Reprinted from PHYSIOLOGICAL REVIEWS
Vol. 39, No. 4, 1959
Printed in U.S.A.

Metabolism of Epinephrine and Other Sympathomimetic Amines

JULIUS AXELROD

Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

THE PHYSIOLOGICAL ACTIONS of epinephrine and other sympathomimetic amines are well established, yet the metabolism of these compounds is poorly understood. Although several enzymes have been shown to transform these amines *in vitro*, the part that they play in the intact organism has been conjectural. In the past few years considerable information concerning the fate of sympathomimetic amines has become available. Many new enzymes have been found that are of importance in the transformation of these amines. This review will describe these newer developments in the metabolism and physiological disposition of catecholamine hormones and related compounds of therapeutic interest.

METABOLISM OF CATECHOLAMINES

When epinephrine or norepinephrine is administered to man (65, 66) and other species (131) only a small percentage of these compounds is excreted unchanged, indicating that they undergo almost complete transformation in the body. Many pathways have been proposed for the metabolism of catecholamines *in vivo*, but until recently, no convincing evidence has been presented showing to what extent, if any, they are involved in the transormation of exogenous or endogenous catecholamine hormones. In the past, the routes postulated for the metabolism of these amines included deamination, oxidation and conjugation. Some of these pathways will be dealt with only briefly, since they have been extensively covered in past reviews (23, 27, 30, 84).

Oxidation

In 1938, Green and Richter (79) demonstrated that cytochrome oxidase can convert epinephrine to adrenochrome. Because of the widespread occurrence of this enzyme, it was assumed that it also metabolizes epinephrine in the body. However, Schayer has shown that the formation of adrenochrome from epinephrine in the body is unlikely (136). He observed that after the administration of C^{14}-adrenochrome to rats, the urine contained an intensely colored yellow pigment which showed a smear of radioactivity on paper chromatograms, with no distinct peaks (136). After the administration of C^{14}-epinephrine, five radioactive peaks were found (135). This dissimilarity in chromatographic behavior suggested that adrenochrome was not a metabolite of epinephrine. The presence of adrenochrome in the plasma of man was reported by Hoffer (86). Using a specific and sensitive method, Szara *et al.* failed to confirm this (148). In man, over 90 per cent of ad-

ministered epinephrine can be accounted for by a number of metabolites, none of which have been identified as adrenochrome (102).

The dangers of translating *in vitro* enzyme activity to metabolic events occurring in the intact animal might be pointed out at this time. Cytochrome oxidase metabolizes catecholamines *in vitro* more rapidly than any known enzyme (Axelrod, unpublished observations) but in the body it attacks catecholamines to a negligible extent. The question of how closely the results of controlled *in vitro* reactions parallel reactions in the body depends not only on enzyme activity, but on other competing reactions, localization and degree of binding of substrates, affinity of substrate for the enzyme, naturally occurring inhibitors, and passage of the compound across many membrane barriers to the intracellular site of metabolism.

Imaizumi and co-workers have described an enzyme, epinephrine dehydrogenase, in rabbit plasma that reversibly oxidizes epinephrine to adrenalone, diphosphopyridine nucleotide serving as the hydrogen acceptor (90, 91). These investigators have also reported that after the administration of epinephrine to guinea pigs, compounds were found in the urine that had the same R_F on chromatograms as adrenalone, protocatechuic aldehyde, protocatechuic acid and norepinephrine (99). Treatment of guinea pigs with iproniazid resulted in an increased excretion of adrenalone. These findings indicated that the guinea pig not only oxidized epinephrine to adrenalone, but also demethylated it to norepinephrine. Weil-Malherbe and Bone did not succeed in finding the epinephrine dehydrogenase reported above (160). They have looked for this enzyme in rabbit and human plasma, and in extracts of rat liver, heart, spleen and brain. Using β H^3-epinephrine, no evidence for the formation of adrenalone or norepinephrine has been found (13, 102).

Conjugation

Richter ingested large amounts of epinephrine, 3,4-dihydroxyephedrine, and epinine and noted that these catecholamines were excreted as sulfo-conjugates (124, 125). These observations were confirmed in dogs (152). After the oral administration of epinephrine to rabbits, it was excreted mostly as epinephrine glucosiduronic acid. On the basis of these results, it was concluded that conjugation constituted an important pathway for the metabolism of catecholamines. According to more recent studies, the intravenous administration of physiological doses of catecholamines led to the excretion of only small amounts of conjugated catecholamines (65, 66, 131). It is now generally believed that conjugation plays a minor role in the inactivation of physiological amounts of catecholamines. In the earlier experiments, the massive amounts of the catecholamines given may have overwhelmed the normal enzymatic mechanisms. Furthermore, after oral ingestion, the intestinal flora may have conjugated these amines before they were absorbed.

Deamination by Monoamine Oxidase

A number of investigators (31a, 116, 122) have demonstrated that epinephrine serves as a substrate for monoamine oxidase. Since this observation was made,

monoamine oxidase has been considered by many to be the enzyme chiefly concerned with the metabolism of epinephrine and other catecholamines in the intact organism (see refs. 30 and 53 for reviews on this subject). Burn (41) postulated that monoamine oxidase acts at the sympathetic nerve endings in the same manner as acetylcholine esterases in the parasympathetic system. This assumption rested mainly on the fact that monoamine oxidase is present in the autonomic nervous system and that after denervation of the nictitating membrane, iris and blood vessels in cats, a decrease in the enzyme was found in these tissues (43). A correlation between the fall in enzyme activity and increased sensitivity to norepinephrine was also observed. Armin *et al.* (3), however, showed that after sectioning of the sympathetic nerves amine oxidase persisted undiminished, while acetylcholine esterase activity was markedly reduced. They attributed the hypersensitivity to epinephrine after denervation to a reduction of acetylcholine esterase rather than amine oxidase. Subsequent work of Burn *et al.* (42) did not confirm the previous findings of Burn and Robinson (43) that degeneration of sympathetic nerves results in a fall in monoamine oxidase activity. In contrast to the high localization of acetylcholine esterase in cholinergic fibers there appears to be no selective association of monoamine oxidase with adrenergic nerves (101).

The introduction of the potent monoamine oxidase inhibitors iproniazid (165) and choline-*p*-tolyl ether (40) made possible a reevaluation of the role of monoamine oxidase in the metabolism of catecholamine hormones. In an important experiment Griesemer *et al.* (80) found that inhibition of monoamine oxidase *in vivo* markedly prolonged the contraction of the cat's nictitating membrane produced by phenylethylamine and tyramine, but no prolongation of the actions of administered epinephrine was observed. Following this report a number of investigators confirmed these findings and reported that the *in vivo* inhibition of monoamine oxidase had no effect on a variety of actions of epinephrine and norepinephrine (24, 42, 52, 120). Moreover, Kamijo *et al.* (95) showed that any potentiating action of iproniazid on the effects of adrenergic nerve stimulation and of injected epinephrine were unrelated to its ability to inhibit monoamine oxidase. Exposure of isolated strips of rabbit aorta to iproniazid until monoamine oxidase was completely inhibited neither potentiated nor depressed the response of strips to epinephrine or norepinephrine, yet in these preparations the response to tyramine was potentiated (72). The administration of monoamine oxidase inhibitors also increased the excretion of administered tyramine (132), tryptamine (137), and serotonin (52) but had little or no effect on the excretion (52, 71) or rate of disappearance (48, 71) of administered catecholamines.

Stimulation of postganglionic sympathetic fibers to the spleen elevated the norepinephrine concentration of the venous blood leaving the spleen, but monoamine oxidase inhibitors did not affect this increase (39). It was also noted that the adrenergic blocking agents Dibenamine and Dibenzyline increased the norepinephrine concentration in venous blood after sympathetic stimulation. As a result of these observations, it was suggested that the receptors inactivate norepinephrine, and that adrenergic blocking agents not only antagonize the actions of sympathomimetic amines, but also prevent the receptors from metabolizing

the amines. Von Euler and co-workers (63) found that the administration of monoamine oxidase inhibitors had no effect on the catecholamine content of cat tissues. All these observations strongly suggest that the metabolism of administered as well as endogenous catecholamines is unaffected by the inhibition of monoamine oxidase.

Evidence for the participation of monoamine oxidase in the metabolism of norepinephrine in the central nervous system has been presented by Brodie and co-workers (141, 145). After the administration of iproniazid to rabbits, a threefold increase in brain norepinephrine was noted. In addition, pretreatment with iproniazid completely blocked the metabolism of amines released by reserpine. From these observations, it was proposed that monoamine oxidase is the enzyme chiefly responsible for the transformation of norepinephrine in the nervous system. Carlsson *et al.* reported that iproniazid treatment elevated dopamine (46) but not norepinephrine (47) in the rabbit brain. Treatment with monoamine oxidase inhibitors did not increase the norepinephrine content in the brain of dogs and cats (63, 144, 156). These conflicting findings might be due to species differences in the metabolism of catecholamines in the central nervous system.

Using radioactive epinephrine, Schayer and co-workers demonstrated that monoamine oxidase was involved at some step in the metabolism of epinephrine (136). After the administration to rats of C^{14}-epinephrine labeled on the β carbon, almost all of the radioactivity was found in the urine (130), but when N-methyl C^{14}-epinephrine was injected, only 50 per cent of the radioactivity was excreted (136). However, rats receiving N-methyl C^{14}-epinephrine after treatment with iproniazid excreted almost all of the radioactivity. Similar results were obtained with epinephrine in man (121) and with norepinephrine in rats (134). It was concluded that iproniazid blocks the cleavage between the β carbon and methyl carbon, presumably by inhibiting deamination, and that half of the administered catecholamines are metabolized by monoamine oxidase (136). Whether deamination occurred on the epinephrine molecule itself, or an amine-containing metabolite, was not established.

When the urine of rats that received β C^{14}-epinephrine was subjected to paper chromatography, five radioactive metabolites retaining the β carbon were found (130). Except for epinephrine, none of these compounds was identified. Paper chromatograms of urine of rats to which N-methyl C^{14}-epinephrine was given showed only three radioactive metabolites, one of which was epinephrine (135). From these experiments it appeared that epinephrine formed two major metabolites that retained the methyl amino group and two in which this group was removed.

Metabolism of Dopa and Dopamine In Vivo

The discovery of a heretofore unrecognized pathway for catecholamines stemmed from the observation of MacLaglan and Wilkinson (107), who found that phenolic compounds are O-methylated in the body. DeEds and co-workers also demonstrated that ingested catechol flavanoids were excreted as 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, ref. 33). Following this, Shaw

et al. (140) and DeEds *et al.* (54) independently reported that homovanillic acid and 3,4-dihydroxyphenylacetic acid were metabolites of dihydroxyphenylalanine (dopa). Much smaller amounts of these compounds were formed from D- than from L-dopa (140). Armstrong *et al.* also identified homovanillic acid as a normal constituent of human and rat urine (5). The latter compound was proposed as a metabolite of endogenous L-dopa. Von Euler *et al.* have also detected a compound chromatographically identical with dihydroxyphenylacetic acid in human urine (58). This compound has been reported to be present in plasma, vascular wall (64), splenic nerve (62) and brain (110). In rabbits and rats DeEds *et al.* (54) found that L-dopa is also transformed to *m*-hydroxyphenylacetic acid. The latter reaction, which involves the removal of a hydroxyl group from an aromatic nucleus, is a new type of metabolic transformation. Shaw *et al.* reported that dehydroxylation of 3,4-dihydroxyphenylacetic acid did not occur to any significant extent in man or rat (140).

