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Catechol-O-Methyl Transferase: Pharmacological Aspects and Physiological Role

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Introduction

In the second half of the 1950's work on the metabolism of noradrenaline and adrenaline performed at the National Institute of Mental Health led Julius Axelrod to postulate that these catecholamines undergo an O-methylation reaction (22). An impetus to this work, later emphasized by Axelrod in his 1970 Nobel lecture (25), was the identification by Armstrong and co-workers (15) of 3-methoxy-4-hydroxymandelic acid in the urine of patients with the adrenaline-forming tumour, phaeochromocytoma. Previously it had been shown that the administration of some catechol derivatives to rabbits resulted in the appearance of 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid) in the urine (66).

When a rat liver fraction was incubated with S-adenosylmethionine, magnesium ions and adrenaline, the disappearance of the catecholamine was noted and the product, metanephrine, was isolated and identified, thereby demonstrating that S-adenosylmethionine could donate its methyl group to one of the hydroxy groups of catecholamines (34). Animal tissues were then examined for the normal occurrence of O-methylated amines and the presence of normetanephrine, metanephrine and 3-methoxytyramine, formed from the endogenous catecholamines, noradrenaline, adrenaline and dopamine, respectively, was established (33). The enzyme responsible for the O-methylation of catecholamines: catechol-O-methyl transferase (COMT)—S-adenosylmethionine: catechol-O-methyl transferase, EC 2.1.1.6—was partly purified and preliminarily characterized (34).

The demonstration of the inhibition of COMT by polyphenols in 1959 (29) furnished an explanation for the findings of Bacq made in 1936 (39) that pyrogallol prolonged the physiological effects of adrenaline by inhibition of the O-methylating enzyme (41). Investigations were extended to show that pyrogallol was less active in prolonging the effect of noradrenaline (441) and although the consensus of opinion at the time supported the view that COMT contributed to the removal of circulating catecholamines, doubts were raised regarding its role in inactivation of noradrenaline at receptor sites (127).

The search for new inhibitors of COMT led to the description in 1963 of the tropolones as a new class of COMT inhibitors (58) and the dopacetamides as amide derivatives of catechol (93). Some pharmacological properties of these drugs represented improvements on the earlier COMT inhibitors, such as pyrogallol and catechol, but their impact as tools in the field of experimental work on adrenergic mechanisms, was limited.

In addition to the biological inactivation processes for catecholamines through biotransformation by monoamine oxidase (MAO) and COMT, uptake and binding of circulating noradrenaline in tissues was shown in the early 1960's to represent an important mechanism for the inactivation

of this substance (430). In the subsequent decade, studies have been concerned with defining the properties of the uptake processes involved and the importance of these mechanisms. In our context the relation of uptake to the metabolism of catecholamines will be of particular interest.

The application of knowledge from animal laboratory studies to studies in man should ultimately lead to a better understanding of the aetiology, diagnosis, and treatment of human disease. For example, the initiation of treatment of Parkinson's disease with orally administered L-dopa was based on the knowledge of a biochemical observation, namely the reduced concentration of dopamine in the basal ganglia (143) found in this disease. In turn, this has stimulated renewed interest in the metabolism of catecholamines, including O-methylation and not only as an inactivating process but also in producing some of the actions of L-dopa.

The purpose of the present review is to discuss critically methods used in the determination of COMT activity as an attempt to delineate problems encountered in the enzyme assays. The use of reliable methods for given experimental situations will in turn form the basis for the subsequent discussions on enzyme properties and kinetics of reaction and the occurrence of COMT in animals and plants. COMT inhibitors and their pharmacological properties will be reviewed comprehensively since this has not been done before. The physiological significance of O-methylation will be discussed in relation to COMT and lastly some clinical implications will be mentioned.

II. Methodology

A. Principles for Determination of COMT Activity

The methods used for the determination of COMT activity are based on the principle that the enzyme catalyses the transfer of methyl groups to substrates containing the catechol grouping in the presence of S-adenosylmethionine (S-AMe) as methyl donor and magnesium ions (Mg^{2+}) as activator.

Substrates employed include adrenaline (34), noradrenaline (123), 3,4-dihydroxybenzoic acid (137), pyrocatecholphthalein (9), 3,4-dihydroxyphenylacetic acid (17), 3,4-dihydroxypropiophenone (325) and 2-hydroxyestradiol-17β (50). In principle, almost any substrate containing the catechol grouping can be used.

End products of the enzymatic reaction are measured spectrophotofluorometrically or radiochemically and, rarely, colourometrically. The first procedure described for the assay of COMT activity measured the end product fluorometrically (34). Colourometric methods are generally unsuitable for application to COMT assays, and a lack of sensitivity of end product detection is evident in early work (58). But this type of detection can be used in screening tests during purification stages of the enzyme (10) or for determinations of COMT in tissues with a high enzyme activity (207). In 1973, a simple spectrophotometric assay for COMT was described in which the COMT activity can be monitored continuously with a coupled enzyme assay where the product S-adenosylhomocysteine is converted to S-inosylhomocysteine (115). The introduction of radioassay techniques to the measurements of COMT activity has led to improvements in methods for enzyme determination.

Two approaches for radiometric assay are used. The first uses isotopically labelled catecholamine such that if D,L-^{3}H-adrenaline is employed as the substrate, the product, ^{3}H-metanephrine, is extracted and assayed radiometrically (26). In the second technique, S-AMe-^{14}C-methyl or ^{3}H-methyl is used as the methyl donor. This approach can be used with various types of substrates: 1) catechol-acids—3,4-dihydroxybenzoic acid (295) and 3,4-dihydroxyphenylacetic acid (84); 2) catecholamines—adrenaline (16) and noradrenaline (144); 3) catechol—3,4-dihydrox-

ypropiophenone (120); and 4) catechol oestrogen—2-hydroxyoestradiol-17β (50).

B. Enzyme Preparations

For most tissues, liver, kidney, heart, salivary gland, *etc.*, the COMT activity is determined in the 100,000 $\times$ *g* supernatant fraction of homogenates (34) or, instead of this crude soluble enzyme preparation, in a purified enzyme preparation (23).

For some tissues, such as brain, a crude supernatant fraction obtained after low speed centrifugation (<14,000 $\times g$) may be used. This applies equally well to tissues such as liver since ultracentrifugational separation does not appear to alter significantly the specific enzyme activity (336).

For tissues with relatively low enzyme activity such as brain a crude homogenate preparation is adequate (295).

Less commonly other types of tissue preparations have been employed. *Tissue slices* from rat and rabbit livers have been used (306). *Tissue cultures* have been applied to the study of COMT in cultures of fibroblasts (234) and in cultured rodent astrocytoma cells (382).

Although the main COMT activity resides in the soluble fractions, the use of differential centrifugation has demonstrated enzyme activity in other fractions. The *microsomal fraction*, prepared by resuspension of the carefully washed 105,000 $\times$ *g* precipitate from liver, has been used for the demonstration of O-methylation of monophenols after hydroxylation (221). *Cell "ghost" preparations* from adipose cells, which are formed by gentle rupture of the cells producing a preparation of isolated plasma membranes which sediments at low speeds (404), and erythrocyte "ghosts" (20) have been used in kinetic studies and determination of COMT. *Solubilization by Triton X-100* has been used in order to increase the COMT activity in the soluble fraction from rat erythrocytes (20) and the rat brain synaptosomal fraction (83), and a membrane-bound form of COMT has been solubilized from rat liver microsomes (71).

C. Evaluation of Laboratory Procedures

For details on laboratory procedures the reader is referred to an excellent review published in 1971 (120).

1. Radioassay techniques and substrates. The introduction of radioassay techniques has increased the sensitivity and greatly simplified the measurement of COMT. The technique has basically two approaches: either with radioactively labelled S-AMe (16, 144) or labelled substrate, (nor)-adrenaline (26). In the first technique, after enzymatic transfer of the isotopically labelled methyl group to the substrate, the O-methylated product is separated and measured radiometrically. With this assay technique and 3,4-dihydroxybenzoic acid as substrate the product can be separated by paper chromatography (137) or more simply by extraction of the product into an organic layer (295) after washing the ethyl acetate extract with weak hydrochloric acid (239) to remove a foreign fraction resulting from the nonspecific breakdown of the coenzyme (84). Similar techniques have employed other substrates such as 3,4-dihydroxyphenylacetic acid (84), 3,4-dihydroxypropiophenone (120), adrenaline (18), noradrenaline (36) and 2-hydroxyestradiol-17β (50). The relative *in vitro* activities (34, 325) of the various substrates used do not differ to such an extent that the choice of substrate is of decisive importance. Other factors may have to be considered in choice of substrate such as its stability, extractability and possible interference of extractable radioactive products when using inhibitors that also are substrates for COMT.

In the second type of radioassay, the substrate has been ^{3}H-adrenaline or ^{3}H-noradrenaline and the product is extracted into toluene-isoamyl alcohol and assayed (23, 26). With crude tissue preparations this technique must be used with caution since catecholamines and their O-

methylated products are deaminated by MAO, thus introducing a possible source of error unless MAO is inhibited with, for instance, pargyline or tranylcypromine (120). This technique is suitable in studies of the inhibition of COMT by catechols and pyrogallols, since the products formed from these competitive substrates will not be radioactively labelled.

A radiochemical enzyme assay has the advantages over chemical assays of higher sensitivity and, generally, more rapid and simplified procedures. However, radiochemical methods, as opposed to, for instance, spectrophotofluorometric methods, lack specificity of end product detection. Impurities in radiochemical reagents, nonspecific breakdown or transfer of labelled groups, *etc.*, can lead to erroneous results (84). Values obtained for COMT activity by a radioactive method were about twice those obtained by a fluorometric method (18). This discrepancy might to a significant extent be accounted for by the finding (84) that iodide inhibits COMT, since the investigators used S-AMe-iodide for the fluorometric method while the labelled coenzyme contained sulphate.

2. *Kinetic factors*. When determining COMT activity it is of paramount importance that sufficient attention is paid to the kinetics of the enzyme reaction. A great deal of published work on COMT lacks sufficient data on the enzyme kinetics under the experimental conditions prevailing and thus make interpretations or extrapolations of the results difficult.

Linearity between COMT activity and incubation time and between enzyme activity and amount of tissue (or enzyme preparation) must be observed in any given experimental conditions. Creveling and Daly (120) pointed out that regardless of the type of assay employed for COMT, the final concentrations of substrate as well as Mg^{2+} and S-AMe should be such that maximal reaction rates are obtained. Since the optimum concentrations of these substances needed will depend upon a number of factors, such as type and kind of preparation of tissue, incubation medium, ionic strength and composition of ions, pH, temperature, *etc.*, they must be determined experimentally under the prevailing conditions.

Standardization (controlling carefully such factors as temperature, pH, buffer, ionic strength, substrate concentrations, *etc.*) of methods for COMT determination makes it possible or easier to compare results from different laboratories. Schwabe and Flohé (374) have recommended a substrate concentration in the medium of about three times its K_m value in order to avoid substrate or end product inhibition of the reaction.

The concentration of S-AMe used in the reaction is of special importance since it has been shown that S-adenosylhomocysteine (SAH), a product from S-AMe, is a potent inhibitor of COMT (134). These results would suggest a possible problem in working with commercial batches of S-AMe which are not sufficiently purified Moreover, data in the literature (114) indicated that routine kinetic analyses of the COMT catalyzed reaction involved greater than 5% conversion of S-AMe to SAH and, therefore, considerable inhibition by SAH occurred. Data should be obtained under saturating concentrations of S-AMe as at a S-AMe concentration of 6×10^{-4} M conversion to product was always kept below 5% (114). It has been stated that a higher concentration of S-AMe (1×10^{-3} M) is required for saturation of COMT (120).

The reaction rate is dependent on Mg^{2+} concentrations and it has been stated (120) that marked inhibition of COMT activity is produced by Mg^{2+} concentrations greater than 2×10^{-3} M. The optimum concentration of Mg^{2+} needed for maximal activity is dependent on experimental conditions, *vis.*, type of tissue, the extent of dilution in the homogenizing media (120), pH and other factors of the incubation medium (374) . For crude homogenates from brain tissue a Mg^{2+} concentration of

4.8×10^{-3} M is required for optimal conditions (84), thus the optimum concentration of Mg^{2+} must be determined under the particular assay conditions used.

Attention to the effects on the kinetics of COMT reactions of additional factors such as choice of buffer, ions, *etc.* is required (374) and future work along these lines is needed.

Finally, a critical note on the presentation of kinetic data on COMT inhibitors is required. Webb (426) has suggested that "inhibitors should either be used carefully and thoroughly, or not used at all." Although inhibition studies may well be done under correct experimental conditions, it is difficult, if not impossible, to compare a great deal of the published work on inhibitors of COMT from different laboratories. For instance, presentation of results using percentage inhibition without detailed kinetic data is of limited value and often renders comparisons of different studies impossible.

3. Biological factors. Circumstances seldom allow one to relate quantitatively the action of an enzyme in an extract to its activity in the intact cell. Up to the present time, methods used for studying COMT have involved primarily the obtaining of information on the enzyme and enzyme inhibitors under experimental conditions where the enzyme has been isolated from cells in extracts and studied in artificial media. It is possible that methods for COMT determinations may have to be modified to obtain additional information on the enzyme in studies in which one is concerned with its role in physiological or pathological conditions, and not primarily with its *in vitro* biochemical properties. There seems to be a need for studies of COMT under conditions where, as far as it is practical, the normal configuration and milieu of the enzyme are maintained. However, in many situations the technique of proper *in vitro* measurement cannot be duplicated or improved for use under *in vivo* conditions.

Here the mode of preparing the tissue may be important. So far most of the COMT determinations have been made on high speed supernatants or purified enzyme preparations under various experimental conditions but it also seems desirable to obtain results under conditions in which the cellular architecture is maintained to varying degrees with crude homogenates, tissue slices, cell cultures, *etc.*

It is unphysiological to have large amounts of enzyme present in the incubation medium as well as large excesses of the substrate. With the co-substrate, S-AMe, in amounts that saturate COMT also may be unphysiological in that the COMT activity *in vivo* may be considerably inhibited by endogenous adenosylhomocysteine (159).

COMT is an intracellular enzyme and the ionic concentration and composition of the incubation medium should logically approximate the intracellular environment. However, these factors may not always be easy to determine. For instance, the intracellular Mg^{2+} concentration is about 20 mM, a concentration which would cause marked inhibition to COMT, but it is, of course, the free Mg^{2+} concentration that will be the determining factor. There is often a reciprocal relation between Mg^{2+} and Ca^{2+} in terms of physiological mechanisms taking place across the cell membrane. Although the intracellular Ca^{2+} concentration is low, Ca^{2+} may, nevertheless, play a role in the determination of COMT activity (374). Moreover, because of the intracellular localization of COMT, it seems logical to select K^+ buffers rather than Na^+ buffers for the incubation medium. Similarly, there is little rationale in the use of an odd assortment of unnatural buffers in the incubation medium and if experiments were made nearer the physiological pH (intracellular pH of 6.8) there would be no need for buffers effective outside this range.

Under physiological conditions, it is likely that O-methylation takes place

within certain cells subsequent to uptake of the substrate by the cell. Ideally, therefore, one should use a substrate, *e.g.*, noradrenaline, which is taken up by cells by physiological processes. In practice, this is difficult since concentrations of the substrates used are so high that diffusion rather than selective uptake will influence the apparent enzyme activity.

The use of COMT inhibitors under biological conditions as compared to their use *in vitro* test tube conditions raises a number of additional problems. Many factors, apart from the enzyme-inhibitor complex formation, must be considered, namely, penetrability of inhibitor into the cells, choice of optimal inhibitor concentration for intracellular inhibition, possibilities for nonspecific effects of the inhibitor, metabolism of the inhibitor, the presence of a significant degree of endogenous metabolism, *etc.* The criteria for COMT assays under these conditions have apparently attracted little attention in published works.

D. Additional Methods with COMT

1. Catechol-forming enzymes. The ability of COMT to O-methylate catechols has been found useful in studying the group of catechol-forming enzymes (24). These enzymes hydroxylate monophenols to catechols and are localized in the microsomes of the liver. Since the catechol metabolites formed are unstable and the amounts formed are too small to measure, the catechol is converted to the O-methylated compound by the action of COMT. A number of monophenolic biogenic amines are hydroxylated and O-methylated by these enzymes: tyramine, octopamine, synephrine, and N-acetylserotonin (24). In addition, the enzyme system can form O-methylated metabolites from such phenolic substrates as morphine, levorphanol, phentolamine, oestradiol, diethylstilboestrol (131), and hydroxychlorpromazine (132).

A number of reports have appeared (28, 131, 221) in which COMT and ^{14}C-S-AMe have been used to measure the microsomal formation of catechols from phenols as, for instance, the formation of dopamine from tyramine and noradrenaline from octopamine.

2. Catecholamine determinations. Purified COMT with ^{14}C-S-AMe has been used for radiometric assays of endogenous levels of noradrenaline (148, 324) and dopamine in the presence of other biogenic amines in the tissue extracts (129). The methods appear sensitive and sufficiently specific for the determination of catecholamine content of biological specimens containing low levels of catecholamines.

III Characteristics of COMT Reactions

A. Substrates

1. Specificity. In spite of the broad substrate specificity of COMT, all known substrates are characterized by a catechol configuration regardless of other substituents on the aromatic nucleus.

Physiological substrates, *i.e.*, endogenously occurring substances in animals, include adrenaline, noradrenaline, dopamine, 3,4-dihydroxyphenylalanine (dopa), 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylethanol, 3,4-dihydroxyphenylglycol, N-acetyldopamine and N-acetylnoradrenaline (34, 119). In addition to these catechol compounds mentioned, ascorbic acid (63) which like the catechols contains the enediol group and the catechol oestrogen 2-hydroxyestradiol (266) are O-methylated by COMT.

Foreign catechols, such as arterenone, adrenalone, 3,4-dihydroxycinnamic acid (119, 130), triphenols, such as pyrogallol (34), and substituted catechols, such as 3,4-dihydroxypropiophenone (325), can serve as substrates for COMT.

2. Isomers, m- and p-methylation, enzyme/substrate interactions. It has been shown that COMT shows no stereospecificity with respect to D- and L-isomers of

adrenaline (34) and other catecholamines (169) and it has been shown that the K_m values for D- and L-adrenaline are fairly similar, 2.8×10^{-4} M and 6.3×10^{-4} M respectively (112). However, for various isomeric catechols the distribution of *m*- *versus* *p*-methylated metabolites was shown to be quite different (122).

O-Methylation of catecholamines and related physiological substrates *in vivo* results almost exclusively in the formation of *m*-O-methylated derivatives (24). Administration of xenobiotic catechols such as arterenone and adrenalone results in the formation and excretion of *p*-O-methylated metabolites (130). Physiological substrates tested *in vitro* produce significant amounts of *p*-O-methylated derivatives by the catalytic action of COMT (119, 122, 130, 275, 376, 434).

