0031-6997/85/3704-0333$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 37, No. 4
Printed in U.S.A.

Catecholamine Metabolism: Basic Aspects and Clinical Significance

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I. Introduction

THE three naturally occurring catecholamines, EPI,* NE, and DA, play important roles as brain and peripheral sympathoadrenal medullary neurohumoral transmitters (fig. 1). EPI was discovered first as the hormone released from the adrenal medulla in response to a variety of physiological or environmental stresses. NE, its immediate precursor, was later identified as the neurotransmitter substance released from peripheral sympathetic nerves throughout the body. Because NE was not distributed uniformly throughout the brain, Vogt (277) suggested that it was also a central neurotransmitter. Although DA was long recognized as the precursor of these catecholamines, it was the last of the three to be suspected of having a physiological role. Since the concentration of EPI in brain is very low and it is localized to a relatively few areas, only recently has this catecholamine been considered to have a role as a transmitter substance in brain.

Understanding the metabolism of the catecholamines is important for gaining insights into the mechanisms which are responsible for their inactivation, for evaluating the level of activity of the neural pathways in which they play a role, and for determining how drugs might act through altering their metabolism or disposition. It has been realized for nearly half a century that catecholamines were substrates for MAO, but it became apparent that, although the enzyme acted on these compounds in vivo and that at least a portion of each of these amines was metabolized by deamination, other mechanisms were important for terminating their action. The subsequent discoveries of COMT and the active transport processes for neuronal and extraneuronal uptake of the amines accounted for the various metabolic products of the catecholamines and adequately explained their inactivation. Over the years there have been a number of reviews of the state of knowledge of metabolism and disposition of catecholamines (1, 15, 16, 19, 88, 115, 117, 147). A large (1000 page) volume, Catecholamines, edited by Blaschko and Muscholl (24), provides a detailed account of the remarkable advances that had been made in understanding the disposition, metabolism, and biochemical effects of catecholamines and their interactions with drugs.

TABLE 1
*Abbreviations used in text**

Catecholamines and metabolites	
DOPA	3,4-Dihydroxyphenylalanine
DA	Dopamine (3,4-dihydroxyphenylethylamine)
NE	Norepinephrine (noradrenalin)
EPI	Epinephrine (adrenaline)
3MT	3-Methoxytyramine (4-hydroxy-3-methoxyphenylethylamine)
NMN	Normetanephrine
MN	Metanephrine
DOPAC	3,4-Dihydroxyphenylacetic acid
DOPET	3,4-Dihydroxyphenylethanol
DHMA	3,4-Dihydroxymandelic acid (DOMA)
DHPG	3,4-Dihydroxyphenyl(ethylene)glycol (DOPEG)
HVA	Homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid)
MOPET	4-Hydroxy-3-methoxyphenylethanol
VMA	Vanillyl mandelic acid (4-methoxy-3-hydroxymandelic acid, MOMA)
MHPG	4-Hydroxy-3-methoxyphenyl(ethylene)glycol (MOPEG)
Enzymes and cofactors	
MAO	Monoamine oxidase
COMT	Catechol-O-methyltransferase
PST	Phenolsulfotransferase
S-AMe	S-Adenosylmethionine
UDPGT	Uridine diphosphoglucuronyl transferase
UDPG	Uridine diphosphoglucose
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced form of NAD
Other	
CSF	Cerebrospinal fluid
6-OH-DA	6-Hydroxydopamine (3,4,6-trihydroxyphenylethylamine)
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

* In those compounds derived from dopamine, "hydroxy" has been designated as "O," whereas in those compounds derived from NE, hydroxy is translated as "H." Thus, it is immediately apparent that MOPET is derived from DA, whereas MHPG is derived from NE, etc.

* Abbreviations used are: EPI, epinephrine; NE, norepinephrine; DA, dopamine; MAO, monoamine oxidase; COMT, catechol-O-methyltransferase; PST, phenolsulfotransferase; S-AMe, S-adenosylmethionine; NMN, normetanephrine; MET, methionine; MHPG, 4-hydroxy-3-methoxyphenyl(ethylene)glycol; PAPS, phosphoadenosinephosphosulfate; HVA, homovanillic acid; VMA, vanillyl mandelic acid; TS, thermostable; TL, thermolabile; DOPA, 3,4-dihydroxyphenylalanine; UDPG, uridine diphosphoglucose; DOPAC, 3,4-dihydroxylphenylacetic acid; DOPET, 3,4-dihydroxyphenylethanol; DHPG, 3,4-dihydroxyphenyl(ethylene)glycol; DHMA, 3,4-dihydroxymandelic acid; OMDA, 4-hydroxy-3-methoxydopamine; 3MT, 3-methoxytyramine; CSF, cerebrospinal fluid; GC-MS, gas chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; ED, electrochemical detection; GC-EC, gas chromatography-electrochemical; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OH-DA, 6-hydroxydopamine; IOH, idiopathic orthostatic hypotension; MN, methanephrine; MOPET, 4-hydroxy-3-methoxyphenylethanol; UDPGT, uridine diphosphoglucuronyl transferase.

Most of the metabolic studies performed up to about that time depended on radioisotope-labeled compounds. The most widely used was tritiated NE ([^{3}H]NE) prepared as the racemic catecholamine. Attention was focused on identifying and quantifying the urinary excretion products of the catecholamines. In addition to diagnosis of catecholamine-producing tumors, attempts were made to define abnormalities in neuropsychiatric disorders and other disease states or to determine the effects of drugs and thereby draw inferences regarding their mode of action. While many of the observations obtained with (±)-[^{3}H]NE are generally valid, the availability of (−)-[^{3}H]NE made possible examination of stereospecificity of the various enzymes, storage sites, and transport processes involving the catecholamines. Furthermore, these could be examined in isolated tissues, and the kinetic parameters governing metabolism and transfer of the amines and their metabolites could be described in detail (263, 264).

With the development of highly sensitive and specific assays for exogenous catecholamines and their metabolites and the unfolding of additional information about the activities and location of enzymes involved in catecholamine O-methylation and deamination, the factors influencing metabolite diffusion and transport, and the intermediary processes which transform the initially formed metabolites into the final excretion products, there has been a continuing evolution in our concepts regarding the origins and routes of formation, distribution, and excretion of catecholamine metabolites. It is the purpose of this review to integrate the information which has been acquired about catecholamine metabolites during the last decade with what had been known previously and to indicate how this newer information influences interpretation of data on the levels of catecholamine metabolites in the various body fluids.

II. Catecholamine Metabolizing Enzymes

COMT, MAO, and PST act on the catecholamines to form their 3-O-methylated, deaminated, and sulfoconjugated derivatives, respectively. In some species, glucuronide conjugation occurs and may exceed sulfation. There appear to be membrane-bound and cytoplasmic forms of COMT and two distinct types of MAO, both of which are mitochondrial enzymes, and several types of PST. The relative importance of these enzymes in metabolic inactivation of catecholamines depends on their cellular and subcellular locations, their concentrations and relative affinities for their substrates, and the amounts of catecholamines to which they are exposed. These factors vary with species and with the tissue examined. Furthermore, the aldehyde product produced by the action of MAO may be reversibly reduced to the alcohol or irreversibly oxidized to the acid metabolite. The amine products of O-methylation are also substrates for MAO and conjugating enzymes; the deaminated catechols can be O-methylated by COMT. The metabolites resulting from these reactions are shown in fig. 1. In addition to these, small amounts of vanillic acid are formed as a final metabolite of NE. Before considering the various metabolic pathways and their quantitative importance, it is useful to review the properties and localization of the enzymes involved.

A. Cellular and Subcellular Distributions of COMT

COMT is a magnesium-dependent enzyme which catalyzes the transfer of the methyl group from S-AMe to one of the hydroxyl groups (usually the hydroxyl group in position 3) of a catechol (fig. 2). Although (18) originally purified COMT from the soluble fraction of rat liver homogenates, in many tissues, including liver and brain, COMT occurs in both soluble and membrane-bound forms. Generally the soluble form predominates, but in some tissues, such as human lymphocytes (253), as much as 50% of the COMT may be membrane bound.

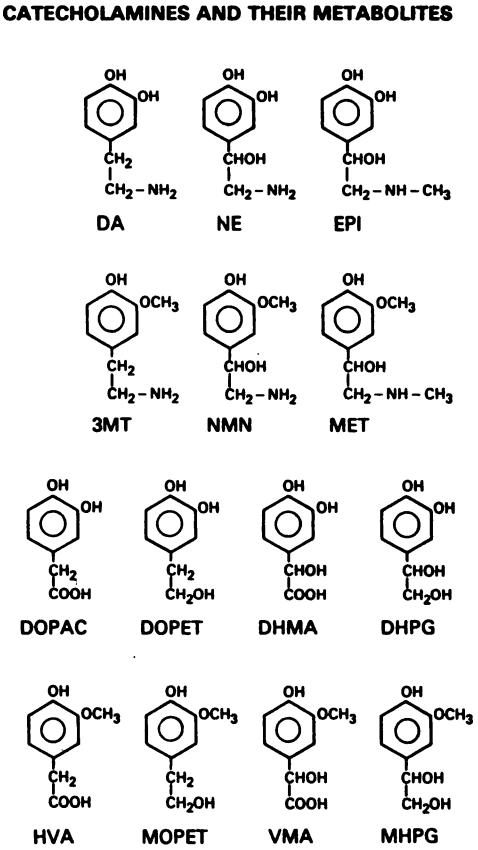

FIG. 1. Chemical structures of catecholamines and their major metabolites. Abbreviations are defined in table 1 and footnote *.

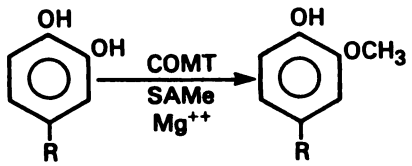

FIG. 2. COMT is a magnesium-dependent enzyme which catalyzes the transfer of the methyl group from S-AMe predominantly to position 3 of 3,4-dihydroxyphenyl derivatives, including catecholamines and their metabolites.

There are many similarities between the two forms of COMT, and they are probably closely related structurally, but there are also important differences. Antibodies prepared against soluble rat liver COMT react with COMT in liver, kidney, brain, and choroid plexus (132), but immunoreactive COMT does not necessarily represent a single protein. Gel electrophoresis of solubilized COMT from various rat tissues revealed the presence of three major immune-specific proteins with apparent molecular weights of 23,000, 26,000, and 66,000 (99). The M_r 26,000 protein appeared to be associated with a particulate fraction obtained by centrifugation at 100,000 × *g* for 1 h, whereas the others remained in the supernatant fraction. The larger of the soluble forms was believed to be an aggregate of smaller form(s). Isoelectric focusing resolved the M_r 23,000 protein into three bands, but the M_r 26,000 protein remained a single band. The major form of COMT in all rat tissues was the soluble M_r 23,000 protein with a pI of 5.2. COMT obtained from the outer mitochondrial membranes from rat liver had different properties, appearing identical to the M_r 26,000 protein (pI 6.2).

In spite of differences in the properties of soluble and membrane-bound COMT, there appear to be many similarities between the two forms of the enzyme (see ref. 122 and the references therein). Thus, although the pH optimum for the membrane-bound form of COMT is near 7.0, and that of the soluble form is close to 8.0 (14), solubilization of the membrane-bound COMT with Triton X-100 shifts the pH optimum to that of the soluble form. Furthermore, the high affinity of the membrane-bound form of COMT is reduced by solubilization, but not to the low level of the soluble form(s). The reaction mechanisms of the two forms of COMT also appear to be similar, with formation of an enzyme-S-adenosylmethionine complex before interaction with the catechol to be methylated (123). These similarities and the immunological cross-reactivity of the soluble and bound forms of COMT suggest that they are structurally similar, but not identical.

COMT is not distributed equally among the cells in a tissue. In the rat heart, COMT immunoreactive protein is present in the capillary endothelium and myocardial cells but appears to be absent in the smooth muscles of the coronary arteries (172). On examination by an electron microscopic technique, COMT immunoreactivity was found in the cytoplasm and on the plasma membranes of these cells, but not on intracellular membranes. Since membrane-bound COMT has a much higher affinity for catecholamines than does the soluble form (14,224), at lower concentrations of released catecholamines, the smaller quantity of the high affinity form of the enzyme might account for a substantial portion of O-methylation. The role of plasma membrane-associated COMT in O-methylation was examined by Head et al. (104a) by incubating segments of rabbit thoracic aorta with [*methyl*-^{3}H]S-AMe and isoproterenol. They found preferential O-methylation of the (+)- rather than the (−)-isomer and 8-fold stimulation of the methylation by removal of calcium ions. O-methylation of [^{3}H]isoproterenol by endogenous intracellular S-AMe was unaffected. These results indicate that, while there was some COMT available to extracellular sites, the lack of S-AMe and its inhibition by physiological concentrations of calcium ions made unlikely a role for this enzyme site in O-methylation of extracellular catecholamines.

In the brain, COMT immunoreactivity is demonstrable only in nonneuronal cellular elements, i.e., glia, ventricular ependymal cells, choroid plexus, and circumventricular organs, (131, 133, 134). Although the results of these studies suggest that the predominant localization of COMT is nonneuronal, the authors emphasize with appropriate caution that the presence of small quantities of the enzyme in neurons cannot be excluded. The location in association with endothelium and interfaces of the blood with the tissues, particularly brain, suggest a role for COMT as an enzymatic barrier to free catecholamine diffusion. Rivett et al. (225) found that, after kainic acid-induced neuronal degeneration and gliosis, there was a decrease in membrane-bound and an increase in soluble COMT. This change was attended by a decrease in glutamic acid decarboxylase (a neuronal marker) and an increase in glutamine synthetase (an astroglial marker), suggesting that membrane-bound COMT is localized predominantly to neurones, whereas soluble COMT is found in glial cells. They proposed that membrane-bound COMT in brain might be located on the outer surface of postsynaptic membranes, situated to inactivate neuronally released catecholamines. The immunocytochemical localization of COMT to cytoplasm and plasma membranes noted above is also consistent with these observations, but as indicated above, it is unlikely that extraneuronal O-methylation can play a physiologically important role in terminating the effects of released norepinephrine.

B. MAO-A and MAO-B

MAOs catalyze the oxidation of amines, presumably via an intermediate imine, to their corresponding aldehydes and ammonia with the formation of hydrogen peroxide (fig. 3). They are generally mitochondrial enzymes and are found throughout the body, but in highest concentration in the liver and kidney. Differences in the substrate affinities and specificities of MAOs derived

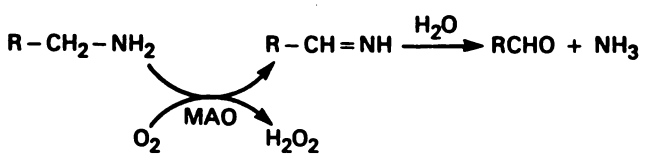

FIG. 3. MAO is a flavin-containing enzyme which reacts with monoamines and oxygen to yield hydrogen peroxide and an aldhyde. It has been postulated that an intermediary compound, perhaps an imine as shown, is involved.

from different tissues and the development of drugs which appear to inhibit selectively the differing MAO activities led to the conclusion that there are at least two types of MAO (see ref. 201). Although many amines appear to be substrates for both, they often differ in their affinity towards the two types of MAO. MAO-A is more active in deaminating 5-hydroxytryptamine, and MAO-B is more active towards benzylamine and phenylethylamine. MAO-A has a higher affinity for norepinephrine (297), so that at low concentrations of this amine, if exposed equally to both forms of the enzyme, MAO-A would be expected to be more important than MAO-B in its metabolism, but at high concentrations, the B form of MAO could become increasingly important.