Many years ago, Holtz *et al.* showed that dopa is converted to dopamine by the enzyme dopadecarboxylase (88). These investigators, using bioassay techniques, also found that man and animals excreted large amounts of dopamine after the administration of dopa (87), and that dopamine is a normal constituent of human urine. More recently, Pellerin and D'Iorio (114) identified free and conjugated dopamine in the urine when C^{14} dopa was given to rats. In addition, 3,4-dihydroxyphenylpyruvic acid, as well as two unidentified metabolites, were also present. Since L-dopa can participate in transamination reactions (44), it appears likely that the pyruvic acid derivative arises from this type of transformation.

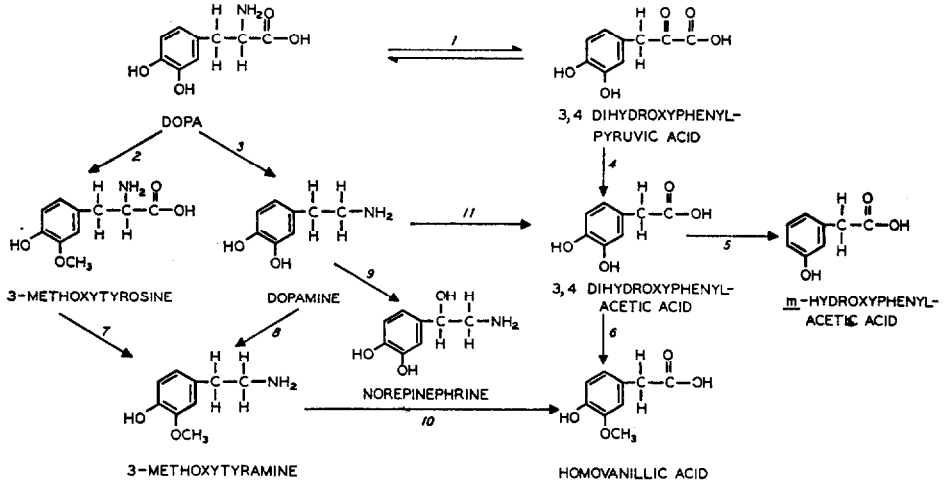

FIG. 1. Metabolism of dopa. *1:* This reaction is presumably catalyzed by a transaminase. *3, 7:* Dopadecarboxylase and pyridoxal are required for *step 3* and possibly *step 7*. *2, 6, 8:* Catechol-O-methyl transferase catalyzes these reactions in the presence of S-adenosylmethionine and a divalent cation. Only *reaction 8* has been shown to occur *in vivo*. *4:* This step has never been demonstrated *in vivo*. *5:* This interesting pathway is found in certain species. The enzyme catalyzing this step has not yet been described. *10, 11:* These reactions are catalyzed by monoamine oxidase and aldehyde dehydrogenase. *9:* The enzyme concerned with this reaction is not well characterized.

According to Shaw *et al.* (140), deamination precedes O-methylation in the formation of homovanillic acid from dopa. However, after the administration of dopamine to rats, Axelrod *et al.* found free and conjugated 3-methoxytyramine as well as homovanillic acid in the urine (18). Pretreating the animals with iproniazid caused a fivefold increase in the excretion of 3-methoxytyramine (18). These observations indicate that catecholamines are O-methylated prior to deamination, and that 3-methoxytyramine is deaminated to homovanillic acid by monoamine oxidase. Goldstein *et al.* (77) found that the administration of dopamine to rats results in the excreton of normetanephrine (3-O-methyl norepinephrine). Since dopa is O-methylated *in vitro* (20), the possibility of the formation of 3-methoxytyrosine should also be considered.

The routes of metabolism of dopa are summarized in figure 1.

O-Methylation of Epinephrine and Norepinephrine In Vivo

In a recent study, Armstrong *et al.* reported that 3-methoxy-4-hydroxymandelic acid is a normal constituent of human urine (4). The excretion of this compound was increased after the administration of norepinephrine or 3,4-dihydroxymandelic acid, and in subjects with pheochromocytomas. It was concluded by these investigators that 3-methoxy-4-hydroxymandelic acid is an important metabolic product of norepinephrine and that deamination of norepinephrine precedes O-methylation. Axelrod (11) found that metanephrine (3-O-methylepinephrine) and normetanephrine (3-O-methylnorepinephrine), in free and conjugated form, occur normally in rat urine, and that the excretion of these compounds was markedly elevated when epinephrine and norepinephrine were given to rats. These results demonstrated that the catecholamines undergo O-methylation before deamination.

The extent to which O-methylation of epinephrine occurs was examined after intraperitoneal injection of physiological amounts of β H^3-epinephrine to rats (13, 16). After collecting the urine for 24 hours, the O-methylated metabolites were extracted into organic solvents (18). About 87 per cent of the administered radioactivity appeared in the urine, 55 per cent of which was identified as free metanephrine and metanephrine glucosiduronic acid. Twelve per cent of the radioactivity was present as 3-methoxy-4-hydroxymandelic acid and only traces as 3,4 dihydroxymandelic acid. After the administration of large amounts of epinephrine or metanephrine to rats, smaller percentages of metanephrine (free and conjugated) appeared in the urine (16).

To study the sequential steps in the biotransformation of epinephrine, metanephrine was given to rats and its metabolic fate examined (13, 16). About the same amounts of metanephrine (free and conjugated) and 3-methoxy-4-hydroxymandelic acid were excreted after metanephrine as were found after epinephrine, indicating that 3-methoxy-4-hydroxymandelic acid is derived from metanephrine rather than 3,4-dihydroxymandelic acid. When rats were pretreated with iproniazid, virtually all the administered epinephrine or metanephrine was excreted as free and conjugated metanephrine. The metanephrine excretion increased at the expense of 3-methoxy-4-hydroxymandelic acid. A major metabolic product found

after the administration of norepinephrine to rats was normetanephrine (16). Pretreatment with iproniazid resulted in a twofold increase in the excretion of normetanephrine. These observations provided strong evidence that, in rodents, O-methylation is the first step in the metabolism of catecholamines *in vivo*, and that monoamine oxidase is concerned in the deamination of the O-methyl amines.

Normetanephrine and metanephrine were found to occur normally in adrenal gland, spleen (18), pheochromocytoma tumors (142) and urine (18, 103). Normal occurrence of O-methylated amines in tissues rich in epinephrine and norepinephrine as well as in urine would indicate that the endogenous catecholamines are also O-methylated. However, the extent to which endogenous catecholamines are O-methylated remains to be established.

The presence of large amounts of 3-methoxy-4-hydroxymandelic acid (4) and normetanephrine (103) in the urine of human subjects with pheochromocytomas suggests that O-methylation is an important route of metabolism of catecholamines in man. After the intravenous infusion of physiological doses of β H^3-epinephrine, about 90 per cent of the radioactivity appeared in the urine. Fifty-five per cent of the radioactivity was free and conjugated metanephrine and 30 per cent was 3-methoxy-4-hydroxymandelic acid (102). Kirshner *et al.* found essentially similar results in man (98). In rats, metanephrine is conjugated with glucuronic acid, while in man it is conjugated presumably with sulfuric acid. An additional metabolite, present in small quantities, was tentatively identified as 3,4-dihydroxymandelic acid (98, 121). When metanephrine was administered to man, the same fraction was excreted as metanephrine and 3-methoxy-4-hydroxymandelic acid as was observed after epinephrine (102), indicating that the acid arises mainly from the deamination of metanephrine. In iproniazid-treated subjects the excretion of metanephrine is increased, while that of 3-methoxy-4-hydroxymandelic acid is decreased (130).

Normetanephrine and metanephrine possess relatively weak physiological and psychological activity (68, 102) suggesting that O-methylation of catecholamines is an inactivation process.

In the light of these findings, it can be concluded that the principal pathway for the metabolism of epinephrine and norepinephrine in man and rodents is O-methylation to metanephrine or normetanephrine, the latter amines being in turn either deaminated by monoamine oxidase or conjugated. Direct deamination of these catecholamines appears to be a minor route of transformation. The pathways for the metabolism of epinephrine and norepinephrine are shown in figure 2. A new metabolic product of epinephrine and norepinephrine, 3-methyoxy-4-hydroxyphenylglycol, has been identified in this laboratory (Axelrod, Kopin and Mann, unpublished observations). The metabolite arises from the deamination of metanephrine or normetanephrine followed by reduction of the resulting 3-methoxy- 4-hydroxyphenylglycol aldehyde.

O-Methylation of Catecholamines In Vitro

An enzyme, catechol-O-methyl transferase, that O-methylates epinephrine and other catechols has been found in this laboratory (11, 20). The presence of

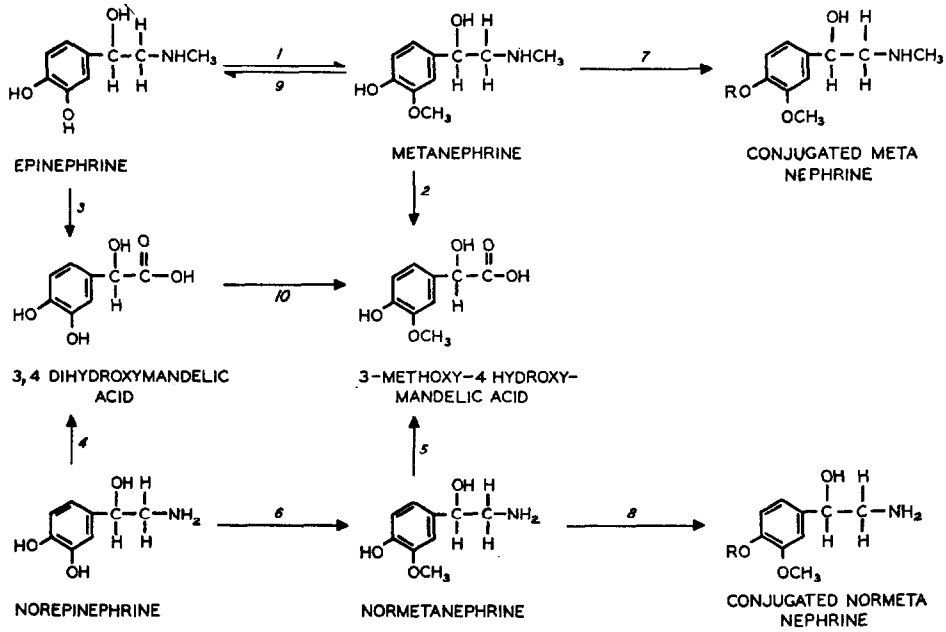

FIG. 2. Metabolism of epinephrine and norepinephrine. *1, 6, 10:* Catalyzed by catechol-O-methyl transferase, S-adenosylmethionine serving as the methyl donor. Divalent cations are required. *2, 3, 4, 5:* These reactions take place in two steps, deamination by monoamine oxidase to form the corresponding aldehyde, followed by oxidation by aldehyde dehydrogenase and diphosphopyridine nucleotide. *7, 8:* In may species, conjugation occurs by the transfer of glucuronic acid from uridine diphosphate glucuronic acid by a transferring enzyme in the liver microsomes. *9:* This reaction has been shown to occur *in vitro* in certain species.

this enzyme has been confirmed by other investigators (75, 105, 115). Incubating the soluble supernatant fraction of rat liver with epinephrine, S-adenosylmethionine and Mg^{++} resulted in the disappearance of one mole of the catecholamine and the appearance of one mole of metanephrine. In the absence of S-adenoslymethionine or Mg^{++} negligible metabolism occurred (11, 20). These observations demonstrated that the enzyme transfers the methyl group of S-adenosylmethionine to the 3-hydroxy group of epinephrine.