The reason for the anomaly between different isomeric ring substitution *in vivo* and *in vitro* is not known. It has been suggested that it could either be due to cellular disruption which alters the nature of the active site of COMT so that *p*-methylation is facilitated or that the *p*-O-methyl metabolites are further metabolized *in vivo* by some other route, *e.g.*, demethylation (119). Experiments with human blood indicate that it contains enzymatic system(s) able to degrade *o*-methoxy phenols specifically (163). It was shown that normeta- and norparanephrine disappear at similar rates when incubated with human blood and the simultaneous appearance of noradrenaline indicates that at least a part of this degradation was caused by O-demethylation. Rat blood cells O-methylate noradrenaline to normetanephrine without detectable norparanephrine while purified COMT from blood or rat liver O-methylates noradrenaline in both the *p*- and *m*-positions (165). The absence of *p*-O-methylated products when noradrenaline was incubated with whole blood probably can be accounted for by a preferential action of O-demethylating enzymes on norparanephrine. A portion of 2-hydroxyestradiol is converted *in vivo* to the 2-methyl ether while *in vitro* experiments yield a mixture of the isomeric 2- and 3-methyl ethers and it has been shown that sulfate conjugates are involved in the *in vivo* selectivity of COMT (157).

The ratio of *p*- and *m*-O-methylated products synthesized enzymatically *in vitro* is strongly dependent on the nature of the aromatic substrate and the pH of the reaction mixture. The *m*-methylated isomer predominates with substrates which contain a highly polar substituent as is the case with most physiological substrates (119). With nonpolar substituents the ratio of the *m*- and *p*-O-methylated products was found to be close to unity. Phenolic substrates having three adjacent hydroxy groups, *e.g.*, pyrogallol and gallic acid, are methylated on the middle hydroxy group regardless of whether the hydroxy group occupies a *p*- or *m*-position (307).

Findings reported in 1972 (122) extend and amplify earlier conclusions (119, 121) that the presence of a nonpolar region in the catechol-binding site of COMT militates against binding of polar substrates in the orientation necessary for *p*-methylation, while nonpolar substrates appear to bind in a more random fashion, resulting in the formation of nearly equal amounts of *m*- and *p*-O-methylated products. The high *meta:para* ratios observed with the amines is due to the presence of the ionized ammonium function in the side chain.

The variation in *meta:para* ratios as a function of side chain structure may also be rationalized in terms of probable preferred conformations of the various dopamine and noradrenaline derivatives and their interaction with hydrophobic centres in COMT. Studies reported in 1971 (387) have suggested that the staggered conformer of dopamine analogues and the gauche conformer of noradrenaline analogues are preferred respective conformations for COMT.

The additional factor of stereoisomerism may provide further information on the

geometry of the active site of COMT. Thus, in all pairs of D- and L-isomers, the L-isomer affords a higher *meta:para* ratio.

B. Kinetics

There are relatively few reports in the literature containing kinetic data of COMT which express adequately factors such as affinities of various substrates for the enzyme and data on a particular substrate under different experimental conditions. Interest lies not only in factors which may affect enzyme reactions primarily as a consequence of altering the *in vitro* conditions but also in factors which may throw light on results which are conditioned principally by the *in vivo* properties of the enzyme itself.

The apparent affinities of commonly employed catechol substrates do not seem to differ markedly in that K_m values for amines such as noradrenaline (2.6×10^{-4} M) and adrenaline (3.8×10^{-4} M) and acids such as 3,4-dihydroxybenzoic acid (2.5×10^{-4} M) and 3,4-dihydroxyphenylacetic acid (3.1×10^{-4} M) do not deviate much (119). Under comparable experimental conditions the K_m values for dopamine, 3,4-dihydroxymandelic acid, 3,4-dihydroxypropiophenone and catechol were 1.6×10^{-4} M, 9.4×10^{-4} M, 1.7×10^{-3} M and 6×10^{-4} M, respectively (119, 325). The K_m value for the catechol-oestrogen 2-hydroxyestradiol-17-β has been determined to 1.4×10^{-5} M (51).

The kinetic data of COMT reported from different laboratories are varied. Taking the examples of adrenaline and noradrenaline, the extreme K_m values reported for the former are 10^{-5} M (26) and 6.3×10^{-4} M (122), and for the latter, 6×10^{-6} M (20) and 1.6×10^{-3} M (122). Some of the factors which can affect the K_m value for a substrate have been investigated in recent years and may to some extent explain the variations. Factors include the composition, ionic strength and pH of the incubation medium, the instability of the substrates, a possible influence of the concentration of one substrate on the K_m value for the second substrate or *vice versa*, difference in enzyme source and preparations, a possible heterogenity of COMT, the instability of the enzyme itself and the presence or absence of a COMT stabilizing agent such as dithiothreitol.

It has been shown that the nature of the ionic medium and the pH has a marked effect on the reaction rate. A 3-fold increase in rate was observed at pH 9.1 in Tris buffer over that with phosphate buffer at pH 7.8. This change in rate was accompanied by a change in the apparent K_m value for noradrenaline from 2.6×10^{-4} M to 7.2×10^{-5} M (324). Experiments reported in 1972 revealed that the K_m value for adrenaline decreased markedly with increasing pH and that at a pH above 7 the K_m was influenced by the concentration of Mg^{2+} so that it did not coincide with the dissociation constant of the complex of the enzyme and the substrate (159). It was suggested earlier that it is not adrenaline itself but its magnesium complex that is bound to COMT (58, 377) and the pH of the incubation medium has an influence on the formation of the enzyme-magnesium-substrate complex. According to Flohé and Schwabe (159) the K_m values cited in the literature are generally too high since it is evident from their investigations that the K_m is dependent on the Mg^{2+} concentration; thus in Mg^{2+} chelating buffers the K_m values may be hard to interpret.

Early reports seemed to indicate that the K_m might vary with the source of the enzyme since for adrenaline it was 1.2×10^{-4} M for the liver enzyme (34) but less than 10^{-5} M in brain (26). A similar trend in the differences was observed in other studies when using 3,4-dihydroxybenzoic acid as substrate which gave a K_m of 6.0×10^{-5} M for crude homogenates from rat brain (295) and 2.5×10^{-4} M for purified enzyme from rat liver (325). However, after approximately 30-fold purification of COMT from the liver, brain and heart of the rat, the K_m for noradrenaline was found to be about 3×10^{-4} M regardless of the source of the enzyme (123). With 3,4-dihy-

droxyphenylacetic acid as substrate there is relatively good agreement between the K_m values obtained on enzyme from rat liver soluble fraction, purified rat liver and rat brain homogenates: 2×10^{-4} M, 3.1×10^{-4} M, and 1.9×10^{-4} M, respectively (17, 84, 119).

The K_m's for L-noradrenaline were the same (2×10^{-4} M) for human liver and red blood cell COMT (27). On the other hand, starch block electrophoretic fractions from supernatants of rat liver gave two protein peaks corresponding to COMT activity with K_m values for L-noradrenaline of 4.3×10^{-5} M and 2×10^{-4} M which were statistically significantly different (36). Moreover, with the soluble fraction from rat red blood cell and noradrenaline as substrate a K_m of 3.7×10^{-4} M was obtained while the K_m obtained with the membrane fraction from "ghost" red blood cells was 6.0×10^{-6} M (20). It is of interest that the membrane-bound COMT showed a 70 times greater affinity for noradrenaline and other catecholamines than the soluble fraction enzyme. It is likely that the differences in K_m values are associated with different forms of COMT.

Instability of COMT is particularly encountered on purification of the enzyme (10, 34). It is evident that discrepancies in K_m values can arise when working with an unstable enzyme, which can, for instance, happen even with a crude preparation at high pH (158). In 1972, it was reported that the absence or presence of a protective reagent for sulfhydryl groups, such as dithiothreitol, affect the apparent K_m for a catecholamine (122).

An important finding, which can affect kinetic analyses of COMT catalyzed reactions, is that S-adenosylhomocysteine, a product from S-AMe, is a potent inhibitor of COMT (114, 134). Errors in kinetic analyses may result when working with commercial batches of S-AMe which are not sufficiently purified (134) and when working under nonsaturating conditions for S-AMe such that conversion to product is greater than 5% (114). Moreover, interesting physiological consequences may arise (159) in that the COMT activity *in vivo* may be inhibited considerably since the adenosylhomocysteine content of the liver is of the same order as the S-AMe content (363).

There is a considerable variation in the kinetic data given in the literature depicting the dependency of the O-methylation reaction rate on the S-AMc concentration. With a purified enzyme preparation and adrenaline in the concentration range 0.5×10^{-4} M to 10^{-3} M, the K_m for S-AMe was calculated to be 3.2×10^{-6} M (158). The K_m value for S-AMe remained constant at about 10^{-6} M to 3×10^{-6} M between pH 6.5 and 9.5, while it was influenced by the concentration of Mg^{2+} at pH values above 7 (159). Under various experimental conditions other investigators have calculated K_m values for S-AMe of the order of 3.3×10^{-6} M for both soluble and "ghost" fractions of rat erythrocytes (20), 6×10^{-6} M (296), 8.5×10^{-6} M (51), 4×10^{-5} M (123) and up to as high as 1.7×10^{-4} M (325). An early report indicated that the relation between COMT activity and substrate concentration had a sharp optimum concentration at about 6×10^{-5} M with a marked inhibition at higher concentrations of S-AMe (295) but this observation has not been confirmed by others (17, 51, 84, 158, 325).

There is scant information in the literature on the exact requirements for Mg^{2+} of the COMT catalyzed reactions (58, 377). By expressing graphically the relation between enzyme activity and magnesium ion concentration, a straight line relationship was obtained up to a magnesium ion concentration of 2.4×10^{-5} M, with the apparent K_m for Mg^{2+} of 4.3×10^{-6} M (324). As the Mg^{2+} concentration was increased to 1.6×10^{-3} M, there was a marked activation of the reaction rate followed by progressive inhibition at concentrations of Mg^{2+} greater than 1.2×10^{-3} M. In 1972, it was been shown that COMT was inhibited at high concentrations of Mg^{2+} in a mixed type noncompetitive manner (159). It was

also shown that the K_m value for Mg^{2+} strongly decreased with increasing pH and that the K_m values for all substrates have to be re-examined in the light of Mg^{2+} inhibition (159).

IV. Some Properties of COMT

A. Purified COMT

Liver is the best source of COMT and attempts to purify and characterise COMT have been limited to rat (10, 19, 34, 158) and human liver (50).

Axelrod and Tomchick (34) purified COMT from rat liver about 30-fold by using ammonium sulphate fractionation and gel filtration. By applying the additional technique of ion-exchange chromatography to the purification of the enzyme, it was possible to obtain a 200-fold purification (10). However, the lability of this preparation prevented further attempts at purification. The instability of the enzyme during the last stages of purification could be reduced significantly by the presence of dithiothreitol, indicating that the inactivation of purified COMT resulted from the oxidation of sulfhydryl groups on the protein molecule, and a 450-fold purification was achieved with about a 12% yield in activity (19). An enzyme preparation with relatively high specific activity and fairly good yield has also been prepared by other workers, the purification being about 300-fold without reactivation, although the enzyme was protected by dithiothreitol (158). Although dithiothreitol was the most suitable of the common SH-compounds to prevent oxidative inactivation of COMT, mercaptoethanol also protected and partially reactivated the enzyme but a slow steady loss of activity was observed even in the presence of these SH-compounds (158). Disc electrophoretic studies of the enzyme did not reveal any sign of impurity after diethylaminoethyl (DEAE)-Sephadex chromatography (158). A 380-fold purification of human liver COMT has been obtained by fractionation with ammonium sulphate, gel filtration on Sephadex columns and ion-exchange chromatography on CM-Sephadex and DEAE-cellulose columns (50). The enzyme preparation, representing the major peak of the DEAE-cellulose eluate, was apparently homogenous when checked by further chromatography, analytical ultracentrifugation, and disc electrophoresis at pH 9.5. The COMT from human liver was also very unstable in its purer forms and was not stabilized by dithiothreitol, when added at the beginning of storage, but was by $MgCl_2$ and ethylenediamine tetraacetate (EDTA), in equimolar concentrations (50).

B. Molecular Weight of COMT

The molecular weight of COMT with pooled eluates of highest COMT activity from Bio-Gel P-60 columns was estimated as 29,000 from elution volume data obtained with a column of Sephadex G-100 and calculating the column constants graphically with the aid of two proteins of known molecular weight (10). The molecular weight of the enzyme determined by gel filtration of a 130-fold purified preparation, was calculated to be 24,000 (19) and this is in reasonable agreement with the earlier finding of 29,000. However, the molecular weight calculated from velocity sedimentation measurements appeared to be approximately 40,000 (19). This discrepancy was not investigated further but it was suggested that it might have arisen from dimerisation of the molecule. However, similar discrepancies in results with different techniques were not reported by other workers (50) in that the molecular weight of purified COMT from human liver was estimated by gel filtration through Sephadex G-100 and by analytical ultracentrifugation with the CsCl-density gradient method to be 25,000 ± 3,000 and 29,000 ± 2,000, respectively. In 1974, the molecular weight of the enzyme protein was calculated to 23,000 ± 700 as determined by SDS-acrylamide disc gel electrophoresis, gel filtration on Sephadex G-50, 75 and 100, amino acid analysis and sedimentation equilibrium analysis while at least two

other forms of COMT with molecular weights of 11,250 and 37,000 also were present in the soluble fraction from rat liver (117).

C. Different Forms of the Enzyme

Early work had shown that COMT is highly localized in the soluble fraction of the cell (34). Later investigations, separating microsomes from the soluble fraction by centrifuging at 105,000 × *g* for 45 minutes and subsequently washing the microsomal fraction carefully but not using marker enzymes or electronmicroscopy for control, contained reports of an enzyme in liver microsomes which could O-methylate catechols (221). Furthermore, it was found that the microsomal COMT differed from soluble COMT in its response to 3,4-benzpyrene and cold stress and pH optima. The pH optimum for microsomal O-methylation was 7.0 compared to 7.9 for the soluble enzyme. In a study reported in 1974, a membrane-bound form of COMT was solubilized and partially purified from liver microsomes of the rat but the microsomal COMT represented only about 0.1% of the total COMT activity in the liver and did not appear to differ in biochemical and immunological characteristics from the soluble COMT (71).

COMT is also generally assumed to be a cytoplasmic enzyme in the brain (34) but some of the enzyme is recovered in a particle bound form (3). Results reported in 1972 on the subcellular localization of COMT in the rat brain suggested that the enzyme is present in the cytoplasm and that the portion of enzyme present in the synaptosomal fraction might be accounted for by the cytoplasm being trapped within the nerve terminals (83).

During purification procedures for COMT, investigators have reported different fractions with COMT activity in polyacrylamide electrophoretic studies (10), during chromatography on hydroxyapatite (158) and upon elution of the DEAE-cellulose column (50).

The results, obtained from using either differential centrifugation or chromatography/electrophoresis fractionation, suggest that the heterogenity of COMT might be of biological significance. Thus, the occurrence of isoenzymes, or at least different functional states, of COMT in some tissue preparations must be considered. However, the studies have not provided kinetic data to support the notion that COMT exists in different functional forms.

Axelrod and Vesell (36) identified biochemically distinguishable forms of COMT possessing different catalytic properties. The electrophoretic mobilities on starch blocks of COMT from heart, kidney, brain and liver from the rats and liver from cats, dogs and human beings were examined. The rat tissues exhibited two isoenzymes of COMT after starch block electrophoresis. In the rat, isoenzymes from one tissue had the same electrophoretic mobility as the corresponding isoenzymes from the other tissue. The heat stability and K_m values of the two COMT isoenzymes from rat liver were found to differ. Two electrophoretically distinguishable forms of COMT were also separated from human, dog, and cat livers. These isoenzymes appeared to have different electrophoretic mobilities, heat stabilities, and K_m values from those of the rat, indicating that species differences exist in the physicochemical properties of the COMT.

There is also some evidence for the existence of COMT isoenzymes in the soluble and "ghost" fractions of rat erythrocytes (20). Preparations of COMT from the two fractions differed in their pH optimum, heat stability, kinetic property, and immunochemical reactivity. It is of interest that the membrane-bound COMT showed a 70-fold greater affinity for catecholamines than the soluble fraction COMT (K_m 3.3 × 10^{-6} M for the membrane fraction and 2.2 × 10^{-4} M for the soluble fraction). After solubilization with Triton X-100 a smaller difference in K_m values was found, which were approximately 20-fold lower than the K_m values found for the soluble enzyme.

Salt precipitation of the proteins in supernatants from brain homogenates yielded two peaks of COMT activity with different kinetic constants, and subcellular fractionation of brain homogenates indicated the presence of COMT fractions with different kinetic constants (82).

Isoenzymes are commonly encountered in biological systems. The role performed by possible isoenzymes of COMT is not known. Certain isoenzymes may represent synthetic or degradative by-products without additional physiological significance and some may even be artifacts. However, the markedly different physicochemical properties of COMT isoenzymes suggest their existence within the cell (36).

D. *Production of Antibodies to COMT*

Immunization of rabbits with purified (450-fold) COMT from supernatants of rat liver homogenates can yield a potent antibody (18). Antigen-antibody interaction was demonstrated by specific precipitation and immunoelectrophoretic analysis. Antisera had a potent inhibitory effect on the COMT activity from rat liver. The antibody exhibited cross-reactivity with the enzyme from various tissues including liver, spleen, heart, muscle and lung from the rat, while brain COMT was inhibited to a much smaller extent. Furthermore, cross-reactivity was observed between the antibody and enzyme from several species, but the antiserum did not inhibit bovine COMT. Interestingly the antiserum did not inhibit rat liver microsomal COMT, which indicates that rat liver soluble and particle-bound COMT are different proteins with specific antigenic properties. The relatively high specificity of the antibody was further shown by the lack of inhibition of other O-methylating enzymes such as phenol-O-methyl transferase and hydroxyindole-O-methyl transferase. Results reported in 1974 indicate that after solubilization of a partially purified form of liver microsomal COMT it exhibits immunological properties similar to those of the soluble COMT (71, 117).

The preparation of specific antibodies to soluble COMT may, in future studies, be made use of in techniques to investigate differences between different forms of COMT and also possible differences existing between COMT of various tissues, such as rat liver and brain. The immunochemical principle, as used for COMT in important studies (18, 117), might also be the basis for the development of fluorescence histochemical techniques for the localization of COMT. Thus, results from *in vivo* studies with purified antibodies to COMT are awaited.

V. Distribution of COMT

The occurrence of COMT in various plants and animals is summarized in table 1 and the occurrence of COMT in various mammalian tissues is summarized in table 2.

A. *Plants*

The enzyme is found in various monocotyledonous and dicotyledonous plants (154–156) (table 1). The enzymatic methylation of substrates by polyphenol oxidase prevents oxidation from occurring. Oxidation is the cause of fruit discolouration (156).