Although most MAO inhibitors block the actions of both forms of the enzyme, the discovery by Johnston (129) that increasing concentrations of clorgyline had a biphasic effect on MAO activity in the ability of rat liver to deaminate tyramine led him to conclude that there were two forms of MAO, types A and B. Clorgyline was highly selective for MAO-A. The subsequent demonstration by Knoll and Magyar (146) that deprenyl is a much more effective inhibitor of MAO-B strengthened the case for the existence of two forms of MAO. Using these drugs to inhibit selectively one of the forms of MAO, there have been numerous studies of substrate specificity and effects on amine metabolism in vivo to determine the physiological importance of the MAO types. The results of such studies suggest that dopamine, like NE, is metabolized mainly, but not exclusively, by MAO-A (see, e.g., ref. 84). The physical separation of the two forms of MAO in human liver by immunoaffinity column chromatography (65, 66) is consistent with the significant structural differences between the two [^{3}H]pargyline-labeled MAOs (47). Levitt et al. (168), using antibodies against bovine MAO-B, found that in rat brain, immunoreactivity, presumably representing the B form of the enzyme, was localized in astrocytes and serotonergic neurones, but it could not be detected in noradrenergic neurones. This observation has been confirmed and extended in primate brain by Westlund et al. (289, 290). They demonstrated, by use of monoclonal antibodies directed towards each of the types of MAO, distinct populations of cells containing either MAO-A or MAO-B. They found that MAO-B was contained in serotonergic neurones in the median raphe and in astrocytes, particularly around large blood vessels and ventricles, whereas MAO-A was in neurones in the catecholaminergic cell areas (A1–A14). The highest concentrations of MAO-A were found in the locus coeruleus and subcoeruleus in the lateral pontine area. While both MAO-A and MAO-B were present in the hypothalamus, double staining indicated that the enzymes were localized to distinct, although intermingled, cell populations.

C. Conjugating Enzymes

The importance of conjugation in the disposition of p.o. administered catecholamines had been established since the early observation by Richter (223) that ingested EPI was almost completely recovered in urine as a conjugate, presumed to be an ethereal sulfate on the basis of the ease of its acid hydrolysis. Häggendal (101) was the first to report the presence of acid hydrolyzable conjugates of NE and EPI in plasma and noted that the levels were 2 to 3 times as great as those of the free amine. Furthermore, he reported that physiological procedures which elevated levels of the free catecholamines did not much influence the concentrations of the conjugates, whereas p.o. administration of EPI markedly increased plasma levels, as well as urinary excretion, of the conjugate. As might be expected, ingestion of foods, such as bananas or certain other fruits, which contain relatively high quantities of DA or NE, results in marked increases in urinary excretion (54) and plasma concentrations (61) of conjugated DA and NE. Although sulfoconjugation appears to be an extremely efficient means of protecting the body from the effects of ingested catecholamines, only a small proportion of i.v. administered EPI is excreted as a sulfate conjugate (15). In both rats and humans, however, glucuronide conjugates of catecholamine metabolites, NMN, MET, and MHPG, have been identified as major excretion products of administered labeled catecholamines and are also produced from the endogenous compounds.

1. Sulfoconjugation. The finding, that MHPG sulfate is the major NE metabolite in rat and guinea pig brain and is present in the brain and spinal fluid of the African green monkey (244), indicated that sulfation could occur in the brain of at least some species. The enzyme responsible for sulfation of MHPG and other phenols, PST, is present in higher concentration in rat liver than in any other tissue (82). Inorganic sulfate, derived from food or from catabolism of sulfur-containing amino acids or highly sulfated glycosaminoglycans, is activated in two enzymatic steps resulting in the formation of PAPS. PAPS is the sulfate donor for the sulfations catalyzed by PST.

Rat brain contains higher levels of PST than most other tissues, but adrenal, lung, kidney, and testes have measurable levels of the enzyme. Rabbits, rats, and guinea pigs have high brain levels; mice and frogs, low levels; and humans and chicks, intermediate levels of PST activity (82). Wong (294) found that HVA and VMA could also be sulfated by supernatant fluids from homogenates of liver and brain of mice, rats, and rabbits, but only about one-tenth as rapidly as was MHPG. In rabbit and dog, small intestinal homogenates contained about as much sulfating activity as did liver.

The presence in human brain of PST activity (222) and the occurrence of DA, NE, and EPI sulfoconjugates in human plasma (128) and cerebrospinal fluid (161, 269) stimulated considerable interest in sulfoconjugation and the enzymes involved in the formation of these conjugates (121, 240, 199).

PST activity is also present in whole blood, mostly in the platelets as a soluble enzyme (104). Although red cells appear to contain an endogenous inhibitor(s) of PST, none was demonstrable in the platelets, gut, or kidney (10). Platelet PST exists in two forms (46, 218, 220), one having a greater affinity for phenol ("P" form) and the other more active towards sulfation of monoamines and their metabolites ("M" form). The M form has the higher affinity for catecholamines, but some phenols are almost equally good substrates for both forms of the enzyme. The two forms of PST are distributed differently in the body and show different susceptibilities to inhibition by 2,6-dichloro-4-nitrophenol. The P form is more heat stable than the M form, and they have also been termed the TS and TL forms, respectively.

Rat brain contains at least two forms of PST, only one of which conjugates DA (226). After kainic acid lesioning of the striatum, there was a parallel decrease of both forms of PST with the decrease in neuronal markers, i.e., glutamic acid decarboxylase. Buu (35), however, found that 6-hydroxydopamine pretreatment did not alter the accumulation in the striatum (or hypothalamus and brainstem) of DA sulfate induced by administration of *l*-DOPA with a monoamine oxidase inhibitor. Taken together, these results suggest that PST is in non-catecholamine-containing neurones. In human brain, there are also two forms of PST activity having properties similar to the P (or TS) and M (or TL) forms (219, 299). Platelet and brain (temporal or frontal cortex from surgically treated epileptics) TS-PST activities were found to be closely correlated, whereas TL-PST activities showed no such correlation (298). There was a good correlation of human platelet PST activity in sulfating MHPG with activities found in the renal cortex or jejunal mucosal tissue (10). Since MHPG is a substrate for both forms of PST, these results suggest that platelet PST activity may reflect sulfating ability of at least the TL (or P) form of PST in a variety of tissues, including brain, but caution must be taken with regard to relating amine sulfation capacity in platelets to that in brain.

2. Glucuronide formation. The synthesis of glucuronides is an important means of metabolizing a variety of foreign and endogenous compounds. There does not appear to be any special function associated with glucuronidation of catecholamines or their metabolites. Glucuronide formation occurs mainly in the liver but may also take place in the skin, gastrointestinal tract, kidney, and possibly other tissues. Glucuronic acid is formed from glucose-1-phosphate after it is enzymatically converted to UDPG. UDPG is enzymatically oxidized by a specific dehydrogenase to uridine diphosphoglucuronic acid. A transferase is responsible for the reaction in which active glucuronic acid is converted to the phenolic hydroxide of catecholamine metabolites. Glucuronides of catecholamine metabolites have been found in the bile, plasma, and urine (see below). Although some hydrolysis of these conjugates by tissue glucuronidase may occur, they may be considered end products of metabolism.

D. Relative Activities of Catecholamine Metabolizing Enzymes

Although PST has a high affinity for the catecholamines, particularly for DA, the role of sulfation in the metabolism of endogenous catecholamines is still in question. Rivett et al. (224) attempted to estimate in homogenates of crude homogenates of human brain frontal cortex the total capacity of sulfoconjugation relative to O-methylation or deamination. On the basis of the kinetic constants, they calculated the relative activities of the three enzymatic pathways at different substrate concentrations and concluded that deamination would predominate for DA, whereas at low concentrations of NE, O-methylation and deamination would be about equally predominant over conjugation. Sulfation accounted for a maximum of 15% of DA and 7% of NE metabolism. It was acknowledged that this approach does not take into account selective localization of the enzymes and substrates nor the accessibility of the catecholamines to the enzyme sites within the cells. Furthermore, the enzyme assays were performed at saturating concentrations of cofactors; in vivo, the availability and accessibility of cofactors could limit enzymatic activity. Their study did point out, however, that many factors, in addition to the number of enzyme molecules in a cell, might be important in determining the fate of catecholamines in a tissue.

E. Aldehyde and Alcohol Metabolizing Enzymes

The products of oxidative deamination catalyzed by either MAO type A or B are aldehydes which are further metabolized by oxidation to the corresponding acids or reduction to the alcohol. Irreversible oxidation of aldehydes is carried out by aldehyde dehydrogenase, whereas their reduction can be mediated by aldehyde reductase. Alcohol dehydrogenase may reverse aldehyde reduction. Formation of the final metabolic products of the catecholamines involves each of these enzymes to varying extents depending on organ and species.

1. Aldehyde oxidation. Oxidation of aldehydes to acids is mediated by several aldehyde dehydrogenases which are found in many organs, including brain, but is present in highest concentration in liver. Two forms of aldehyde dehydrogenase are present in rat liver mitochondria, one of which plays a predominant role in oxidation of the aldehyde formed by deamination of DA (262). Acetaldehyde does not appear to compete with this oxidation, whereas rotenone blockade of mitochondrial reoxidation of NADH diminishes DOPAC and enhances DOPET formation from dopamine. The ratio of NAD^+/NADH appears to play an important role in determining the degree of predominance of dehydrogenase activity in this situation. There is also a cytosolic aldehyde dehydrogenase, but the mitochondrial enzyme has an order of mag-

nitude lower K_m and is probably responsible for oxidation of catecholamine-derived aldehydes (9). The aldehyde dehydrogenase level is about 10-fold higher in human liver than in brain and is more active (has a higher V_{max}) towards phenylacetaldehydes than for the β-hydroxylated derivatives, although there appears to be little difference in substrate affinity (K_m) for the enzyme (285, 286).

2. Aldehyde reduction. Transformation of aldehyde to their corresponding alcohols is mediated by aldehyde reductases. Theoretically, aldehyde reduction could result from the reverse of the reaction catalyzed by alcohol dehydrogenases. Both enzymes catalyze the same reversible reaction, but the equilibrium constants differ; the enzymes are named for the predominance of the direction of the reaction they catalyze. Thus, alcohol formation is overwhelmingly favored in the reaction catalyzed by aldehyde reductase, whereas aldehyde formation predominates in that mediated by alcohol dehydrogenase. Alcohol dehydrogenase is usually coupled to NADH, whereas aldehyde reductase prefers NADPH as its coenzyme. The enzymes also differ in their susceptibility to inhibitors; pyrazole does not inhibit aldehyde reductase but is an effective inhibitor of the dehydrogenase (260). There are at least two forms of aldehyde reductase, both of which appear to be cytosolic (238). The low K_m form appears to be relatively insensitive to inhibition by valproate and benzodiazepines, whereas the high K_m form is inhibited by these drugs. The low K_m form shows a preference for phenylglycolaldehydes over corresponding phenylacetaldehydes (261, 266), and the V_{max} of the low K_m form is enhanced 5-fold by O-methylation of a catechol, whereas the low K_m form is uninfluenced by O-methylation (260).

The properties of these enzymes and the factors which regulate their activities influence the proportions of oxidized and reduced metabolites which appear in the body fluids. Thus, the ratio of acid to alcohol as aldehyde products depends on the activities of aldehyde dehydrogenase and aldehyde reductase, the relative affinity of the aldehyde towards the available enzymes, and the levels of the appropriate cofactor and its oxidation-reduction.

3. Alcohol oxidation. Once formed, the alcohol derivative of the aldehyde product of MAO may be further metabolized by alcohol dehydrogenase. There are three major classes of alcohol dehydrogenases which differ in substrate specificity, susceptibility to inhibition by pyrazole derivatives, and immunologically (271). Mardh et al. (187) have recently reported that the class I, but not classes II or III, alcohol dehydrogenases are able to catalyze the oxidation of the glycol derivatives of NE deamination, DHPG and MHPG. The liver was reported to be the major site for this oxidation; the enzyme is competitively inhibited by ethanol in vivo, as well as in vitro. This has major implications in explaining the metabolic fate of NE in humans. Dietary, hormonal, or neurogenic influences might mediate alterations in the activity of alcohol dehydrogenase or PST with resultant shifts in metabolism of MHPG from conjugation to oxidation or vice versa. Such changes could account for depressed MHPG excretion in depression or apparent increased MHPG formation with alcohol ingestion (see below).

III. Metabolism of Catecholamines in Vitro

The fate of catecholamines in individual tissues determines the overall rate of entry of catecholamines and their metabolites into the body fluids, after which they are further metabolized or excreted. Individual tissues have been studied in vitro to examine the factors which influence the fate of catecholamines formed and released from sympathetic nerve terminals.

A. Metabolism of NE in Vitro

The availability of high specific activity tritiated racemic NE made possible studies of the disposition of the catecholamine in isolated tissues. The first of such studies was performed in isolated perfused rat hearts (152) and showed that uptake and storage of NE were quantitatively more important than metabolism, that O-methylation of the exogenous catecholamine exceeded deamination, and that in hearts of reserpine-pretreated rats storage was rapidly saturated and deamination became the predominant route of metabolic inactivation. These studies and studies of the metabolic excretion products of i.v. administered [^{3}H]NE immediately after its administration, 10–13 h later, and during depletion of stored [^{3}H]NE after administration of tyramine or reserpine, provided the first evidence that intraneuronal NE was deaminated, whereas NE released from the nerves was O-methylated (147). The kinetic studies of Iversen (117) detailed the high affinity neuronal and lower affinity extraneuronal uptake processes in isolated perfused rat hearts. As indicated above, racemic (±)-[^{3}H]NE was used in these early studies so that stereospecific processes influenced some of the results.

1. DHPG formation. When (−)-[^{3}H]NE became available, comparison of the physiological isomer with racemic [^{3}H]NE showed that neither neuronal nor extraneuronal uptake of NE is stereospecific (although extraneuronal uptake shows stereospecificity for N-substituted catecholamines; see, e.g., ref. 98a), but that only the physiological isomer is retained in the storage vesicles. Furthermore, DHPG was the predominant metabolite of (−)-[^{3}H]NE, whereas considerable amounts of DHMA were formed from the (+)-isomer. These observations were reported in cat nictitating membrane (95), rabbit aorta (70, 167), and rat vas deferens (97) and were confirmed in guinea pig atria incubated with unlabeled (−)- or (+)-NE (254).

DHPG has been found to be the major metabolite formed in vitro from previously stored (−)-[^{3}H]NE in a

variety of isolated tissues during incubation or perfusion (table 2). Although the specific activity of (−)-[^{3}H]NE released into the medium is different from that retained in the tissue (229), indicating that the (−)-[^{3}H]NE taken up into the tissue does not mix uniformly with the endogenous amine, reasonably valid conclusions appear to have been reached on the basis of the metabolism of the labeled amine, since similar results were obtained when it became possible to measure the metabolism of endogenous NE (table 2).

A number of earlier studies with (−)-[^{3}H]NE had shown that [^{3}H]DHPG appearing in the medium during nerve stimulation was largely derived from intraneuronally deaminated NE. Dubocovitch and Langer (68) reported that inhibition of NE uptake by cocaine or phenoxybenzamine prevented [^{3}H]DHPG formation from [^{3}H]NE released during stimulation of perfused cat spleens and concluded that a large portion of the released amine was recaptured by nerve endings and metabolized to [^{3}H]DHPG rather than being stored. Consistent with this conclusion, cocaine diminishes field stimulation-induced efflux of [^{3}H]DHPG from dog saphenous vein (27, 29) and from rabbit pulmonary artery (79) preloaded with [^{3}H]NE and amitriptyline, which, like cocaine inhibits uptake of NE, also decreases the outflow of DHPG during electrical stimulation of canine venous strips preloaded with [^{3}H]NE (51). Furthermore, in rabbit mesenteric artery, cocaine or imipramine increases stimulation-induced outflow of endogenous NE while decreasing DHPG outflow (197). Rorie et al. (228) noted that the diminution in ^{3}H-DHPG formation in dog saphenous vein when uptake was blocked was less than the enhancement of [^{3}H]NE release and concluded that the difference (5–20%) was a measure of the proportion of the recaptured NE which is sequestered in the vesicles for reuse.