Catechol-O-methyl transferase is widely distributed among a variety of species and tissues (table 1) and is localized in the soluble fraction of the cell (14, 20). Of all the tissues examined, enzyme activity is found to be consistently highest in the liver, while the activity in monkey liver is greater than in any other mammalian species examined. Particularly noteworthy is the high catechol-O-methyl transferase in such glandular tissues as salivary gland, pancreas and adenohypophysis (14). The magnitude of enzyme activity is the same in thyroid gland, spleen, blood vessels and brain in the monkey. Catechol-O-methyl transferase activity is also found in all peripheral nerves (14). The presence of this enzyme in those organs upon which norepinephrine and epinephrine exert their effects suggests that it might act locally in the transformation of these hormones.

TABLE 1. DISTRIBUTION OF CATECHOL-O-METHYL TRANSFERASE

SPECIES	TISSUE	CONC. UNITS*	SPECIES	TISSUE	CONC. UNITS*
Monkey	Liver	1700	Monkey	Aorta	120
Rat	Liver	900	Monkey	Inf. vena cava	80
Cow	Liver	430	Monkey	Submax. gland	820
Pig	Liver	380	Monkey	Pancreas	680
Mouse	Liver	300	Monkey	Adenohypophysis	420
Guinea pig	Liver	130	Monkey	Thyroid	115
Man	Liver	100	Monkey	Brain	105
Rabbit	Liver	38	Rat	Brain	26
Rat	Kidney	260	Monkey	Neurohypophysis	190
Rat	Spleen	63	Monkey	Sup. cerv. gangl.	130
Rat	Sm. intestine	40	Monkey	Vagus nerve	128
Rat	Lung	40	Monkey	Saphenous nerve	88
Rat	Skel. muscle	0	Monkey	Splanchnic nerve	87
Rat	Heart muscle	25	Monkey	Sciatic nerve	80
			Monkey	Sacral sympath. nerve	65
			Monkey	Splenic nerve	60

* 1 unit = μg metanephrine formed from epinephrine in 1 hr. per gm tissue (values obtained from refs. 14, 20).

Catechol transferase was purified about 30-fold from rat liver by procedures involving ammonium sulfate precipitation and adsorption and elution on calcium phosphate gel (20). The purified enzyme has an absolute requirement for S-adenosylmethionine and Mg^{++}. However, a number of divalent cations such as Co^{++}, Mn^{++}, Cd^{++}, Fe^{++} and Ni^{++} could be substituted for Mg^{++}. All catechols, regardless of the substituents on the aromatic nucleus, were O-methylated. The enzyme showed no stereospecificity toward the D or the L isomers of epinephrine, nor did it O-methylate monophenols. Among the catechols methylated were normally occurring compounds such as norepinephrine, dopamine, 3,4-dihydroxyphenylalanine, 3,4-dihydroxymandelic acid and dihydroxyphenylacetic acid, as well as many synthetic catechols (20). The optimal pH for enzyme activity ranged from 7.5 to 8.2. In the presence of *p*-chloromercuric benzoate and iodoacetic acid, O-methylation was inhibited (20), pointing to a sulfhydryl group as the active site on the enzyme surface. Pyrogallol and other catechols were found to block the O-methylation of catecholamines *in vitro* (13a). Pretreating mice with a catechol-O-methyl transferase inhibitor (pyrogallol) markedly slowed the metabolism of epinephrine. A monoamine oxidase inhibitor (iproniazid), on the other hand, did not affect the rate of disappearance of epinephrine or norepinephrine.

The specificity of the O-methyl transferase with respect to catechols and the requirement for divalent cations suggests a role for the cation (20). The metal could link the adjacent hydroxy groups of the catechol to the enzyme surface. O-methylation would then proceed by a nucleophilic attack of the hydroxy group by the electrophilic methyl carbon of S-adenosylmethionine. Since the 3-hydroxy group is strongly nucleophilic, the transfer takes place on the 3 position. Further support of such a mechanism is provided by the finding that catechols having a

strongly nucleophilic group on position 4 are O-methylated on this position by the enzyme (139a).

Metabolism of Metanephrine and Other O-Methylated Amines

The O-methylated metabolites of catecholamines undergo further metabolic change *in vivo* and *in vitro*. The administration of metanephrine results in the excretion of 3-methoxy-4-hydroxymandelic acid (13, 102). When epinephrine, norepinephrine, dopamine, metanephrine or normetanephrine were given to animals in which monoamine oxidase was inhibited, the excretion of the O-methylated amines was increased at the expense of O-methylated acids (13, 16, 18). Consequently, deamination and oxidation of the O-methylated amines constitute a pathway in the metabolism of these compounds *in vivo*. O-methylated amines, metanephrine, normetanephrine and 3-methoxytyramine also serve as substrates for monoamine oxidase preparations (11, 12, 105). In addition, deaminated products can be oxidized to their corresponding acids by aldehyde dehydrogenase (11, 12, 105). Normetanephrine was found to be a better substrate for monoamine oxidase than either epinephrine or norepinephrine (105).

An enzyme has been found in the microsomes of rabbit liver that can O-demethylate metanephrine to epinephrine (19). The enzyme requires reduced triphosphopyridine nucleotide and appears to be similar to other O-demethylating enzymes (10).

A major route of metabolism of O-methylated amines in some animal species involves conjugation with glucuronic acid on the hydroxy group. The synthesis of glucuronides has been shown to be catalyzed by an enzyme system in the microsomes of liver, and requires uridine diphosphate glucuronic acid as the glucuronide donor (55, 92). Incubation of metanephrine or normetanephrine with microsomes of rat liver and uridine diphosphate glucuronic acid resulted in the formation of the corresponding glucosiduronic acid of these amines (unpublished).

Formation and Metabolism of Catecholamines in Nervous System

The presence and role of norepinephrine in the autonomic nervous system has been well established (61). Although norepinephrine has been shown to be highly localized in certain regions of the central nervous system (155), the functions of this catecholamine in the brain are not clear. In the sympathetic nervous system norepinephrine presumably arises from dopa via dopamine. Holtz and Westermark (89) have found dopa decarboxylase activity to be high in postganglionic sympathetic nerves, sympathetic ganglia and in the sympathetic trunk and brain stem. Furthermore, Schümann has shown that sympathetic nerves contain dopamine in relatively high concentrations (138). Both dopadecarboxylase and its metabolite, dopamine, are highly localized in the cytoplasm of the nerves, while norepinephrine is found in the particulate fraction of the nerve cell (139). Recently Goodall and Kirshner (78) reported that the incubation of C^{14} dopa with sympathetic nerves and ganglia led to the formation of radioactive dopamine and norepinephrine. Negligible amounts of radioactive epinephrine were found. The

vagus nerve, on the other hand, formed no significant amounts of radioactive norepinephrine and only small amounts of radioactive hydroxytyramine. Using paper chromatographic techniques, Schümann found norepinephrine and dopamine, but no epinephrine, in the splenic nerve and spleen (139). On the basis of these observations, this investigator postulates that norepinephrine is the only transmitter substance at the sympathetic nerve endings.

The concentration of H^3-epinephrine was determined in various regions of the cat brain after its intravenous infusion (159). It did not exceed that expected from the blood concentration of the tissues, except in the hypothalamus, where small amounts of H^3-epinephrine were found (159). It was concluded from these experiments that epinephrine is unable to cross the blood-brain barrier, except to a small extent in the hypothalamus (159). Since epinephrine is present in the brain (155), it must be formed from precursors which are able to cross the blood-brain barrier. It is probable that norepinephrine in the brain is formed from dopa, a compound that can cross the blood-brain barrier (46). Both dopa (110) and dopamine (46, 110) were found to occur normally in the brain of a number of mammalian species. Moreover, the intravenous injection of dopa to rabbits caused a considerable rise in the dopamine content of the brain (46). Pretreatment with iproniazid markedly enhanced the dopamine levels, but had little or no effect on norepinephrine levels in the brain (47). The presence of the deaminated product of dopamine, dihydroxyphenylacetic acid, in the splenic nerve (62) and brain (110) has also been reported. These observations would suggest that in the nervous system dopamine is readily attacked by monoamine oxidase.

The manner in which the actions of the catecholamines in the nervous system are terminated is poorly understood. For many years, monoamine oxidase has been considered to be the principal enzyme involved in the inactivation of norepinephrine and epinephrine. However, since the introduction of monoamine oxidase inhibitors, considerable doubt has been cast upon the role of this enzyme in the metabolism of epinephrine and norepinephrine. (See above for a review of this subject.)

Recently, O-methylation has been shown to be the principal pathway for the metabolism of administered epinephrine and norepinephrine (16, 102). Normetanephrine has been found to be present in organs rich in sympathetic fibers (18) and in the brain extract of rats treated with iproniazid (12). When normetanephrine was incubated with brain mitochondria and aldehyde dehydrogenase, it was oxidatively deaminated to 3-methoxy-4-hydroxymandelic acid (12). This indicates that the endogenous norepinephrine could be O-methylated in the nervous system and then further metabolized. The enzyme, catechol-O-methyl transferase, which catalyzes the first reaction, has been found to be present throughout the nervous system, including the brain, sympathetic ganglia and postganglionic sympathetic fibers, as well as parasympathetic, motor and sensory nerves (table 1 and ref. 14). Within the central nervous system differences of enzyme activity ranging up to fourfold were found (14). The lowest values occurred in the cerebral cortex and amygdala, and the highest in the neurohypophysis. There

was no direct relationship between catechol-O-methyl transferase activity found in various regions of the monkey brain and norepinephrine levels reported in different areas in the dog brain. The Michaelis constant (Km) of catechol-O-methyl transferase with respect to brain epinephrine is less than 10^{-5} M, which places it in the physiological range of tissue catecholamine concentrations (14). Although direct evidence of the main pathway of metabolism of the catecholamines in the nervous system is lacking, it appears likely that catechol-O-methyl transferase is an important enzyme.

S-adenosylmethionine, the donor of active methyl groups (45), is assuming considerable importance in the metabolism of biologically active amines. The cofactor is involved in the O-methylation of catecholamines (11), the N-methylation of norepinephrine to epinephrine (97), and more recently it has been shown to be required for the conversion of histamine to methyl histamine (38). The latter reaction is of paramount importance in the inactivation of histamine (133). The ability of the nervous system to synthesize S-adenosylmethionine from adenosine triphosphate and methionine has been established (14).

Physiological Disposition of Epinephrine

There is considerable information available concerning the localization of endogenous catecholamines (61). However, relatively little is known about the disposition of these hormones when they are released from the adrenal medulla and sympathetic nerve endings, or when administered intravenously. Raab and Gigee (117, 118) reported that epinephrine and norepinephrine were selectively taken up by heart muscle and other vascular tissues when massive doses of these compounds were administered to cats or dogs, but there was no demonstrable increase in the catecholamine content with doses below 2 milligrams per kilogram. Von Euler (60), on the other hand, did not find any significant changes in the catecholamine content of heart, spleen, liver, kidney or skeletal muscle of the cat when large or small amounts of catecholamines were given. These contradictory results might be due to the lack of precision and specificity of the methods employed. More recently, the physiological disposition of epinephrine and its melabolite, metanephrine, was examined in cats and mice after an intravenous infusion of physiological amounts of tritium-labeled β DL H^3-epinephrine of high specific activity (22). The amines were separately determined by specific procedures involving column chromatography and extraction in organic solvents. Immediately after the end of a 30-minute infusion (3 μg/min/kg) the concentration of H^3-epinephrine in heart, spleen, adrenal and pituitary gland exceeded that of the plasma several-fold. In kidney, liver, lung and intestines, its concentration was of the same magnitude as that of the plasma, while it was lower in skeletal muscle. After an intravenous infusion of radioactive epinephrine, Weil-Malherbe *et al.* (159) found negligible amounts of radioactive epinephrine in the brain of the cat, while Schaepdryver (129) reported significant amounts of the radioactive catecholamine in the brain of the dog. Two hours after the administration of H^3-epinephrine, large quantities were found in heart and spleen, indicating that

these tissues not only accumulate the hormone, but also retain it for long periods of time.