B. *Invertebrates*

In the gastropod snail *Helix aspersa* COMT occurs in the circumoesophageal ganglia, the hepatopancreas and the heart (303). The oesophageal ganglia contain high concentrations of dopamine (263). The enzyme is also found in the ganglia of the squid *Aplysia californica* (296). There is evidence for the presence of an enzyme similar to COMT in the muscle tissue of the polychaeta *Ophelia neglecta* (400). The much greater N-acetyl transferase than MAO or COMT activity in the fly *Drosophila* indicates that the major en-

TABLE 1
The distribution of COMT in plants and animals

Species	Tissue	COMT activity[a]	Reference
Plants			
Cortaderia selloana (pampas grass)	Shoot tissue	Present	154
DICOTYLEDONS			
Pitosporum crassifolia (apple)	Cambial scrapings	Present	155
Coffee plant	Green beans	Present	156
Animals			
INVERTEBRATES			
ANNELIDS: *Ophelia neglecta*	Muscle	Present (indirect evidence)	400
ARTHROPODS			
Crustaceans: *Upogebia littoralis*	Hepatopancreas	Present	302
Insects: *Drosophila melanogaster*	Head homogenate	0.1 μmol/g protein/hr	135
	Whole fly	0.3 μmol/g protein/hr (3,4-dihydroxybenzoic acid substrate)	135
MULLUSCS			
Gastropods			
Helix aspersa	Cerebral ganglia	0.19 μmol HVA/g/hr	303
	Visceral ganglia	0.22 μmol HVA/g/hr	303
	Hepatopancreas	1.39 μmol HVA/g/hr	303
	Heart	0.50 μmol HVA/g/hr	303
Aplysia californica	Buccal ganglia	1.47 μmol/g protein/hr (3,4-dihydroxybenzoic acid substrate)	296
	Cerebral ganglia	2.70 μmol/g protein/hr	296
	Parietal-visceral ganglia	2.64 μmol/g protein/hr	296
	Pleural ganglia	2.18 μmol/g protein/hr	296
	Pedal ganglia	3.51 μmol/g protein/hr	296
	Single nerve cells	1.70–4.22 μmol/g protein/hr	296
	Non-nerve cells	3.16–4.20 μmol/g protein/hr	296
	Neurophil	5.1 μmol/g protein/hr	296
VERTEBRATES			
ELASMOBRANCHS			
Squalus acanthas (Spiny dogfish)	Liver, kidney, sympathetic nerve, interrenal body, muscle	Present	75, 405
Carcharhinus milberti (brown shark)	Liver, kidney, heart, spleen, brain	Present	75, 405
Gynglymostoma cirratum (nurse shark)	Interrenal body	Present	75, 405
Carcharhinus limbatus (small black-tipped shark)	Interrenal body	Present	75, 405
Potamotrygon (sting ray)	Interrenal body	Present	75, 405
AMPHIBIANS: Frog	Skin	Present	53
BIRDS			
Quail	Pineal gland	0.12 nmol normetanephrine/hr	30

[a] When possible the actual values are given, with the relevant information, to indicate comparative distribution.

TABLE 1—*Continued*

Species	Tissue	COMT activity[a]	Reference
Turkey	Pineal gland	0.03 nmol normetanephrine/hr	30
Kahki-Cambell (duck)	Pineal gland	0.25 nmol normetanephrine/hr	30
Leghorn chicken	Pineal gland	0.17 nmol normetanephrine/hr	30
Pigeon	Pineal gland	0.04 nmol normetanephrine/hr	30
Chicken	Red blood cells	Present	215
	Embryonic heart	Present from 4th day of incubation	218
	Embryonic liver	Present	218

zymatic regulation of amines in insects is by N-acetylation (135).

C. Vertebrates

1. Peripheral system. In the vertebrates the greatest activity is found in the liver (34), but there is a considerable species variation in the level of activity, with the following descending order: rat > cow > pig > mouse > guinea pig > man > cat > rabbit (2, 28, 265, 341, 393, 425) though this is probably partly due to assay conditions. In the Elasmobranchs (sharks) the greatest activity is, however, found in the interrenal body, the analogue of the mammalian adrenal cortex (75, 405). The enzyme is also found in the mammalian spleen (34), intestines (34), adrenal gland (361), thyroid, pituitary and salivary glands (34), ocular tissues (425) including the inferior and medial muscles of the cat nictitating membrane (238), cultured guinea pig fibroblasts (234, 235), adipose tissue including isolated adipocytes (393, 404), peripheral nerves (34), uterus (174, 361, 436), ovaries and testis (28), heart muscle (34, 127, 174, 181, 218), aorta and the inferior vena cava (34). There is COMT in erythrocytes from mammals (20, 27, 191, 301, 342) and from the chicken (215) but not in plasma (27). The activity in erythrocytes is low (107, 191) although very considerable activity has been reported (300). It is suggested, however, that the high values obtained were due to insufficient identification of the incubation product (191). The enzyme is also found in normal and chronic melanocytic human leucocytes although the activity did not differ in the two types (46). In the dog kidney there is a significantly greater activity in the medulla than in the cortex (323). Axelrod and Tomchick (34) in 1958 found no activity in skeletal muscle although later work demonstrated catechol-O-methylation in rat, mouse (16), and rabbit (402) skeletal muscle, and very low activity was found in the retractor penis muscle of the bull (265).

The enzyme activity in the skin of mammals is associated with the epidermis (52) and hair bulbs but not the shaft, and the activity is the same in the depigmented skin of patients with vitiligo and pigmented normal skin (53). In patients with psoriasis the activity of COMT is much greater in involved areas of skin than in uninvolved areas, which may be related to the observed local vasodilatation (54).

There is COMT activity in the pineal glands of birds (30) and rat (38) and in the latter there is a 24-hour rhythm in activity; the activity is greatest during the light period, lowest in the dark, and the rhythm is abolished when rats are kept in continuous light.

2. Central nervous system. In the monkey brain highest COMT activity is found in the neurohypophysis with the pons, corpus striatum, occipital cortex, hippocampus and hypothalamus having activ-

TABLE 2
Tissue distribution of COMT in mammals

Tissue	Species	COMT value	Reference
Liver	Rat[a]	5.0 μmol metanephrine/g/hr	34
		22.0 μmol normetanephrine/g/hr	127
		2.8 μmol normetanephrine/g/hr	393
Liver	Cow	3.3 μmol metanephrine/g/hr	34
Liver	Pig	2.9 μmol metanephrine/g/hr	34
Liver	Mouse[a]	2.3 μmol metanephrine/g/hr	34
		1.5 μmol normetanephrine/g/hr	393
Liver	Guinea pig	1.0 μmol metanephrine/g/hr	34
Liver	Man	0.4 μmol metanephrine/g/hr	34
Liver	Cat	0.3 μmol metanephrine/g/hr	34
Liver	Rabbit[a]	0.1 μmol metanephrine/g/hr	34
Liver	Monkey	8.6 μmol metanephrine/g/hr	26
Liver	Rat	7.0 μmol metanephrine/g/hr	34
Liver	Man (stillborn)	3.0 μmol metanephrine/g/hr	2
Liver	Man 1-12 days	23.0 μmol metanephrine/g/hr	2
Liver	Man 1-9 months	38.0 μmol metanephrine/g/hr	2
Liver	Man 1-6 years	58.0 μmol metanephrine/g/hr	2
Liver	Man 20-50 years	233.0 μmol metanephrine/g/hr	2
Liver	Man over 60 years	71.0 μmol metanephrine/g/hr	2
Aorta	Monkey	0.6 μmol metanephrine/g/hr	26
Aorta (adventitia)	Rabbit	0.31 nmol normetanephrine/g/hr	420
Aorta (tunica media)	Rabbit	0.69 nmol normetanephrine/g/hr	420
Blood	Rat	Present	165
Erythrocytes	Rat	5-8 nmol normetanephrine/ml/hr	27
Erythrocytes (reticulocytes)	Rat	Present	342
Erythrocytes (soluble fraction)	Rat	Present	20
Erythrocytes (ghost membrane)	Rat	Present	20
Erythrocytes	Cat	22 nmol normetanephrine/ml/hr	27
Erythrocytes	Dog	2.0-6.8 nmol normetanephrine/ml/hr	27
Erythrocytes	Guinea pig	9.0 nmol normetanephrine/ml/hr	27
Erythrocytes	Man	1.0-10.0 nmol normetanephrine/ml/hr	27
Erythrocytes	Man	Present	301
Plasma	Man	Absent	27, 189
Leucocytes	Man	Present	46
Fibroblasts (cultured)	Guinea pig	1.16-2.94 nmol/mg protein/hr (3,4-dihydroxybenzoic acid substrate)	235
Adipose tissue	Rat	0.08 μmol normetanephrine/g/hr	393
Adipose tissue	Rat	0.11 μmol normetanephrine/g/hr	215
Adipose tissue	Mouse	0.03 μmol normetanephrine/g/hr	393
Adipose tissue	Guinea pig	Absent	393
Kidney	Rat[b]	2.1 μmol metanephrine/g/hr	34
Kidney	Bull	0.12 μmol metanephrine/g/hr	265
Kidney (cortex)	Dog	1.13 nmol metanephrine/mg protein/hr	323
Kidney (medulla)	Dog	2.22 nmol metanephrine/mg protein/hr	323
Heart	Rat	0.2 μmol normetanephrine/g/hr	127
Heart	Rat	0.1 μmol metanephrine/g/hr	34

[a] See also refs. 28, 276, 341.
[b] See also ref. 341.
[c] See also ref. 341.

TABLE 2—*Continued*

Tissue	Species	COMT value	Reference
Heart	Rat	"low activity"	181
Heart	Human (biopsy)	"low activity"	181
Heart	Cat	"high activity"	181
Heart	Rat	0.01 μmol normetanephrine/g/hr	393
Heart	Mouse	0.04 μmol normetanephrine/g/hr	393
Heart	Guinea pig	Absent	393
Heart	Mouse	0.015 μmol/mg protein/hr (3,4-dihydroxybenzoic acid substrate)	238
Heart (right atrium)	Human (foetal)	0.83 μmol metanephrine/mg protein/hr	170
Heart (left atrium)	Human (foetal)	7.73 μmol metanephrine/mg protein/hr	170
Heart (ventricles)	Human (foetal)	8.21–9.71 μmol metanephrine/mg protein/hr	170
Isolated adipocytes	Rat	0.3 μmol normetanephrine/g/hr	404
Skeletal muscle	Rat	Absent	34
Skeletal muscle	Rat, mouse	Present	16
Retractor penis muscle	Bull	Absent	265
Extraocular muscle	Rabbit	3.8 nmol normetanephrine/g/hr	425
Nictitating membrane			
a) Inferior muscle	Cat	Present	238
b) Medial muscle	Cat	Present	238
Retina-choroid	Rabbit	13.1 nmol normetanephrine/g/hr	425
Iris-ciliary body	Rabbit	12.0 nmol normetanephrine/g/hr	425
Iris	Rabbit	1.16 nmol/mg protein/hr (3,4-dihydroxybenzoic acid substrate)	238
Submaxillary gland	Monkey	4.2 mμmol metanephrine/g/hr	26
Submaxillary gland	Mouse	18.9 nmol/mg protein/hr (3,4-dihydroxybenzoic acid substrate)	238
Submaxillary gland	Rabbit	0.06 nmol/mg protein/hr (3,4-dihydroxybenzoic acid substrate)	238
Submaxillary gland	Rat	0.33 μmol homovanillic acid/g/hr	304
Adrenal gland	Rabbit	Absent (oestradiol substrate)	28
Adrenal gland	Rat	6.3–9.2 μmol metanephrine/g/hr	361
Thyroid gland	Monkey	0.6 μmol metanephrine/g/hr	26
Pancreas	Monkey	3.5 μmol metanephrine/g/hr	26
Small intestine	Rat	0.3 μmol metanephrine/g/hr	34
Small intestine	Rabbit	6.7 nmol/g/hr (oestradiol substrate)	28
Spleen	Rat	0.5 μmol metanephrine/g/hr	34
Spleen	Monkey	0.4 μmol metanephrine/g/hr	26
Spleen	Mouse	18.9 nmol/mg protein/hr (3,4-dihydroxybenzoic acid substrate)	238
Spleen	Rabbit	1.3 nmol/g/hr (oestradiol substrate)	28
Vas deferens	Rat	27.2 nmol/mg protein/hr (3,4-dihydroxybenzoic acid substrate)	239
Vas deferens	Rabbit	5.5 nmol/mg protein/hr	239
Vas deferens	Guinea pig	3.4 nmol/mg protein/hr	239

TABLE 2—*Continued*

Tissue	Species	COMT value	Reference
Testes	Rabbit	3.3 nmol/g/hr (oestradiol substrate)	28
Uterus	Rat (pregnant)	12.8 μmol metanephrine/g/hr	436
Uterus	Rat (postpartum)	10.0 μmol metanephrine/g/hr	436
Uterus	Rat (control)	5.2 μmol metanephrine/g/hr	436
Uterus	Rat (oestrus)	90 nmol metanephrine/uterus/hr	174
Uterus	Rat (dioestrus)	56 nmol metanephrine/uterus/hr	174
Uterus	Rat (oestrus)	0.58 μmol metanephrine/g/hr	361
Uterus	Rat (dioestrus)	3.60 μmol metanephrine/g/hr	361
Ovaries	Rat (oestrus)	6.0 μmol metanephrine/g/hr	361
Ovaries	Rat (dioestrus)	8.58 μmol metanephrine/g/hr	361
Lung	Rat	0.3 μmol metanephrine/g/hr	34
Lung	Rabbit	Absent (oestradiol substrate)	28
Skin	Rat	Present	52, 53, 198
Skin	Mouse	Present	52, 53, 198
Skin	Rabbit	Present	52, 53, 198
Skin	Man	Present	52, 53, 198
Hair bulb	Man	Present	53
Hair shaft	Man	Absent	53
Peripheral nerves	Monkeys	0.66–0.31 μmol metanephrine/g/hr	26
Optic nerve	Rabbit	12.3 nmol normetanephrine/g/hr	425
Pineal gland	Monkey	Very low activity	32
Pineal gland	Rat	0.03–0.04 nmol normetanephrine/gland/hr	38
Pineal gland	Man	0.19–0.23 μmol/g/hr (3,4-dihydroxybenzoic acid substrate)	421
Pituitary			
a) Adenohypophysis	Monkey	2.1 μmol metanephrine/g/hr	26
b) Neurohypophysis	Monkey	0.97 μmol metanephrine/g/hr	26
Whole brain	Rat[c]	0.2 μmol metanephrine/g/hr	34
Whole brain	Rat[c]	0.14 μmol normetanephrine/g/hr	127
Astrocytoma cells (cultured)	Rat/hamster	Present	382

ities very similar to one another (26). A postmortem study of COMT in various regions of human brain revealed no striking regional variations in the activity of the enzyme (table 3) and no marked variations in the values obtained in the four subjects who had suffered from different mental illnesses (421). In the rat brain a specific regional distribution of the enzyme is also absent and there is no correlation between the distribution of catecholamines and COMT as there is with tyrosine hydroxylase and dopa decarboxylase (83).

D. Cellular Distribution

1. Peripheral system. COMT is an intracellular enzyme, *i.e.* there is no activity in blood plasma but there is in erythrocytes (27) and cultured fibroblasts (235).

For many years COMT was considered as exclusively extraneuronal (24, 91, 271). This belief was supported by studies with surgically sympathetically denervated (238, 323, 340, 425) or immunosympathectomized rat and mouse tissues (226) in which there was no change in the COMT activity after denervation. Moreover, Jonason (242, 243) in studies of the *in vitro* metabolism of catecholamines in the normal, atrophied, and sympathectomized rat salivary gland found that O-methylation was drastically reduced in the atrophied gland. He concluded that COMT was localized in the extraneuronal parenchymal

TABLE 3

Regional distribution of COMT in mammalian CNS

Area	Rat[a,b]	Monkey[c]	Man[d,e]
	μmol HVA/g/hr	*μmol metanephrine/g/hr*	*μmol 3-methoxy-4-hydroxy-benzoic acid/g/hr*
Frontal cortex	0.099		0.24–0.26
Occipital cortex	0.095	0.49	
Precentral cortex		0.39	0.22–0.24
Postcentral cortex			0.14–0.23
Bulbus olfactorius	0.088		
Thalamus	0.094		0.18–0.27
Hypothalamus	0.128[f]	0.49	0.18–0.22
Hippocampus	0.086	0.31[g] 0.47[h]	
Amygdala		0.25	
Caudate nucleus		0.52	0.15–0.23
Putamen		0.55	0.21–0.36
Globus pallidus			0.27–0.61
Corpus striatum	0.075		
Substantia nigra			0.18–0.25
Ventral midbrain		0.55	
Medulla oblongata	0.102[i]	0.50	
Pons		0.53	0.12–0.21
Cerebellar cortex	0.108	0.23	0.18–0.21
Corpora quadrigemina	0.116		
Corpus callosum	0.072	0.55	

[a] Ref. 83.
[b] See also ref. 361.
[c] Ref. 26.
[d] Ref. 421.
[e] Range of four values given.
[f] See also ref. 413.
[g] Ventral hippocampus.
[h] Dorsal hippocampus.
[i] Includes pons.

cells which showed marked atrophy after ligation of the excretory duct while the adrenergic nerve terminals remain unaffected (5, 304). However, Crout and Cooper (126) showed a fall in COMT activity in the denervated cat heart and reports in 1971 indicate that COMT activity is decreased in denervated smooth muscle of the cat nictitating membrane (240), vas deferens of the rat and rabbit but not guinea pig (239), and the rat (304) and rabbit submaxillary gland (238). It is often assumed that when the activity of an enzyme is reduced after sympathetic denervation, the proportional reduction of the total activity represents the neuronal localization of the enzyme. Thus the reduction in COMT activity after sympathetic denervation may indicate that COMT is both intra- and extraneuronally located. An alternative suggestion is that while COMT has an extraneuronal localization, the activity of the enzyme is partially dependent on an intact nerve supply and the availability of noradrenaline as a substrate (304). In this respect the fall in COMT activity after administration of reserpine and α-methyl-p-tyrosine may be due to a reduction in available substrate for COMT (304). Jarrott and Iversen (239) point out that postsynaptic changes take place slowly and that these cannot explain the rapid fall in COMT activity which occurs after denervation of the rat vas deferens. In 1974, Broch (81) obtained some evidence for the occurrence of two forms of COMT in rat salivary gland, one form being intraneuronal and susceptible

to sympathectomy and another being extraneuronal. Reserpine, however, caused a similar reduction in both enzymes so the result gives no direct evidence for the theory of substrate dependence of COMT. α-Methyl-*p*-tyrosine, which depletes both neuronal and circulating catecholamines, produces a greater loss in COMT activity than sympathetic denervation which only depletes neuronal catecholamines (304). COMT is involved in the inactivation of circulating catecholamines so if the activity of the enzyme reflects availability of substrate one would predict a greater fall in activity after reserpine or after α-methyl-*p*-tyrosine than after sympathectomy. It would be interesting to see what effect tyramine has on peripheral COMT activity since catecholamines released by tyramine are principally metabolized by O-methylation (271) rather than by deamination as with reserpine (366).

There is no convincing evidence in the literature that COMT belongs to the type of enzymes showing the characteristics of induction and/or suppression, though long-term administration of pyrogallol led to elevated COMT activity in rat liver (328). It has been reported that in the hypophysectomized rat the hepatic, but not the cardiac, COMT activity rose slightly (281). In 1973, it was reported that glucocorticoids affected COMT activities both *in vivo* and *in vitro* and that there was roughly an inverse proportionality between COMT activities and the amount of glucocorticoids present in the body. The results were discussed in the light of the regulatory factors which affect the rate of synthesis as well as breakdown of enzyme protein (337, 338).

2. *Central nervous system*. McCaman and Dewhurst (296) dissected out individual neuronal and non-neuronal cells from the squid *Aplysia californica* and found similar COMT activity in both types of cells (table 1) though the ubiquitous distribution may be due to contamination by glial cells. COMT activity in the white, primarily non-nervous, matter of the corpus callosum and the lateral wall of the lateral ventricles is identical with that in the corpus striatum and similar to the activity in the hypothalamus. This is indicative of a nonspecific localization of the enzyme in the brain (83). Furthermore, neither MAO nor COMT activities are significantly changed by lesions in the substantia nigra which destroy the dopaminergic pathways to the striatum (305). The intraventricular injection of 6-hydroxydopamine into the brain reduced dopa decarboxylase activity in the striatum and hypothalamus but produced no change in the COMT activity (413).