TABLE 2
*DHPG as a major NE metabolite in vitro**

Species	Tissues	Refs.
Rat	Perfused heart (reserpinized)	81
	Atrium	49
	Vas deferens	96
	Vas deferens (endogenous)	206
	Mesenteric artery	98
	Tail artery (endogenous)	207
Guinea pig	Atrium	3
	Atrium (endogenous)	254
Rabbit	Perfused heart	94
	Perfused (endogenous)	184
	Aortic strip	70, 167
	Mesenteric artery (endogenous)	197
	Pulmonary artery	79
Cat	Perfused spleen	55, 68
	Nictitating membrane	166, 173
	Perfused heart	94
Dog	Saphenous vein	27, 28, 200
	Pulmonary artery	230

* DHPG has been shown to be the major metabolite of [^{3}H]NE which was previously taken up and stored in the sympathetic neurones, during spontaneous or stimulation-induced release of the neurotransmitter. Those studies in which endogenous DHPG was measured are so indicated.

2. DHMA formation. In spite of occasional difficulties with labeling sites (255) and small isotope effects (265), the results obtained regarding DHPG formation from labeled NE generally are similar to those later obtained with measurements of endogenous NE metabolites. However, this has not been true for DHMA. Inhibition by cocaine of neuronal uptake of (−)-[^{3}H]NE by perfused rat hearts markedly diminished formation of [^{3}H]DHPG, but almost completely abolished that of [^{3}H]DHMA (81). Blockade of extraneuronal uptake with corticosterone almost abolished the formation of [^{3}H]NMN and diminished the formation of OMDA and [^{3}H]DHPG without affecting [^{3}H]DHMA. Similar effects were seen in the cat nictitating membrane (173). These results indicate that O-methylation of [^{3}H]NE occurs extraneuronally, whereas deamination which is followed by aldehyde oxidation to [^{3}H]DHMA occurs mainly intraneuronally. Similar results were obtained with (−)-[^{3}H]NE in perfused rabbit and cat hearts (94). Formation of [^{3}H] DHMA appears to be related to the relatively high levels of exogenous (−)-[^{3}H]NE, since different results were obtained when metabolites of endogenous NE were examined. Although [^{3}H]DHMA was present when exogenous [^{3}H]NE was perfused, endogenous DHMA was not detected in the outflow from perfused rabbit hearts even during stimulation-induced NE release (184). Cocaine almost abolished formation of both oxidized and reduced deaminated metabolites of exogenous NE. Similar differences between exogenous and endogenous NE were obtained in isolated guinea pig atria by Starke et al. (254), who found no significant DHMA formation from endogenous NE, even during release by tyramine or by a reserpine-like drug. DHMA was formed only when atria were incubated with relatively high concentrations of (−)-[^{3}H]NE, and since this was blocked by amezinium (an inhibitor of intraneuronal MAO) or by cocaine, it was concluded, in agreement with Fiebig and Trendelenburg (81), that the acid metabolite was formed in the nerve terminal. Differences from earlier results by others in regard to DHMA formation in guinea pig atria were attributed to use of racemic [^{3}H]NE and to retention in the tissue of the poorly diffusible acidic metabolite which had been formed before MAO was inhibited.

While these factors may have contributed to the discrepancy in the results in atria, they do not explain the differences between exogenous (−)-[^{3}H]NE and endogenous NE seen in experiments with the perfused hearts. Apparently significant quantities of DHMA are formed only from exogenous NE taken up into the nerves in amounts sufficient to form large enough quantities of dihydroxyphenylacetaldehyde to saturate the aldehyde reductase responsible for DHPG formation. The

amounts of available endogenous NE, whether derived from the vesicles in the nerve endings or from reuptake of released transmitter, do not appear to form the aldehyde at a rate which exceeds the capacity of the reductase.

3. MAO-A and MAO-B. When specific inhibitors of MAO subtypes became available, it was possible to examine their roles in deaminating NE and its O-methylated metabolite. Both MAO-A and MAO-B are present in dog saphenous vein, MAO-B exclusively extraneuronally and MAO-A both intra- and extraneuronally (41, 42). Inhibition of MOA-A with clorgyline markedly depressed [^{3}H]DHPG formation from (−)-[^{3}H]NE by dog saphenous vein strips (43, 273). Formation of [^{3}H]OMDA metabolites was also decreased, with increased recovery of [^{3}H]NMN. Clorgyline pretreatment markedly depressed stimulation-induced outflow of DHPG and slightly decreased OMDA metabolites, while increasing NE. Inhibition of MAO-B with deprenyl, however, had no apparent effect on the formation of any of the metabolites under basal conditions or during stimulation. These results are consistent with the conclusions that DHPG is formed in nerve terminals, whereas almost all NMN and OMDA are formed extraneuronally. NMN formed extraneuronally can be deaminated by either MAO-A or MAO-B and in dog saphenous vein strips preferentially is converted to MHPG (44).

4. O-methylation sites. As indicated above, COMT is not distributed equally among the cells of a tissue. It might be expected that predominance of O-methylation or deamination would depend on the enzyme contents of the cells. Thus, Levin (167) found that the adventitia of rabbit thoracic aorta, which contains over 95% of the endogenous norepinephrine, formed primarily [^{3}H]DHPG during incubation with (−)-[^{3}H]NE, whereas [^{3}H]NMN was the major metabolite formed by the medium (which may have also included the intima and endothelium). In the intact aorta, [^{3}H]NMN and [^{3}H]DPHG were the major metabolites formed from (−)-[^{3}H]NE, the former in the smooth muscle (or endothelium) and the latter primarily in the nerve terminals. Rorie (227) found striking decreases in [^{3}H]NE O-methylation by dog pulmonary arteries denuded of their epithelium. Then Rorie and Tyce (232) showed that removal of the endothelium from dog pulmonary artery reduced by about 40% the release into the superfusate, before or during electrical stimulation, of O-methylated metabolites of preloaded [^{3}H]NE. These results are consistent with the cellular localization of COMT in the vascular endothelium (172) and suggest that both the endothelium and smooth muscle play a role in O-methylation of released NE. Kinetic analysis of the extraneuronal O-methylating systems (263), present in several tissues, but examined in particular detail in the perfused rat heart, reveals a low K_m, low V_{max}, corticosterone-sensitive and a high K_m, high V_{max}, and corticosterone-insensitive (in some cases) O-methylating system. The different cellular and subcellular distributions of COMT discussed above might be related to the differences in the affinities and saturability of these O-methylating systems.

During hypoxia, there is a reduction in uptake of released NE and virtual elimination of intraneuronal deamination in superfused dog pulmonary artery preparations (231). It is possible that the importance of O-methylation *versus* deamination may vary widely in different parts of an organ depending upon availability of cofactors (S-adenosylmethionine and oxygen, respectively) as well as upon enzyme activities and access of the substrate to the enzyme.

Thus, in vitro, in a variety of tissues from a number of species, DHPG is the major metabolite formed from NE, during either basal or stimulation-induced release of the transmitter. This metabolite is formed from NE which leaks out of the storage vesicles or which is recaptured after its release. O-methylation is confined mainly to extraneuronal sites and is responsible for formation of NMN from NE which escapes recapture and for conversion of DHPG to MHPG. The small amounts of VMA which are formed are probably derived mainly from deamination of NMN rather than O-methylation of DHMA. In the intact animal, COMT may participate as an enzymatic barrier between the tissue and the circulation, whereas intraneuronal MAO may limit cytoplasmic levels and metabolically inactivate recaptured NE.

5. Metabolism of NE by brain tissue. As in peripheral tissues, the first studies of brain catecholamine metabolism were undertaken in intact animals by injecting radiolabeled norepinephrine into the cerebral ventricles (see ref. 88). Rutledge and Jonason (236) examined the metabolic fate of norepinephrine in rabbit cerebral cortex slices and found that DHPG and MHPG were the major deamination products obtained from NE, along with considerable amounts of NMN, although no NMN could be detected from NE formed during incubation with dopamine.

In rats, Farah et al. (80) found that slices of hypothalamus, cerebral cortex, and cerebellar cortex, after preincubation with labeled NE, spontaneously released [^{3}H]DHPG. During stimulation with elevated K^+, about 80% of the released tritium was as NE, but [^{3}H]DHPG was the major metabolite. Exposure to cocaine abolished [^{3}H]DHPG formation, indicating that it was being formed after reuptake into the adrenergic neurones. Similar results have been reported in slices of mouse hypothalamus (2) using bretylium to inhibit neuronal monoamine oxidase rather than cocaine to inhibit uptake. Uptake and metabolism of NE into slices of possum brain are similar to that seen in mammals, including diminution of DHPG formation by cocaine (196). All of these results are consistent with the conclusion that DHPG is formed inside the nerve, whereas NMN is formed extraneuronally.

B. Metabolism of DA in Vitro

In isolated perfused rat hearts, DA is accumulated by two distinct uptake processes which differ in their affinity for the amine and yield different patterns of metabolite formation (106). At low (5 nM) perfusion concentrations, most DA is taken up and stored unchanged; the main metabolite is NE. At higher concentrations (260 μM), however, deamination predominates. O-methylation appears to play only a minor role in the metabolism of DA in the perfused rat heart, even if the metabolites in the perfusate are included. When hearts from rats pretreated with 6-hydroxydopamine were perfused (107), NE formation and deamination were both decreased, whereas O-methylation was increased. These results support the notion that O-methylation occurs extraneuronally, β-hydroxylation intraneuronally, and deamination at both sites.

In rabbit brain slices, Rutledge and Jonason (236) found that exogenous DA was deaminated to the two acid derivatives, DOPAC and HVA, whereas NE, as indicated above, was converted to the corresponding glycols. After pretreatment to inhibit MAO, rabbit brain slices produced 6-fold greater amounts of 3MT, but only 1.5-fold greater NMN. MAO inhibition enhanced conversion of DA to NE, suggesting that uptake into vesicles (and subsequent β-hydroxylation) competes with MAO for cytoplasmic DA.

Tyce and Rorie (267) demonstrated the formation of an acid-hydrolyzable DA conjugate in the superfusates of rat striatal slices. The conjugate represented about 10–20% of the free DA, both under basal conditions and during tyramine-evoked DA release. The amounts of conjugate were similar to HVA, both being less than DOPAC, but during tyramine-evoked release, the conjugate increased more than did either of the acid metabolites. Surprisingly, inhibition of MAO did not increase formation of the conjugate.

C. Significance of Studies of Catecholamine Metabolism in Vitro

The metabolites formed in individual tissues and the relative ease with which they diffuse into the surrounding tissue fluids or are further metabolized to other products determine their rates of entry and egress from the body fluids and influence the final excretion products of the catecholamines. Trendelenburg and coworkers (263) have reviewed several aspects of the kinetic parameters regulating catecholamine metabolite formation and overflow into surrounding medium or perfusates from isolated animal organs. Although the apparent compartmental models which were proposed to explain the observed kinetics may, after 5 yr, require some modification to take into account new information about localization of enzymes, the data and the principles regulating diffusion of the metabolites remain completely valid. Interpretation of the significance of catecholamine metabolite concentrations in tissues or body fluids in intact animals or in humans must consider these factors.

In general, glycol metabolites diffuse freely and leave the tissues more rapidly than do the O-methylated amines. Acid metabolites, however, having lowest lipid solubility, cross cell membranes most slowly and have a more limited distribution volume. This distortion of the metabolic pattern reflected in the plasma (or CSF) is further distorted by differences in hepatic metabolism and renal excretion of the metabolites from plasma.

IV. Renal and Hepatic Handling of Catecholamines and Their Metabolites

The kidney and liver have special roles in relation to catecholamine metabolism since they excrete as well as metabolize the amines and their metabolites.

A. Renal Handling of the Catecholamines and Their Metabolites

Rennick et al. (221) were the first to examine the renal excretion of catecholamines. They chose to do this in chickens because, in that species, there is a renal portal system which bypasses the glomeruli, and they were able to demonstrate that the catecholamines are secreted by the renal tubule and appear in the urine almost entirely unchanged. Quebbemann and Rennick (215) later showed that the secretory mechanism involved an organic base transport system. In mammals, renal clearance methods applicable to most substances do not provide unambiguous data, because catecholamines and their metabolites are formed in the kidney (258) as well as entering via the circulation and are removed by metabolism as well as into the urine and by venous outflow. ^{14}C-labeled EPI, NE, and DA infused into the renal artery of dogs appear in the urine unchanged mainly as a result of glomerular filtration, but a major portion is O-methylated and secreted into the urine by the renal tubules (109). In the cat, however, DA administered into the renal artery is excreted largely as deaminated metabolites (257).

[^{3}H]NE or [^{3}H]DA or their O-methylated metabolites, administered by microinjection into the proximal tubules of rat kidney, were recovered almost completely unchanged in the urine of the kidney receiving injection (22). After injection into the capillaries, DA and its O-methylated metabolite were the major urinary excretion products, with twice as much of each appearing on the injected side. Equal amounts of the acidic metabolites were excreted from both injected and uninjected kidneys, indicating that these metabolites were not made in the kidney. The secretion of NE and its O-methylated metabolite appears to be less effective than for DA and 3MT. In isolated perfused rat kidney, Silva et al. (252) found that excretion of (±)-[^{3}H]EPI and (−)-[^{3}H]NE exceeded the amounts filtered, indicating tubular secretion. More O-methylated metabolites were formed from racemic NE than from the physiological isomer, due

primarily to formation of VMA. There was no evidence of glucuronide or sulfate formation, although about one-third of the catecholamines were metabolized. In anesthetized greyhounds, the renal venous concentrations of DOPA were found to be about half the arterial level, although no DOPA appeared in the urine (23). About 75% of EPI was cleared by the kidney, whereas there were higher levels of DA (4-fold) and NE (2-fold) in renal venous than arterial blood. Ball et al. (23) concluded that the appearance of the catecholamines in urine was due to both filtration and secretion.

B. Hepatic Handling of Catecholamines

The importance of the liver in inactivating circulating catecholamines had been recognized (174) long before the discovery of COMT and the high levels of this enzyme in the liver. This was confirmed biochemically when Vendsalu (272) found that the levels of plasma catecholamines in blood obtained from the hepatic vein were only about 20% of the arterial concentration. When Hertting and LaBrosse (110) injected [^{3}H]EPI into the portal vein of anesthetized rats, they found that only 2% of the injected dose was recovered in the urine unchanged, whereas after i.v. injection of the same dose, about 13% of the injected tritium appeared in the urine as EPI. This suggested that only 15% of the injected amine reached the systemic circulation. Similar results were obtained by Carlsson and Waldeck (45) who showed, using cardiac NE uptake as means for capturing circulating NE, that only about 30% as much i.p. injected [^{3}H]NE reached the heart as did i.v. injected [^{14}C]NE. Hertting and LaBrosse (110) found that about 30% of the intraportally injected EPI was excreted in the bile, consisting mainly of MET glucuronide (27% of the dose) with small amounts of MHPG sulfate (3%). This was about 3-fold greater than after i.v. administration of the amine. More MHPG sulfate and VMA were excreted after i.v. than after intraportal injection, but the urinary MET glucuronide was about the same after either route of administration. The results showed that intraportally injected EPI was mainly O-methylated and the product was conjugated with glucuronide which was in part excreted in the bile and in part released into the circulation.

The biliary excretion of NE metabolites by isolated perfused rat livers confirmed the importance of glucuronide formation from circulating NE (301). During 5 h of perfusion with blood containing (−)-[^{14}C]NE, about 19% of the catecholamine was recovered from the bile as MNM glucuronide and 11% as MHPG sulfate. More MHPG (18%) and less NMN (12%) were excreted when (±)-NE was used. These results confirm that O-methylation is the major metabolic route for NE in the liver and that the NMN formed there is converted to the glucuronide conjugate which enters the bile or the blood.