Immediately after H^3-epinephrine infusion, the concentration of metanephrine in plasma was about the same as that of epinephrine (22). The O-methylated metabolite accumulated in heart, spleen, adrenal gland and liver, but insignificant amounts were found in the brain, indicating that metanephrine crosses the blood-brain barrier with difficulty.

After the intravenous injection of epinephrine in rabbits and dogs, Pekkarinen (113) and Lund (106) reported that the amine was completely eliminated from the blood within 10 minutes. Norepinephrine disappeared from the plasma even more rapidly than epinephrine (106). An intravenous injection of H^3-epinephrine was followed by a precipitous fall in the plasma levels of the amine within 5 minutes (22a). In contrast to the earlier findings, however, small but significant amounts of epinephrine were present in the plasma for more than 2 hours (22a, 129). Within 2 minutes after the intravenous injection of H^3-epinephrine, its metabolite, metanephrine, reached a peak level in the plasma, with approximately the same or higher concentrations as that of epinephrine (25). Only small amounts of 3-methoxy-4-hydroxymandelic acid and 3,4-dihydroxymandelic acid (less than one-tenth that of metanephrine) were present in the plasma at that time (22a). These observations indicate that during the period of physiological inactivation of epinephrine, catechol-O-methyl transferase is the predominating enzyme. The disappearance of the metanephrine from the plasma then closely paralleled that of epinephrine (fig. 3). The initial drop in the plasma level was presumably due to passage into the tissues, as well as metabolic transformation.

The dynamic factors involved in the elimination of epinephrine in the intact organism were examined in the mouse (22a). After the intravenous injection of 3

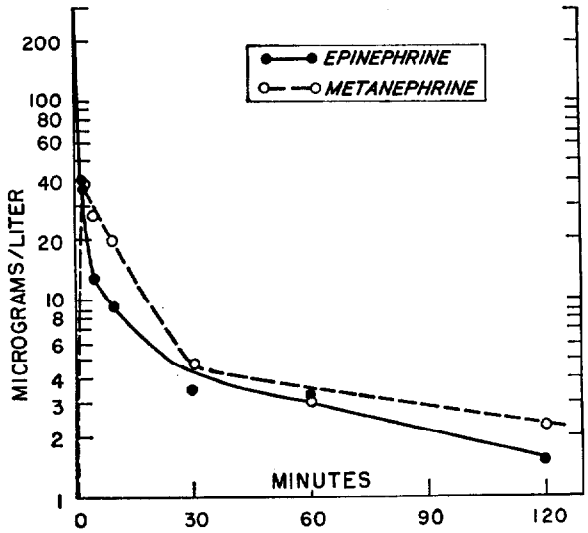

FIG. 3. Plasma levels of epinephrine and metanephrine after intravenous injection of 70 μg/kg epinephrine to a cat (22).

micrograms of H^3-epinephrine to mice, the catecholamine and its metabolite were determined in the whole animal at various time intervals. Within 10 minutes, more than half of the epinephrine disappeared. After 10 minutes, the amine disappeared much more slowly; more than 10 per cent of the administered epinephrine was still found in the mouse after 3 hours. The rate of elimination of epinephrine in the whole mouse was independent of the amount of epinephrine administered over a range of 1–10 micrograms of injected epinephrine per mouse. In the initial phase, 1 mole of metanephrine appeared in the whole mouse for every mole of epinephrine eliminated. These observations, together with those described above, suggest the following sequence of events in the elimination of circulating epinephrine: first phase (0–5 minutes), rapid dilution by redistribution into tissues, O-methylation by catechol-O-methyl transferase and binding onto certain tissues; second phase (5–180 minutes or longer), slow release of epinephrine from binding sites, followed by attack by catechol-O-methyl transferase.

Metabolism of Phenylethylamines

Studies on the metabolism of amines with a phenylethylamine nucleus were initiated in 1910 by Ewins and Laidlow (69) who found that the administration of phenylethylamine and tyramine to dogs, cats and rabbits resulted in the excretion of large amounts of phenylacetic acid and *p*-hydroxyphenylacetic acid, respectively. Guggenheim and Löffler confirmed these findings in rats and mice (81). More recently, Schayer reported that about 90 per cent of C^{14} tyramine was excreted as free and conjugated *p*-hydroxyphenylacetic acid and 10 per cent as free and conjugated tyramine (132). These observations suggested the presence of an enzyme that could deaminate these amines *in vivo*.

In 1928 Hare-Bernheim demonstrated an enzyme that catalyzes the oxidative deamination of tyramine to yield *p*-hydroxybenzaldehyde, peroxide and ammonia (82). The aldehyde is further oxidized to the corresponding carboxylic acid by aldehyde dehydrogenase and diphosphorpyidine nucleotide (161).

It was subsequently observed that this enzyme, monoamine oxidase, deaminates many alkyl and aromatic amines, including epinephrine and norepinephrine, to their corresponding aldehydes (31a, 116, 122). The substrate specificity of monoamine oxidase has been studied extensively, since so many amines have marked biological activity and are of therapeutic interest (see ref. 30 for an extensive review of the subject). *In vitro*, monoamine oxidase acts on amines which have an amine group attached to a terminal carbon atom, e.g. compounds of the type $R—CH_2—NH_2$. Aromatic amines such as aniline, in which the amine is attached to the aromatic ring, are not deaminated, but benzylamine and α-phenylethylamine are metabolized. Secondary amines are deaminated as rapidly as the corresponding primary amines. Introduction of N-methyl groups reduces the rate considerably, but does not abolish the reaction. Hordinine ($HOC_6H_4CH_2CH_2N(CH_3)_2$) is a naturally occurring tertiary amine which is slowly oxidized by amine oxidase. Phenylamines in which the amino group is not attached to a terminal carbon atom are not deaminated by monoamine oxidase,

e. g. ephedrine and amphetamine. Not only are the phenylisopropylamines stable with respect to monoamine oxidase, but they also inhibit this enzyme *in vitro* (73). Gaddum and Kwiatkowski postulated that phenylisopropylamines delay the destruction of epinephrine *in vivo* by inhibiting monoamine oxidase (74). However, the arguments put forward by these investigators have been challenged (23).

Alles and Heegard (1) have determined the relative rates of deamination of a number of phenylamines. From the data obtained in these studies, it was suggested that more than one enzyme may be involved in the deamination of monoamines. This is also the view of Werle and Roewer (163), who believe that in ox liver, tyramine and *n*-butylamine are oxidized by two enzyme systems. Recently other amine oxidases have been identified, one isolated from ox plasma which shows marked activity towards spermine and spermidine (149), and another enzyme localized in liver microsomes which deaminates phenylisopropylamines (9). The further purification of monoamine oxidase (161) may make it possible to uncover other closely related amine oxidases.

The evaluation of monoamine oxidase in the metabolism of phenylethylamine derivatives *in vivo* was made possible by the introduction of potent monoamine oxidase inhibitors such as iproniazid (165) and choline-*p*-tolyether bromide (40). Pretreatment with iproniazid markedly prolonged the contraction of the nictitating membrane of the cat when phenylethylamine or tyramine was administered (80). The toxic action of phenylethylamine and tyramine, but not of ephedrine, was increased by pretreating guinea pigs with iproniazid (120). In mice, iproniazid or choline-*p*-tolylether bromide caused a pronounced increase in the excretion of C^{14} tyramine after the administration of the latter substance (132). Several compounds, such as ephedrine, amphetamine and cocaine, which inhibit monaomine oxidase *in vitro*, were found to have no effect *in vivo* (132).

The metabolism of mescaline (3,4,5-trimethoxy phenylethylamine), the active psychotomimetic principle of 'mescal buttons,' has been the subject of many studies. After its administration to dogs and rabbits, Slotta and Muller (143) recovered large amounts of 3,4,5-trimethoxy phenylacetic acid in the urine. These results could not be confirmed by Cochin *et al.* (49). In man and dog, a major fraction of mescaline is excreted unchanged (49, 123, 127). An interesting minor metabolite found in the urine of man after the ingestion of mescaline was identified as a conjugate of 3,4-dihydroxy-5-methoxyphenylacetic acid, indicating that this molecule can undergo O-demethylation. Despite the fact that mescaline contains a phenylethylamine nucleus, it is metabolized by diamine oxidase, rather than monoamine oxidase (166). Mescaline is also O-demethylated by an enzyme localized in the microsomes of the liver (10). After the administration of mescaline to the dog, the amine is widely distributed in all organ tissues, including the brain (49). Block *et al.* (32) have made the interesting observation that ingested mescaline is incorporated into liver proteins, and that the mescaline-containing protein may be involved in the production of hallucinations.

Recently the incorporation of a variety of C^{14}-labeled amines in the soluble protein fraction of mammalian tissues has been reported (111, 128). This system

is activated by Ca^{++} and consists of a protein that accepts the amines as well as an enzyme catalyzing the incorporation. The participation of protein amide groups in this reaction was proposed by these investigators.

Metabolism and Physiological Disposition of Phenylisopropylamines

Phenylisopropylamines constitute an important segment of the sympathomimetic amines, yet until recently little was known about the disposition and metabolism of these compounds. Richter (123) reported that when the phenylisopropylamines ephedrine and amphetamine were administered to man, they were excreted in the urine almost entirely unchanged. He ascribed the stability of the drugs *in vivo* to the failure of monoamine oxidase to attack these compounds. Other investigators (6, 7, 29, 93, 96) did not corroborate these findings. They found that considerable amounts of administered phenylisopropylamines were metabolized in man, dog and rabbit. Moreover, when the liver function of dogs was impaired by the administration of carbon tetrachloride, the urinary excretion of amphetamine was increased (29).

In the past, studies on the fate of phenylisopropylamines have been hampered by the lack of sensitive and specific methods for the estimation of these compounds in biological materials. The development of methods for the estimation of amphetamine, *p*-hydroxyamphetamine (Paradrin), methamphetamine, ephedrine, norephedrine (Propadrine), *p*-hydroxyephedrine (Suprifen) and *p*-hydroxynorephedrine has made possible a study of the metabolism of these compounds *in vivo* and *in vitro* (6, 7).

The accompanying scheme (fig. 4) describes the metabolic transformation of amphetamine *in vivo* (7).

The main route of metabolism of D-amphetamine in the dog involves hydroxylation to *p*-hydroxyamphetamine, a potent pressor agent. After the administration of L-amphetamine, however, only small amounts of *p*-hydroxyamphetamine are excreted. The differences in the metabolism of the optical isomers of amphetamine are presumably due to a stereospecific enzyme (9) which can deaminate the levo isomer of amphetamine more readily than the dextro isomer. In the dog

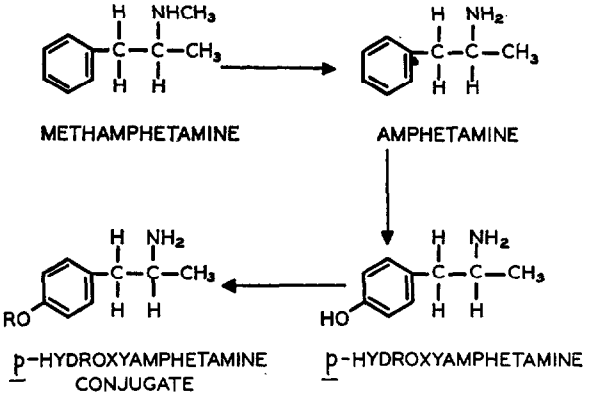

FIG. 4. Metabolism of amphetamine.