The limiting membranes of nerve endings are disintegrated by the addition of 0.1% Triton X-100 to brain homogenates thus destroying the synaptic structure and releasing cytoplasm trapped within the synaptosomes. The structural components, synaptic vesicles and mitochondria of the synapse are left essentially intact (113). When rat brain is homogenized in the presence of 0.5% Triton the COMT activity measured is increased by 100% (83, 305) while similar treatment to salivary gland tissue had no effect. This suggests that an occluded form of the enzyme present in brain is either absent or accounts for only a very small part of the total COMT activity in the salivary gland. In brain the occluded form is associated with the cytoplasm of nerve endings since the COMT activity in the crude mitochondrial fraction of brain homogenates is released by Triton (83).

A useful addition to the study of the cellular localization of COMT would be the development of a histochemical method for the enzyme. This could either be based on a colourimetric COMT assay (9) or the use of a COMT antibody (18) to develop an immunohistochemical technique. A major problem, however, is the cytoplasmic nature of the enzyme. Information is needed about the effects of various fixatives on the activity of the enzyme, as fixation would appear to be a prerequisite for the successful histochemical localization of COMT. Another approach for studying the cellular

localization of COMT in the brain may be the use of brain fractions enriched with either intact neurons or glia (206). In 1972, COMT and MAO were reported to be present in rat and hamster neoplastic astrocytes grown in culture (382), thus giving direct evidence for the occurrence of the enzyme in glial cells.

E. Subcellular Distribution

1. Peripheral system. COMT was found only in the 78,000 × *g* supernatant fraction of rat liver homogenates by Axelrod and Tomchick (34) who termed the enzyme soluble. Similar results are obtained with human liver (50) and rat salivary gland (304). The term "soluble" might be misleading and perhaps a more accurate description is "cytoplasmic." The fact that COMT is found only in the supernatant does not, however, explain the enzyme's precise localization within the cell or whether it is free in the cytoplasm or loosely bound to membranes and easily released during homogenization. Only a small fraction of COMT appears to be firmly bound to membranes (71).

In the liver and kidney of the spiny dogfish, in contrast to mammalian liver, there is more COMT activity in the microsomal fraction than in the 9,000 × *g* supernatant and the enzyme is not Mg^{++} dependent. As microsomal enzymes are considered fairly primitive the microsomal COMT may represent a phylogenetically primitive stage of the enzyme (75). In the snail *Helix aspersa*, however, COMT is found primarily in the supernatant with none in the microsomal fraction (303) but it can be argued that in terms of invertebrate evolution the snail cannot be considered phylogenetically primitive. There is considerable COMT activity in the microsomal fraction of rabbit thoracic aorta though most activity is found in the supernatant (420). Since the microsomal fraction in the aorta consists of endoplasmic reticulum, plasmalemma, and micropinocytic vesicles, COMT in this fraction could be associated with any of these membranes (419). The microsomal fraction, however, under these conditions contained considerable MAO activity implying the presence of small mitochondria or broken mitochondrial membranes. Axelrod and Cohn (27) found the COMT in red blood cells in the supernatant fraction, while Assicot and Bohuon (20) found two distinct O-methylating activities associated with rat erythrocytes, one associated with the supernatant fraction and the other with erythrocyte ghosts and so membrane bound. The membrane bound activity was released by Triton X-100 but differed from the activity found in the supernatant with respect to heat stability, pH optimum, K_m value and immunochemical reactivity. It also had a 70 times greater affinity for catecholamine substrates. The membrane fraction is increased remarkably in reticulocyte-rich blood while the cytoplasmic activity was only insignificantly increased (342). The COMT activity in adipocyte cells (404) is also mainly associated with particulate material and in fat cell "ghosts" the major part of the activity occurs in the "ghosts" rather than the supernatant. These results may give some indication of the intracellular locus of COMT and it would be interesting to know whether COMT in other cell "ghosts" remains membrane bound after gentle rupture of the cell membranes.

In 1973 a study was reported of the axonal transport of COMT and other enzymes involved in the synthesis and metabolism of catecholamines; tyrosine hydroxylase and dopamine-β-hydroxylase both showed fast transport rates while MAO and COMT were slowly transported, and DOPA decarboxylase had an intermediate rate. The transport of all the enzymes was inhibited by vinblastine and colchicine (435). The results suggest the transport and inactivation of fast and slow moving enzymes are under different controls, and that axonal transport characteristics may be a more physiological approach to a clarification of the intracellular localization of these enzymes. The rate of transport

does not appear to be related to "apparent" subcellular distribution since cytoplasmic tyrosine hydroxylase is transported rapidly but "cytoplasmic" COMT is not.

2. Central nervous system. Of the COMT activity of rat brain 58.7% was found in the crude mitochondrial fraction (3) indicating that part of the activity was particulate and associated with synaptic components. Further separation of the mitochondrial fraction showed that the COMT was associated with nerve endings and not free mitochondria. Osmotic shock released more than 60% of the COMT in the crude mitochondrial fraction while MAO remained associated with mitochondria. The particulate fraction of brain COMT is associated with the synaptosomal subfraction of the crude mitochondrial fraction. Resuspension of the crude mitochondrial fraction in distilled water followed by separation of the subfractions in a sucrose gradient demonstrated the release of COMT and no significant activity in the membraneous, vesicular, or mitochondrial fractions (83). This confirms the cytoplasmic nature of the enzyme, but does not indicate whether it is loosely membrane-bound as is suggested for peripheral tissues.

F. Relationship between COMT and Age

1. Chick. In the chick embryo heart COMT is apparent from the 4th day of incubation. The activities of MAO and COMT are at a maximum on the 19th day of incubation but decline to minimal values by the 2nd or 3rd day after hatching. There is a rapid increase in the activities of the enzymes during the first half of incubation, a decline in the second half of incubation and then a gradual rise in the concentration of COMT, and a rapid rise in the concentration of MAO after hatching (218). The activities of the enzymes were expressed in relation to unit weight of protein. Since the protein concentration remained at a steady level during incubation, with a gradual increase during the immediate posthatching period, the variations in the activities of MAO and COMT cannot be explained in terms of changes in the protein concentration. Dopa and dopamine undergo similar fluctuations in the embryonic and newly hatched chick heart, which suggests a possible relationship between the two parameters (219).

2. Rat. There is a linear increase in the weight of the rat brain during the first two weeks after birth together with a 2-fold increase in the protein concentration (116). The rise in protein is restricted to the precipitate fractions, especially the mitochondrial fraction, with no significant change in the supernatant. The total COMT and lactate dehydrogenase activities of brain increase in the three weeks after birth when related to brain weight but not when related to protein concentration (79). The activity (about 33%) in the crude mitochondrial fraction (83) is, however, related to the protein concentration and probably reflects the development of nerve endings (79).

Other workers found no increase in brain COMT in 2- to 9-week-old rats, some increase in liver COMT during the 9 to 35-week period, but no further change after 35 weeks (341).

3. Human. MAO but not COMT has been found in the paraganglia of the mid-term human foetus (171) though there is activity in the liver of the stillborn human foetus. There is also a 10-fold increase in activity from birth to adulthood (20–50 years) and the greatest increase occurs from 6 to 20 years with a decline in the group over 60 years of age (2). COMT from human neonatal liver has a pH optimum of 7.1 whereas the pH optimum for liver from adults is 7.7 (2). The latter influences the values obtained at the different age groups as all determinations were made at pH 7.8. For liver from the newborn human infant the values are 15 to 20% below those that would be obtained with the pH optimum for that age. This cannot account for the large increase in activity between birth and the sixth decade, but such a consideration

would reduce it from a 10-fold to an 8-fold increase. In human foetal heart COMT is distributed uniformly in the left atrium, the ventricles, and intraventricular septum but the activity in the right atrium is very low. On average the COMT activity is only 1/10 to 1/100 that of MAO (170). These authors point out that the low COMT is in the area containing the cardiac pacemaker and may be related to a non-neuronal adrenergic regulation of the foetal heart rate *via* the sinus node. If such a regulation occurs it means the non-neuronal amines reach the receptors at the sinus node, *via* the circulation, at far lower concentrations than the noradrenaline released from adrenergic nerves. The concentration of the amine at the receptors certainly would be enhanced by low COMT activity since this enzyme is responsible to a large extent for the enzymatic inactivation of circulating catecholamines.

G. Uterine COMT

During pregnancy there is a 2-fold increase in the COMT activity in the rat uterus but none in the heart (436). The activity in the nonpregnant uterus is very low compared with the liver and heart (table 2). The MAO activity is much greater than COMT activity but shows no increase during pregnancy. Uterine adrenaline is found mainly in the supernatant whereas adrenaline in the heart is microsomal. Oestrogen treatment increases the accumulation of ^{3}H-adrenaline in the uterus but not the heart (436).

The relation between uterine adrenaline and COMT activity has been further studied by measuring COMT activity at different stages of the rat sexual cycle (174, 361). The adrenaline concentration of the uterus is elevated during oestrus (358) and there is a 2-fold increase in metanephrine formation in uterine slices taken from rats during oestrus as compared with dioestrus (188). COMT activity has been reported as greater in the uterus during oestrus than dioestrus (174), and greater during dioestrus than oestrus (361). The latter result has been related to changes in catecholamine levels during the oestrus cycle. The conflicting results cannot be explained by differences in method or manner of expressing the velocity of metanephrine formation. Immunosympathectomy reduces uterine noradrenaline, but not adrenaline, and inhibition of COMT with U-0521 raises the concentration of uterine adrenaline but neither the concentration of uterine noradrenaline nor cardiac adrenaline (174). These results indicate that COMT activity and adrenaline concentration show cyclical changes in the uterus which are probably related, but this requires further study.

VI. COMT Inhibitors and Their Pharmacological Properties

A. Pyrogallol and Derivatives (Fig. 1)

1. Pyrogallol. Pyrogallol (1,2,3-trihydroxybenzene) was reported several years ago to inhibit COMT (29, 41, 127). Pyrogallol and related compounds are substrates for COMT and are methylated to form 1,3-dihydroxy-2-methoxybenzene (14, 65, 130). Although pyrogallol probably acts mainly as a competitive substrate, a noncompetitive component of its action has been demonstrated *in vitro* (123). The degree of noncompetitive inhibition was greatest in the most purified enzyme preparations, and preincubation of COMT with pyrogallol increased the noncompetitive component of the inhibition. Pyrogallol is a potent inhibitor of COMT and under *in vitro* conditions the K_i value has been calculated to be as low as 8×10^{-6} M (123) although the results from other investigations have indicated somewhat higher values, 2.6×10^{-5} M (48) and 1.3×10^{-5} M (table 4).

Pyrogallol shares with COMT inhibitors as a whole a number of limiting factors for its pharmacological usefulness. The most important of these are its relatively high toxicity and its low potency *in vivo* which is linked to its *pharmacokinetic properties*. Although pyrogallol is an excellent substrate for COMT (34), its inhibitory effects *in vivo* are of short duration, and effective

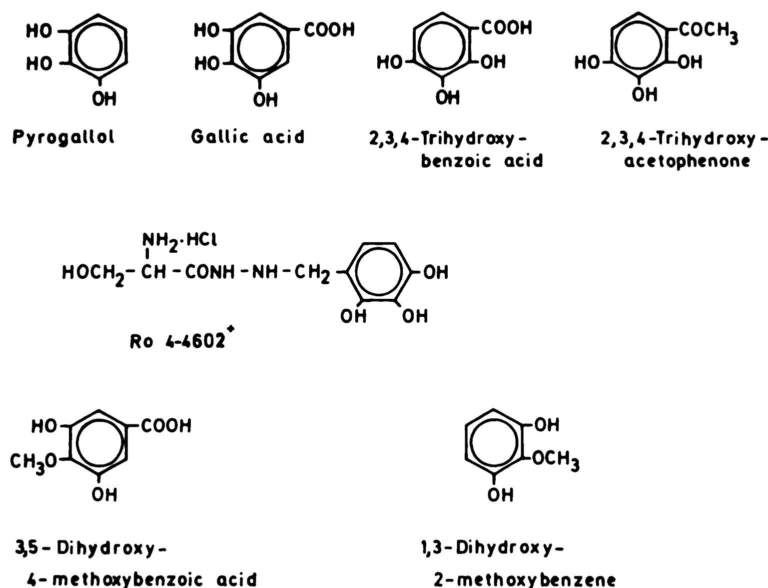

FIG. 1. COMT inhibitors of the group: pyrogallol and derivatives.
The abbreviation used is: ⁺RO4-4602, N^1-(D,L-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine hydrochloride.

TABLE 4
Inhibitory constants (K_i-values) for some inhibitors of COMT[a]

Inhibitor	K_i-value	Inhibitor	K_i-value
	M		*M*
Pyrogallol	1.3×10^{-5}	L-Dopa	9.8×10^{-4}
Gallic acid	7.0×10^{-5}	Tetrahydropapaveroline	5.5×10^{-5}
Phloroglucinol	$>10^{-2}$	Apomorphine	7.0×10^{-4}
Rutin	3.0×10^{-5}	Isoprenaline	1.5×10^{-4}
Quercetin	8.4×10^{-6}	Tropolone	2.7×10^{-5}
Catechin	3.0×10^{-5}	L-Ascorbic acid	1.4×10^{-2}

[a] Crude extracts (homogenates in isotonic KCl) of rat liver were assayed for COMT activity (120) and the compounds tested for their ability to prevent the formation of ^{3}H-normetanephrine from ^{3}H-noradrenaline (concentration range: 10^{-5} M-10^{-4} M), in the presence of S-AMe (0.5×10^{-3} M) and Mg Cl_2 (2×10^{-3} M) and various concentrations of inhibitor. The ^{3}H-normetanephrine produced was estracted into toluene:isoamyl alcohol (3:2, by volume) and counted by scintillation spectrometry. The K_i values were determined by plotting (concentration range: 10^{-5} M-10^{-4} M), in the presence of S-AMe (0.5×10^{-3} M) and $MgCl_2$ (2×10^{-3} M) and various concentrations of inhibitor. The ^{3}H-normetanephrine produced was extracted into toluene:isoamyl alcohol (3:2, by volume) and counted by scintillation spectrometry. The K_i values were determined by plotting l/v against inhibitor concentration at different substrate concentrations.

inhibition of the enzyme could be achieved for only 30 to 60 minutes with a single, high dose of 200 mg/kg. A repeated dose of 50 mg/kg every 30 minutes was required to maintain more prolonged enzyme inhibition (127). The drug is distributed widely in the body. An early report suggested that pyrogallol may not be able to cross the blood-brain barrier (309) but after systemic administration it was shown to have inhibited COMT in the mouse brain (355) and after an intraperitoneal injection it readily entered the brain of mice (348).

EFFECTS ON METABOLISM OF CATECHOLAMINES. Brain catecholamines are not elevated in rats after repeated administration of pyrogallol, 50 mg/kg intraperitoneally every 30 minutes for 18 hours (127), nor after a single injection (348, 427) or even after daily administration for several weeks

(298). Pyrogallol treatment (12 mg/kg or 50 mg/kg every 30 minutes), rather than causing an increase in brain noradrenaline, produced a decrease which was significant at both dose levels (127). It may seem contradictory that pyrogallol (10 mg/kg) increased catecholamines in various regions of mouse and rat brains within 20 to 40 minutes after an intraperitoneal injection (231) and in rabbit brain within 1 hour after intracisternal injection (309). An explanation for the transient rise followed by a fall in brain catecholamines after pyrogallol may be that the initial inhibition of COMT leads to a feedback inhibition of catecholamine synthesis.

The simultaneous administration of other drugs affecting brain catecholamines may elucidate the action of pyrogallol. Although pyrogallol had no effect on the concentrations of noradrenaline and dopamine in the brain and did not inhibit the effect of reserpine, it potentiated the inhibition of the reserpine effect by the monoamine oxidase inhibitor β-phenylisopropylhydrazine (Catron) (427). Here the effects are not seen until the alternative metabolic pathways are both blocked by respective enzyme inhibitors. Similarly, after pretreatment of animals with β-phenylisopropylhydrazine and pyrogallol, administration of DOPA caused a greater increase in brain noradrenaline concentration than with the MAO inhibitor alone (427).

Merely measuring steady-state concentrations of brain catecholamines may not give sufficient data on the effect of pyrogallol on brain catecholamines. Determinations of cerebral metabolites have shown that the inhibitor has a profound effect on catecholamine metabolism. Rats given pyrogallol showed a marked increase in the retention of ^{3}H-L-dopa and accumulation of labelled catecholamines and deaminated catechols, but a decrease in O-methylated metabolites in the brain (47). Administration of pyrogallol (3–15 mg/kg) and ^{14}C-noradrenaline into the lateral ventricle of the rat led to a considerable fall in ^{14}C-3-methoxy-4-hydroxyphenylglycol sulphate formation (397).

In the periphery, pyrogallol, after long-term administration, had no effect on catecholamines in adrenals and heart (298) but short-term administration elevated the concentration of ^{3}H-noradrenaline and lowered that of ^{3}H-normetanephrine in several tissues (211). In the rat heart, pyrogallol prevented the extraneuronal formation of O-methylated derivatives and elevated the concentration of noradrenaline (146). Field stimulation of the cat isolated nictitating membrane in the presence of pyrogallol and ^{3}H-noradrenaline caused a marked fall in the O-methylated metabolites and an increase in deaminated metabolites (282). These data demonstrate the effect of pyrogallol on the levels of endogenous catecholamines in some tissues, and its peripheral effects cannot be confined merely to those on exogenous catecholamines (269).

PHARMACOLOGICAL EFFECTS. The vasopressor response of adrenaline is prolonged by pyrogallol (dose range 10–40 mg/kg) in the cat (39) and the spinal cat (441); the latter investigations also showed that pyrogallol is less active in prolonging the effect of noradrenaline and that the COMT inhibitor caused some small pressor effects when given alone. The pressor effects of both adrenaline and noradrenaline on the blood pressure of dogs are potentiated and prolonged (220, 232) by pyrogallol (20 mg/kg) but when given alone in doses of 1 to 10 mg/kg intravenously the inhibitor does not affect blood pressure (220). Pyrogallol (40–50 mg/kg i.v.) in the rat enhanced and/or prolonged the blood pressure response not only to the catecholamines but also to tyramine (416). Similarly, the pressor action of guanethidine, by the release of noradrenaline from nerve endings, was prolonged after the administration of pyrogallol (440). After cocaine the potentiating effect of pyrogallol on the adrenaline-induced pressor effect of spinal rats and cats was not further enhanced (287).

These results tend to support the original suggestion (41) that the inhibition of COMT by pyrogallol is probably responsible for the potentiation of the effects of catecholamines on smooth muscles. However, some doubt may be expressed as to the specificity of action of pyrogallol on COMT in mediating the vasopressor effect since the doses of pyrogallol used in most experiments did not correspond to the doses which give effective *in vivo* inhibition (124). On the other hand, this might not be a serious objection since evidence for the *in vivo* inhibition of COMT by pyrogallol was obtained primarily by *in vitro* assay techniques (127) and, as has been pointed out (325), some observations suggest that the inhibition of COMT inhibitors is perhaps more effective *in vivo* than might be indicated by *in vitro* assays.