V. Catecholamine Metabolites in Body Fluids

Studies involving assessment of catecholamine neuronal activity in humans or intact experimental animals frequently depend on measurement of catecholamine metabolite urinary excretion or levels in the CSF or plasma. The validity of the assumptions implicit in such measurements is often untested, and the significance of the data obtained is poorly understood. During the last decade, we have gained much new information regarding the relationships of catecholamine metabolite formation, distribution, and excretion to activity status of catecholaminergic systems. Such information helps understanding the significance of changes in metabolite levels in response to stress, after administration of drugs, or in disease states and provides rationale for appropriate experimental designs to obtain meaningful data.

A. Metabolism of Catecholamines in Vivo

The major metabolites of the catecholamines were first identified in urine of patients with catecholamine-producing tumors or after administration of the catecholamines of their precursor, DOPA. If administered labeled catecholamines adequately mix with the endogenous compounds, it would be expected that the specific activities of all the metabolites would be equal if sufficient time were allowed for complete excretion of the administered isotope. When isotopically labeled catecholamines became available, the various metabolic products were identified, but because of differences in the fate of administered and endogenous catecholamines, only labeled epinephrine could be considered an adequate tracer molecule with which to assess metabolism of the endogenously released amine (see refs. 147 and 148). Thus, after i.v. administration of racemic radiolabeled NE, the specific activity of urinary NE was found to be over twice that of urinary NMN and more than 7-fold greater than that of VMA; the specific activity of VMA was about twice that of MHPG (86). Similar differences in relative specific activities of norepinephrine metabolites after [^{3}H]NE infused can be seen in the data reported by Maas and Landis (183). There is excellent agreement between the two modes of administration of labeled NE (table 3). Discrepancies between the distribution of products of administered isotopically labeled dopamine (89) and metabolites of endogenous DA are also evident (table 4). Clearly there are limitations in the use of labeled catecholamines to quantify routes of their metabolism. These limitations are due to differences in distribution of the administered compound as well as to any isotope effects and stereospecific selectivity. At times, such differences may be used to advantage, but they must always be taken into account in interpreting results obtained with labeled catecholamines.

1. Methods for assay of catecholamine metabolites. During the last 15 yr, there have been introduced several new highly sensitive and specific methods for assay of

TABLE 3

Relative specific activities on [^{3}H]NE metabolites in humans. Relative specific activities were calculated by dividing the specific activity of each metabolite by the specific activity of MHPG.

Metabolite	Maas and Landis (183)*	Gitlow et al. ((87)†
NE	11.4	14.0
NMN	8.5	6.3
DHPG and DHMA	0.48	—
MHPG	1.0	1.0
VMA	1.72	1.95

* [^{3}H]NE was administered as a slow i.v. infusion during a 48-h interval, and urine was collected for 96 h beginning with the onset of the infusion.

† [^{3}H]NE was administered i.v. over a short interval, and urine was collected for the next 24 h.

TABLE 4

Urinary metabolites of administered ^{14}C- and endogenous DA

	[^{14}C]DA (% of administered dose)*	Endogenous (pmol/day)†	Relative specific activity (% of administered/ pmol/day)‡
DA			
(F)§	3.35	4.8	0.67
(C)	1.53	14.8	0.10
DOPAC			
(F)	2.02	12.5	0.16
(C)	6.61	4.2	1.57
3MT	6.76	0.89	7.60
HVA			
(F)	27.6	30.7	0.90
(C)	5.11	3.68	1.38

* Data from Goodall and Alton (89) are mean values for six normal young men.

† Data from Muskiet et al. (203–205) are mean values for normal adults.

‡ Calculated from administered [^{14}C]DA metabolite excretion (expressed as percentage of administered dose) divided by an estimate of the endogenous secretion rate of the metabolite.

§ F, free; C, conjugated.

endogenous catecholamines and their metabolites. While it is beyond the scope of this review to detail and critically evaluate these methods, it is important to recognize that their availability has made possible much of the research being discussed. Radioenzymatic assays depend on the transfer of a labeled (usually with tritium) methyl group from S-adenosylmethionine to the catechol or nitrogen of the catecholamine metabolite, separation of the products by a chromatographic procedure, and quantification by liquid scintillation spectrometry. Appropriate standards are used to correct for recovery. These methods can be used only when the substance to be assayed is the substrate for an available enzyme which can add a methyl group (or another labeled group) to the molecule and the products can be separated quantitatively. Thus, radioenzymatic assays are not available for final metabolic products such as VMA, HVA, and MHPG.

Another group of methods combines chromatographic separation methods with highly sensitive detection devices. In these, the specificity is determined by the chromatographic characteristics of the compound to be assayed and the sensitivity dependent upon the detection device. Gas chromatographic techniques require conversion of the metabolites to volatile derivatives; detection by electron capture provides high sensitivity but does not further enhance specificity. The combination of GC-MS provides the most specific methods because detection is based on mass of the ion being measured. This has the additional advantage that deuterated compounds can be simultaneously quantified and serve as internal standards or as tracer molecules in metabolic studies or both. GC-MS requires expensive instruments and time-consuming sample preparation, but its highly sensitive, specific, and reliable assays provide a standard against which other more convenient and less costly methods can be compared. HPLC combined with ED has proven to be versatile, specific, and highly sensitive (see ref. 145). In this method, the sample preparation procedures are often minimal, and the metabolite separations provide specificity and the electrochemical reactions necessary for sensitivity and some additional specificity based on the ease of oxidation of the substance assayed. HPLC-ED is becoming increasingly popular for assay of all the catecholamines and their metabolites. It has largely replaced older fluorimetric and GC-EC assays and is competing with the radioenzymatic assays.

2. *Kinetic considerations in measurements of catecholamine metabolite levels.* With the introduction of the newer sensitive and specific assays, catecholamines and most of their metabolites have been measured in the tissues, CSF, and plasma as well as in urine of experimental animals and in humans. Depending on the origin of the catecholamine and the site examined, the major metabolites of DA are its conjugate, DOPAC, and HVA. Those of NE are its conjugate, DHPG, MHPG, and VMA. The O-methylated metabolites of the endogenous catecholamines are usually present in low concentrations unless MAO has been inhibited. The levels of a metabolite in a tissue or body fluid clearly are dependent on its rate of formation and/or entry and the rate of its metabolism or egress. In a steady state, these are equal, and the concentration of the metabolite remains constant. In most studies involving measurement of catecholamine metabolites in tissues or fluids, there is an implicit assumption that removal rates remain unaltered first-order processes. Only under these conditions can a change in the level of a metabolite be considered to be proportional to a change in the rate of its formation. In general, however, a change in rate of formation of a metabolite is reflected by a corresponding, but not necessarily proportional, change in the levels of the metabolite in a tissue or body fluid. The relative concentrations of different metabolites of the same parent amine may not, however,

reflect the relative rates of their formation, since their rates of loss may differ. As indicated above, the principles which have been established by kinetic studies of metabolites in isolated tissues should be considered in the interpretation of data obtained in vivo.

A special word must be said regarding conjugates. Conjugates of catecholamines and their metabolites are distinct compounds having different pharmacological activities, physical properties, and metabolic fates than their parent compounds. At this time, no unique physiological role has been established for any of the conjugates of catecholamines. Although the conjugates are conveniently measured by calculating the difference between the "total" and the "free" forms, the distinctions among conjugates and their parent compounds should not be forgotten nor ignored. Levels of each compound, conjugates as well as the parent, are dependent on their separate rates of entry and egress from tissues or body fluids. Comparisons of levels may not accurately reflect the relative importance of the pathway represented by the compound, because low levels of metabolites in plasma or CSF may reflect rapid turnover or large distribution volume. Combined measurements of free and conjugated metabolites treated as a single "total" should be avoided, particularly if turnover rates are to be measured, since the free compound must be considered as a precursor to its conjugate. In urine, conjugates represent metabolites and may be regarded as excretion forms of the parent compound. They should, of course, be included among the metabolites in determining the total production rate of the unconjugated compound and original parent amine.

B. DA Metabolites

Three major metabolites of DA are found in the tissues and body fluids. The conjugates of DA are major products of the ingested amine and its precursor, DOPA, and DOPAC and HVA, the deaminated derivatives of DA. The relative quantities of these substances and their conjugates vary with species and tissues and whether cerebrospinal fluid, plasma, or urine is examined. Since foods may contain considerable quantities of DOPA and DA, their metabolites may be of dietary origin, and studies of catecholamine metabolites in plasma or urine must be adequately controlled.

1. DA conjugates. As indicated above, conjugates were the first urinary metabolites of catecholamines to be discovered. After ingestion by humans of DA-containing foods or DOPA, relatively large amounts of DA sulfate are excreted, but over two-thirds of administered DOPA is metabolized to deaminated products (see ref. 239). L-DOPA (125 mg) p. o. results in up to 100-fold increases in plasma DA sulfate and 10-fold increments in EPI but no change in free EPI or free or conjugated NE (56). In rats, the major conjugate of DA in plasma is the glucuronide, and this compound decreases with stress (6). 3-*O*-DA sulfate predominates (about 20-fold) over 4-*O*-DA sulfate in the urine of parkinsonian patients treated with *l*-DOPA (4.0 g/day), but when a tracer dose of radiolabeled DOPA was given, there was a lower ratio (3:1) of 3- to 4-*O*-sulfation (30). After i.v. administration of [^{14}C] DA, less than 2% of the administered dose was recovered as a DA conjugate in human urine (89). In other species, however, conjugation appears to play a more important role. After p.o. administration of [2-^{14}C]DA to dogs, over three-fourths of the radioactivity recovered in urine (84% of the administered dose) in 24 h was found to be DA sulfate, whereas after i.v. administration, about 20% of the urinary radioactivity was DA sulfate (194). In rats, DA glucuronide is reported to be a major metabolite of DA (50,279); the rate of excretion of this DA conjugate (100–110 nmol/rat/day) was remarkably high and similar to the rate of excretion of HVA (113, 155), even after 3 days on 20% sucrose instead of the routine diet.

Formation of DA sulfate does not appear to be confined to the gut, since there is considerable evidence that PST capable of sulfating DA is present in rat and human brain (see above) and since DA sulfate has been found in rat (37) and human (73) brain and in spinal fluid from humans as well as from cats and monkeys (103, 246, 249, 268, 269). In spite of the presence of PST in rat brain, however, neither administration of DOPA (36) nor inhibition of MAO resulted in detectable levels of DA sulfate in rat hypothalamus or brainstem, and only after MAO inhibition was any DA sulfate detected in the striatum (34). Combined MAO inhibition and *l*-DOPA administration were required to elevate significantly DA sulfate levels in these brain regions, and even then the conjugate represented only a small proportion (1–8%) of the total DA present. Presumably, most excess DA can be removed by another route of metabolism, probably O-methylation.

Conjugated DA has been reported to have been found in rat kidney, liver, and adrenal gland and in the mouse kidney (250), but the conjugates may have been glucuronides, since BUU et al. (36) found no DA sulfate in rat adrenals. Small amounts of DA sulfate were found in bovine adrenal cortex and medulla (216), but in no region, including the adrenal glands, were significant arteriovenous differences in plasma DA sulfate levels detected in human hypertensives (158).

There are considerable species differences in the plasma content of DA conjugates. In rats, the predominant DA conjugate in plasma is the glucuronide, whereas in dogs, DA 3-O-sulfate predominates, and in humans this appears to be the major if not the sole conjugate (50, 270, 279, 280, 295). After s.c. administration of *l*-DOPA to rats, DA sulfate levels in plasma are markedly increased, reaching levels 7-fold higher than the free catecholamine, but this is not reflected in the kidney or urine, where free DA levels are higher than those of the conjugate (36). In ducks, DA glucuronide is by far the major conjugate of DA in plasma, but in chickens, there

are almost equal amounts of sulfate and glucuronide conjugates of DA (293).

Conjugates are not necessarily inert final excretion products, nor should they be considered physiologically similar to their parent compounds. Small doses of [^{14}C] DA 3-O-sulfate administered i.v. to guinea pigs or rats were extensively deconjugated and excreted largely as DOPAC and HVA and, in the rat, as glucuronides of DA and its O-methylated derivative (194). Deconjugation also occurs in dogs; p.o. administration of [^{14}C]DA 3-O-sulfate resulted in excretion of 11% of the urinary radioactivity (67% of the radioactivity administered) as HVA. In spite of deconjugation of DA sulfate, Ackerman et al. (1) found no effects on blood pressure or renal blood flow in dogs receiving i.v. doses of up to 34 μg of DA 4-O-sulfate pr kg. The conjugate was also devoid of activity in the isolated guinea pig atrium and perfused rabbit ear. Buu and Kuchel (38, 39) found that purified bovine dopamine-β-oxidase could transform DA 3- and 4-O sulfates into free NE, but there is little evidence that this occurs to a significant extent in vivo. After adrenalectomized rats were given [^{3}H]DA sulfate, Buu et al. (40) reported [^{3}H]EPI in the kidney and [^{3}H]DA and [^{3}H]NE in the urine, but the high concentrations of conjugated DA found in plasma after ingestion of DA or DOPA, or after i.v. infusion of dopamine, are not attended by any evident physiological effects (159). Conjugated DA may, however, have some pharmacological actions, since DA sulfate produces bicuculline-like convulsions when administered into the cerebral ventricles of rats (35). DA and DA 3-O-sulfate, but not DA 4-O-sulfate, inhibit angiotensin II-stimulated aldosterone release from cultured bovine adrenal cells (217). As indicated earlier, conjugates are different compounds than their parents and have different properties. The physiological importance of the pharmacological properties of DA sulfate remains, however, to be established (233).

2. Deaminated metabolites of DA. Since the major urinary metabolites of endogenous DA are DOPAC and HVA, it was expected that these substances would be formed at sites in which DA was metabolized. Most of the areas having a high concentration of DA are in the brain, but DA is also the precursor of the other catecholamines and occurs in lower levels in many areas of the body. In experimental animals, it is possible to examine directly the content and turnover rates of DA and its metabolites, but in humans and intact animals, indirect methods, based on determining levels of metabolites in body fluids, have been required.

a. BRAIN AND CSF. Relatively high concentrations of HVA are found in areas of brain containing the highest DA levels (see refs. 4 and 15). Measurements of the turnover rates of DA and its metabolites in brain tissues supported the notion that increased neuronal activity with enhanced release of DA would be reflected in elevated levels of the metabolites. Thus, stimulation of the neuronal pathways to the DA-containing brain areas produced increases in the HVA content of cerebroventricular perfusates (212). There have been numerous studies demonstrating changes in the levels of HVA or of DOPAC after administration of drugs which directly or indirectly alter dopaminergic neuronal activity (see ref. 234 and references therein). Generally the results of these studies are consistent with the view that increases in impulse flow enhance release of DA and are attended by increased levels of the metabolites, particularly HVA. The relative concentrations of DOPAC and of HVA and their conjugates, however, differ among the various brain regions and with species. In rat brain, HVA and DOPAC levels are similar, and conjugation plays an important role (259), whereas in many other species, free HVA predominates (table 5).

Determination of the relative magnitudes of alternative pathways for the disposition of DOPAC is possible if the turnover rates of the products are estimated taking into account the precursor-product relationships. For example, Dedek et al. (63) examined the apparent turnover of the DA metabolites in the rat striatum using probenecid blockade of acid transport or inhibition of MAO, with and without inhibition of COMT. They attempted to quantify each of the pathways for removal of each of the metabolites and concluded that most DA was converted to DOPAC (at a rate of 20–23 nmol/g/h). About two-thirds of the DOPAC was O-methylated to HVA, with most of the remainder being conjugated (fig. 4). About 40% of the HVA was conjugated, and the remainder was eliminated as free HVA. Elimination appeared to be due to active acid transport, because probenecid administration increased the levels of the various compounds. Blockade of acid transport produced greater increases in the conjugates than of the free acids, presumably because the free compounds may be conjugated when their egress is blocked, whereas conjugates can only accumulate. At higher doses of probenecid, however, conjugation may also be inhibited, and there was an increase in free HVA and finally, at very high doses, DOPAC levels increased. The results of this study indicate that sulfoconjugated DOPAC and HVA as well as free HVA are the metabolic products of DA which are released from brain into the rat circulation. The rate of outflow of HVA into perfused cerebroventricles of rats accounted for only a very small fraction of the calculated HVA production rate in brain, and Aizenstein and Korf (5) concluded that a maximum of 3.5% of HVA formed in rat brain is released into the CSF. Curzon and coworkers (59, 241) also found that probenecid blockade of acid transport elevated DOPAC and HVA conjugates more rapidly than the free compounds. They found 3- to 4-fold differences in apparent turnover of the metabolites among their animals in spite of precautions to diminish variance. Furthermore, the pre-probenecid metabolite concentrations in the cisternal CSF in individual animals

TABLE 5

Species differences in dopamine metabolite concentrations in striatum and CSF. CSF was obtained from the lateral ventricles, except in rats, in which CSF was obtained from the third ventricle.