D-amphetamine disappears more slowly than its hydroxylated metabolite, suggesting that in this species at least, the major pressor effect is mediated by the parent compound. When hydroxyamphetamine is administered, it appears in the urine partly free and partly conjugated. After the administration of methamphetamine to a dog, about half the compound is demethylated to amphetamine, part of which is hydroxylated to *p*-hydroxyamphetamine. From these observations, it appears that the pharmacological action of methamphetamine is mediated through its metabolic product, amphetamine.

There are marked differences in the metabolism of D-amphetamine in various species. Dogs and rats hydroxylate considerable amounts of the amine, while rabbits almost completely metabolize amphetamine by deamination. Walkenstein *et al.* (157) have shown that congeners of amphetamine also undergo N-demethylation and *p*-hydroxylation in the dog, rat and rabbit. At present, no studies on the metabolic fate of amphetamine have been made in man. Previous work has shown that a number of aromatic compounds, including acetanilid, aniline (34) and antipyrine (35) are hydroxylated in man; it seems likely that amphetamine would also hydroxylate in this species.

Studies at a cellular level have uncovered an enzyme system in the microsomes of liver that can deaminate amphetamine to phenylacetone and ammonia in the presence of reduced triphosphopyridine nucleotide (TPNH) and oxygen (9). The enzymatic deamination of amphetamine is shown in the following equation:

$$C_6H_5CH_2CH(NH_2)CH_3 \xrightarrow[O_2]{TPNH} C_6H_5CH_2COCH_3 + NH_3$$

The enzyme which deaminates amphetamine differs from other deaminating enzymes such as amine oxidase, D-amino acid oxidase, L-amino acid oxidase and glutamic acid dehydrogenase with respect to its substrate specificity, intracellular localization and cofactor requirements (9).

The role of TPNH in an enzyme system catalyzing the oxidative deamination of an amine is not understood. TPNH could conceivably act by the generation of hydrogen peroxide through the transfer of hydrogen by an intermediate electron transport system to molecular oxygen. However, hydrogen peroxide generated from D-amino acid oxidase was unable to replace TPNH in the deamination of amphetamine. Gillette *et al.* (76) have found that TPNH is oxidized by an enzyme in liver microsomes in the absence of amine substrate to form a 'peroxide.' These investigators believe that the oxidation of TPNH is necessary to carry out other metabolic reactions in the microsomes.

In addition to amphetamine, a number of other amines are deaminated by the TPNH-dependent enzymes in the microsomes (9). Amines having a phenylisopropylamine or phenylbutylamine structure are extensively transformed by the enzyme, while phenolic-substituted amines, phenylethylamines and aliphatic amines are metabolized slightly or not at all. The enzyme also has some degree of stereospecificity, since the L-isomers of amphetamine are transformed more rapidly than the D-isomers. The deaminating activity of rabbit liver microsomes is

considerably depressed when rat microsomes are added. Heating of the rat liver microsomes unmasks the presence of a heat-stable activator for this reaction. The nature of this activating factor is unknown. Species differences in the metabolism of amphetamine may in part be explained by the presence of inhibitory and activating factors in liver microsomes (9).

Following the observation that an enzyme in the microsomes of liver requiring TPNH and oxygen can deaminate amphetamine, many TPNH-dependent enzyme systems have been found that carry out a wide variety of reactions. Metabolic transformations catalyzed by these enzyme systems include N-demethylation (8, 104), hydroxylation of aromatic compounds (109), side chain oxidation (51) and cleavage of ethers (10). A review of this subject has been published (37). The relative nonspecificity of the substrates atacked by the microsomal enzymes would suggest that they act as 'scavenger' enzymes and perform normal roles as well. Another property common to microsomal enzyme systems is their inhibition by β-diethylaminoethyl diphenylpropylacetate (SKF 525A, refs. 17, 50). This compound has little pharmacological activity of its own, but prolongs the action of sympathomimetic amines as well as other pharmacological agents by inhibiting microsomal enzyme systems which are concerned with the inactivation of these compounds.

Beyer (25) has observed that ascorbic acid can deaminate a number of phenylethylamines and phenylisopropylamine derivatives nonenzymatically. This was substantiated *in vivo* for amphetamine when it was reported that the excretion of the amine in dogs could be markedly diminished by the subcutaneous injections of large amounts of ascorbic acid (26). When the injections were discontinued, the excretion of amphetamine increased again. It has been reported that ascorbic acid in the presence of Fe^{++}, versene and oxygen hydroxylates aromatic compounds in the test tube (36, 151). Thus, in the model chemical system, tyramine is oxidized to dopamine (36) and amphetamine and ephedrine are hydroxylated to their corresponding para derivatives (36). In ascorbic acid-deficient guinea pigs the rate of the hydroxylation of aromatic compounds was reduced (21). The precise role that ascorbic acid plays in the metabolism of amines in the organism is not known.

Figure 5 shows the route of metabolism of ephedrine (6).

The principal pathway of biotransformation of ephedrine in the dog involves

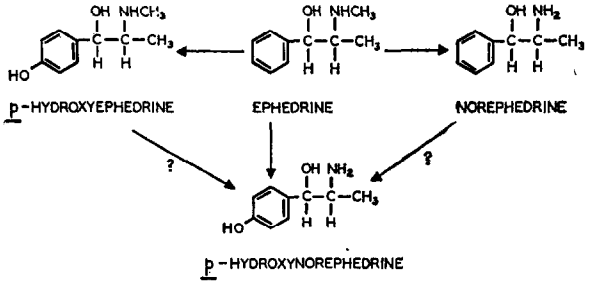

FIG. 5. Metabolism of ephedrine.

rapid N-demethylation to norephedrine, a relatively stable and potent pressor agent. It is probable that the activity of ephedrine is mediated to a considerable extent through its metabolite, norephedrine. Other minor routes of metabolism involve hydroxylation of both ephedrine and norephedrine to yield the corresponding *p*-hydroxy derivatives, both of which are potent pressor agents. No evidence has been found for the excretion of 3,4-dihydroxyephedrine. However, these catecholamines are readily O-methylated by catechol-O-methyltransferase, and if formed, they might be further metabolized to their corresponding O-methyl derivatives. Norephedrine is relatively stable, and is excreted in the urine mainly unchanged. Calculations of the renal excretion of norephedrine from plasma levels and urinary excretion yielded values which were considerably higher than the glomerular filtration rate, suggesting that a secretory transport mechanism is involved in its excretion (6).

There are differences in the metabolism of ephedrine in mammalian species. In the dog and guinea pig, demethylation proceeds rapidly, constituting a major route of biotransformation. The rat, on the other hand, demethylates ephedrine slowly and considerable amounts of the drug are excreted both unchanged and as hydroxylated derivatives. Only very small quantities of norephedrine and ephedrine are eliminated by the rabbit, indicating that in this species ephedrine is demethylated to norephedrine, which in turn is extensively metabolized by pathways other than hydroxylation.

After the subcutaneous administration of 3,4-dihydroxynorephedrine to man, only small amounts of the compound are excreted unchanged (67), indicating almost complete metabolic alteration of the molecule. Although the metabolic products of this catechol have not been identified, it is probable that it is O-methylated and excreted as free or conjugated 3-methoxy-4-hydroxynorephedrine.

Ephedrine is N-demethylated to norephedrine and formaldehyde by a TPNH-dependent enzyme system in the microsomes of the liver (8). The over-all reaction can be represented by the following equation:

$$C_6H_5CHOHCH_2(NHCH_3)CH_3 \xrightarrow[O_2]{TPNH} C_6H_5CHOH(NH_2)CH_3 + HCHO.$$

The microsomal preparation also metabolizes ephedrine by pathways other than demethylation (8). The ability of the microsomal enzyme system to demethylate other sympathomimetic amines is dependent on the structure of the amine. Substrates possessing a phenylisopropylamine nucleus are extensively demethylated, while those with a phenylethylamine nucleus are not. Amines with hydroxy groups on the aromatic nucleus are demethylated slightly or not at all. Species differences in the metabolism of ephedrine can be explained in part by the activity of the TPNH-dependent microsomal demethylating enzyme as compared to the deaminating enzyme. When ephedrine or norephedrine are administered to the rabbit, both compounds are extensively metabolized. The transformation of these amines is catalyzed by enzymes which demethylate ephedrine and deaminate norephedrine. In the dog the activity of the norephedrine-deaminating enzyme is

low, so that the demethylated ephedrine, once formed, is excreted unchanged. The activity of the enzymes which demethylate ephedrine and deaminate norephedrine in the rat is negligible, so that administered ephedrine in this species is excreted unchanged or as *p*-hydroxyephedrine.

The tissue concentration of the phenylisopropylamines amphetamine, ephedrine and norephedrine, shows a similar pattern of distribution (6, 7). After their administration, these compounds are localized in organ tissues to a considerable extent, with small amounts in the bile and fat. The high concentration of the amines found in the brain and cerebrospinal fluid suggests that there is little hindrance to their passage across the blood-brain barrier. Catecholamines, on the other hand, cross the blood-brain barrier with difficulty or not at all (159).

Metabolism of Indolamines

The metabolism of the indolamine, tryptamine, has been elucidated by Ewins and Laidlow many years ago (70). After its administration, it is deaminated to 3-indolacetic acid, which is then excreted as a glycine conjugate. Since tryptamine is a substrate for monoamine oxidase (7, 31, 31a) it has been assumed that this enzyme deaminates the amine in the intact organism. More recently, Schayer *et al.* (137) have shown that the inhibition of monoamine oxidase *in vivo* increases the excretion of administered tryptamine.

An indole base with an R_F value corresponding to that of tryptamine has been found in normal urine (126). Tryptamine has also been found in tissues, including brain and liver, following the administration of a monoamine oxidase inhibitor and tryptophan (85). Although the decarboxylation of tryptophan has been demonstrated *in vitro* (158, 162) the origin and physiological significance of tryptamine in the body have yet to be established.

In the past 10 years, tryptamine derivatives have acquired a new significance when serotonin (5-hydroxytryptamine) was found to be a normal constituent in the body (119). The presence of serotonin in the brain (2, 150) and the provocative proposal of Wooley (164) and Gaddum (73) that this amine may be involved in the maintenance of mental health, stimulated great activity in this area of research. An exhaustive review of this subject has been published recently by Page (112), and the metabolism of serotonin will be covered only briefly here. Serotonin has been shown to arise from tryptophan in the body (154). Although tryptophan and 5-hydroxytryptophan form serotonin in man, tryptamine does not (152), indicating that hydroxylation of the indole nucleus precedes decarboxylation. A major pathway for the metabolism of serotonin is deamination by monoamine oxidase and aldehyde dehydrogenase to 5-hydroxyindolacetic acid (153) which is then conjugated with glycine and excreted in the urine. Other, though minor, routes for the metabolism of serotonin involve N-acetylation and conjugation with glucuronic acid (108).