It has been suggested that COMT contributes to the removal of catecholamines in the circulation but not from the receptor site (127). Evidence for a role of COMT in the metabolism of catecholamines at receptor sites has been obtained with the aortic strip preparation (248, 255–258) (for details see section VII). Inhibitors of COMT, including pyrogallol, potentiated the responses of aortic strips to adrenaline much more than to noradrenaline (248). The COMT inhibitors abolished the enhancing effects of hydrocortisone on responses of rabbit aortic strips to catecholamines (248). Pyrogallol was shown to increase the response of the lateral saphenous vein of the dog to electrical stimulation of the lumbar sympathetic chain, but did not influence the vasoconstrictor effect of local cooling (415).

The inhibition of COMT by pyrogallol (37.5 mg/kg i.v. times two, 15 minutes apart) produced moderate prolongation of the cardiac effects of noradrenaline injected into anaesthetized dogs (124). The duration of the isoprenaline-induced tachycardia in mice was prolonged in the presence of pyrogallol (100 mg/kg i.p.) while the effects of adrenaline and noradrenaline were prolonged only slightly (352, 353). Other workers have claimed that pyrogallol significantly enhances the duration of tachycardia induced by adrenaline and noradrenaline both in the intact animal (232) and in isolated rabbit and guinea pig hearts (229, 246). This suggests that the effects were mediated through the result of a reduced rate of metabolism of endogenous catecholamines caused by the inhibition of cardiac COMT. However, it is more likely that the potentiating effect of pyrogallol on the positive inotropic and chronotropic response of isolated rabbit atria is caused by exogenous, and not endogenous catecholamines (220).

The effect of adrenaline on urinary bladder contractions and the rhythmic contraction of the isolated guinea pig ureter are potentiated by pyrogallol. Pyrogallol in concentrations above 10^{-7} M decreased the rhythmic contraction of the isolated rat uterus, while the isolated rat intestine responded to pyrogallol above 10^{-6} M by relaxation, decreased amplitude of contraction and potentiation of the inhibitory action of adrenaline (220). Similarly, pyrogallol exerted an initial depressor effect on the dog duodenum *in situ* and also enhanced the duration of the inhibitory action of adrenaline on duodenal motility (230). Finally, the effect of adrenergic nerve stimulation on the contractile response of the cat nictitating membrane *in vivo* was prolonged by pyrogallol (39, 441).

However, doubt concerning the specificity of action of COMT inhibition by pyrogallol on smooth muscle must be raised since phenol-like compounds, including pyrogallol, possess a general spasmolytic property similar to papaverine on smooth muscle (241), and a local anaesthetic property common to phenols has not been ruled out as part of the mechanism of action of pyrogallol.

For selective inhibition of COMT in the brain pyrogallol and some of its derivatives appeared to be the most effective among a number of COMT inhibitors tested (355).

Nevertheless, the central nervous system (CNS) effects of pyrogallol appear to be modest. Pyrogallol (60 mg/kg) did not produce convulsive activity (11) except at higher doses and was a much less active convulsant than catechol. Pyrogallol in very high doses, 200 mg/kg intraperitoneally, produced no motor disturbance or other apparent neurological symptoms (233), though intracisternal injection of the drug (20 mg/kg) in the rabbit resulted in hypotonia, ataxia, and eventually abolition of motor activity (309). In mice there is dose-dependent suppression of exploratory behaviour (314) and in rats establishment of a conditioned reflex is slowed (233).

TOXIC EFFECTS. Some of the toxic effects of pyrogallol may be considered purely as extensions of its pharmacological effects. For instance, pyrogallol augments the toxicity of intravenously administered adrenaline in the rat. The augmented toxicity is adrenergic in nature and is presumably due to inhibition of COMT (441). The effects with noradrenaline were less pronounced (441) indicative of the higher effectiveness of oxidative deamination as an alternative pathway for the inactivation of noradrenaline (255). Other pharmacological effects, which may lead to serious toxic reactions, such as the convulsant property of pyrogallol in very high doses did not appear to be related to a specific effect on COMT (12).

Other mechanisms for a biochemical basis of pyrogallol toxicity may be related to the fact that the drug, as an inhibitor of COMT, is a substrate for the enzyme and, in the large doses required for COMT inhibition *in vivo*, may utilize methyl groups and depress tissue levels of the methyl donor (47). In addition to the enzymatic effect on COMT and coenzyme depletion, pyrogallol may affect other enzyme systems such as has been recently described for a sulphotransferase enzyme (142). The toxic manifestations of such mechanisms of action of pyrogallol are not known.

Marked toxicity to pyrogallol occurs at doses sufficient to approach complete inhibition of COMT activity. Pyrogallol, after its absorption into the blood, may form methaemoglobin, disrupt the blood corpuscles and lead to intense acute nephritis (390). Animals treated by injection with pyrogallol, in doses of up to 50 mg/kg every 30 minutes for periods of up to 18 hours, developed pronounced methaemoglobinaemia, marked lethargy after 8 to 10 hours and finally paralysis of the hind legs (127). Long term administration of pyrogallol (50 mg/kg/day) produced some tissue necrosis at the injection site (298).

The relative high toxicity of pyrogallol makes it quite unsuitable for use in man and the main use of the drug in pharmacology has been as a research tool, in particular, in relation to studies on adrenergic mechanisms.

2. Gallic acid (3,4,5-trihydroxybenzoic acid) undergoes O-methylation to form 4-O-methyl gallic acid which is the major metabolite in the urine of rats or rabbits ingesting this substance (65). Gallic acid is a considerably less potent inhibitor of COMT than pyrogallol with respective K_i values of 7×10^{-5} M and 1.2×10^{-5} M (table 4). Although gallic acid and pyrogallol are both trihydroxybenzene derivatives, they have quite different physicochemical properties which make the former compound much less toxic to cells.

Other trihydroxy derivatives, such as 2,3,4-trihydroxybenzoic acid (65) and 2,-3,4-trihydroxyacetophenone (354) would be expected substrates for COMT but a publication (202) claiming the trihydroxybenzene, phloroglucinol—a nonpyrogallol derivative—to be an inhibitor of COMT, was surprising. We could not confirm these results in that phloroglucinol did not inhibit significantly rat liver COMT *in vitro* (table 4).

GALLIC ACID ESTERS. The main interest in gallic acid as an inhibitor of COMT stems from its potential use as such a drug in man.

Gallic acid occurs naturally in foods and lauryl and propyl gallates have been used for a long time as safe food additives for

their antioxidant properties (286). The LD50 of propyl gallate administered to rats orally was about 3.8 g/kg and when administered intraperitoneally it was about 0.38 g/kg. Death from these doses occurred usually within 1 hour and was due to cardiorespiratory failure (333).

In laboratory studies, it was shown that some of the cardiovascular responses to L-dopa in the dog were augmented by the administration of butyl gallate and it appeared that the peripherally-mediated pressor effect was enhanced more than the hypotensive effect which is a manifestation of the central effect of L-dopa (345).

N-Butyl-gallate (GPA-1714) in a maximal daily dose of 750 mg appeared to have some beneficial effect in patients with parkinsonism and in a small group of patients with Huntington's chorea and spasmodic torticollis (149). Possibly, the most significant finding in this trial was that GPA-1714 given together with a reduced dose of L-dopa, diminished the number of side-effects of L-dopa therapy in patients with parkinsonism. GPA-1714 given to chronic schizophrenic patients in a maximum daily dosage of 5 g (384) and to patients with endogenous depression in a mean daily dose of about 3.5 g (13) had no beneficial therapeutic effects. None of these clinical studies on GPA-1714 reported any serious toxicity or troublesome side-effects to the drug, although closer examinations may reveal serious toxicity.

3. N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine (RO4-4602), a decarboxylase inhibitor, is also an active inhibitor of COMT with an apparent ID50 close to that for pryogallol, of which it is a structural analogue (45). The kinetics of the inhibitory effect appear to involve a mixed competitive and noncompetitive mode of inhibition. The *in vivo* inhibition of COMT by RO4-4602 may be partly responsible for its beneficial effects in patients treated with L-dopa (48).

4. 3,5-Dihydroxy-4-methoxybenzoic acid and related compounds were described recently as a new class of inhibitors of COMT (325), based on the observation that the product of the O-methylation of pyrogallol, 1,3-dihydroxy-2-methoxybenzene, contributes to the inhibition of COMT (123). A series of compounds related to 1,3-dihydroxy-2-methoxybenzene were compared as inhibitors of purified COMT and the results expressed as percentage of inhibition of the O-methylation of 3,4-dihydroxybenzoic acid, obtained at a ratio of inhibitor to substrate of 1:10 (325). No K_i values for various inhibitors were given. Most derivatives of the parent compounds 1,3-dihydroxy-2-methoxy-, 1-hydroxy-2,3-dimethoxy-, 2-hydroxy-1,3-dimethoxy-, and 1,3-dihydroxy-4-methoxybenzene strongly inhibited the enzymatic reaction. Lineweaver-Burk plots of kinetic data were applied to the relation between 3,5-dihydroxy-4-methoxybenzoic acid and the substrates of the reaction. A reversible noncompetitive or mixed type of inhibition was obtained with 3,4-dihydroxybenzoic acid, noradrenaline, and catechol as substrates but no inhibition was obtained with 3,4-dihydroxypropiophenone as substrate. A similar type of inhibition was obtained against S-AMe but a complex relation existed between the magnesium ion concentration, inhibitor concentration, and enzymatic activity.

This study is of greatest interest from the point of view of inhibition of COMT *in vivo* and the potential use of these compounds as pharmacological tools. However, data obtained in 1972 for the inhibitor 3,4-dimethoxy-5-hydroxybenzoic acid suggest that the compound is a weak *in vivo* inhibitor of COMT, particularly in the brain (47).

B. Catechols and Derivatives (Fig. 2)

1. Catechol, protocatechuic acid, caffeic acid, etc. Many catechols and derivatives have been reported to be inhibitors of COMT. The parent compound *catechol* is a competitive substrate for COMT (41) and an *in vivo* COMT inhibitor (355). Catechol shares many pharmacological properties with pyrogallol such as potentiating the effect of adrenaline on various smooth

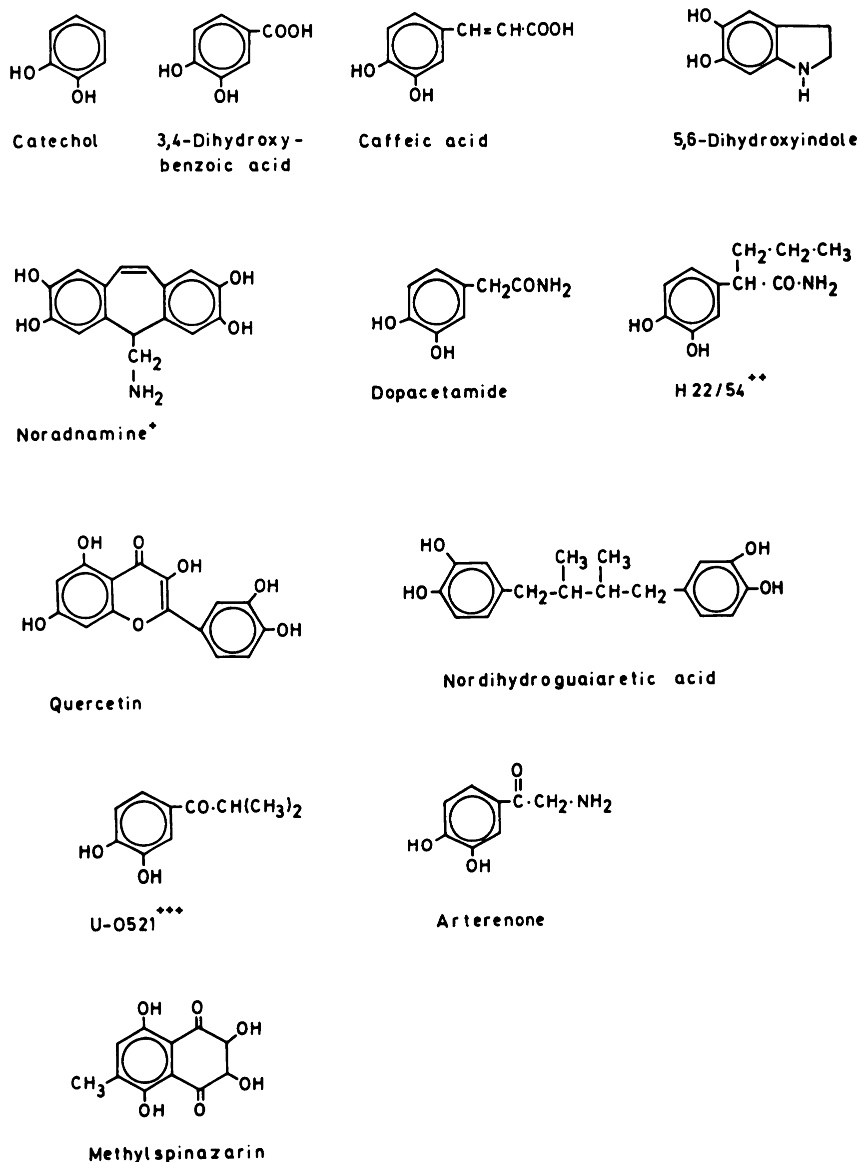

FIG. 2. COMT inhibitors of the group: catechol and derivatives.
The abbreviations used are: +Noradnamine, 5-aminomethyl-2,3,7,8-tetrahydroxydibenzo-(a,e)-cycloheptatriene; ++H22/54; α-propyl-3,4-dihydroxyphenylacetamide; +++U-0521, 3,4-dihydroxy-2-methylpropiophenone; ++++MK486, L-α-methyldopahydrazine (carbidopa).

muscle (41, 441) but some of its actions are not adrenergic (386). A disadvantage of catechol is its high toxicity, the most noteworthy of which is its convulsive property which is unrelated to COMT inhibition (12, 42, 348). Catechol elicited convulsions within 15 to 20 seconds with a peak at 2 to 3 minutes after intraperitoneal injection and this correlated well with its penetration into brain (348).

Incubation of liver COMT or plant COMT with the orthodiphenolic acids *3,4-dihydroxybenzoic acid* (protocatechuic acid) and *3,4-dihydroxycinnamic acid* (caffeic acid) gave rise to the corresponding O-methylated metabolites 3-methoxy-4-

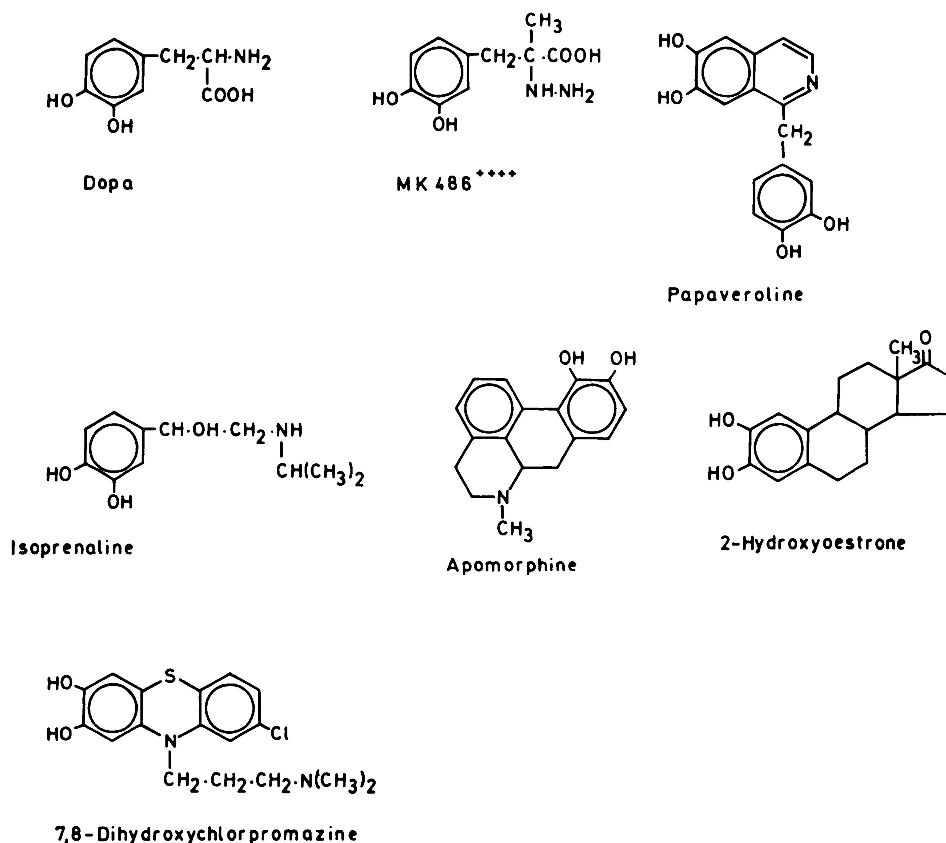

FIG. 2.—*Continued*

hydroxybenzoic acid (vanillic acid) and 3-methoxy-4-hydroxycinnamic acid (ferulic acid) (154, 306). *5,6-Dihydroxyindoles* are both catechols and hydroxyindoles and they can be O-methylated by COMT (31).

Adnamine and *noradnamine*, acid degradation products of adrenaline and noradrenaline, have been shown to be quite potent inhibitors of COMT (1). Under the experimental conditions employed, concentrations of 2.5×10^{-4} M produced a 30% inhibition of COMT, concentrations at which catechol and pyrogallol caused 10% and 70% inhibition respectively.

Metanephrine under *in vitro* conditions is a weak inhibitor of COMT (4, 325, 374) but only at very high concentrations (10^{-3} M-10^{-2} M) and no inhibition has been found *in vivo* (4).

2. *2-(3,4-Dihydroxyphenyl)-acetamide (dopacetamide)*. This catechol and derivatives such as 2-(3,4-dihydroxyphenyl)-pentanoylamide and 2-(3,4-dihydroxyphenyl)-hexanoylamide inhibit COMT *in vitro* (354). There are no published kinetic data for this group of COMT inhibitors but in potency, measured by the ability of the substances *in vitro* to cause 50% inhibition of COMT, several of the 3,4-dihydroxyphenylacetamide derivatives which were tested were equal to pyrogallol (354). Although there is little information in the literature on the *in vitro* kinetic analyses of these compounds, they are relatively potent and nontoxic *in vivo* inhibitors of COMT and in these respects compare favourably with pyrogallol (93, 355).

In screening a series of *alpha*-substituted 3,4-dihydroxyphenylacetamides (dopacetamides) for *in vivo* COMT inhibitory potency, the α-propyl-3,4-dihydroxyphenylacetamide (H22/54) was found to

inhibit the enzyme most effectively (90). The 2,3-dopacetamides did not block COMT (90) presumably because 2,3-dihydroxyphenolic compounds are poor substrates for COMT. However, both 3,4- and 2,3-dopacetamides decrease monoamine biosynthesis (90) by inhibiting the hydroxylation step (322).

Pharmacokinetic studies of H22/54 have shown that after intravenous administration (50–100 mg/kg) the drug is eliminated from the blood by first order kinetics, that there existed considerable species differences in the elimination rate of the amide and that the most important pathway of elimination was a conjugation, though 3-O-methylation was also found (429).

After an intraperitoneal injection of ^{3}H-noradrenaline the labelled amine recovered from the tissues increased severalfold in dopacetamide-treated mice while noradrenaline derived from an intravenous injection, and hence less vulnerable to liver COMT, showed only a moderate increase (94). Furthermore, the dopacetamides considerably reduced the tissue content of labelled normetanephrine. In brain dopacetamides caused a marked fall (90%) in the levels of normetanephrine and methoxytyramine while noradrenaline and dopamine levels were unchanged (197).

Dopacetamides prolonged the isoprenaline-induced tachycardia in mice (355).