Species	Caudate (striatum) (nmol/g)			(Ventricular) CSF (nmol/ml)		
	HVA	DOPAC	(Ref.)	HVA	DOPAC	(Ref.)
Human	35.6 ± 0.3*	0.70 ± 0.01	(256)	1.35 ± 0.5		(60)
	16.0 ± 2.5	1.3 ± 0.2	(292)	2.19 ± 0.03		(291)
	51.0 ± 5.0	1.8 ± 0.4	(21)	0.80 ± 0.08	0.058 ± 0.01	(135)
Monkey						
Macaca mulatta	68 ± 15	2.1 ± 1.6	(248)	6.37 ± 0.59	0.13 ± 0.01	(92)
M. arcoides	69.7 ± 12.0	3.0 ± 1.0	(21)			
Cebus appella	44–58	20–36	(102)			
Dog	91.8 ± 39.2	15.7 ± 2.7	(193)	2.6 ± 2.7	1.5 ± 0.4	(13)
Rat	3.6 ± 0.1	5.9 ± 0.5	(63)	0.88 ± 0.06	1.29 ± 0.60	(195)
	3.9 ± 1.0	4.9 ± 1.2	(283)	0.97 ± 0.02	1.04 ± 0.34	(208, 278)
	7.5 ± 0.6	9.2 ± 0.6	(21)			
Mouse	3.18 ± 0.10	1.72 ± 0.10	(202)			

* Mean ± SE.

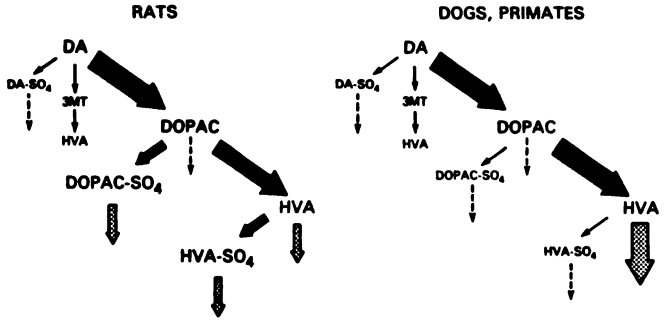

FIG. 4. The pathways of metabolism of DA in brain are different in the rat and in dogs or primates, primarily because of the difference in the relative importance of conjugation. In the rat, substantial amounts of DOPAC are protected from O-methylation by conjugation, and even the O-methylated derivative, HVA, is largely conjugated, whereas in the other species, conjugation is minimal and HVA is the major final metabolite accounting for most DA metabolized in the brain. *Stippled arrows* indicate urinary excretion.

did not parallel the apparent turnover rates of the metabolites. They attributed this discrepancy to differences in metabolite egress. Changes in DOPA transport from brain have also been suggested to explain the absence of increased brain DA metabolites with increased DA turnover during operant performance in rats (105).

In dogs and primates, including man, the concentration of DOPAC in various dopamine-containing brain areas and in CSF is much lower than that of HVA (see tables 5 and 6). In these species, sulfoconjugates of DOPAC and HVA appear to be minor metabolites (fig. 4). As in rats, levels of the O-methylated derivative of DA, 3MT, are quite low unless MAO has been inhibited. It would be expected, therefore, that in these species free HVA is the major DA metabolite released from the brain.

The concentrations of HVA and DOPAC in the CSF obtained from the lateral ventricles are about one-tenth of those found in the caudate and are probably maintained by diffusion of the acid metabolites from the adjacent brain tissue (table 5). There is a marked gradient in HVA concentration between the lateral ventricular CSF and lumbar CSF. HVA concentrations fall exponentially as the CSF flows through the ventricles and down the spinal column (fig. 5). In primates, DOPAC levels are much lower than those of HVA (tables 5 and 6), but lumbar CSF levels of this metabolite also are much lower than in ventricular CSF. A gradient in HVA

TABLE 6

Catecholamine metabolite concentrations in human plasma and CSF.*

Metabolite	Plasma (pmol/ml)	CSF (pmol/ml)	Refs.
DOPAC	17.4 (15–20)	12.6 (2.6–16)	92, 118, 135, 247, 268
HVA	69.4 (59–79)	218 (148–280)	11, 74, 92, 135, 141, 176, 268
NMN			
(F)†	3.6–4.7	1.0	83, 296
(C)	7.7–20.2		83, 276
DHPG			
(F)	6.2 (3.4–9.7)	6.6 (5.3–8.8)	67, 78, 118, 129, 276
(C)	7.3–18		67, 125
DHMA	10.1–11.4	12.7	118, 276
MHPG			
(F)	21.9 (18–29)	43.1 (31–51)	78, 125, 189, 191, 211
(C)	38	4.3	211
VMA	27.5	2	186, 284

* Values shown are representative; they are averages of the mean values of three or more studies (with the range of mean values in parentheses), two values from two studies, or a single mean value from one study. The standard deviations may be as high as 50% of the mean values and, in general assays performed by GC-MS, are lower than those obtained by other methods (see ref. 126).

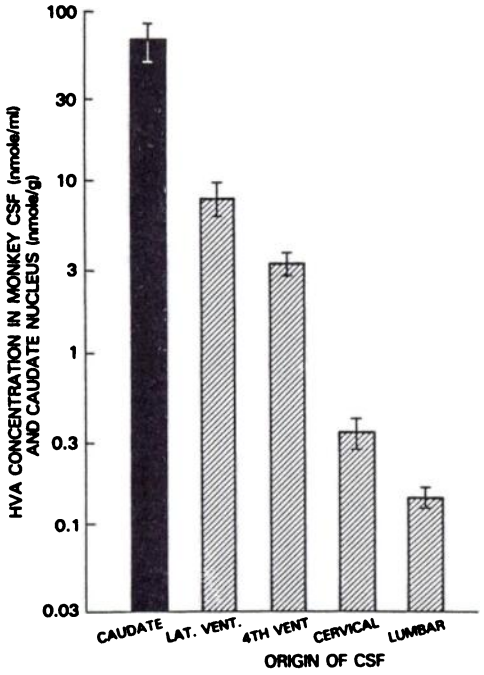

FIG. 5. Concentrations of HVA in the caudate and in CSF obtained from various levels of the monkey central nervous system. There is a marked, exponential decrease in HVA content of CSF with increasing distance from the caudate nucleus. Data obtained from Sharman et al. (248) and Gordon et al. (93) in *Macaca mulatta*. *Lat. Vent.*, lateral ventricle *vent*, ventricle.

concentrations was demonstrated in sequential 5-ml samples of lumbar CSF (251). Removal of HVA from the brain tissue via the capillaries (5) is inhibited by probenecid, indicating that there is an active transport of the acid metabolites from the brain and CSF into the blood. There appear to be considerable species differences in the anatomical extent of the probenecid-sensitive transport system (see ref. 142). In the monkey, in the region of the spinal cord, clearance of HVA from CSF (21 ± 4 μl/min) is unaltered by probenecid, whereas spinal transport of HVA in cats is rapidly inhibited by this drug. In humans, a time lag of 4 h after probenecid treatment would be expected before the rise of HVA levels in human lumbar CSF begins, because of the time required for bulk flow of the CSF. The rise in HVA earlier than this (138) is consistent with the probenecid-sensitive active acid transport system in the human spinal cord. In monkeys, spinal cord production of HVA occurs but contributes only a relatively small amount of the metabolite compared to the amount derived from more caudal areas (144). The markedly lowered CSF HVA levels in patients with combined spinal cord transection and CSF blockade compared to those without blockade (213) support the view that HVA in lumbar CSF is largely derived from the brain. The rise in HVA levels in lumbar CSF after probenecid has been used to enhance the sensitivity of lumbar CSF HVA measurements as an index of DA production in the human brain (see refs. 48 and 91).

Reservations have been expressed regarding the precision with which CSF HVA levels reflect brain DA metabolism. The efficacy of probenecid in blocking acid metabolite transport is dependent upon the drug levels, and blockade is generally incomplete. Thus, differences among individuals or between patient groups may be due to differences in characteristics of the transport system rather than in rate of metabolite formation (52). When there is severe damage to DA neurones, CSF levels of HVA appear to reflect DA metabolism in at least that portion of brain which is adjacent to the cerebral ventricles. The selective damage to the nigrostriatal dopaminergic neurones by exposure to the neurotoxin, MPTP is attended by a marked diminution of the HVA content of lateral ventricular CSF in monkeys (32) and of lumbar CSF in humans (33). The neuronal degeneration produced by the toxin is more limited than that seen in Parkinson's disease, but the decrease in lumbar CSF HVA levels is similar in the two movement disorders. With less severe impairment of DA neurones, however, factors such as differences in the transport of the acid metabolites, the rate of CSF flow, etc., can affect HVA levels, and such measurements must be interpreted with appropriate caution (see ref. 58).

b. PLASMA DA METABOLITES. The products of DA metabolism are removed from brain into the blood so that in humans it might be expected that plasma levels of HVA metabolites would reflect brain DA metabolism, particularly since plasma HVA levels are lower than those in brain, and CSF (table 5) and HVA in plasma, unless present in very high concentrations, do not penetrate into CSF (214). In addition to its role as the neurotransmitter for several important neuronal pathways in brain, however, DA is formed as the precursor of NE and EPI and has been considered as a candidate for a neurotransmitter in the peripheral tissues (164). The biosynthetic pathway for all three of the catecholamines originates with (and is rate-limited by) conversion of tyrosine to DOPA through the action of tyrosine hydroxylase. DOPA is rapidly converted to DA by aromatic amino acid decarboxylase. Formation of DA appears to occur in the cytoplasm, after which the amine is transported into storage vesicles, the site from which the neurotransmitters are released. The vesicles of noradrenergic neurones contain dopamine-β-hydroxylase which readily converts DA to NE.

There is considerable evidence, however, that not all of the DA formed in the NE-containing neurones is captured and sequestered in the vesicles. DA which is not taken up into, or which escapes from, the vesicles is exposed to mitochondrial MAO and is deaminated, mainly to DOPAC. Depending on the preparation or animal studied, the DOPAC may escape, undergo conjugation, or be O-methylated to form HVA. In isolated atria from reserpine-treated rabbits incubated with [^{3}H] DA, formation of NE was less, and formation of DOPAC and HVA was greater than in atria from untreated rabbits (237). This effect was attributed to the inhibition by

reserpine of DA uptake by the storage vesicles and its rapid metabolism, primarily by MAO. This action of reserpine reduced the amounts of both DA and NE recovered from [^{3}H]tyrosine and [^{3}H]DOPA in perfused hearts from reserpine-treated rabbits. A similar effect is found in vivo in rats treated with reserpine; excretion of HVA is markedly increased, whereas MHPG excretion is greatly reduced (155). After destruction of peripheral sympathetic neurones by i.v. (113) or i.p. (20) administration of 6-OH-DA, there is a significant decrease (by about 25%) in urinary excretion or plasma levels of HVA. Furthermore, peripheral tissue content of HVA and of DOPAC exceeds the concentration of these metabolites in plasma and the tissue levels of DA (165). DOPAC levels were found to be decreased after treatment with 6-OH-DA, and DA was elevated after MAO inhibition. All these results suggest that considerable amounts of DA are deaminated in peripheral sympathetic neurones, rather than taken up into their vesicles and converted to NE.

More recently, evidence has been provided that DA metabolites are formed also in noradrenergic neurones in brain. Anden and Grabowska-Anden (7) showed that yohimbine and reserpine elevated the concentrations of DOPAC and HVA both in NE- and DA-predominant regions of brain. In NE-containing regions, MHPG concentrations were increased by yohimbine but decreased after reserpine. Furthermore, in NE-predominant brain regions of reserpine-treated mice, DOPAC and HVA levels were lowered by clonidine, but not by apomorphine. Thus, the levels of DA metabolites in NE-predominant regions of brain paralleled the rate of DA synthesis in NE neurones. Similarly, electrical stimulation of the locus coeruleus or ascending noradrenergic pathways produces increases in hippocampal DA and DOPAC which parallel the increases in MHPG (243), and antidromic stimulation increases levels of DOPAC and HVA, as well as MHPG, in the locus coeruleus (57). Decreasing neuronal activity by local infusion of tetrodotoxin or by systemic administration of clonidine decreased levels of both the NE and DA metabolites. Anden et al. (8) demonstrated a rapid turnover of the DA present in the locus coeruleus and in the noradrenergic neurones of the superior cervical ganglion and estimated that only about half of the DA was converted to NE, the remainder presumably metabolized to deaminated products. At noradrenergic nerve terminals, perhaps because of a greater density of vesicles, there appears to be more efficient conversion of DA to NE. Westernik et al. (288) estimated from measurements of DOPA accumulation (after inhibition of the decarboxylase) and DOPAC removal rate (after MAO inhibition) that, in nondopaminergic areas, 85–90% of the dopamine formed is converted to NE, and only 10–15% is deaminated to DOPAC. These observations further support the view that HVA is formed in central and peripheral noradrenergic as well as dopaminergic neurones, but there are wide variations in the relative rates of deamination and β-hydroxylation.

After i.v. administration of deuterium-labeled HVA, estimates of the production rate of the metabolite can be calculated from the kinetic parameters of the decline in levels of the isotope and steady-state level of the endogenous metabolite (11). Angaard et al. (11) used as elimination rate constants the plasma half-lives of the administered labeled compound and calculated HVA production rates which far exceeded urinary excretion rates. Although they had recovered 60% of administered HVA in urine within 7 h and would probably have recovered more HVA had the collection period been extended, they concluded that about half of HVA is eliminated by mechanisms other than renal excretion. Elchisak et al. (74) used the polyexponential curve obtained for plasma d_2-HVA levels to calculate the production rates in humans and obtained lower values. In rhesus monkeys, the calculated production rates and observed urinary excretion rates were almost identical (75, 76). It is likely that most HVA is not further metabolized.

c. URINARY EXCRETION OF DA METABOLITES. The rates of urinary excretion of catecholamine metabolites are shown in fig. 6. HVA is by far the major urinary metabolite of DA. Only a small fraction appears to be excreted as a conjugate. The excretion of DA conjugates may be quite high, but these are probably derived largely from ingested DA or DOPA which is present in many foods. Free urinary DA appears to be derived mainly from the decarboxylation of circulating DOPA (31), but it does not contribute greatly to the total of the metabolites. It

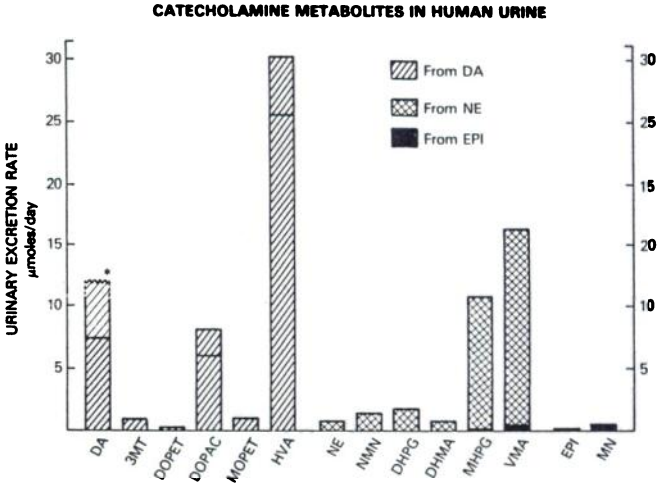

FIG. 6. Urinary excretion of catecholamine metabolites. Data are representative values obtained from weighted mean excretion rates reported in refs. 154, 160, 171, 190, 203–205, 235, and 300 and found in laboratories at the National Institutes of Health. The amounts of DA and DA conjugates excreted may vary widely with diet, and ingestion of DOPA- and DA-containing foods influences the other DA metabolites. The *horizontal lines* in the columns for DA, DOPAC, and HVA indicate the level of the unconjugated metabolites. Most 3MT, NMN, MHPG, and MHPG is excreted in conjugated forms (sulfate and/or glucuronide). The amounts of MHPG and VMA formed from EPI are estimated as indicated in table 7.

should be clear from the consideration of the origins of plasma HVA that the excretion of this metabolite (and probably some of the other metabolites) is not totally representative of dopaminergic neuronal activity, since considerable amounts of DA are formed and deaminated in noradrenergic neurones in the brain and in the peripheral sympathetic nervous system.

