Schwartz, M. A., *J. Pharmacol. Exptl. Therap.*, **130**, 157–65 (1960)
159. Schwartz, M. A., *Proc. Soc. Exptl. Biol. Med.*, **107**, 613–16 (1961)
160. Sjoerdsma, A., Lovenberg, W., Oates, J. A., Crout, J. R., and Udenfriend, *Science*, **130**, 225 (1959)
161. Smith, M. E., *Nature*, **190**, 273–74 (1961)
162. Smith, M. E., *J. Pharmacol. Exptl. Therap.*, **134**, 233–37 (1961)
163. Smith, M. E., and Newman, H. W., *Proc. Soc. Exptl. Biol. Med.*, **104**, 282–84 (1960)
164. Sterling, W. R., Henderson, J. F., Mandel, H. G., and Smith, P. K., *Biochem. Pharmacol.*, **11**, 135–45 (1962)
165. Stevenson, I. H., and Dutton, G. J., *Biochem. J. (London)*, **82**, 330–40 (1962)
166. Takemori, A. E., *J. Pharmacol. Exptl. Therap.*, **130**, 370–74 (1960)
167. Tephly, T. R., Mannering, G. J., and Parks, R. E., Jr., *Federation Proc.*, **20**, 65 (1961)
168. Tephly, T. R., Mannering, G. J., and Parks, R. E., Jr. *J. Pharmacol. Exptl. Therap.*, **134**, 77–82 (1961)
169. Tephly, T. R., Parks, R. E., Jr., and Mannering, G. J., *J. Pharmacol. Exptl. Therap.*, **131**, 147–51 (1961)
170. Tephly, T. R., Parks, R. E., Jr., and

Mannering, G. J., *Federation Proc.*, **21**, 45 (1962)
171. Theorell, H., and Chance, B., *Acta Chem. Scand.*, **5**, 1127–44 (1951)
172. Theorell, H., and McKinley-McKee, J. S., *Acta Chem. Scand.*, **15**, 1797–1810 (1961)
173. Theorell, H., and McKinley-McKee, J. S., *Acta Chem. Scand.*, **15**, 1811–33 (1961)
174. Theorell, H., and McKinley-McKee, J. S., *Acta Chem. Scand.*, **15**, 1834–65 (1961)
175. Theorell, H., and McKinley-McKee, J. S., *Nature*, **192**, 47–50 (1961)
176. Touster, O., Hester, R. W., and Siler, R. A., *Biochem. Biophys. Res. Commun.*, **3**, 248–52 (1960)
177. Touster, O., and Hollmann, S., *Ann. N. Y. Acad. Sci.*, **92**, 318–23 (1961)
178. Treble, D. H., *Biochem. J. (London)*, **82**, 129–34 (1962)
179. Tsutsumi, S., *Chem. Pharm. Bull.*, **9**, 432–36 (1961)
180. Tsutsumi, S., *Chem. Pharm. Bull.*, **9**, 437–41 (1961)
181. Vane, J. R., Wolstenhome, G. E. W., and O'Connor, M., *Adrenergic Mechanisms*, 6–56 (Little, Brown and Company, Boston, Mass., 632 pp., 1960)
182. Vestermark, A., and Boström, H., *Exptl. Cell Res.*, **18**, 174–77 (1959)
183. Vestermark, A., and Boström, H., *Acta Chem. Scand.*, **13**, 2133 (1960)
184. Villela, G. G., *Nature*, **190**, 807–8 (1961)
185. Wada, E., Bowman, E. R., Turnbull, L. B., and McKennis, H., Jr., *J. Med. Pharm. Chem.*, **4**, 21–30 (1961)
186. Waddell, W. J., and Butler, T. C., *J. Pharmacol. Exptl. Therap.*, **132**, 291–94 (1961)
187. Walaszek, E. J., Kocsis, J. J., LeRoy, G. V., and Geiling, E. M. K., *Arch. Intern. Pharmacodyn.*, **125**, 371–82 (1960)
188. Walkenstein, S. S., Knebel, C. M., MacMullen, J. A., and Seifter, J. *J. Pharmacol. Exptl. Therap.*, **123**, 254–58 (1958)
189. Wartburg, J. P., Von, and Eppenberger, H. M., *Helv. Physiol. Pharmacol. Acta*, **19**, 303–22 (1961)
190. Wartburg, J. P., Von, and Rothlisberger, M., *Helv. Physiol. et Pharmacol. Acta*, **19**, 30–41 (1961)
191. Watson, W. C., and Gordon, R. S., Jr., *Biochem. Pharmacol.*, **11**, 229–36 (1962)
192. Way, E. L., and Adler, T. K., *Pharmacol. Rev.*, **12**, 383–446 (1960)
193. Way, E. L., Kemp, J. W., Young, J. M., and Grassetti, D. R., *J. Pharmacol. Exptl. Therap.*, **129**, 144–54 (1960)
194. Welles, J. S., Root, M. A., and Anderson, R. C., *Proc. Soc. Exptl. Biol. Med.*, **107**, 583–85 (1961)
195. Williams, R. T., *Detoxication Mechanisms*, 2nd ed. (Chapman & Hall, Ltd. London, 796 pp., 1959)
196. Winer, A. D., *Acta Chem. Scand.*, **12**, 1695–96 (1958)
197. Winer, A. D., and Theorell, H., *Acta Chem. Scand.*, **14**, 1729–42 (1960)
198. Witter, A., *Acta Chem. Scand.*, **14**, 1717–28 (1960)
199. Woronick, C. L., *Acta Chem. Scand.*, **15**, 2062–64 (1961)
200. Yard, A. S., and McKennis, H., Jr., *J. Med. Pharm. Chem.*, **5**, 196–203 (1962)

CONTENTS