3. Flavonoids. Since earlier reports on the inhibition of COMT by *flavonoids* (35, 273, 354), two studies reported in 1972 and 1973 tested a number of different flavonoids for their COMT-inhibiting potency and determined some of the structural characteristics responsible for this inhibition (190, 375). A wide range of flavonoids of different basic structure (flavon, flavanon, flavonol, *etc.*) can inhibit COMT *in vitro*. The most powerful inhibitors are catechol-configurated flavonoids such as quercetin and rutin. The inhibitor constant K_i was calculated to average 5.3×10^{-6} M for varying concentrations of quercetin and 10.8×10^{-6} M for rutin (190) and these substances proved to be more potent as COMT inhibitors than pyrogallol (375); our results gave K_i values for pyrogallol, quercetin, and rutin of 1.3×10^{-5} M, 8.4×10^{-6} M, and 3.0×10^{-5} M, respectively (table 4). Both quercetin and rutin inhibit the enzymatic methylation of noradrenaline competitively by serving as substrates for COMT (190). Flavonoids, such as isorhamnetin, which are O-methylated in o-position to a side ring group, are less potent than the catechol flavonoids and appear to inhibit COMT by a mixed type inhibition (190). Flavonols such as caempferol, which lack neighboured OH-groups, are not metabolized by COMT, but effectively inhibit the enzyme in a mixed type noncompetitive manner while flavon or flavanon derivatives, devoid of the 3-OH-group, exhibit a less pronounced, but significant, inhibition (375).

Flavonoids have been used in man, but the importance that can be attributed to the interaction between flavonoids and catecholamines is not known (190).

4. Nordihydroguaiaretic acid. The phenolic antioxidant *nordihydroguaiaretic acid* is an *in vitro* inhibitor of COMT, and 6×10^{-6} M inhibited rat liver COMT 50% with noradrenaline as substrate; it is also a substrate for COMT with the O-methylation of both catechol rings (85).

The safety of adding nordihydroguaiaretic acid as an antioxidant to foodstuffs has been questioned in view of its interaction with catecholamines (85).

5. U-0521 and ketone analogues. Catechol ketones inhibit COMT (316) and *3,4-dihydroxy-2-methylpropiophenone* (*U-0521*) is a competitive inhibitor of the enzyme. It has a K_i value calculated to be 7.8×10^{-6} M which makes this compound a better inhibitor of COMT than pyrogallol (173). U-0521 (100 mg/kg and 65 mg/kg 1 hour and 0.5 hour respectively before killing) subsequently was used *in vivo* (174) and it increased the adrenaline content of the uterus and the noradrenaline concentration of the brain but had no significant effect on cardiac catecholamines.

U-0521 potentiates the positive inotropic

action of adrenaline and isoprenaline of isolated rabbit atria by inhibition of COMT (173), but does not potentiate the responses to noradrenaline (3×10^{-7} M) (see section VIII). It also has been suggested that U-0521 has a direct *beta*-receptor stimulating effect on the heart and effects unrelated to inhibition of COMT on other tissues, such as rat aortic strips and cat and rat splenic capsules (411).

The ketone analogues of adrenaline and noradrenaline, *adrenalone* (ω[N-methylamino]-3,4-dihydroxyacetophenone) and *arterenone* (ω-amino-3,4-dihydroxyacetophenone), are *in vitro* inhibitors with greater affinity for the enzyme than, for instance, catechol (441).

6. *Methylspinazarin (2,3,5,8-tetrahydroxy-6-methyl-1,4-naphthoquinone)* and *6,7-dihydromethylspinazarin* were reported in 1973 to inhibit COMT *in vitro* (101). Although these substances are not catechols they are included here since it is likely that their COMT inhibitory property resides in the ortho-dihydroxy structure and their dimethoxy derivatives show no inhibition at 100 μg/ml whereas, the ID50 of methylspinazarin and dihydromethylspinazarin are 0.80 μg/ml (3.3×10^{-6} M) and 0.78 μg/ml (3.2×10^{-6} M), respectively (101). These two inhibitors of COMT were obtained from a cultured broth of *Streptomyces*, and although they are potent *in vitro* inhibitors of COMT they have not been tested for their *in vivo* potency. Both compounds exhibit a marked hypotensive effect in spontaneously hypertensive rats (101).

7. *COMT inhibitors*. A number of commonly used drugs are inhibitors of COMT including L-dopa, L-α-methyl-dopa-α-hydrazine, isoprenaline, apomorphine and desmethylpapaverine, and metabolites such as 2-hydroxyestrone and 7,8-dihydroxychlorpromazine. All these compounds are catechol derivatives and would be expected to inhibit COMT by competing with the substrate for the active sites of the enzyme.

Dopa (3,4-dihydroxyphenylalanine) is a substrate for COMT (34) and inhibits the enzyme reaction *in vitro*, in competition with noradrenaline, with an inhibitory constant (K_i) of 4.2×10^{-4} M (48) and 9.8×10^{-4} M (table 4). Dopa is thus a fairly weak inhibitor of COMT and when the D- and L-isomers were compared D-dopa was found to be slightly less potent than the natural isomer (48, 119, 122).

When animals were given L-dopa (100 mg/kg i.p.) a major portion of the administered catechol amino acid is O-methylated to form 3-O-methyldopa (438). Thus, some COMT inhibition by substrate competition may occur *in vivo* after large doses of L-dopa but this is probably transitory and COMT inhibition was not detected in most tissues of rats fed a diet containing 1% L-dopa for a long period of time (379). There is a report of reduced COMT activity in the rat liver and erythrocytes after chronic administration of L-dopa (278), which is thus at variance with the just mentioned report (379). This discrepancy might be explained by procedural differences in the COMT assays. The choice of 3,4-dihydroxybenzoic acid as substrate (278) is an unfortunate one when studying L-dopa since different phenolic acid metabolites could be formed in the incubation medium.

L-Dopa as a substrate of COMT can deplete tissue concentrations of the methyl donor S-AMe and interfere with the methylation of substances *in vivo* (97). There is some indirect evidence that COMT activity is lowered in this way since prior administration of L-dopa decreased O-methylation of intracisternally administered noradrenaline and increased the percentage of deaminated metabolites (437). To test whether availability of cofactor, S-AMe, influences COMT activity, the dietary methionine content to rats was doubled for 12 days; this had no effect on the COMT activity (379). However, there is evidence that the methionine concentrations in serum and tissue of rats treated chronically with sufficient L-dopa to depress hepatic S-AMe are unaltered and hence sources of methyl groups other than

dietary methionine must be available to the body during L-dopa therapy (332).

Hydrazine derivatives of catechols, such as *L-α-methyl-dopa-α-hydrazine* (*MK-486*), used clinically to inhibit L-aromatic amino acid decarboxylase, have also been tested *in vitro* for COMT inhibition (45). MK-486 has a COMT inhibitory potency similar to L-dopa, with apparent ID50 values about 10^{-3} M, and appears to be a competitive inhibitor. The effect of MK-486 on COMT might have some significance *in vivo* since doses of 1000 mg or more can be given to animals without apparent ill effects.

Papaveroline or desmethylpapaverine inhibits COMT competitively *in vitro* and papaveroline produced a 50% inhibition at a concentration of approximately 8×10^{-6} M (86). This inhibition might account for some of the pharmacological effects of papaveroline, and possibly also of papaverine, after its *in vitro* demethylation (86). However, the coronary vasodilator activity of a number of analogues of papaverine were not related to the saturation of the 3,4- and 1,2-double bonds to give 3,4-dihydroxypapaverine and 1,2,3,4-tetrahydropapaverine (201).

In 1973, a report was published of some tetrahydroisoquinoline alkaloids such as papaveroline, formed by the Pictet-Spengler condensation of dopamine with an aldehyde, having been demonstrated *in vivo* in the urine of patients with Parkinson's disease during oral treatment with L-dopa (365). It has been shown that tetrahydroisoquinoline alkaloids are converted *in vivo* to O-methylated metabolites (109, 356). Salsolinol and tetrahydropapaveroline are competitive inhibitors of COMT *in vitro*, their K_i values being 1.3×10^{-4} M and 2×10^{-5} M respectively (109). The K_i value for tetrahydropapaveroline on rat liver COMT is 5.5×10^{-5} M (table 4).

Isoprenaline is an excellent substrate for COMT (172) and *in vitro* inhibits the enzyme at fairly low concentrations (K_i 1.5×10^{-4} M; table 4). Since there was no evidence of a change in the urinary excretion of O-methylated products from either noradrenaline or adrenaline after isoprenaline administration (317), it is unlikely that isoprenaline exerts a significant effect on COMT clinically.

Apomorphine contains a catechol group and is a substrate for COMT with an apparent K_m of 1.4×10^{-3} M for liver COMT and K_i value of 7×10^{-4} M (table 4). Apomorphine may inhibit O-methylation of adrenaline administered parenterally to cats since it reduces urinary excretion of 3-methoxy-4-hydroxymandelic acid (56). Apomorphine (10 mg/kg to cats) given 20 minutes before adrenaline (0.5 mg/kg) also reduced the urinary excretion of 3-methoxy-4-hydroxymandelic acid during a 3-hour collection period (56). Apomorphine is O-methylated by rat liver COMT *in vitro* and the products identified as apocodeine and isoapocodeine (315); this O-methylation *in vitro* is prevented by COMT inhibitors (297). COMT inhibitors, such as pyrogallol and 3,4-dimethoxy-5-hydroxybenzoic acid, prolonged apomorphine-induced stereotyped behaviour in the rat indicating the importance of O-methylation in the biotransformation of apomorphine *in vivo* (315).

2-Hydroxylated oestrogens, principal metabolites of oestrogen hormones, competitively inhibit the *in vitro* formation of metanephrine and normetanephrine, when using rat liver COMT, with total inhibition at 4-fold molar excess of 2-hydroxyoestradiol-17β to the substrate (adrenaline and noradrenaline) (266). Isomeric monomethyl ethers of 2-hydroxyoestrogens also inhibit O-methylation of catecholamines but it is noncompetitive. The O-methylation of 2-hydroxyoestradiol-17β, to the corresponding 2- and 3-methylethers, is not inhibited by adrenaline or noradrenaline, even with a 20-fold molar excess of the catecholamines (266).

Chronic oestradiol administration to female rats reduces hepatic COMT activity

(106) probably a result of a decrease in elective food consumption in the oestradiol-treated animals (103).

7,8-Dihydroxychlorpromazine formed through a minor pathway of chlorpromazine metabolism by hydroxylation to 7-hydroxychlorpromazine and hydroxylation of this major metabolite leads to the extremely labile 7,8-dihydroxychlorpromazine which is further metabolized by COMT to 7-hydroxy-8-methoxychlorpromazine and 8-hydroxy-7-methoxychlorpromazine (132, 133). Further investigations will be needed to determine whether this represents a significant pathway *in vivo* and whether the administration of chlorpromazine in high doses will indirectly affect COMT activity.

C. Tropolones

The tropolones (fig. 3), tropolone, 4-methyl- or 4-isopropyltropolone and tropolone-4-acetamide, are isosteric with catechols and are inhibitors of COMT (58, 310, 354). The *in vitro* inhibition of COMT by 4-methyltropolone was originally proposed to be noncompetitive (57) but on the basis of subsequent results a competitive mechanism for tropolone inhibition was proposed (58) which was supported by other workers (310). Competitive inhibition also has been proposed for enzyme preparations from brain (78), liver, and salivary gland (80). In an attempt to clarify the mechanism by which tropolones inhibit COMT, careful kinetic studies with COMT purified from rat liver (67) indicated that tropolones (tropolone, 4-methyltropolone, and β-thujaplicin) are linear noncompetitive inhibitors with respect to various commonly used substrates. They were noncompetitive with respect to S-AMe but a complex relationship existed with respect to Mg^{2+}. The Ki_s value of 2.2×10^{-5} M was close to the reported K_i values for tropolone obtained under slightly different experimental conditions: 1.5×10^{-5} M (310), 3.0×10^{-5} M (78), 3.4 and 2.7×10^{-5} M (80), and 2.7×10^{-5} M (table 4). The inhibition

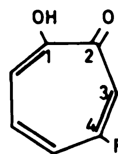

FIG. 3. COMT inhibitors of the tropolone group.

constants, Ki_s for 4-methyltropolone and β-thujaplicin were calculated to be $8.9 \times 10^{-6} \times 1.01 \times 10^{-5}$, respectively, and these derivatives are thus more potent *in vitro* than tropolone. The type of linear noncompetitive inhibition with dead-end inhibitors such as tropolones demonstrated in the extensive results of Borchardt (67) provides further information on the mechanism of enzyme/inhibitor reaction. It is thus unlikely that two or more molecules of the inhibitor add simultaneously to the same form of the enzyme. This, however, does not rule out the possibility that tropolone may be combining with two different forms of the enzyme. The noncompetitive pattern observed could result from tropolone binding with one form of the enzyme to produce competitive kinetics and with another form to produce noncompetitive kinetics. The patterns of inhibition and the kinetic parameters of tropolone inhibition remained constant through various stages of enzyme purification which might argue against the involvement of more than one form of the enzyme (67). It is believed that the inhibition of COMT by tropolones is by chelation, not of the free metal (67), but the formation of an enzyme-Mg^{2+}-tropolone complex (58).

In vivo COMT inhibition by tropolones has been studied by: 1) measuring the effect on the *in vivo* concentrations of O-methylated metabolites from catecholamines (90, 310); 2) assessing the ability of tropolones to modify adrenergic responses (58); and 3) by measuring COMT activity

in vivo after the administration of the drug to the animal (78, 355). The results indicate that tropolone and its derivatives (4-methyltropolone, β-thujaplicin, and tropolone-4-acetamide) inhibit COMT *in vivo*, but are short acting, presumably, due to the reversible and easily releasable binding of the inhibitor to the enzyme. The reduction in COMT activity in brain does not correlate with the brain cencentration of tropolone (78). The enzyme activity falls to 73% of normal after 1 hour while the theoretical reduction, as calculated from the K_m and K_i values found in the brain at 1 hour, was found to be 45%. The reason for this discrepancy may be a redistribution of tropolone within the subcellular particles of the homogenate. Moreover, the measurement of COMT *in vitro* may not be an adequate measure of enzyme inhibition *in vivo* (78). Tropolone (100 mg/kg i.p.) given to rats caused a modest inhibition when measured by essentially *in vitro* assay techniques (80), while a small dose of tropolone (6.25 mg/kg) in mice pretreated with L-dopa lowered the O-methylated metabolites of dopamine in the striatum (320).

PHARMACOKINETIC DATA. There is a high concentration of tropolone in the brain, liver, and salivary glands 0.5 hour after administration of the inhibitor (100 mg/kg i.p.) to rats (78, 80). The drug concentrations in brain and liver were of the same order, thus indicating that tropolones do not have difficulties in penetrating the blood-brain barrier as had been suggested (355). The high concentrations of tropolone at 0.5 hour were reduced markedly in all the tissues 2 to 3 hours after its administration and by 24 hours very little tropolone could be detected (78, 80). No evidence was found for the presence of any metabolite of tropolone in the rat (78).

EFFECTS ON CATECHOLAMINE METABOLISM. In brain, tropolone (40 mg/kg i.p.) 2 hours and 30 minutes prior to ^{14}C-dopa enhanced accumulation of cerebral catecholamines (182). Ventricular perfusion experiments with ^{14}C-noradrenaline showed that tropolone (50×10^{-6} g/ml) added to the perfusion fluid increased the labelled noradrenaline concentration of the cat hypothalamus but reduced the 3-methoxy-4-hydroxymandelic acid concentration in the perfusion fluid (339). Tropolone-4-acetamide markedly reduced the formation of O-methylated metabolites without changing the levels of the parent catecholamines in brain (179, 320, 360). In rat brain, tropolone (100 mg/kg i.p.) caused a rapid 40 to 60% fall in the normetanephrine concentration with a parallel fall in the noradrenaline concentration (80) while the striatal dopamine concentration rose initially with a simultaneous reduction in the O-methylated dopamine metabolites (78). The differences, in response to tropolone, in noradrenaline and dopamine might reflect differences in the relative importance of uptake and biotransformation mechanisms of the two amines. Tropolone (50 mg/kg) caused only a small fall in the mouse striatal homovanillic acid concentration after 2 hours while tropolone (25 mg/kg) given to animals treated with probenecid, to prevent the egress of the acid from brain, appeared to prevent formation of homovanillic acid completely (347). In mice treated with L-dopa, a low dose of tropolone (6.25 mg/kg) reduced the concentration of homovanillic acid by 50% (320). These results demonstrate the profound effect of inhibiting O-methylation with tropolone on dopamine metabolism in the CNS.

Rats treated with β-thujaplicin (40 mg/kg) have a reduced urinary output of labelled O-methylated metabolites formed from ^{14}C-dopamine after its systemic administration (321). Similarly, the administration of ^{14}C-labelled adrenaline to rats pretreated with 4-methyltropolone (30 mg/kg) decreased the excretion of labelled metanephrine (310) and tropolone-4-acetamide reduced the formation of O-methylated metabolites in the salivary gland (6).

When a high dose of tropolone is used, in excess of 100 mg/kg in mice, factors other than COMT inhibition may affect metabolism of catecholamines such as transport of metabolites across membranes, interference by tropolone by chelation of many metabolic reactions (320) and inhibition of other enzyme systems *in vivo* including tyrosine hydroxylase (183, 335) and dopamine-β-hydroxylase (185).

PHARMACOLOGICAL EFFECTS. The earliest results on COMT inhibition by tropolone suggested that in addition the compounds exerted a *beta*-receptor blocking property (58). This was supported by the results of some other workers (319) who found that a low dose of methyltropolone (10 mg/kg) increased mortality in mice given adrenaline whereas a high dose (100 mg/kg) protected the animals. Mortality after isoprenaline administration was increased at both dose levels though the vasodepressor effect of isoprenaline in anaesthetized cats was reduced. However, subsequently it was found that a low dose of 4-methyltropolone and β-thujaplicin increased the height of the adrenaline-stimulated contraction of rabbit aortic strips and potentiated the toxic effects of adrenaline in mice, whereas large doses antagonized these effects most likely through nonspecific actions of the tropolones (318). The specific, potentiating effect of tropolone and tropolone-4-acetamide on the catecholamine response of rabbit aortic strips has been demonstrated by other workers (248, 256, 290). No adrenergic *beta*-receptor blocking action of tropolone was observed with the isoprenaline induced tachycardia of mice as test (355). The specificity of the haemodynamic effects of thujaplicin in dogs (311) is difficult to assess since the data were inadequate to draw any conclusions. Tropolones appeared to have a papaverine-like action (58) and this might contribute to a nonspecific effect. Tropolone and catechol rings are biochemically isosteric and this might also apply to adrenergic receptors (58) which may be identical with membrane-bound COMT (71), but pharmacological effects may not be detectable as a result of the theoretical tropolone-COMT interaction because true adrenergic receptors are very scarce compared to membrane protein and probable COMT binding sites (128).

TOXICITY. Tropolones are quite toxic and cannot be given to man. In the laboratory they have been used extensively but without systematic toxicity studies. The nature of the substituents on the tropolone ring have a marked influence on the toxicity of the tropolone derivative (58). For instance, in mice the LD50 values for 4-methyltropolone and 4-isopropyltropolone after intraperitoneal administration were 535 mg/kg and 85 mg/kg respectively. In rat, LD50 for tropolone itself was calculated to be about 100 mg/kg (82).