C. NE Metabolites

The major metabolites of NE and of EPI were unknown until Armstrong et al. (12) found that VMA is the major urinary excretion product of NE in humans. Subsequently, conjugated MHPG was identified (17) and together with VMA shown to account for almost all of the metabolites of endogenous NE. In the tissues and body fluids, however, the major metabolites of NE include both reduced and oxidized products of NE deamination, DHPG and DHMA, as well as their respective O-methylated derivatives, MHPG and VMA. Generally, the neutral metabolites predominate, often as the conjugates. Although the initial metabolism of NE in brain and sympathetic nerves appears to be similar, with deamination and reduction in DHPG predominating in most species, there are interesting and important species differences in the subsequent metabolism of this initial metabolite.

1. Brain and CSF. The early studies on brain catecholamine metabolism focused on the relative importance of COMT and MAO in metabolic inactivation of catecholamines and concluded that intraneuronal deamination and extraneuronal methylation were important in brain as well as in the peripheral sympathetic nervous system (88). After Schanberg et al. (245) found that conjugated MHPG was the major metabolite of NMN in the brain, they demonstrated that MHPG is present in the brain and CSF (244). These observations and the in vitro studies in brain and a wide variety of tissues from several species, as described above, supported the view that the initial pathway for metabolism of NE involves deamination followed by reduction to DHPG and subsequent MHPG formation. This is consistent with the cellular localization of COMT (see above). In rats, drugs and procedures which enhance or diminish brain NE release produce corresponding changes in brain free and conjugated DHPG levels (see, e.g., refs. 242 and 282). There are, however, interesting species differences in the subsequent metabolism of DHPG; in mice the glycol metabolites are almost all unconjugated, whereas in rats, most of the metabolites are present in the conjugated form (281). Li et al. (169) found that, in brains of rats treated with probenecid, conjugated DHPG accumulates more rapidly (650 pmol/g/h) than does conjugated MHPG (320 pg/mol/h). This relationship appeared to occur in many areas of brain and indicated that conjugated DHPG was the major route of egress of brain NE metabolites in rats.

In mice, conjugation of DHPG and MHPG appears to be minor pathways; almost all DHPG formed is rapidly O-methylated to MHPG (170). The turnover rate of MHPG (500–600 pmol/g/h) was less than half that of DHPG (1200–1260 pmol/g/h). The discrepancy between DHPG and MHPG turnover rates in mouse brain was thought to result from invalid assumptions regarding a single MHPG compartment. The production rate calculated for DHPG accounted for 70-100% of the rate of NE formation (depending on the method for estimating NE turnover). The rate of disappearance of MHPG was more rapid after inhibition of COMT and MAO than after COMT inhibition alone, suggesting that some MHPG was formed via NM. Using HPLC-ED, Westernik (287) found that the levels of NM in various areas of the brain of decapitated rats were similar to those of free DHPG. These values were not very different than others found using GC-MS (see refs. in 287). The results obtained in turnover studies, however, indicate that free MHPG is a major means of elimination of NE metabolites in the mouse. These studies illustrate the usefulness of determining turnover rates rather than just metabolite levels when deciding which metabolite should be measured to provide the most meaningful index of changes in noradrenergic activity.

In primates, as in mice, free MHPG appears to be the major NE metabolite released from the brain into the blood. As seen from table 6, unconjugated MHPG is the major NE metabolite found in brains and CSF of primates (78, 136). Furthermore, Maas et al. (180–182) have been able to demonstrate significant differences in free MHPG concentrations between arterial and jugular venous blood. In primates, including humans, almost all of the MHPG in CSF is unconjugated (136). The role of conjugation of the NE metabolites appears to be similar to its role in metabolism of DA metabolites in the different species. Conjugation is important in rats, but not dogs or primates (fig. 4). Perhaps conjugation is less important because of the low levels of PST in the brain of these species and the relatively low affinity of the enzyme for this substrate (see above).

2. Relationship of plasma and CSF catecholamine metabolites. The blood-brain barrier is usually thought to prevent entrance of catecholamines and their metabolites into the brain and CSF, and for most metabolites at physiological concentrations, this is a valid assumption. Thus, 3-fold elevation of conjugated DA did not alter CSF levels of DA sulfate (161), and radiolabeled amines are not found to easily enter the brain (88). If high plasma concentrations of HVA are maintained for long periods, however, CSF HVA levels slowly increase (214) and may even approach the plasma HVA level. With marked increases in plasma levels of conjugated NMN in patients with phaeochromocytoma, levels of the conjugate in CSF can reach about 10% of the plasma level, which increases CSF levels about 10-fold (83). Thus, the blood-brain barrier cannot be considered ab-

solutely impermeable, and to varying degrees, depending on the compound and its physical properties, plasma metabolites may affect CSF metabolite levels.

3. Relationship of plasma and CSF MHPG levels. Since MHPG is a neutral metabolite, it readily diffuses through tissue membranes (143, 264). It is expected, therefore, that free exchange of free MHPG in plasma and in CSF and brain would result in a significant correlation between MHPG concentrations in these fluids (151). The influence of plasma MHPG levels on the concentration of the metabolite in CSF can be predicted and appropriate corrections applied.

a. THEORETICAL CONSIDERATIONS OF PLASMA CSF MHPG EXCHANGE. Free exchange of CSF and plasma MHPG can be represented as a two-compartment system, as shown in fig. 7, and the relationship of the MHPG concentration in CSF, $[C]$, to that in plasma, $[P]$, defined by the following equation:

$$[C] = (k_1/k_2) \cdot [P] + M_c/k_2 \qquad \text{eqn 1}$$

where M_c is the rate of entry into the CSF of MHPG from the central nervous system, and the rate constants, k_1 and k_2, are expressed in units of clearance (volume/time), since $[C]$ and $[P]$ are concentrations (amount/volume) rather than amounts. The MHPG concentration in plasma is defined by the equation:

$$[P] = (M_p + M_c)/k_3 \qquad \text{eqn 2}$$

where M_p is the rate of formation of MHPG in the peripheral tissues, and k_3 is the plasma clearance rate of MHPG. From these equations it can be seen that

$$[C] = M_c/k_2 + k_1 \cdot (M_c + M_p)/k_2 \cdot k_3. \qquad \text{eqn 3}$$

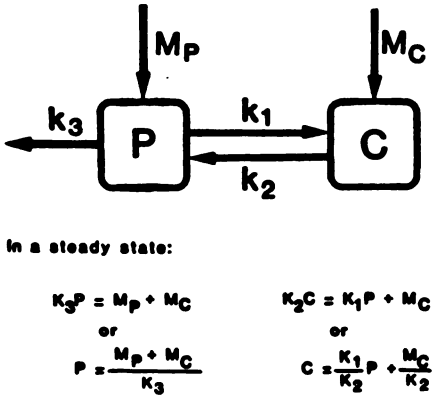

FIG. 7. Plasma and CSF considered as a two-compartment system: k_1 an k_2, rate constants for transfer of MHPG from plasma to CSF and vice versa (with a net flux of MHPG into plasma from CSF); $[C]$, level of MHPG in CSF; $[P]$, its level in plasma; M_p, its rate of entry from the tissues into plasma; M_c, rate of entry of MHPG into the CSF; and k_3, sum of the rates of removal of MHPG from plasma by excretion, conjugation, or oxidation. From Kopin et al. (151).

Since M_p is greater than M_c, it is apparent in eqn 2 that P is more dependent upon M_p than on M_c. Because k_3 in eqn 3 is large compared to k_1 and k_2, $[C]$ is largely determined by M_c. Even if M_c and M_p are completely independent, some degree of correlation between $[P]$ and $[C]$ is to be expected because of the contribution of the central and peripheral MHPG formation rates to both, but the correlation may be weak. As M_p increases relative to M_c, the correlation between the concentrations should increase, because the last term in eqn 3, which contains M_p, has an increasingly important influence on $[C]$ as well as being the major determinant of $[P]$.

Whether or not there is a sufficiently small variance to determine k_1/k_2 from $[C]$ and $[P]$ when a group of normal individuals is studied, eqn 1 remains valid for each subject, and if the value of k_1/k_2 can be assumed to be similar among the subjects and is known, M_c/k_2 can be calculated for any individual.

An estimate of k_1/k_2 can be obtained most precisely if M_p is large and can be assumed to vary independently of M_c. This occurs in patients with phaeochromocytoma, since the tumor is not under nervous control and contains large amounts of MHPG (149) which can diffuse freely into the circulation. In a study which included patients with this tumor and normal subjects, there was a highly significant ($P < 0.0001$) linear relationship between $[C]$ and $[P]$ having a slope of 0.9 (151). This slope is consistent with expectations, since k_1 is due to clearance by diffusion, and k_2 is similar, but possibly slightly larger, due to bulk flow of the CSF as it circulates from the site of its formation at the choroid plexus to sites of its absorption at the arachnoid villi or via the lympathics (62).

When deuterated MHPG was infused i.v. (M_c is zero for the labeled compound) at a constant rate for 12 h, the CSF levels of the labeled metabolite approximated those in plasma (69), confirming the validity of the assumption that MHPG can diffuse readily from plasma into CSF and consistent with a value for k_1/k_2 close to unity. Since M_c is defined as the rate of entry of MHPG into CSF from the central nervous system, M_c/k_2 should be a useful parameter with which to assess central noradrenergic activity. From eqn 1 and the empirical value for k_1/k_2, we obtain

$$M_c/k_2 = [C] - 0.9[P] = [C]_{corr} \qquad \text{eqn 4}$$

which may be considered the CSF MHPG level corrected for the plasma MHPG contribution to CSF.

If, as has been suggested (175), the rate of MHPG formation in the brain is a determinant of the rate of its formation in the peripheral tissues, then M_p is a function

of M_c. In such a case, clearly $[P]$ will be strongly correlated with $[C]$. Suppose

$$M_p = F \cdot M_c, \qquad \text{eqn 5}$$

then from eqns 2 and 4,

$$M_c = k_3 \cdot [P]/(F + 1) \qquad \text{eqn 6}$$

and eqn 1 can be expressed

$$[C] = k_1/k_2 + k_3/k_2/(F + 1) \cdot [P]. \qquad \text{eqn 7}$$

Thus, if M_p is a function of M_c, as in eqn 4, clearly there would be a strong correlation between $[C]$ and $[P]$ defined by a straight line having a slope greater than 0.9 (k_1/k_2) and intercepting the origin. Since CSF MHPG levels corrected by subtraction of $k_1 \cdot [P]/k_2$ yields M_c/k_2, the corrected value, $[C]_{corr}$, should be significantly correlated with $[P]$ by virtue of the relationship described by eqn 5. In 30 normal subjects combined from 2 separate studies (124, 211), there were a highly significant correlation and CSF MHPG levels (fig. 8). There was, however, no significant correlation between $[C]_{corr}$ and $[P]$, suggesting that overall central nervous system noradrenergic activity, as measured from lumbar CSF MHPG, does not greatly influence peripheral sympathetic neuronal function. This is consistent with the report by Crawley et al. (53) that destruction by 6-OH-DA treatment of the noradrenergic neurones in the locus coeruleus does not interfere with central nervous system stimulation-induced activation of the peripheral adrenergic neurones. Of course, selected portions of adrenergic neurones of the central nervous system may indeed play an important role in regulating autonomic function, but peripheral measures, such as plasma MHPG levels, cannot be assumed to reflect all, or even a major segment, of brain noradrenergic activity.

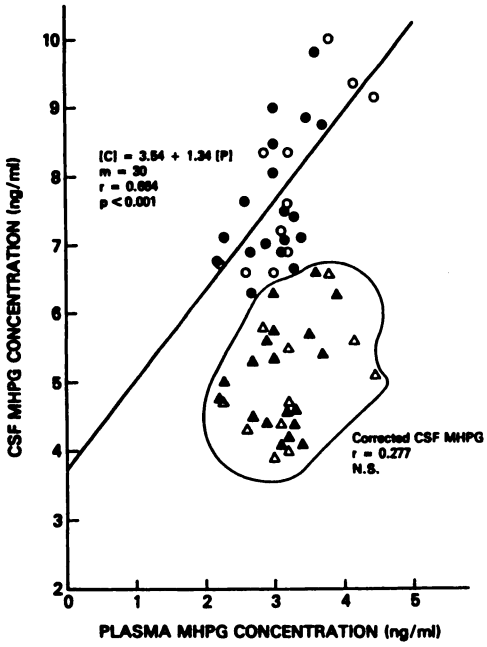

FIG. 8. Relationship between concentrations of MHPG in plasma and in CSF of normal subjects. Uncorrected CSF levels are shown with data from Jimerson et al. (124) (●) and data from Polinsky et al. (211) (○). The CSF_{corr} values are shown (▲, △). The highly significant ($P < 0.001$) correlation between plasma and CSF MHPG levels becomes insignificant if the CSF levels are corrected for the plasma contribution. *N.S.* not significant.

b. DISSOCIATION OF CENTRAL AND PERIPHERAL MHPG PRODUCTION. The value of M_c/k_2 obtained after correcting the CSF MHPG level by subtracting $0.9 \cdot [P]$ appears to be a useful clinical index of MHPG production in the central nervous system, although it may, in part, reflect spinal cord production of MHPG. Independent alterations in apparent M_c/k_2 and peripheral MHPG production have been demonstrated in two forms of orthostatic hypotension and in idiopathic and neurotoxin-induced parkinsonian syndromes.

Neurogenic orthostatic hypotension may result from peripheral sympathetic neuronal dysfunction (as in diabetic neuropathy or in idiopathic orthostatic hypotension) or secondarily from dysregulation of the autonomic nervous system in association with a central neurological disorder (multiple system atrophy or some patients with Parkinson's disease). Polinksy et al. (211) showed that CSF levels of MHPG were significantly lower than normal in both types of orthostatic hypotension, but that only IOH patients had low plasma levels of this catecholamine metabolite. In these patients, the low CSF MHPG levels were due to the low plasma MHPG concentration, whereas in patients with multiple system atrophy, the low CSF MHPG levels reflected diminished NE metabolism in the central nervous system.

A toxic metabolite of MPTP has been shown to selectively destroy nigrostriatal dopaminergic neurones and to produce the primates, including man, a syndrome which closely resembles Parkinson's disease. Burns et al. (33) found that the plasma levels of MHPG were abnormally low in patients with MPTP-induced parkinsonism as well as those with Parkinson's disease. In CSF, however, MHPG levels were lower than normal in patients with the spontaneous disorder, whereas in MPTP parkinsonism, they were elevated. After correction of the CSF levels for the plasma contribution of MHPG, there was complete separation of the two groups of patients; no patient with MPTP-induced parkinsonism had a corrected CSF MHPG level below 6.0 ng/ml, and no patient with Parkinson's disease had greater than that level. Patients in both groups had low CSF levels of HVA. These biochemical abnormalities are consistent with the pathological changes in the brain. Neurones containing norepinephrine are involved in the degenerative process of Parkinson's disease, whereas they are spared and appear to be hyperactive in patients with MPTP-induced parkinsonism.