Szara has described the psychotomimetic actions of dimethyltryptamine (146). After its administration, this compound is excreted in the urine as 3-indolacetic acid in the rat (57) and man (146). Recently, new pathways for the metabolism of

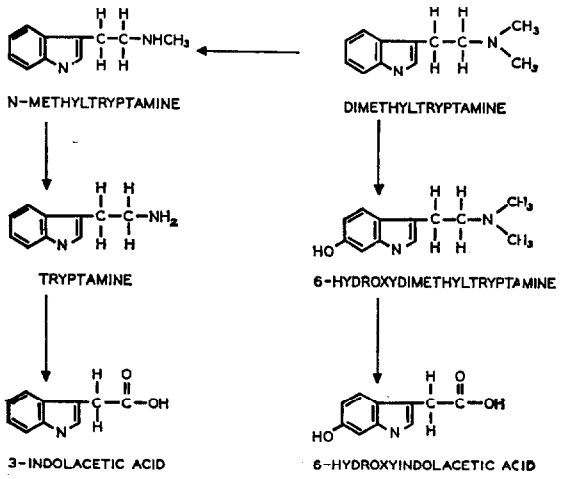

FIG. 6. Metabolism of dimethyltryptamine.

indolamines have been described (147). The accompanying scheme (fig. 6) has been proposed for the biotransformation of dimethyltryptamine (147). Dimethyltryptamine is metabolized along two pathways involving N-demethylation and hydroxylation on the 6 position. The metabolites are further transformed by deamination. *In vitro* studies have shown that N-demethylation (147) and 6-hydroxylation (94, 147) of the indolamine is catalyzed by TPNH-dependent enzymes in the liver microsomes.

Although the indole-containing ergot alkaloids are widely used as pharmacologic agents, little is known about their biologic fate. One of these compounds, lysergic acid diethylamide (LSD), a potent hallucinogenic compound, is readily hydroxylated to 2-oxy-LSD by a microsomal enzyme in the liver of a number of mammalian species (15). Other ergot alkaloids may be hydroxylated in the same manner. When administered, LSD is almost completely altered in the body, and there are species differences in the rate of biotransformation of the compound (15). From the amount of LSD found in the brain of cats, it was calculated that the drug exerts its psychological effect at an exceedingly minute concentration.

SUMMARY

Routes for the metabolism of catecholamines *in vivo* have been established the past few years. The major pathway for the metabolism of epinephrine and norepinephrine involves O-methylation to yield metanephrine and normetanephrine. These physiologically inactive metabolites are then conjugated or deaminated to 3-methoxy-4-hydroxymandelic acid. A considerable body of evidence has accumulated indicating that monoamine oxidase is mainly concerned with the deamination of the O-methylated metabolites, rather than the catecholamines themselves.

An enzyme, catechol-O-methyl transferase, catalyzes the O-methylation of epinephrine, norepinephrine and other normally occurring catechols. This enzyme is widely distributed in most organs and tissues, including the autonomic

and central nervous system. Catechol-O-methyl transferase is chiefly concerned in the metabolism of circulating epinephrine and norepinephrine, but its role in the inactivation of endogenously released catecholamines remains to be established.

Sympathomimetic phenylethylamines, phenylisopropylamines and indolamines are metabolized by a variety of pathways, including deamination, N- and O-demethylation, hydroxylation and conjugation.

REFERENCES

1. ALLES, G. A. AND E. V. HEEGAARD. Substrate specificity of amine oxidase. *J. Biol. Chem.* 147: 487–513, 1943.
2. AMIN, A. H., T. B. B. CRAWFORD AND J. H. GADDUM. Distribution of substance P and 5-hydroxytryptamine in central nervous system in dog. *J. Physiol.* 126: 596–618, 1954.
3. ARMIN, J., R. T. GRANT, R. H. S. THOMPSON AND A. TICKNER. An explanation for the heightened vascular reactivity of the denervated rabbit's ear. *J. Physiol.* 121: 603–622, 1953.
4. ARMSTRONG, M. D., A. McMILLAN AND K. N. F. SHAW. 3-Methoxy-4-hydroxy-D-mandelic acid, a urinary metabolite of norepinephrine. *Biochim. et biophys. acta* 25: 442–423, 1957.
5. ARMSTRONG, M D., K. N. F. SHAW AND P. E. WALL. Phenolic acids of human urine. *J. Biol. Chem.* 218: 293–303, 1958.
6. AXELROD, J. Studies on sympathomimetic amines. I. Biotransformation and physiological disposition of *l*-ephedrin and *l*-norephedrine. *J. Pharmacol. & Exper. Therap.* 109: 62–73, 1953.
7. AXELROD, J. Studies on sympathomimetic amines. II. Biotransformation and physiological disposition of *d*-amphetamine, *d-p*-hydroxyamphetamine and *d*-methamphetamine. *J. Pharmacol. & Exper. Therap.* 110: 315–326, 1954.
8. AXELROD, J. Enzymatic demethylation of ephedrine. *J. Pharmacol. & Exper. Therap.* 114: 430–438, 1955.
9. AXELROD, J. Enzymatic deamination of amphetamine. *J. Biol. Chem.* 214: 753–763, 1955.
10. AXELROD, J. Enzymic cleavage of aromatic ethers. *Biochem. J.* 63: 634–639, 1956.
11. AXELROD, J. O-Methylation of catechol amines *in vitro* and *in vivo*. *Science* 126: 400–401, 1957.
12. AXELROD, J. Presence, formation and metabolism of normetanephrine in the brain. *Science* 127: 754–755, 1958.
13. AXELROD, J. Metabolism of catechol amines *in vivo* and *in vitro*. *Pharmacol. Rev.* 11: (Pt. 2): 402, 1959.

13a. AXELROD, J. AND LAROCHE. *Science*. In press.

14. AXELROD, J., R. W. ALBERS AND C. D. CLEMENTE. Distribution of catechol-O-methyl transferase in the nervous system and certain tissues. *J. Neurochem.* In press.
15. AXELROD, J., R. O. BRADY, B. WITKOP AND E. V. EVARTS. Distribution and metabolism of lysergic acid diethylamine. Ann. New York Acad. Sc. 66: 435–444, 1957.
16. AXELROD, J., J. K. INSCOE, S. SENOH AND B. WITKOP. O-Methylation, the principal pathway for the metabolism of epinephrine and norepinephrine in the rat. *Biochim. et biophys. acta* 27: 210–211, 1958.
17. AXELROD, J., J. REICHENTHAL AND B. B. BRODIE. Mechanism of potentiating action of β-diethyl aminoethyl diphenylpropylacetate (SKF525A) *J. Pharmacol. & Exper. Therap.* 112: 49–54, 1954.
18. AXELROD, J., S. SENOH AND B. B. WITKOP. O-Methylation of catechol amines *in vivo*. *J. Biol. Chem.* 233: 697–701, 1958.
19. AXELROD, J. AND S. SZARA. Enzymic conversion of metanephrine to epinephrine. *Biochim. et biophys. acta* 30: 188–189, 1958.
20. AXELROD, J. AND R. TOMCHICK. Enzymatic O-methylation of epinephrine and other catechols. *J. Biol. Chem.* 233: 702–705, 1958.
21. AXELROD, J., S. UDENFRIEND AND B. B. BRODIE. Ascorbic acid in aromatic hydroxylation. III. Effect of ascorbic acid on hydroxylation of acetanilide, aniline and antipyrine *in vivo*. *J. Pharmacol. & Exper. Therap.* 111: 176–181, 1954.
22. AXELROD, J., H. WEIL-MALHERBE AND R. TOMCHICK. Physiological disposition of H^3-adrenaline and its principal metabolite, metanephrine. *Fed. Proc.* 18: 364, 1959.

22a. AXELROD, J., H. WEIL-MAEHERBE AND R. TOMCHICK. *J. Pharmacol. & Exper. Therap.* In press.

23. BACQ, Z. M. The metabolism of adrenaline. *Pharmacol. Rev.* 1: 1–26, 1949.
24. BALZER, H. AND P. HOLTZ. Beeinflussung der Wirkung biogener Amine durch Hemmung der Aminoxydase. *Arch. exper. Path. u. Pharmakol.* 227: 547–558, 1956.
25. BEYER, K. H. Action of vitamin C and phenoloxidase in the inactivation of β-phenylpropylamines. *J. Pharmacol. & Exper. Therap.* 71: 394–401, 1941.
26. BEYER, K. H. Ascorbic acid in the inactivation of sympathomimetic amines. *J. Pharmacol. & Exper. Therap.* 76: 149–155, 1942.
27. BEYER, K. H. Sympathomimetic amines: Relation of structure to their action and inactivation. *Physiol. Rev.* 26: 169–197, 1946.
28. BEYER, K. H. AND S. H. SHAPIRO. Excretion of conjugated epinephrine and related compounds. *Am. J. Physiol.* 144: 321–330, 1945.
29. BEYER, K. H. AND J. T. SKINNER. Detoxication and excretion of β-phenylisopropylamine (Benzedrine). *J. Pharmacol. & Exper. Therap.* 68: 419–432, 1940.
30. BLASCHKO, H. Amine oxidase and amine metabolism. *Pharmacol. Rev.* 4: 415–458, 1952.
31. BLASCHKO, H. AND F. J. PHILPOT. Enzymic

oxidation of tryptamine derivatives. *J. Physiol.* 122: 403–408, 1953.

31a. BLASCHKO, H., D. RICHTER AND H. J. SCHLOSSMAN. Inactivation of adrenaline. *J. Physiol.* 90: 1–17, 1937.

32. BLOCK, W., K. BLOCK AND B. PATZIG. Zur Physiologic des C^{14}-radioaktiven Mescalins in Tierversuch. II. Mitteilung Verteilung der Radioaktivitat in den Organen in Abhangigkeit von der Zeit. *Ztschr. Physiol. Chem.* 290: 230–243, 1952.

33. BOOTH, A. N., C. W. MURRAY, F. DEEDS AND F. T. JONES. Metabolic fate of rutin and quercitrin. *Fed. Proc.* 14: 321, 1958.

34. BRODIE, B. B. AND J. AXELROD. Fate of acetanilide in man. *J. Pharmacol. & Exper. Therap.* 94: 29–38, 1948.

35 BRODIE, B. B. AND J. AXELROD. Fate of antipyrine in man. *J. Pharmacol. & Exper. Therap.* 98: 97–104, 1950.

36. BRODIE, B. B., J. AXELROD, P. A. SHORE AND S. UDENFRIEND. Ascorbic acid in aromatic hydroxylation II. Products formed by reaction of substrate with ascorbic acid, ferrous ion and oxygen. *J. Biol. Chem.* 208: 741–750, 1954.

37. BRODIE, B. B., J. R. GILLETTE AND B. N. LA DU. Enzymatic metabolism of drugs and other foreign compounds. *Ann. Rev. Biochem.* 27: 427–454, 1958.

38. BROWN, D. D., J. AXELROD AND R. TOMCHICK. Enzymatic N-methylation of histamine. *Nature* 183: 680, 1959.

39. BROWN, G. L. AND J. S. GILLESPIE. Output of sympathetic transmitter from the spleen of the cat. *J. Physiol.* 138: 81–102, 1957.

40. BROWN, B. G. AND P. HEY. Choline phenyl ethers as inhibitors of amine oxidase. *Brit. J. Pharmacol.* 11: 58–65, 1956.

41. BURN, J. H. Mechanism of action of chemical substances at nerve endings. *Acta physiol. scandinav.* 29: 40–49, 1953.

42. BURN, J. H., F. J. PHILPOT AND U. TRENDELENBURG. Effect of denervation on enzymes in iris and blood vessels. *Brit. J. Pharmacol.* 9: 423–429, 1954.

43. BURN, J. H. AND J. ROBINSON. Effect of denervation on amine oxidase in structures inervated by the sympathetic nerves. *Brit. J. Pharmacol.* 7: 304–318, 1952.

44. CAMMARATA, P. S. AND P. P. COHEN. Scope of transamination in tissues. *J. Biol. Chem.* 187: 439–452, 1950.

45. CANTONI, G. L. S-Adenosylmethionine; a new intermediate formed enzymatically from l-methionine and adenosinetriphosphate. *J. Biol. Chem.* 204: 403–416, 1953.

46. CARLSSON, A., M. LINDQVIST, T. MAGNUSSON AND B. WALDECH. Presence of 3-hydroxytyramine in brain. *Science* 127: 471, 1958.