D. *Miscellaneous* (Fig. 4)

1. 8-Hydroxyquinolines. A potential class of COMT inhibitors belonging to the nonhydroxyphenyl substituted compounds is that in which another hetero-atom has been substituted for a hydroxyl in the basic catechol molecule, as for example the 8-hydroxyquinolines. These compounds have had little attention since it was reported in 1964 that 8-hydroxyquinoline was a 1.5 times more potent *in vitro* inhibitor of mouse brain COMT than pyrogallol (354). An extensive investigation reported in 1973 on the *in vitro* inhibition of COMT by substituted 8-hydroxyquinolines (68) disclosed some of the most potent *in vitro* inhibitors of COMT yet reported. For example, the K_i value for 7-iodo-8-hydroxyquinoline-5-sulfonic acid was found to be 8.93×10^{-7} M. Rate studies indicate the inhibition is linear and noncompetitive with respect to catechol substrate, uncompetitive with respect to S-AMe, has a complex relationship with respect to Mg^{2+}. This is similar to tropolone (67) and hence 8-hydroxyquinoline might produce its noncompetitive pattern as a result of interacting with two different forms of the enzyme, one producing competitive kinetics and the

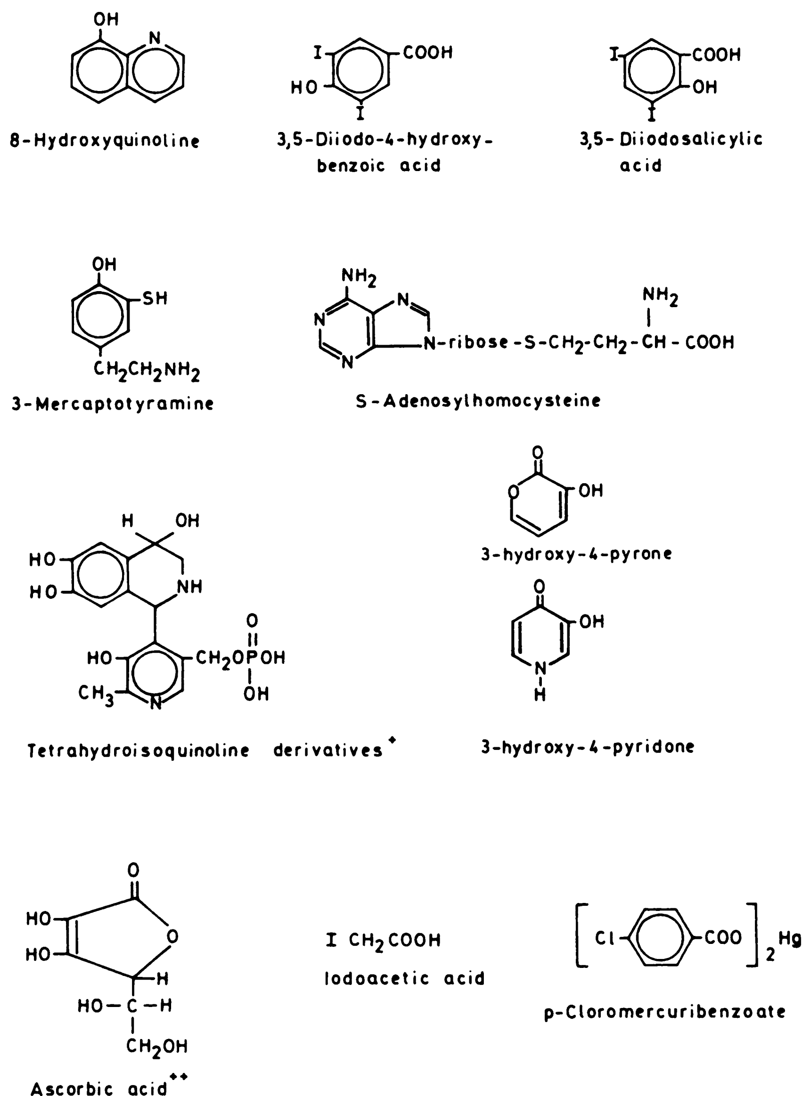

FIG. 4. COMT inhibitors of the miscellaneous group.
+ Tetrahydroisoquinoline derivative formed from the reaction of L-noradrenaline and pyridoxal-5'-phosphate; ++ L-ascorbic acid drawn in the enediol form of 3-keto-L-gulonic acid.

other producing uncompetitive kinetics with the overall results being of a noncompetitive pattern (68). The inhibition appears not to be a direct result of the metal chelating properties of 8-hydroxyquinolines but might be through chelation with an enzyme bound Mg^{2+}. Delineation of the structure-activity relationship requirements for *in vitro* inhibition of COMT by 8-hydroxyquinolines demonstrate that another hetero-atom or functional group can be substituted for one of the hydroxyl groups of the catechol substrate resulting in inhibitors of COMT which bind reversibly and more tightly to the enzyme than the substrate itself.

The pharmacological significance of COMT inhibition by 8-hydroxyquinolines is not known but it is worth noting that certain compounds in this general class have been used in the chemotherapy of amoebiasis, compounds such as chiniofon and vioform (186).

2. *Iodophenol derivatives*. The urinary

excretion of 3,5-diiodo-4-methoxybenzoic acid after the injection of 3,5-diiodo-4-hydroxybutylbenzoate (DIHBA) to rats prompted an *in vitro* study of its inhibition effect on COMT (137). No evidence was found *in vitro* for the formation of O-methylated metabolites of DIHBA with a crude rat liver enzyme preparation, though competitive inhibition of COMT by DIHBA and 3,4-diiodosalisylic acid occurred with K_i values of 1.3×10^{-5} M and 3.4×10^{-4} M, respectively (138). 3,4-Diiodotyrosine had no effect, while the transaminated derivative 3,5-diiodo-4-hydroxyphenylpyruvic acid produced a progressive and partly reversible inhibition. Attention was drawn to experiments indicating the possible enzymatic O-methylation of the structurally analogous thyroid hormones (139) which was stimulated by the earlier finding (136) that thyroxine injected into rats resulted in a marked fall in the activity of liver COMT even though thyroxine *in vitro* did not inhibit liver COMT (310). One might speculate that *in vivo* inhibition of COMT by thyroxine might have a nutritional basis.

3. *3-Mercaptotyramine*. This is an analogue of dopamine in which the *m*-hydroxy group of the catechol moiety has been replaced by a mercapto group. 3-Mercaptotyramine effectively inhibits the enzymatic O-methylation of various catechols, and it is not a substrate for COMT and according to kinetic analysis the inhibition is of a mixed type, *i.e.*, the presence of the inhibitor interfered to some extent with the binding of substrate as well as producing a noncompetitive inhibition (293). The K_i value for the initial competitive reaction calculated from the slopes of the double reciprocal plots is 6×10^{-4} M. The inhibition is essentially irreversible by dialysis but readily can be reversed or prevented by dithiothreitol, a selective reagent for the reduction of disulfides. On the basis of this evidence 3-mercaptotyramine was suggested to inhibit COMT by reacting with a cysteine residue in or near the active site by the formation of a disulfide bond (293). Whether 3-mercaptotyramine and other mercaptophenols are also active as inhibitors of COMT *in vivo* awaits testing.

4. *S-Adenosylhomocysteine*. It was reported in 1972 that the hydrolysis product of S-AMe S-adenosylhomocysteine (SAH) is a potent inhibitor of COMT (114). The kinetic constants derived from their data were a K_i of approximately 3×10^{-5} M obtained for SAH acting as an inhibitor of S-AMe (K_m = *ca.* 6×10^{-5} M) and for 3,4-dihydroxybenzoic acid (K_m = *ca.* 2×10^{-4} M). An alternative method of evaluating the effectiveness of SAH as an inhibitor of COMT shows the concentration of SAH required to effect a 50% inhibition of the reaction (I50) is 3.0×10^{-5} M. The authors stress that these constants should be considered as only approximate values since saturating conditions of S-AMe, so that conversion to SAH stays below 5%, could not be met at low concentrations of the substrate and coenzyme. For the same reason it was not considered possible to state with certainty the exact nature of the SAH inhibition of COMT. The sulfoxide analogue of SAH is a less potent COMT inhibitor than SAH (I50: 8.6×10^{-4} M) (114), suggesting that more stable analogues of SAH might be useful as inhibitors of COMT.

5. *Pyridoxal-5'-phosphate*. A recent communication described the competitive inhibition of COMT (K_i 5.4×10^{-5} M) *in vitro* by pyridoxal-5'-phosphate (PLP) (62) which over a range of 10^{-5} M to 10^{-4} M produced up to 90% inhibition of COMT. From the kinetic data, competition of PLP with noradrenaline for enzyme binding site(s) was indicated although the mechanism of inhibition was not defined.

A detailed study of the mechanism of PLP inhibition of COMT has been carried out (69). Since difficulty was encountered in constructing linear reciprocal plots for PLP inhibition of COMT, nonenzymatic studies were carried out which revealed that a facile chemical reaction took place between noradrenaline and PLP resulting in the formation of a tetrahydroisoquino-

line. This tetrahydroisoquinoline was shown to account for the majority of the enzyme inhibition observed with PLP. PLP was a very poor inhibitor of COMT with substrates not capable of forming this tetrahydroisoquinoline. Thus it appears that the inhibition of COMT-catalyzed O-methylation of noradrenaline by PLP can be accounted for by three separate mechanisms: 1) removal of the substrate by a chemical reaction between noradrenaline and PLP; 2) production of a potent inhibitor, tetrahydroisoquinoline; and 3) weak inhibition by PLP itself (69).

There is no evidence of *in vivo* COMT inhibition by pyridoxine hydrochloride (62).

6. L-Ascorbic acid. L-Ascorbic acid is believed to exist in the enediol form of 3-keto-L-gulonic acid, thus involving a 5-membered ring with two adjacent OH-groups. L-Ascorbic acid is methylated *in vitro* by COMT and the O-methylated product was identified as 2-methyl-L-ascorbic acid (63); it is, however, a poor inhibitor (K_i value of 1.4×10^{-2} M) (table 4).

7. Sulfhydryl binding agents. The active site on the COMT appears to contain a sulfhydryl group since the enzyme was inhibited by SH-binding compounds such as *p*-chloromercuribenzoate and iodoacetic acid; the respective concentration of each needed to inhibit enzyme activity about 50% is 3×10^{-5} M and 2×10^{-3} M (34).

8. Iodide. With coenzyme preparations from two different commercial sources it was discovered that S-AMe iodide gave considerably lower values for the COMT activity than the chloride. Iodide inhibits the enzymatic reaction and behaves as a mixed type inhibitor with a K_i value of the order 5.2×10^{-4} M, but the mechanism of inhibition has not been elucidated (84).

9. Pyrones and pyridones. An interesting group of COMT inhibitors comprising the prototypes 3-hydroxy-4-pyrone, 3-hydroxy-2-pyridone, and 3-hydroxy-4-pyridone was reported in 1970 and the mechanism of COMT inhibition appears to be similar to that of tropolone (70).

VII. O-Methylation and the Metabolism of Catecholamines in Peripheral Systems

Endogenous adrenaline originates mainly from the adrenal medulla, its site of synthesis and storage, and probably no significant metabolism occurs until it is released into the circulation (268). Endogenous noradrenaline, on the other hand, is found within neurons of the sympathetic nervous system where it is concentrated in the terminals (327) and its inactivation after release is at sites in the immediate vicinity of the receptors.

After the release of noradrenaline from the terminals into the synaptic space two processes may influence the concentration of the transmitter in this space: re-uptake into the terminals (153, 224, 225, 408) and uptake into non-neuronal sites (extraneuronal uptake) (222, 291). COMT is an intracellular enzyme mostly found in extraneuronal cells, though a proportion of the activity is neuronal in the nictitating membrane, vas deferens, and salivary gland (section V, D). Thus O-methylation is *preceded* by uptake, and metabolism influences the initial uptake process only by reducing the intracellular concentration of the amine and so facilitating the inward transport across membranes.

The importance of neuronal uptake in the regulation of the noradrenaline concentration in the region of the receptor is established (153, 223–225, 412) and the evidence reported in 1972 has been carefully reviewed (408). The purpose of this review is neither to reiterate this evidence nor to discuss the regulation of functional catecholamine concentrations in general terms. Rather it will concentrate on situations in which extraneuronal uptake and subsequent O-methylation is at least an important alternative to neuronal uptake.

Firstly, it is necessary to define the terms used to describe the transfer of cate-

cholamines from the extracellular to intracellular sites and their subsequent fate in both the physiological and/or experimental situation. In this field of study strict use of terminology is essential if one is to avoid misunderstanding and misconceptions.

A. Uptake

1. Neuronal uptake is the unidirectional influx of noradrenaline from the extracellular fluid across the neuronal membrane. This is a high affinity transport system that is Na^+ dependent, stereochemically specific for the *l*-isomer of noradrenaline with a K_m of 0.2 to 0.4 μM, and inhibited by cocaine (223–225). The rate of neuronal uptake is influenced by the K_m, the concentration of amine outside the membrane, and that free in the neuronal cytoplasm; as the latter increases net neuronal uptake falls.

2. Extraneuronal uptake is the unidirectional influx of catecholamines across cellular membranes other than those of neurons. This uptake is not stereochemically specific, has a low affinity (K_m about 250 μM) though it is higher for N-substituted catecholamines *e.g.*, adrenaline and isoprenaline, than for noradrenaline (222, 225, 291). Extraneuronal uptake of noradrenaline *in vitro* occurs into cardiac muscle cells (105, 151), fibroblasts, vascular smooth muscle (236), smooth muscle in vas deferens, spleen, salivary gland, nictitating membrane, and collagen and elastic tissues (21, 140, 175, 200). Inhibitors of extraneuronal uptake include metanephrine, normetanephrine, phenoxybenzamine, phentolamine, 17-β-oestradiol, corticosterone, deoxycorticosterone, and cholesterol (144, 222, 227, 228, 236). Metanephrine and normetanephrine only block extraneuronal uptake (144, 146, 161, 175) while phenoxybenzamine also blocks neuronal uptake (144, 145, 222, 291). Normetanephrine is also a substrate for extraneuronal uptake in the rat heart (383). Extraneuronal uptake of noradrenaline into cardiac muscle cells is not dependent upon an intact sympathetic nerve supply as it is unaffected by chemical sympathectomy (104).

The efflux of catecholamines from neuronal and extraneuronal sites has also been demonstrated under certain experimental conditions (140, 409) and this will be discussed later.

B. Intracellular Mechanisms

1. Metabolism is the intracellular enzymatic deamination and/or O-methylation of catecholamines and is subsequent to uptake.

2. Accumulation of unchanged exogenous catecholamine occurs at intraneuronal sites under experimental conditions that result in a rate of uptake greater than the intracellular metabolic capacity. Thus when the rat heart is perfused with high concentrations of exogenous noradrenaline, accumulation of the noradrenaline occurs at both neuronal and non-neuronal sites (222) and though extraneuronal uptake functions at low perfusion concentrations of noradrenaline there is no intracellular extraneuronal accumulation unless MAO and COMT are inhibited (291).

3. Retention of unchanged noradrenaline implies its storage within vesicles (in the case of neuronal retention) or some other storage organelle. This occurs under physiological and experimental conditions (176, 199) and is an alternative to metabolism in the regulation of the intracellular concentration of free amine. Retention influences the accumulation of exogenous noradrenaline, as the former decreases so will accumulation.

While metabolism and retention are operative under experimental and physiological conditions it is probable that accumulation is an experimental feature since at physiological amine concentrations uptake is followed rapidly by either retention or metabolism. For example, when the rabbit vas deferens is stimulated (2 Hz) the output of endogenous noradrenaline is increased by inhibition of MAO and COMT

but this increase produced by inhibition of metabolism is blocked by inhibition of extraneuronal uptake (216). This indicates that under physiological conditions extraneuronal accumulation does not occur but that the rate of intracellular metabolism and extraneuronal uptake are intimately related.

C. Methodology

Any study of the physiological importance of O-methylation in the regulation of catecholamine concentrations must consider the intimate relationship between uptake and metabolism and the fact that these operate in series (see above). The degree to which an experimental situation with exogenous noradrenaline, very often in an isolated tissue preparation, relates to the physiological state must be carefully analyzed. For example, does the relationship between the agonist and receptor in an isolated preparation remain as *in vivo* (408)?

The role of O-methylation in the regulation of catecholamine concentrations has been studied by measuring the effects of COMT inhibition, alone and in conjunction with that of MAO and uptake inhibitions, on various pharmacological and biochemical parameters.

1. Effect on pharmacological parameters. Studies have been done on the effect on the catecholamine-induced contractions of smooth or striated muscle, *i.e.*, nictitating membrane (140, 406, 407, 410–412), isolated atria and heart muscle (172, 261, 330), blood pressure and the heart (124, 232, 273, 285, 352, 416, 441), aortic strips (86, 203, 204, 289, 290, 380, 411), venous tissue (192, 334, 415), papillary muscle (260, 261), spleen (74, 411) and oesophageal strips (203).

An alternative is to measure the rate of relaxation of isolated smooth muscle in the presence of COMT inhibitors and the interaction with MAO and uptake inhibitors. This has been used as an estimate of the rate of inactivation of the agonist (253). When rabbit aortic strips reach a steady response to the agonist they are immersed in mineral oil, to prevent loss of the agonist into the surrounding medium by diffusion, and the rate of relaxation measured. This method has been used with aortic tissue (248–250, 252–258, 280), venous strips from the saphenous vein of the dog (193, 334) and the isolated cat spleen (71). The method relies on the assumption that there is a causal relationship between rate of relaxation and rate of inactivation. Inhibition of COMT and/or MAO however enhances the intracellular accumulation of noradrenaline (291, 383) resulting in transfer of noradrenaline from the sites of extraneuronal accumulation to the region of receptors (140)—*i.e.*, efflux from extraneuronal sites. This could explain the prolonged muscle contraction and slow decline of the induced extraneuronal noradrenaline fluorescence after exposing vascular tissue to high concentrations of exogenous noradrenaline (21). In a similar manner MAO inhibition during prolonged exposure to exogenous noradrenaline results in the efflux of noradrenaline accumulated within the neuron which may influence the rate of relaxation (409). Thus the assumed correlation between rate of relaxation and rate of inactivation requires careful evaluation especially in the presence of drugs that enhance the intracellular accumulation of the agonist by the inhibition of metabolism (140).

2. Effect on various biochemical parameters. For example, in perfusion studies one can measure the accumulation of exogenous catecholamine, bearing in mind that this is not only a function of the rate of uptake but also the rate of intracellular retention and metabolism, or the elimination of exogenous catecholamine from the perfusion fluid. This enables one to estimate the net neuronal and extraneuronal uptake, with cocaine to inhibit neuronal uptake, without having to consider the intraneuronal fate of the catecholamine (187). However, neither of these methods give information about the type of intracellular metabolism that occurs. To obtain

this, accumulation and elimination measurements can be combined with measurements of the metabolites formed from the exogenous catecholamine (144, 236, 291, 383). There is a possibility that the metabolites measured are only those formed recently as efflux may occur during the perfusion period.