4. Plasma NE metabolites. Norepinephrine released

into the synapse is mainly inactivated by reuptake into the presynaptic nerve terminal where it is deaminated. A portion of the released transmitter escapes and is either O-methylated or reaches the circulation. To sustain a given increment in blood pressure of pithed rats, NE infused i.v. must attain a steady-state level of NE in plasma about 10-fold greater than that attending sympathetic stimulation which produces a similar blood pressure rise (156). This is due to a gradient in concentration between vascular neuroeffector junctions, and plasma suggests that about 70% of the NE is removed before it can reach the circulation; about two thirds of the gradient is due to a desipramine-sensitive uptake process (156). Hoeldtke et al. (112) found that, in normal humans, the apparent NE secretion rate (determined by isotope dilution of infused tritiated NE) is about 22% of the NE production rate (determined from the total of the urinary metabolites of NE). Thus, a major fraction of NE must be metabolized without reaching the circulation. The total NE production rate includes areas which contribute minimally to plasma NE. These areas include the splanchnic sympathetics which release NE into the blood entering the portal vein and liver (see above) and the brain, where almost all NE is metabolized before it reaches the circulation.

Because of the early observations that, in humans, the major NE metabolite in urine is VMA and MHPG is the major brain NE metabolite, it was concluded that MHPG levels in urine or plasma would provide a direct measure of brain NE metabolism, and an impressive body of evidence was accumulated to support this view (see ref. 64). The highly significant positive correlations between MHPG in CSF and in urine (291) or in plasma (77) were taken as further evidence that plasma or urinary content of MHPG reflected brain MHPG production. There were two important implicit assumptions in reaching this conclusion. The first assumption was that MHPG was a final product not further metabolized. The second assumption was that plasma MHPG had no significant effect on CSF (or brain) MHPG levels. Both of the assumptions were unjustified.

As indicated above, because of its lipid solubility, unconjugated MHPG easily exchanges with free MHPG in the CSF; brain MHPG also exchanges (not equilibrates, since fluxes are not equal) with plasma MHPG (unpublished observations), probably as rapidly as does the spinal cord and its surrounding fluid (143). It would be expected that subtraction of the plasma contribution to the free MHPG levels found in brain tissue would diminish (as described above for CSF and plasma MHPG) the strong correlations found by Elsworth et al. (72) between MHPG concentrations in different brain areas, since the correlations can be attributed, at least in part, to exchange with the common plasma pool, and this, in fact, is the case (Elsworth, Redmond, and Roth, personal communication). Thus, it cannot be assumed that plasma MHPG does not influence brain MHPG levels. The effect will be small in NE-rich areas of the brain but may become increasingly important in areas where MHPG production is low.

The assumption that MHPG is a final metabolic product is also unjustified. LaBrosse (162) had found that, in children with neuroblastoma, administered [^{3}H]MHPG was converted to VMA. Blombery et al. (25) and Mardh et al. (185, 188, 189) found that about two-thirds of administered deuterium- or ^{14}C-labeled MHPG is recovered as urinary VMA. Mardh (186) showed that about 10% of administered VMA is metabolized to vanillic acid, consistent with earlier observations that vanillic acid is formed from DHMA (90). Since a major fraction of i.v. administered MHPG is converted to VMA, it is reasonable to assume that MHPG released from the brain is also converted to VMA along with any other endogenous MHPG which reaches the circulation.

As discussed above, DHPG is the major metabolite obtained in isolated tissues, although some MHPG and NE, and, more slowly, acid metabolites, are also released. It is extremely likely that, in humans, in peripheral tissues as well as in the brain, more glycol than acid metabolites are formed and released into the blood. As in the brain, there are important species differences in the subsequent metabolism of the glycol metabolites. In rats the major urinary excretion product from NE metabolism is conjugated MHPG. In humans, however, VMA is the major excretion product. Until recently, it was not widely appreciated that in humans MHPG is the major intermediate in the formation of VMA; it was believed that plasma or urinary MHPG excretion could provide an index of brain NE metabolism. Using kinetic analysis to calculate production rates of VMA from MHPG, Mardh et al. (189) concluded that all VMA formed was derived from plasma MHPG. The production rates of MHPG was calculated from the plasma concentration of endogenous MHPG and the elimination rate constant and apparent volume of distribution (V_d) of the administered labeled MHPG. It was assumed that there was a single compartment from which elimination occurred. It is likely that this assumption or even an assumption of a peripheral and central compartment from which elimination occurs exclusively is not valid. Mardh et al. (189) note that, with the very rapid initial decline in plasma labeled MHPG concentrations, V_d cannot be considered accurate. It is likely, therefore, that V_d was overestimated and that the calculated MHPG production rates were too high. In fact, the calculated production rate of MHPG often exceeded the sum urinary excretion rates of VMA plus MHPG. It was clear, however, that a major portion of VMA must have been derived from plasma MHPG. Isotope dilution analysis of similar data (25) agreed that a large proportion, but not all, of VMA is derived from circulating free MHPG. The presence of unconjugated DHMA in plasma (see table

6), which is known to be metabolized to VMA (90), is strong evidence that not all VMA is derived from free plasma MHPG.

Because both VMA and conjugated MHPG, the major urinary excretion products of NE, are derived mainly from free MHPG (which rapidly exchanges between tissues and plasma), plasma free MHPG levels can be expected to be a useful index of overall NE metabolism, provided there is no alteration in the rates of oxidation or conjugation of free MHPG (150, 189). The central role of plasma free MHPG as a precursor of the major urinary NE metabolites and its free exchange with tissues and CSF (fig. 9) explain the correlations reported among CSF and plasma MHPG levels and urinary excretion rates of conjugated MHPG. Although plasma NE levels correlate better with total MHPG than VMA levels (111), the correlation might have been improved had free MHPG levels been measured. Attention must be given to other factors; e.g., the oxidation of MHPG is markedly inhibited by ethanol. Ethanol ingestion (86) or withdrawal (114) not only increases total NE metabolism, but markedly increases the ratio of MHPG to VMA in urine and elevates plasma free MHPG (unpublished observations).

Plasma DHPG levels are about one-third those of MHPG (table 5) and, as might be expected from in vitro studies described above, are diminished by blockade of NE reuptake with mazindol (120). Infusion of NE to increase plasma NE levels 6-fold produced only a 25% increment in plasma DHPG, similar to that seen with a doubling of plasma NE which attends postural change. These results are explained by the differences in metabolism of i.v. administered and endogenous NE (described above), in diffusion rates, and in rates of tissue O-methylation of the two catechols.

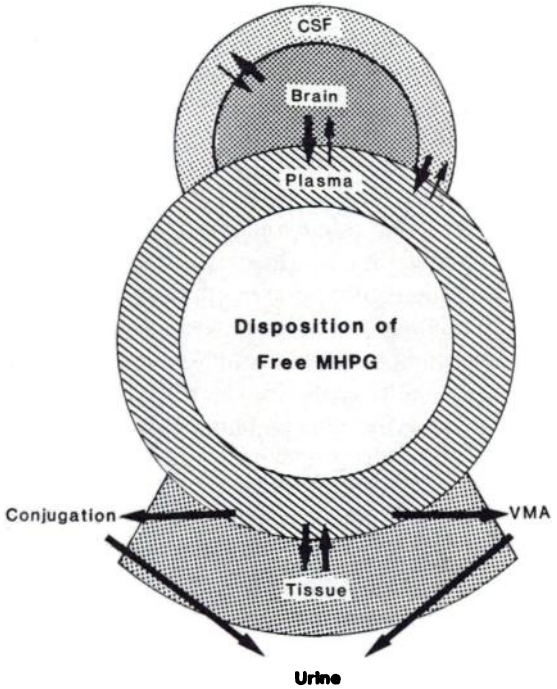

FIG. 9. Diagrammatic representation of the distribution and metabolism of plasma unconjugated MHPG. MHPG enters plasma from tissues in which NE is metabolized and because of its lipid solubility readily enters all tissues. This accounts for at least part of the covariance of plasma MHPG with that in tissues and urine. From Kopin et al. (153).

Both plasma MHPG and DHPG levels are less sensitive indices of rapid changes in sympathetic activity than is plasma NE, because they have slower removal rates (longer half-lives) than the catecholamines. Thus, Vlachakis and Alexander (274) found that, in rats, after 5 min of restraint, stress-induced increments in plasma NE were not attended by a change in plasma catecholamine metabolite levels, but Jimerson et al. (127) reported significant increments in both MHPG and DHPG conjugates in plasma after 15 min of immobilization of intact rats or stimulation of the sympathetic outflow from the spinal cord of pithed rats.

Plasma levels of VMA are similar to those of MHPG, and their turnover rates are similar, but VMA appears to be distributed in a smaller volume so that its apparent total production rate is less than that of MHPG (186). Since VMA is derived from MHPG, its production is at least partly included in the MHPG production rate. The smaller apparent volume of distribution of VMA is consistent with the low apparent lipophilicity (reflected also in the low rate constants for efflux) of acid metabolites (see ref. 263). In humans, VMA does not appear to undergo significant conjugation, but levels of plasma conjugated MHPG are more than twice those of free MHPG (125, 136); urinary MHPG is almost totally in the conjugated forms.

The plasma levels of free DHMA are reported to be about half those of VMA and about twice those of DHPG (table 6). Conjugated DHMA is 4-fold higher in concentration than the free acid (275), whereas conjugated DHPG is 40% (78) to 60% (275) of the total plasma DHPG. As is the case for VMA and MHPG, however, levels in plasma, without knowledge of volumes of distribution and turnover rates, may be misleading, and plasma metabolite levels cannot be considered indicative of the relative importance of any metabolic pathway. In general, it may be expected that acid metabolites will have more difficulty in penetrating membranes and have lower apparent volumes of distribution, but active renal transport of the acids and their conjugates may increase their turnover rates. Conjugation of glycols presumably has the same effect as conversion to the acid metabolite. As in isolated tissues, rapid overflow of the glycol metabolites into the surrounding fluids would be expected to make the levels of these compounds more sensitive indices of changes in NE release than their corresponding acids.

5. Urinary NE metabolites. The rates of excretion of the final metabolic products of NE are shown in fig. 6. The sum of these metabolites reflects total body production of NE, since only a very small portion of the deam-

inated metabolites is derived from EPI. Clearly, VMA and MHPG are the major metabolites, with VMA predominating. The concentrations shown should be considered as representative only; standard deviations as high as 30% of the mean values are to be expected. Diet, drugs, activity, stress, etc., modify the relative proportion as well as the absolute excretion rates of the metabolites. Ethanol, as discussed above, will diminish conversion of MHPG to VMA, and other factors may have the opposite effect. There has been a large number of clinical studies in which MHPG excretion is measured "because MHPG is the major NE metabolite formed in the brain," but it is also the major NE metabolite formed outside of the brain! It should be clear that any abnormality in excretion of MHPG might be explained in terms of peripheral NE metabolism or by alterations in the conversion of MHPG to VMA. There is a highly significant correlation among the rates of excretion of the major NE metabolites (154) as might be expected if they are all derived from the same source. Because NMN is formed primarily from released NE, its proportion of the total metabolites is sensitive to sympathetic neuronal activity. Patients with multiple system atrophy excrete a smaller proportion of the total NE metabolites as NMN (3.1% compared to 4.5% in normal subjects), whereas patients with idiopathic orthostatic hypotension excrete NMN at near the expected proportion of the total (154). As might be expected, the ratios of MHPG to VMA are unaltered in either multiple system atrophy or idiopathic orthostatic hypotension, although the total of the metabolites is markedly diminished in patients with idiopathic orthostatic hypotension.

D. EPI Metabolites

Most EPI in the body is formed in the adrenal medulla and released into the adrenal vein without being first metabolized. Thus, the fate of i.v. administered EPI probably reflects reasonably closely the metabolism of endogenous EPI. Although EPI is present in a few areas of the brain, it is unlikely that the brain is a significant source of urinary EPI or MN. The urinary metabolites of administered EPI are listed in table 7. The estimated specific activities of EPI and MN are in good agreement, and if the other endogenous metabolites can be assumed to also have the same specific activity, then it is clear that only a minor fraction of MHPG and VMA is derived from EPI (fig. 6). At present, measurement of urinary MN and EPI is the only means for assessing the rate of EPI formation in humans. As indicated earlier, in humans, ingested EPI is mainly excreted as a sulfate conjugate, but sulfation of this catecholamine is not confined to the splanchnic area. Joyce et al. (130) found that forearm venous concentrations of EPI sulfate are substantially higher than arterial levels with a corresponding decrease in free EPI concentrations. In rats EPI glucuronide is the predominant conjugate, whereas NE is mainly sulfated (6). The roles of conjugation and possible deconjugation of EPI have not been adequately defined, but the large amounts of the catecholamine conjugates absorbed after p.o. injection do not appear to be physiologically active.

TABLE 7

Urinary metabolites of epinephrine. Data are from reports of LaBrosse et al. (163) and Muskiet et al. (203–205) with relative specific activity calculated as in table 3.

	[^{3}H]Epinephrine (% of administered dose)	Endogenous epinephrine (pmol/day)	Relative specific activity (% of administered/ pmol/day)
EPI	5.8	0.092	73.9
MN	40.8	0.505	80.8
VMA	41.2	0.532* (3.3)†	(77.4)
MHPG	5.3	0.092* (0.9)†	(77.4)
DHMA	1.7	0.021* (8.3)†	(77.4)

* Calculated from mean relative specific activity of urinary EPI and MET (shown in parentheses).

† Percentage of total endogenous metabolites of EPI and NE excreted daily.

VI. Quantifying the Origins of Catecholamine Metabolites

The relative ease and reliability of new assays for catecholamine metabolites in plasma and urine has provoked increasing interest in the use of catecholamine metabolite levels to assess brain neuronal activity in neuropsychiatric disorders and to understand and predict how drugs may be used to correct abnormalities in neuronal function. There must be some assurance, however, that the levels of metabolites measured in body fluids can provide reasonably valid indices of brain neuronal activity. The validity of this assumption depends upon the origins of the metabolites and their relationship to brain catecholamine metabolism. The formation of DOPAC from DA and DHPG from NE in peripheral sympathetic and central adrenergic neurones (and other peripheral tissues, e.g., carotid body) confounds the interpretation of studies attempting to assess brain catecholaminergic neuronal activity by examining plasma levels or urinary excretion of catecholamine metabolites. In attempts to circumvent this problem, several strategies have been devised to estimate the brain contribution to total body production of catecholamine metabolites.

A. Venous-Arterial Differences in Metabolite Concentration

The most direct of the strategies to estimate brain catecholamine production is based on measurement of venous-arterial differences in the concentration of a major brain amine metabolite. If the amount of the metabolite which is derived from brain is significant and the rate of clearance of the metabolite from the blood is reasonably rapid, then blood obtained from the internal jugular vein will have a significantly higher concentration of the metabolite than blood obtained from an artery. This difference in concentration, multiplied by

the cerebral blood flow, provides an estimate of the total amount of the metabolite being formed in the brain:

$$Q = (V - A) \cdot F \qquad \text{eqn 8}$$

in accordance with the Fick principle, where F is the cerebral blood flow, A and V are the concentrations of the metabolite in arterial and venous blood, respectively, and Q is the quantity of the metabolite released in the blood per unit time. The total quantity of the metabolite being released from the brain, divided by the total body production rate (calculated from the plasma half-life, apparent volume of distribution, and steady-state plasma concentration) or the total urinary excretion rate of the metabolite and its products, yields an estimate of the proportion of the total body production of the metabolite which is derived from the brain. This method has been applied by Maas and his coworkers to estimate HVA and MHPG production by the brain of primates and humans.