47. CARLSSON, A., E. ROSENGREN, A. BERTLER AND J. NILSSON. Effect of reserpine on the metabolism of catechol amines. In: *Psychotropic Drugs*, edited by S. Garattini and V. Ghetti. Amsterdam: Van Nostrand, 1958, pp. 363–372.

48. CELENDER, O. AND S. MELLANDER. Elimination of adrenaline and noradrenaline from circulating blood. *Nature* 176: 973, 1955.

49. COCHIN, J., L. A. WOODS AND M. H. SEEVERS. Absorption, distribution and urinary excretion of mescaline in the dog. *J. Pharmacol & Exper. Therap.* 101: 205–209, 1951.

50. COOPER, J. R., J. AXELROD AND B. B. BRODIE. Inhibitory effects of β-diethylaminoethyl diphenylpropylacetate-(SKF525A) *J. Pharmacol. & Exper. Therap.* 112: 49–54, 1954.

51. COOPER, J. R. AND B. B. BRODIE. Enzymatic metabolism of hexobarbital (Evipal). *J. Pharmacol & Exper. Therap.* 114: 409–417, 1955.

52. CORNE, S. J. AND J. D. P. GRAHAM. Effect of inhibition of monoamine oxidase *in vivo* on administered adrenaline, noradrenaline, tyramine and serotonin. *J. Physiol.* 135: 339–349, 1957.

53. DAVISON, A. N. Physiological role of monoamine oxidase. *Physiol. Rev.* 38: 729–747, 1958.

54. DEEDS, F., A. N. BOOTH AND F. T. JONES. Methylation and dehydroxylation of phenolic compounds by rats and rabbits. *J. Biol. Chem.* 225: 615–621, 1957.

55. DUTTON, G. J. AND I. D. E. STOREY. Uridine compounds in glucuronic acid metabolism. I. Formation of glucuronides in liver suspensions. *Biochem. J.* 57: 275–283, 1954.

56. EADE, N. R. Storage and release of catechol amines. *Rev. Canad. Biol.* 17: 299–311, 1958.

57. ERSPAMER, V. Fate of indolalkylamines in the organism. *J. Physiol.* 127: 118–133, 1955.

58. VON EULER, C., U. S. VON EULER AND I. FLODING. Biologically inactive catechol derivatives in urine. *Acta physiol. scandinav.* 33: Suppl. 118: 32–38, 1955.

59. VON EULER, U. S. Nature of adrenergic nerve mediators. *Pharmacol. Rev.* 3: 247–277, 1951.

60. VON EULER, U. S. Catechol amine content of various organs of the cat after injections and infusions of adrenaline and noradrenaline. *Circulation Res.* 4: 647–652, 1956.

61. VON EULER, U. S. *Noradrenaline.* Springfield, Ill.: Thomas, 1956.

62. VON EULER, U. S. Distribution and metabolism of catechol amines. *Recent Progress Hormone Res.* 14: 483–505, 1958.

63. VON EULER, U. S. AND S. HELLNER-BJÖRKMAN. Effect of amine oxidase inhibitors on noradrenaline and adrenaline content of cat organs. *Acta physiol. scandinav.* 33, Suppl. 118: 21–25, 1955.

64. VON EULER, U. S. AND F. LISHAJKO. Catechol amines in the vascular wall. *Acta physiol. scandinav.* 42: 333–341, 1958.

65. VON EULER, U. S. AND R. LUFT. Noradrenaline output in urine after infusion in man. *Brit. J. Pharmacol.* 6: 286–288, 1951.

66. VON EULER, U. S., R. LUFT AND T. SUNDEN. Excretion of urinary adrenaline in normals following intravenous infusion. *Acta physiol. scandinav.* 30: 249–257, 1953.

67. VON EULER, U. S. AND B. ZETTERSTROM. Role of amine oxidase in the inactivation of catechol amines injected in man. *Acta physiol. scandinav.* 33, Suppl. 118: 26–31, 1955.

68. EVARTS, E. V., L. GILLESPIE, T. C. FLEMING AND A. SJOERDSMA. Relative lack of pharmacological action of the 3-methoxy analogue of nor-

epinephrine. *Proc. Soc. Exper. Biol. & Med.* 98: 74–76, 1958.

69. EWINS, A. J. AND P. P. LAIDLAW. Fate of p-hydroxyphenylethylamine in the organism. *J. Physiol.* 41: 78–87, 1910.
70. EWINS, A. J. AND P. P. LAIDLAW. Fate of indolethylamine in the organism. *Biochem. J.* 7: 18–25, 1913.
71. FRIEND, G., M. S. ZILELI, J. R. HAMILIN AND F. W. REUTTER. Effect of iproniazid on the inactivation of norepinephrine in the human. *J. Clin. & Exper. Psychotherap.* 19: 61–68, 1958.
72. FURCHTGOTT, R. F., P. WEINSTEIN, H. HUEBL, P. BOZORGMEHRI AND S. R. MENSENDIEK. Effect of inhibition of monoamine oxidase on response of rabbit aortic strips to sympathomimetic amines. *Fed. Proc.* 14: 341–342, 1955.
73. GADDUM, J. H. In: *Ciba Foundation Symposium on Hypertension.* Boston: Little, 1954.
74. GADDUM, J. H. AND H. KWIATKOWSKI. Action of ephedrine. *J. Physiol.* 94: 87–100, 1938.
75. GAUDETTE, L. E., J. L. SCOTT AND P. KOSKY. Metabolism of DL-C^{14}-epinephrine in mammals. *Fed. Proc.* 17: 52, 1958.
76. GILLETTE, J. R., B. B. BRODIE AND B. N. LA DU. Oxidation of drugs by liver microsomes: Role of TPNH and oxygen. *J. Pharmacol. & Exper. Therap.* 119: 532–540, 1957.
77. GOLDSTEIN, M., A. J. FRIEDHOFF, C. SIMMONS AND N. PROCHOROFF. Conversion of 3-hydroxytyramine *l*-C^{14} to 3-methoxynorepinephrine *in vivo*. *Fed. Proc.* 18: 236, 1959.
78. GOODALL, McC. AND N. KIRSHNER. Biosynthesis of epinephrine and norepinephrine by sympathetic nerves and ganglia. *Circulation* 17: 366–371, 1958.
79. GREEN, D. E. AND D. E. RICHTER. Adrenaline and adrenochrome. *Biochem. J.* 31: 596–616, 1937.
80. GREISEMER, E. C., C. A. BARSKY, C. A. DRAGSTEDT, J. A. WELLS AND E. A. ZELLER. Potentiating effect of iproniazid on the pharmacological actions of sympathomimetic amines. *Proc. Soc. Exper. Biol. & Med.* 84: 699–701, 1953.
81. GUGGENHEIM, M. AND W. LÖFFLER. Das Schicksal protienogener Amine in Tierkörper: *Biochem. Ztschr.* 72: 325–350, 1915.
82. HARE, M. L. C. Tyramine oxidase. I. A new enzyme system in liver. *Biochem. J.* 22: 968–979, 1928.
83. HARLEY-MASON, J., A. H. LAIRD AND J. R. SMYTHIES. Metabolism of mescaline in the human. *Confinia neurol.* 18: 152, 1958.
84. HARTUNG, W. H. Inactivation and detoxication of pressor amines. *Ann. Rev. Biochem.* 15: 593–616, 1946.
85. HESS, S., B. G. REDFIELD AND S. UDENFRIEND. Tryptamine in animal tissues following the administration of iproniazid. *Fed. Proc.* 18: 402, 1959.
86. HOFFER, A. Adrenochrome in blood plasma. *Am. J. Psychiat.* 114: 752, 1958.
87. HOLTZ, P., K. CREDNER AND W. KOEPP. Die enzymatische Entstehung von Oxytyramine in Organismus und die physiologische Bedeutung der Dopadecarboxylase. *Arch. exper. Path. u. Pharmakol.* 200: 356–388, 1942.
88. HOLTZ, P., R. HEISE AND K. LÜDTKE. Fermentativer Abbau von Dopa durch Niere. *Arch. exper. Path. u. Pharmakol.* 191: 87–118, 1938.
89. HOLTZ, P. AND E. WESTERMANN. Ueber die Dopadecarboxylase und Histidinedecarboxylase des Nevengewebes. *Arch. exper. Path. u. Pharmakol.* 227: 538–546, 1956.
90. IMAIZUMI, R. AND K. KAWAMOTO. Adrenaline dehydrogenase in blood. *Med. J. Osaka Univ.* 3: 269–278, 1952.
91. IMAIZUMI, R., K. KAWAMOTO, T. KITA AND H. SATO. Adrenaline dehydrogenase in blood. *Med. J. Osaka Univ.* 3: 279–283, 1952.
92. ISSELBACHER, K. AND J. AXELROD. Enzymatic formation of corticosteroid glucuronides. *J. Am. Chem. Soc.* 77: 1070–1071, 1955.
93. JACOBSEN, E. AND I. GAD. Das Verhalten des β-Phenylisopropylamine in Kaninchenorganismus. *Arch. exper. Path. u. Pharmakol.* 196: 34–42, 1940.
94. JEPSON, J. B., S. UDENFRIEND AND P. SALTZMANN. Enzymic conversion of tryptamine to 6-hydroxytryptamine. *Fed. Proc.* 18: 254, 1959.
95. KAMIJO, K., G. B. KOELLE AND H. H. WAGNER. Modification of the effects of sympathomimetic amines and of adrenergic nerve stimulation by 1-isonicotinyl-2-isopropylhydrazine (IHH) and isonicotinic acid hydrazide (INH). *J. Pharmacol. & Exper. Therap.* 117: 213–227, 1955.
96. KELLER, R. E. AND W. C. ELLENBOGEN. Determination of d-amphetamine in body fluids. *J. Pharmacol. & Exper. Therap.* 106: 77–82, 1952.
97. KIRSHNER, N. AND McC. GOODALL. Formation of adrenaline from noradrenaline. *Biochim. et biophys. acta* 24: 658–659, 1957.
98. KIRSHNER, N., McC. GOODALL AND L. ROSEN. Metabolism of dl-adrenaline-2-C^{14} in the human. *Proc. Soc. Exper. Biol. & Med.* 98: 627–630, 1958.
99. KITA, T. Adrenaline metabolism, especially the formation of adrenalone, protocatechuic aldehyde and protocatechuic acid. *Nippon Yakurigaku Zasshi.* 53: 523–541, 1957. *Chem. Abstr.* 52: 10405, 1958.
100. KOELLE, G. B. Histochemical identification of acetylcholinesterase in cholinergic, adrenergic and sensory neurons. *J. Pharmacol. & Exper. Therap.* 114: 167–184, 1955.
101. KOELLE, G. B. AND A. DET. VALK. Physiological implications of the histochemical localization of monoamine oxidase. *J. Physiol.* 126: 434–447, 1954.
102. LABROSSE, E. H., J. AXELROD AND S. S. KETY. O-Methylation, the principal route of metabolism of epinephrine in man. *Science* 128: 593–594, 1958.
103. LABROSSE, E. A., J. AXELROD AND A. SJOERDSMA. Urinary excretion of normetanephrine by man. *Fed. Proc.* 17: 386, 1958.
104. LA DU, B. N., L. GAUDETTE, N. TROUSOF AND B. B. BRODIE. Enzymatic dealkylation of aminopyrine and other alkylamines. *J. Biol. Chem.* 214: 714–752, 1955.
105. LEEPER, L. C., H. WEISSBACH AND S. UDENFRIEND. Metabolism of norepinephrine, epinephrine and their O-methyl analogoues by partially purified enzyme preparations. *Arch. Biochem. & Biophys.* 77: 417–427, 1958.
106. LUND, A. Elimination of adrenaline and noradrenaline from the organism. *Acta pharmacol. et toxicol.* 7: 297–308, 1951.
107. MACLAGLAN, N. F. AND J. H. WILKINSON. Methylation of a phenolic hydroxyl group in the human body. *Nature* 168: 251, 1951.

108. McISAAC, W. AND I. H. PAGE. New metabolites of serotonin in carcinoid urine. *Science* 128: 537, 1958.
109. MITOMA, C., H. S. POSNER, H. C. REITZ AND S. UDENFRIEND. Enzymatic hydroxylation of aromatic compounds. *Arch. Biochem. & Biophys.* 61: 431–441, 1956.
110. MONTAGU, K. A. Catechol compounds in rat tissues and brains of different animals. *Nature* 180: 244–245, 1957.
111. MYCEK, M. J., D. D. CLARKE, A. NEIDLE AND H. WAELSCH. Enzyme-substrate relationship in amine incorporating system. *Fed. Proc.* 17: 280, 1958.
112. PAGE, I. H. Serotonin (5-hydroxytryptamine): the last four years. *Physiol. Rev.* 38: 277–335, 1958.
113. PEKKARINEN, A. Chemical determination, occurrence and metabolism of adrenaline in blood and urine. *Acta physiol. scandinav.* 16: 54, 1–110, 1948 (suppl.)
114. PELLERIN, J. AND A. D'IORIO. Metabolism of radioactive 3,4-dihydroxyphenylalanine α-C^{14} in the albino rat. *Canad. J. Biochem. & Physiol.* 33: 1055–1061, 1955.
115. PELLERIN, J. AND A. D'IORIO. Methylation of the 3-OH position of catechol acids by rat liver and kidney preparations. *Canad. J. Biochem. & Physiol.* 26: 491–497, 1958.
116. PUGH, C. E. M. AND J. H. QUASTEL. Oxidation of aliphatic amines by brain and other tissues. *Biochem. J.* 31: 286–291, 1937.
117. RAAB, W. AND A. B. GIGEE. Specific avidity of the heart muscle to absorb and store epinephrine and norepinephrine. *Circulation Res.* 3: 553–560, 1955.
118. RAAB, W. AND A. B. GIGEE. Uptake and breakdown of catecholamines in vascular tissue. *Angiology* 9: 283–289, 1958.
119. RAPPORT, M. M., A. A. GREEN AND I. H. PAGE. Serum vasoconstrictor (serotonin). IV. Isolation and characterization. *J. Biol. Chem.* 176: 1243–1251, 1948.
120. RABHUN, J., S. M. FEINBERG AND E. A. ZELLER. Potentiating effects of iproniazid on the action of some sympathomimetic amines. *Proc. Soc. Exper. Biol. & Med.* 87: 218–220, 1954.
121. RESNICK, O., J. M. WOLFE, H. FREEMAN AND F. ELMADJIAN. Iproniazid treatment and metabolism of labeled epinephrine in schizophrenics. *Science* 127: 1116–1117, 1958.
122. RICHTER, D. Adrenaline and amine oxidase. *Biochem. J.* 31: 2022-2028, 1937.
123. RICHTER, D. Elimination of amines in man. *Biochem. J.* 32: 1763–1769, 1938.
124. RICHTER, D. Inactivation of adrenaline *in vivo* in man. *J. Physiol.* 98: 361–374, 1940.
125. RICHTER, D. AND F. C. MACINTOSH. Adrenaline ester. *Am. J. Physiol.* 135: 1–5, 1941.
126. RODNIGHT, R. Separation and characterization of urinary indoles resembling 5-hydroxytryptamine and tryptamine. *Biochem. J.* 64: 621–626, 1956.
127. SALOMAN, K., B. W. GABRIO AND T. THALE. A study of mescaline in human subjects. *J. Pharmacol. & Exper. Therap.* 95: 455–459, 1949.
128. SARKAR, N. K., D. D. CLARKE AND H. WAELSCH. An enzymatically catalyzed incorporation of amines into proteins. *Biochim. et biophys. acta* 25: 451–452, 1957.
129. SCHAEPDRYVER, A. F. *Secretion, Distribution and Excretion of Adrenaline and Noradrenaline.* Bruges, Belgium: St. Catherine Press, 1959.
130. SCHAYER, R. W. Metabolism of β-C^{14} DL-adrenaline. *J. Biol. Chem.* 189: 301–306, 1951.
131. SCHAYER, R. W. Metabolism of adrenaline containing isotopic carbon. *J. Biol. Chem.* 192: 875–881, 1951.
132. SCHAYER, R. W. *In vivo* inhibition of monoamine oxidase studied with radioactive tyramine. *Proc. Soc. Exper. Biol. & Med.* 84: 60–63, 1953.
133. SCHAYER, R. W. Metabolism of histamine in various species. *Brit. J. Pharmacol.* 11: 472–473, 1956.
134. SCHAYER, R. W., R. L. SMILEY, K. J. DAVIS AND Y. KOBAYASHI. Role of monoamine oxidase in noradrenaline metabolism. *Am. J. Physiol.* 182: 285–286, 1955.
135. SCHAYER, R. W., R. L. SMILEY AND E. H. KAPLAN. Metabolism of adrenaline containing isotopic carbon (II). *J. Biol. Chem.* 198: 545–551, 1952.
136. SCHAYER, R. W., R. L. SMILEY AND J. KENNEDY. Metabolism of epinephrine containing isotopic carbon III. *J. Biol. Chem.* 202: 425–430, 1953.
137. SCHAYER, R. W., K. Y. T. WU, R. L. SMILEY AND Y. KOBAYASHI. Monoamine oxidase in intact animals. *J. Biol. Chem.* 210: 259–267, 1954.
138. SCHÜMANN, H. J. Nachweis von Oxytyramine (Dopamin) in sympathischen Nerven und Ganglien. *Arch. exper. Path u. Pharmakol.* 227: 566–573, 1956.
139. SCHÜMANN, H. J. Über die Verteilung von Noradrenalin und Hydroxytyramin in sympathischen Nerven (Milznerven). *Arch. exper. Path u. Pharmakol.* 234: 17–25, 1958.
139a. SENOH, S., J. DALY, J. AXELROD AND B. WITKOP. *J. Am. Chem. Soc.* In press.
140. SHAW, K. N. F., A. McMILLAN AND M. D. ARMSTRONG. Metabolism of 3,4-dihydroxyphenylalanine. *J. Biol. Chem.* 226: 255–266, 1957.
141. SHORE, P. A., J. A. R. MEAD, R. G. KUNTZMAN, S. SPECTOR AND B. B. BRODIE. Physiological significance of monoamine oxidase in the brain. *Science* 126: 1063–1064, 1957.
142. SJOERDSMA, A., W. M. KING, L. C. LEEPER AND S. UDENFRIEND. Demonstration of the 3-methoxy analog of norepinephrine in man. *Science* 127: 876, 1958.
143. SLOTTA, K. H. AND J. MULLER. Uber den Abbau des Mescalin und mescalinanlicher Stoffe in Organismus. *Ztschr. Physiol. Chem.* 238: 217–245, 1936.
144. SPECTOR, S., H. M. MALING AND P. A. SHORE. Effect of JB516, a monoamine oxidase inhibitor, on levels of serotonin and norepinephrine in brain and spinal cord. *Fed. Proc.* 18: 447, 1959.
145. SPECTOR, S., D. PROCKOP, P. A. SHORE AND B. B. BRODIE. Effect of iproniazid on brain levels of norepinephrine and serotonin. *Science* 127: 704, 1958.
146. SZARA, S. Dimethyltryptamine: Its metabolism in man, the relation of its psychotic effects to serotonin metabolism. *Experientia* 12: 441, 1956.
147. SZARA, S. AND J. AXELROD. Hydroxylation and demethylation of dimethyltryptamine *in vitro* and *in vivo*. *Experientia* 15: 216, 1959.
148. SZARA, S., J. AXELROD AND S. PERLIN. Is

adrenochrome present in the blood? *Am. J. Psychiat.* 115: 162–163, 1958.

149. TABOR, C. W., H. TABOR AND S. M. ROSENTHAL. Purification of amine oxidase from beef plasma. *J. Biol. Chem.* 208: 645–661, 1954.
150. TWAROG, B. M. AND I. H. PAGE. Serotonin content in some mammalian tissues and urine. *Am. J. Physiol.* 175: 157–161, 1953.
151. UDENFRIEND, S., C. T. CLARK, J. AXELROD AND B. B. BRODIE. Ascorbic acid in aromatic hydroxylation. I. A model system for aromatic hydroxylation. *J. Biol. Chem.* 208: 731–739, 1954.
152. UDENFRIEND, S., C. S. CREVELING, H. POSNER, B. C. REDFIELD, J. DALY AND B. WITKOP. Inability of tryptamine to serve as a precursor of serotonin. *Arch. Biochem. & Biophys.* In press.
153. UDENFRIEND, S., E. O. TITUS AND H. WEISSBACH. Identification of 5-hydroxy-3-indoleacetic acid in normal urine and a method for its assay. *J. Biol. Chem.* 216: 499–505, 1955.
154. UDENFRIEND, S., E. O. TITUS, H. WEISSBACH AND R. E. PETERSON. Biogenesis and metabolism of 5-hydroxyindole compounds. *J. Biol. Chem.* 219: 335–344, 1956.
155. VOGT, M. Concentration of sympathin in different parts of central nervous system under normal conditions and after administration of drugs. *J. Physiol.* 123: 451–481, 1954.
156. VOGT, M. Catechol amines in brain. *Pharmacol. Rev.* 11 (Pt. 2): 483, 1959.
157. WALKENSTEIN, S. S., N. CHUMAKOW AND J. SHIFTER, Detoxication and excretion of mephentermine. *J, Pharmacol. & Exper. Therap.* 115: 16–20, 1955.
158. WEISSBACH, H., W. KING, A. SJOERDSMA AND S. UDENFRIEND. Formation of indole-3 acetic acid and tryptamine in animals. *J. Biol. Chem.* 234: 81–86, 1959.
159. WEIL-MALHERBE, H., J. AXELROD AND R. TOMCHICK. The blood-brain barrier for adrenaline. *Science* 129: 1226, 1959.
160. WEIL-MALHERBE, H. AND A. D. BONE. The fluorimetric estimation of adrenaline and noradrenaline in plasma. *Biochem. J.* 67: 65–72, 1957.
161. WEISSBACH, H., B. G. REDFIELD AND S. UDENFRIEND. Soluble monoamine oxidase; its properties and actions on serotonin. *J. Biol. Chem.* 229: 953, 1957.
162. WERLE, E. AND G. MENNICKEN. Über die Bildung von Tryptamin aus Tryptophan und von Tyramin aus Tyrosin durch tierisches Gewebe. *Biochem. Ztschr.* 291: 325–327, 1937.
163. WERLE, E. AND F. ROEWER. Über tierische und pflanzilche Monoaminoxydasen. *Biochem. Ztschr.* 322: 320–326, 1952.
164. WOOLEY, D. W. AND E. SHAW. A biochemical and pharmacological suggestion about certain mental disorders. *Proc. Natl. Acad. Sci.* 40: 228–231, 1954.
165. ZELLER, E. A. AND J. BARSKY. *In vivo* inhibition of liver and brain by 1-isonicotinyl-2-isopropylhydrazine. *Proc. Soc. Exper. Biol. & Med.* 81: 459–461, 1952.
166. ZELLER, E. A., J. BARSKY, E. R. BERMAN, M. S. CHERKAS AND J. R. FOUTS. Degradation of mescaline by amine oxidases. *J. Pharmacol. & Exper. Therap.* 124: 282–289, 1958.