1. Jugular venous-arterial differences in HVA concentrations. As indicated above, in primates, HVA appears to be the major metabolic product of DA which is released from the brain into the circulation. In awake monkeys, Maas et al. (182) found a consistent and statistically significant increment of 2.2 ± 0.4 ng/ml in jugular venous over the arterial plasma HVA concentrations (16.0 ± 2.0 ng/ml), but none in DOPAC levels. This difference in HVA, coupled with a measure of cerebral blood flow, indicated a mean net release of about 787 nmol of HVA per day and accounted for about 25% of the total urinary excretion of HVA. In spite of the elegance of this method, a number of practical considerations have limited its application. The difference in HVA concentrations in jugular, venous, and arterial blood is small; thus, small errors in the assay procedures introduce so large an error in the calculated difference in concentration that the determination of net overflow of HVA into the blood from brain loses reliability in an individual subject.

2. Jugular venous-arterial differences in MHPG concentration. Maas et al. (180, 181) introduced this direct method for measuring brain catecholamine metabolite production with attempts to determine the rates of MHPG release from the brain into the blood. Although the increments in MHPG concentrations were small (1.7 ± 0.6 ng/ml) compared to the basal arterial levels (13.0 ± 2.1 ng/ml), they were significant and could be used to estimate the mean MHPG production rate. Furthermore, Maas et al. (180) demonstrated that clonidine, which diminishes brain NE turnover, decreased the apparent release rate of MHPG and that piperoxan produced the expected increase in apparent brain MHPG production. The mean brain MHPG production rate calculated for six monkeys was 1.27 μg/h or 30.5 μg/day. The mean excretion rate of urinary MHPG by similar monkeys determined from data reported by Maas et al. (177) was 365 μg/day. Thus, it appears that only about 8.4% of the urinary MHPG in these monkeys can be attributed to brain NE metabolism. If urine were obtained from larger monkeys than were used for the later study (180), then as much as 15–20% of urinary MHPG could be derived from the brain in monkeys.

When this method was applied to humans (178), there were found similarly small increments (0.7 ± 0.3 ng/ml) in jugular venous above arterial free MHPG concentrations (4.6 ± 3.2 mg/ml), but the mean difference was highly significant ($P = 0.0005$). Based on average human cerebral blood flow, it was estimated that about 38-39 μg of MHPG per h were being released into blood. In six of these subjects from whom urine was obtained, the excretion rate of MHPG was 64.5 μg/h; these six subjects released a mean of 40.9 μg of MHPG per h into the jugular venous blood. On the basis of these observations, it appeared as if 63% of MHPG in the urine was derived from brain NE metabolism. This calculation was made with the assumption that MHPG is not further metabolized. Since, as indicated above, a major portion of plasma free MHPG is oxidized to VMA, this was a gross overestimate. A diagrammatic representation of the distribution and further metabolism of MHPG formed in the brain and peripheral tissues is shown in fig. 10. If the brain was producing 60% as much MHPG as was excreted in the urine, but only 20-30% of i.v. administered MHPG appears in the urine, brain MHPG must contribute less than 20% of urinary MHPG and less than that as a fraction of the total of the urinary NE metabolites (fig. 10). This is more consistent with the calcu-

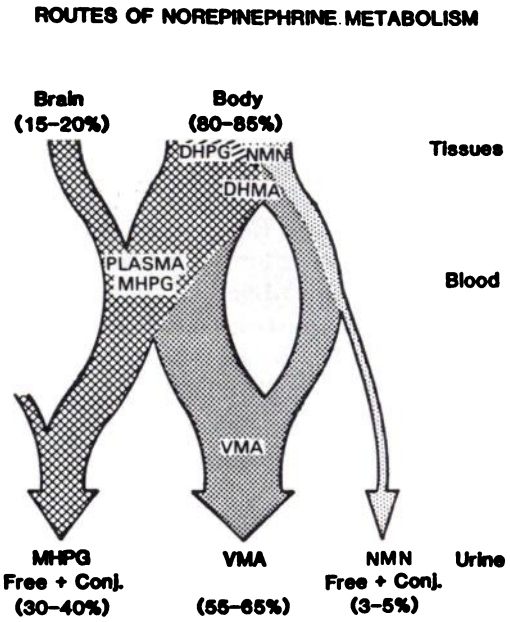

FIG. 10. Diagrammatic representation of the distribution and further metabolism of NE metabolites formed in the brain or at peripheral sympathetic nerve endings. MHPG from brain mixes in plasma with MHPG produced from NE in peripheral tissues; both contribute to the portions converted to VMA or excreted as MHPG conjugates. NMN is excreted (mainly as conjugates) or converted to deaminated metabolites (VMA or MHPG). From Kopin et al. (153). *Conj.*, conjugate.

lated contribution of monkey brain MHPG to urinary MHPG described above.

B. Anatomically Selective Diminution of Metabolite Formation

Selective blockade of peripheral catecholamine formation or destruction of the origin of catecholamines in the brain or peripheral tissues provides a means for distinguishing the origin of catecholamine metabolites. In most experiments of this type, catecholamine-producing neurones have been destroyed using 6-OH-DA. In other studies, formation, release, and intraneuronal deamination of catecholamines in peripheral sympathetic neurones have been blocked by administration of debrisoquin or related compounds.

1. Destruction of catecholamine-producing neurones. 6-OH-DA is taken up by both DA- and NE-containing neurones but does not readily penetrate the blood-brain barrier. When administered systemically, it is concentrated in peripheral sympathetic neurones where its toxicity results in selective destruction of these nerve terminals, and if the dose is sufficient, death of the neurones. In rats, destruction of peripheral sympathetic neurones with 6-OH-DA resulted in a 25% decrease in excretion of HVA and an even greater decrement (38%) in urinary MHPG (113). Intraventricular administration of the neurotoxin reduced brain DA and NE levels by 80% and 67%, respectively, but produced no changes in NE or MHPG excretion and a reduction of urinary HVA excretion by only 27% (H). Plasma HVA and DOPAC levels were reduced by about 45% and 25%, respectively, after central 6-OH-DA (20). Karoum et al. (137) failed to find a significant decrement in urinary excretion of MHPG in rats treated with 6-OH-DA intraventricularly, but by using debrisoquin (see below) and tritiated NE to evaluate the degree of inhibition of peripheral NE metabolite formation, calculated that in rats, about 30% of urinary MHPG is derived from the brain.

Although Maas et al. (177) reported decreased MHPG excretion in monkeys given 6-OH-DA intracerebroventricularly, Kraemer et al. (157) found only transient reduction in MHPG excretion, in spite of persistent diminution in brain NE levels. Edwards et al. (72) found transient decreases in rat urinary MHPG after intraventricular injection of 6-OH-DA, but persistent decreases in HVA excretion. There also appears to be an alteration in DOPAC conjugation after 6-OH-DA administration, since Edwards et al. (72) found elevated brain levels of conjugated HVA in the brain, and Peyrin et al. (210) reported increased urinary excretion of this conjugate; both groups found decreases in DOPAC. Intracisternal administration of 6-OH-DA produced no decrease in rat plasma MHPG in spite of substantial reduction of forebrain MHPG (108). Taken together, the results of all these studies are consistent with the relatively small contribution of brain NE to total body NE metabolism, but they suggest that a large portion of plasma HVA (and DOPAC) derives from catecholamine metabolizing cells in the brain. Use of toxins to selectively destroy brain or peripheral neurones or selective lesioning of brain areas is confined to studies in experimental animals, but anatomically selective drugs may be used in humans.

2. Anatomically selective pharmacological inhibition of metabolite formation. The decreases in plasma levels or urinary excretion of HVA or MHPG after pharmacological suppression of deaminated metabolite formation in peripheral tissues with debrisoquin have been examined in humans and in experimental animals as a means for differentiating brain from peripheral amine metabolism. Debrisoquin was introduced as an antihypertensive agent which acts by producing postganglionic sympathetic blockade (198). Like guanethidine and bretylium, debrisoquin is selectively concentrated in the sympathetic neurones where it reaches sufficiently high concentrations for its weak actions as a MAO inhibitor to block intraneuronal deamination of norepinephrine (85, 209). In hypertensive patients treated with debrisoquin, there was no significant inhibition of MAO in intestinal mucosa nor was there any elevation of urinary excretion of tryptamine or tyramine (209). Excretion of NMN was elevated, consistent with inhibition of sympathetic neuronal MAO, but the decrease in urinary VMA far exceeded the decrement in the amine, suggesting that there is a considerable decrease in norepinephrine formation and metabolism. Since debrisoquin does not enter the brain (192), these metabolic effects are presumably due to actions in sympathetic neurones. Bretylium and related substances are also weak local anesthetics at sensory nerves, and their adrenergic blocking actions have been attributed to buildup of sufficiently high concentrations to stabilize the nerve terminal membranes (100). Horst and Gattamell (116) suggested that debrisoquin decreases the ability of storage granules to retain NE but also inhibits intraneuronal MAO so that NE levels do not change. The combined effects of elevated cytoplasmic NE levels and blockade of NE release result in marked reduction of tyrosine hydroxylation and diminished synthesis of dopamine and NE in peripheral sympathetic nerves. In one of the first attempts to use debrisoquin to distinguish central from peripheral amine metabolism, Karoum et al. (137) found that the drug reduced the urinary excretion by rats of MHPG by 75%. Release of stored [^{3}H]NE was diminished by 40%, deamination by only 17%. Furthermore, with chronic administration cardiac NE was depleted. Thus, although properly debrisoquin should not be considered a "peripheral MAO inhibitor," it does markedly diminish deaminated catecholamine metabolite formation outside the brain.

In monkeys, Maas et al. (179) examined the effects of debrisoquin on the jugular venous-arterial differences in concentrations of HVA and MHPG and estimated the rates of brain production of these metabolites. They

found that the drug reduced apparent MHPG release from the brain but had no effect on the increment in HVA concentration. These results suggested that the decrement in plasma HVA concentration with debrisoquin treatment would be due solely to a reduction in HVA production outside the brain, i.e., those sources destroyed by 6-OH-DA in experimental animals (see above). In rats, Kendler et al. (139) showed that between 4 and 16 h after debrisoquin administration, plasma HVA concentrations were lowered, but striatal HVA levels were unaltered. Furthermore, debrisoquin did not diminish the rise in plasma and striatal HVA produced by haloperidol. Nor did debrisoquin affect the decrements in plasma HVA seen after administration of the DA agonists, apomorphine and bromocryptine (140). By minimizing the contribution of peripheral HVA production, plasma HVA levels can be made to reflect more precisely changes in brain HVA formation (256).

As described above, in the rat, DOPAC formed in the brain is either conjugated or O-methylated to HVA. Unconjugated DOPAC is present in rat plasma, but presumably is not derived from brain, unless DOPAC sulfate is deconjugated. Since conjugated DOPAC is a major product of rat brain metabolism, it would be expected that debrisoquin would lower unconjugated (peripherally derived) DOPAC to a greater extent than conjugated DOPAC. Boudet et al. (26) found that stimulation of central DA metabolism (by administering to rats a long acting neuroleptic) selectively increased plasma levels of DOPAC sulfate, whereas debrisoquin decreased free DOPAC levels only. These results clearly support the view that in rats free DOPAC is derived largely from the peripheral tissues and that a major portion of the sulfate conjugate is formed in brain before the acid enters the circulation. Unfortunately, the differences in metabolism of DOPAC by rats and humans appear to make this metabolite of little value for similar studies in humans.

Urinary excretion of both HVA and MHPG is decreased in rats and guinea pigs given debrisoquin; the reductions in MHPG excretion are proportionately greater than the reductions in urinary HVA (71). This difference could be a result of an effect of debrisoquin on brain MHPG production (as described above in monkeys) or because peripheral catecholamine metabolism accounts for a greater proportion of urinary MHPG than of HVA.

In humans, Izzo et al. (119) showed that quanethidine markedly diminished urinary excretion of MHPG (by 65%) and VMA (by 33%). This drug, like debrisoquin, does not enter the brain, is concentrated in sympathetic neurons, inhibits intraneuronal MAO, blocks NE release, and depletes NE stores. The drug reduces formation of NE and its metabolites outside the brain, but the degree of inhibition is unknown. Recently Maas et al. (176) reported that treatment of humans with debrisoquin lowered plasma, but not CSF levels of HVA, and that after 14 days of treatment with the drug, there was an excellent correlation between the plasma and CSF HVA levels, whereas before treatment they were not significantly correlated. If there can be devised a means for determining the degree of inhibition by a drug of peripheral HVA production, it should become possible to estimate brain HVA metabolism.

VII. Summary and Conclusions

1. Conjugated (mostly sulfated) catecholamines are the major urinary excretion products of p.o. ingested DOPA, DA, NE, and EPI. Although deconjugation is possible, this does not appear to occur at a site which evokes a physiological response, but catecholamine metabolite formation and excretion may be increased by this route.

2. Catecholamines i.v. administered, endogenous EPI released from the adrenal medulla, and NE and DA which escape into the circulation are mainly O-methylated, and then the amine which is formed is conjugated or deaminated.

3. In neuronal cytoplasm, DA or NE derived from uptake of released transmitter, from vesicular leakage, or by decarboxylation of endogenous DOPA is mainly deaminated. A significant portion of the DA formed in noradrenergic neurones appears to escape vesicular uptake and is deaminated. The deaminated product of DA is almost totally oxidized to DOPAC, whereas the aldehyde formed by deamination of NE is mainly reduced to DHPG.

4. In the brain, the metabolism of the deaminated products differs among species. In rats, conjugation with sulfate is a major pathway. Thus major fractions of DOPAC and DHPG are sulfated; the products of O-methylation of these catechols, HVA and MHPG, respectively, are also largely conjugated before leaving the brain. In primates, including humans, free HVA and MHPG are the major metabolites in CSF and are the products which are released from the brain into the blood.

5. The HVA level in CSF is a useful index of DA metabolism in the central nervous system, but requires appropriate precautions regarding the volume of CSF removed because of the steep gradient in HVA concentration between the CSF in the brain and in the lumbar area.

6. Because free MHPG diffuses readily through membranes, CSF MHPG readily exchanges with plasma MHPG; the correlation between plasma and CSF levels of the metabolite are due to this exchange. After correction for the plasma contribution, CSF levels of MHPG appear to reflect central nervous system (perhaps spinal cord) NE metabolism.

7. In peripheral tissues, DA is formed in noradrenergic sympathetic neurones as a precursor to NE, but not all DA is β-hydroxylated. As in brain noradrenergic neurones, DA which escapes uptake into the vesicles and

conversion to NE is deaminated to DOPAC. NE which leaks from the vesicles or is recaptured after release is converted to DHPG. DOPAC diffuses slowly into the surrounding extraneuronal tissues where it is O-methylated to HVA. Although DHPG diffuses rapidly, it is also largely O-methylated in extraneuronal tissue before reaching the systemic circulation. The COMT in arteriolar endothelium may function as an enzymatic barrier to catecholamine entry and egress and be the site of DHPG conversion to MHPG.

8. Plasma HVA is the major final metabolic product of DA. Measurement of HVA in plasma or urine reflects formation of the metabolite in peripheral tissues as well as in the brain, but pharmacologically selective diminution of HVA formation in peripheral tissues might provide a useful means for estimating brain HVA formation.

9. Plasma MHPG is a major intermediate in the formation of both MHPG conjugates and VMA. Measurement of free MHPG in plasma may be a useful index of total body NE metabolism, provided its rate of conjugation or oxidation to VMA is not inhibited. A small fraction of plasma MHPG is derived from the brain; this mixes with MHPG from the rest of the body. Neither plasma MHPG levels nor urinary MHPG sulfate excretion can be considered an index of brain NE metabolism.

10. The fact that measurement of plasma levels or urinary excretion of MHPG is not a valid index of brain NE metabolism does not in any way affect the validity of empirical observations which show differences among patient groups. These observations remain valid, but they require formulation of new testable hypotheses to determine if there is an etiological factor or pathogenetic mechanism to be revealed or if the changes in metabolism are secondary to stress or other factors (see ref. 153).

Acknowledgments. The author wishes to express his appreciation and thanks to Joan Darcey for her great patience and expert assistance in the preparation of this manuscript.

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