3. Effect on stimulated release of noradrenaline. Low current (10 mA) field stimulation of the sympathetically innervated rat atria produces a Ca^{++}-dependent release of noradrenaline and so resembles transmitter release. A higher current produces a Ca^{++}-independent release of noradrenaline (259). The endogenous transmitter stores may be labelled with tritiated noradrenaline and the fate of the released labelled transmitter measured under different stimulation frequencies in tissues such as skeletal muscle (349), cat nictitating membrane (282–284), vas deferens (216, 282, 346, 399), atria (259, 346, 357), arterial strips (396), spleen (40, 176, 209, 270) and the renal sympathetic nerve of the dog (442). There are, however, several precautions needed when interpreting the results. Newly synthesized noradrenaline is released in preference to stored amine (270), though in the guinea pig vas deferens re-uptake is more important than new synthesis for the maintenance of the noradrenaline supply during sympathetic stimulation (399). The preferential release of newly formed transmitter may distort the amount of ^{3}H-noradrenaline released and the formation of its metabolites during stimulation in relation to endogenous noradrenaline, in particular when an important neuronal re-uptake process is inhibited. It is also important to measure both the release of ^{3}H-noradrenaline and the amount of labelled metabolites formed especially when a drug with a multiple mode of action is being used, *i.e.*, phenoxybenzamine, since the *overflow* of ^{3}H-noradrenaline may be increased by more than one mechanism (282). While noradrenaline *release* is the actual output of noradrenaline produced by nerve stimulation, noradrenaline *overflow* is the increase in noradrenaline above the prestimulation resting level collected in the venous effluent of a perfused organ or the bathing solution of an isolated preparation during and after stimulation. When uptake is not inhibited the measurement of *overflow* will give an under-estimation of release as noradrenaline will be lost through uptake and subsequent metabolism or storage, but when uptake is inhibited *overflow* increases. Phenoxybenzamine blocks postsynaptic *alpha*-adrenergic receptors, and inhibits neuronal (401) and extraneuronal uptake (222, 291) so prevents postsynaptic metabolism of released noradrenaline and thus increases noradrenaline *overflow*. However, phenoxybenzamine also increases the output of noradrenaline on nerve stimulation by blockade of presynaptic *alpha*-adrenergic receptors involved in the negative feed-back control of transmitter release (147). When the concentration of the transmitter in the synaptic gap reaches a threshold concentration the presynaptic receptors are stimulated by the transmitter resulting in a reduction in its release. Results obtained with phenoxybenzamine as an inhibitor of uptake (*e.g.*, 176, 346, 396) must therefore be reassessed in the light of its presynaptic receptor activity and no definite conclusions can be reached from such studies.

D. Circulating Endogenous and Exogenous Catecholamines

1. Adrenaline. The half-life of exogenous adrenaline in the circulation is less than 20 seconds so removal is within one circulation (152). After an intravenous infusion of ^{3}H-adrenaline there is a marked initial recovery of ^{3}H-metanephrine indicating the involvement of O-methylation in the metabolism of the circulating amine (37, 367). Only 2% of ^{3}H-adrenaline injected into the hepatic portal system was recovered unchanged, the major metabolites being metanephrine glucuronide and 3-methoxy-4-hydroxyphenyl glycol sulphate (212). Some metanephrine formed is conju-

gated and some deaminated. The contribution of these processes to the total metabolism of administered adrenaline can be assessed with a double isotope technique in which ^{3}H-adrenaline and ^{14}C-metanephrine are given simultaneously (267), when two-thirds of the ^{3}H-adrenaline is converted to metanephrine and only a very small fraction directly deaminated in man (267) and rat (269).

There is no COMT in blood plasma, and while erythrocytes contain the enzyme (27, 301) it is unsufficient to account for the initial O-methylation of administered adrenaline. With the blood-bathed organ assay technique Ginn and Vane (178) and Vane (414) have shown that vascular systems, apart from the portal system, are important removal sites of adrenaline and about 70% of an infusion of adrenaline (50 μg/minute for 10 minutes) is removed during one passage through the hindquarters of the cat and dog (178). There is a similar effective removal from blood in the cat spleen, kidneys, and intestine (96). In contrast adrenaline passes through the pulmonary circulation unchanged (178). Selective and rapid uptake of adrenaline occurs into rat adrenals, heart, pituitary, spleen (37, 367), kidney, and skeletal muscle (414) and the initial removal of adrenaline from the blood is probably by extraneuronal uptake analogous to the extraneuronal uptake of noradrenaline (291). The affinity of adrenaline for extraneuronal uptake is greater than that of noradrenaline (225). The smooth muscle cells of blood vessels are efficient sites for extraneuronal uptake (236) and well situated for the removal of adrenaline from the blood as it passes through the peripheral blood vessels.

The liver is rich in COMT (34) and a greater proportion of a dose of adrenaline administered *via* the portal system is recovered as metanephrine than after systemic administration (212) suggesting that various peripheral vascular beds have a remarkable capacity for removing circulating adrenaline (96, 178). The assumption that hepatic O-methylation is the major means of metabolism for endogenous adrenaline must therefore be considered in relation to the site of administration of exogenous adrenaline. Metabolism of labelled adrenaline is probably a reliable index of the fate of endogenous adrenaline (268), providing that the site of administration is taken into account since this may distort the importance of a particular organ in the inactivation process by placing the adrenaline directly into the blood system of that organ. Adrenaline released by the adrenals into the circulation will pass through the heart and lungs before being distributed within the intestinal tract, hepatic portal system, kidneys, and the other vascular beds. The wide distribution of COMT and tissue uptake of adrenaline is indicative that the extraneuronal uptake and subsequent O-methylation of adrenaline occurs at many peripheral sites.

While it is convenient to postulate that the extraneuronal uptake-O-methylation series is important in the removal of adrenaline released from the adrenal medulla there is no direct evidence. However, the concept raises several interesting questions. For example, are the effects of circulating adrenaline prolonged more by inhibitors of extraneuronal uptake than neuronal uptake? In the physiological context, what is the effect of high blood cholesterol on the removal of adrenaline after its release from the adrenal medulla since cholesterol is a weak inhibitor of extraneuronal uptake (362)? Similarly, do altered blood levels of progesterone and hydrocortisone, both inhibitors of extraneuronal uptake (228), influence the circulating concentration of adrenaline?

2. *Isoprenaline* (isopropylnoradrenaline). Unlike adrenaline or noradrenaline isoprenaline is not deaminated but only O-methylated as the isopropyl group is attached to the N-atom (208). After the oral administration of ^{3}H-isoprenaline to dogs, however, labelled 3-methoxy-4-hydroxymandelic acid has been detected (112) though this has not been confirmed

(111). Furthermore, isoprenaline is not a substrate for neuronal uptake in rat (88) and rabbit heart (7) or the cat nictitating membrane (412) though it is taken up at extraneuronal sites (88), in the guinea pig trachea (162) and cat nictitating membrane (412). This uptake, in common with the extraneuronal uptake of noradrenaline (291), is not inhibited by cocaine or desipramine (88, 160) but is by normetanephrine. This makes the removal of isoprenaline a useful model of the extraneuronal uptake-O-methylation series.

The effects of isoprenaline on the kitten papillary muscle, a tissue devoid of neuronal uptake (261), and dog aortic strip (59) are potentiated by hydrocortisone, an inhibitor of extraneuronal uptake. The COMT inhibitor U-0521 potentiates the effects of isoprenaline on the heart muscle of the cat but the effect is less than that of hydrocortisone (260, 262) and increases the accumulation of ^{3}H-isoprenaline in the perfused rat heart when the concentration of the COMT inhibitor is below 100 μM (64). At concentrations above 100 μM, U-0521 reduces the accumulation of ^{3}H-isoprenaline in the rat heart. Presumably, the drug also inhibits extraneuronal uptake at high concentrations. The effects of U-0521 on the cat heart muscle are prevented by pretreating the tissue with an inhibitor of extraneuronal uptake (260, 261), a clear demonstration that the O-methylation of isoprenaline is preceeded by the extraneuronal uptake of the amine. Bönisch and Trendelenburg in 1974 (64) reported that in the perfused rat heart the rate of O-methylation of isoprenaline (measured by the appearance of ^{3}H-O-methylisoprenaline in the venous effluent) is independent of the accumulation of unchanged ^{3}H-isoprenaline and they propose a two-compartment system in which the O-methylating compartment equilibrates quickly, O-methylates at a constant rate throughout the experimental period (30 minutes) and has a high COMT activity. The second compartment equilibrates slowly, has a high storage capacity for unchanged amine but very little COMT activity so that initially the removal of isoprenaline is by accumulation. As perfusion continues and the second compartment reaches an equilibrium removal is entirely by metabolism. It would be interesting to show whether these different compartments have any morphological basis, indeed, whether they are found in different tissues. It would also be interesting to show whether a similar removal by extracellular accumulation, which is not immediately followed by metabolism, operates under the physiological conditions of the release of adrenaline.

The results *in vitro* show that extraneuronal uptake, accumulation and O-methylation are responsible for the removal of isoprenaline. Initial *in vivo* experiments also indicated that isoprenaline administered intravenously was primarily O-methylated (208) but that orally administered isoprenaline underwent sulphate conjugation (112). In a 1972 study, however (111), 60 to 80% of an intravenous dose of isoprenaline in man and dog was excreted unchanged in the urine while the remaining 20 to 40% appeared as free or conjugated O-methylated isoprenaline. Similarly, in human plasma unchanged isoprenaline was the major fraction recovered 4 minutes after the infusion of 0.44 mg base/kg over 30 minutes, although after 27 minutes the recovery of O-methylated isoprenaline increased. The major portion in plasma and urine of isoprenaline given orally (30 mg sustained release tablet) to man was conjugated isoprenaline with only a small amount of O-methylated isoprenaline and no acidic metabolites (317). From these results it appears that the excretion of conjugated and unchanged isoprenaline are the main means for the rapid removal of isoprenaline administered *in vivo*.

3. Exogenous circulating noradrenaline. In the peripheral nervous system noradrenaline released from nerve endings is removed by structures in the vicinity of the site of release and there are only very low levels of endogenous circulating noradrenaline (150). The removal of high concentra-

tions of exogenous noradrenaline after perfusion or intravenous administration do not relate to the physiological levels of the transmitter. Although neuronal uptake and retention is an important factor in the removal of exogenous circulating noradrenaline (176, 187, 199, 210, 226, 394, 430), the exogenous noradrenaline is accumulated by numerous tissues, including adrenal glands, kidney, lung, and skeletal muscles (37, 94, 211, 217, 430). In the cat the accumulation of ^{3}H-noradrenaline in the heart, spleen, liver, and skeletal muscle is increased by pretreatment with pyrogallol with a parallel decrease in ^{3}H-normetanephrine formed (211). Furthermore, the rapid removal and short half-life of infused noradrenaline (100, 271, 414) can be contrasted with the slower uptake of the amine into nerve endings and the ability of peripheral vascular beds to competently remove exogenous noradrenaline from the circulation (178). One can conclude that the neuronal uptake-retention/metabolism and the extraneuronal uptake-metabolism series are both involved in the removal of exogenous noradrenaline from the circulation. Although extraneuronal uptake sites have a lower affinity for noradrenaline than neuronal uptake sites (225) the former are more numerous so the total amount of the catecholamine removed from the extracellular space by the two processes are probably comparable especially if the concentration of noradrenaline is high.

In conclusion, extraneuronal uptake and subsequent metabolism are important for the removal of exogenous adrenaline and noradrenaline from the circulation, although in the latter case neuronal uptake also influences the concentration.

It is probable that the extraneuronal uptake-metabolism series is the primary means of regulating endogenous levels of circulating adrenaline. There is, however, no direct evidence but the concept raises several physiological and possible clinically important questions.

In vitro extraneuronal uptake and subsequent O-methylation/accumulation remove isoprenaline from the extracellular space. *In vivo*, however, the excretion of unchanged and conjugated isoprenaline are the primary means of removing this clinically important amine from the circulation.

E. COMT and the Regulation of Noradrenaline Concentrations in the Sympathetic Nervous System

Though COMT has a wide distribution (section V, C) there is no simple relationship between the activity of the enzyme and the amount of exogenous noradrenaline accumulated by smooth muscle or the perfused heart (87, 237). The pharmacological effects of COMT inhibition (for details see section VI) indicate an inverse relationship between inhibition and the degree of catecholamine neuronal uptake. Thus the effects of isoprenaline and adrenaline are enhanced more than those of noradrenaline (173, 232, 352, 355, 441). In aortic tissue, however, the effects of exogenous noradrenaline are weakly potentiated by inhibitors of COMT, by 4-tropolone acetamide (258, 259), U-0521 and pyrogallol (204, 248), while the response to noncatechols (phenylephrine, synephrine) is unaffected (204, 248). The effects of noradrenaline on the cat papillary muscle (260), isolated nictitating membrane (411) and rat splenic strips (411) are also potentiated by U-0521 but only in the presence of cocaine, indicating that the potentiation is related to high tissue sensitivity to the agonist.

The following discussion is based on the premise that neuronal uptake is the primary method for removing noradrenaline from the region of the receptors. For example, when the isolated rabbit vas deferens is field stimulated (2 Hz) a large part of the endogenous noradrenaline released is taken up by the neuron, as cocaine (5 μg/ml) produced a 260% increase in output of noradrenaline (216). Inhibition of extraneuronal uptake by corticosterone increased the output by only 30 to 40% while the effects of cocaine and corticosterone were additive. The output was also in-

creased by inhibition of COMT and MAO (100–200%) but this effect was blocked by corticosterone. The results indicate, firstly, that although neuronal uptake is of first importance, extraneuronal uptake also operates at this stimulation frequency and that there is a dynamic relationship between the two uptake processes so that when uptake into neurons is blocked extraneuronal uptake is increased as more substrate becomes available (216). Secondly, extraneuronal uptake preceeds intracellular metabolism. The relative importance of the two uptake processes under experimental conditions can be influenced by the morphology of the adrenergic system or the amount of transmitter released from the nerve endings (*i.e.*, varying stimulation frequency). The possible influence of these factors on the inactivation of noradrenaline in the sympathetic system will be discussed by reference primarily to the heart, arterial tissues, vas deferens, and nictitating membrane.

There are considerable species and tissue variations in the form of the adrenergic innervation (327). This ranges from the uniform dense innervation of the nictitating membrane of the cat through the dense but atypical innervation of the vas deferens to the sparse, very localized distribution of the terminals in the rabbit thoracic aorta. These variations influence the effects of sympathectomy. Thus, while the rat heart, spleen, and salivary gland can be totally sympathectomized by either surgical- or immunosympathectomy (5, 226, 304), the rat vas deferens is resistant to immunosympathectomy but may be surgically denervated (239). The morphological variations may also determine species and organ differences in the neuronal and extraneuronal accumulation of exogenous noradrenaline (87, 175, 177, 237, 239).

The heart has an extensive sympathetic innervation of the sino-atrial and atrial-ventricular nodes while the muscles of the atria, auricles, and ventricles have a rich but patchy innervation. In cat and guinea pig isolated atria, up to 90% of spontaneously released ^{3}H-noradrenaline consists of deaminated metabolites. On field stimulation unchanged ^{3}H-noradrenaline accounts for 60% of the increase, deaminated metabolites 40% and there is no ^{3}H-normetanephrine formed (346). When ^{3}H-noradrenaline is infused into the isolated rat heart the major metabolite recovered within 20 minutes of infusion is ^{3}H-normetanephrine (272). However, this probably represents the rapid initial metabolism of exogenous circulating noradrenaline and not that taken up by and then released from nerve endings. Further indication that removal of the released noradrenaline in the heart is by neuronal uptake comes from the findings that cocaine increases the stimulated output of ^{3}H-noradrenaline (346) and reduces the accumulation of ^{3}H-noradrenaline by over 90% (145). The ionotropic effects on rabbit atria of isoprenaline and adrenaline but not noradrenaline are potentiated by U-0521 (173). Kaumann (260) concluded that in the cat heart neuronal uptake is of first importance but extraneuronal uptake and subsequent metabolism is also an effective way of limiting the concentration of noradrenaline at the receptors especially when the concentration is high. In agreement with this is the observation that exogenous noradrenaline is accumulated by vascular muscle and fibroblasts of the isolated guinea pig atria (236) both of which contain COMT (235, 420). The absence of O-methylated and the presence of deaminated metabolites on stimulation of the cat and guinea pig heart (346) indicates that neuronal uptake in this organ is followed by retention and deaminative metabolism while there is no intraneuronal O-methylation (126). There are, however, species differences in the metabolism of ^{3}H-noradrenaline since ^{3}H-normetanephrine accounts for 40 to 50% of the spontaneously released noradrenaline in the canine heart (100, 340).

In contrast to the heart the rabbit ear artery and thoracic aorta have a marked asymmetrical innervation (327, 418) in that adrenergic terminals are exclusively

located in the adventitia. The accumulation of ^{3}H-noradrenaline occurs in the adventitia, where it is probably neuronal (61, 403), and in the smooth muscle cells of the media (60). There is also a transmural distribution of COMT and MAO with virtually all the COMT and 3.7 times as much MAO in the media compared to the adventitia (418, 420). The asymmetry of the rabbit ear artery is reflected in the difference in pharmacological response to exogenous noradrenaline applied intra- or extraluminally (279, 280). Intraluminal application results in a relative failure to reach the sympathetic nerve terminals in the adventitia in comparison with extraluminal application. There is no difference in the response to exogenous noradrenaline in the saphenous vein of the dog when the agonist is applied intra- or extraluminally (334). The asymmetry of the adrenergic innervation also affects the relative importance of intra- and extraneuronal uptake in the removal of exogenous noradrenaline from the biophase. However, the relatively greater importance of extraneuronal uptake and subsequent O-methylation in the media (290) does not necessarily indicate that extraneuronal uptake is important in the removal of endogenous noradrenaline from the region of the receptors as it is most likely to be removed by structures within the area into which it is released, *i.e.*, the adventitia. Thus in isolated media the major metabolite formed from ^{3}H-noradrenaline is ^{3}H-normetanephrine and in the adventitia 3,4-dihydroxyphenylethyl glycol (288), while in the isolated intact aorta comparable amounts of both metabolites were formed. The effects of exogenous noradrenaline on the contraction of rabbit aortic strips gives further evidence that neuronal uptake and O-methylation occur independently (290, 411). Cocaine and tropolone potentiate the effects of noradrenaline and the effects of tropolone are the same in cocaine pretreated or untreated strips and similarly the effects of cocaine are independent of tropolone. Furthermore inhibition of MAO doubled the tropolone-induced potentiation of the noradrenaline response but had no effect on that of α-methyl noradrenaline which is not a substrate for MAO (290). The inhibition of extraneuronal uptake also blocks the subsequent potentiation of the effects of exogenous noradrenaline by COMT inhibition (248, 249, 251, 290) and reduces the recovery of labelled O-methylated metabolites formed from ^{3}H-noradrenaline (433), with the implication that MAO and COMT function at the same extraneuronal site probably within the media.

With the oil immersion technique the maximum relaxation time of arterial tissue after addition of noradrenaline to the bath occurs in the presence of tropolone which lead to the conclusion that extraneuronal uptake and subsequent O-methylation was the major means of removing noradrenaline from the receptors (258). An alternative explanation is that increased accumulation at extraneuronal sites when COMT is inhibited results in the subsequent *efflux* of noradrenaline back into the region of the receptors so prolonging the state of contraction (408) and that under these conditions rate of relaxation is not the true rate of removal of the agonist.

There is another feature of aortic tissue that may influence the removal of noradrenaline under physiological conditions. The neuromuscular gap in the adventitia of the pulmonary artery is estimated to range between 1900 Å and 4000 Å compared to 300 Å in the cat nictitating membrane (331). Neuronal uptake of an agonist in the biophase decreases as the neuromuscular gap size increases (417). This may influence the removal of noradrenaline released from the nerve terminals that reaches the region of the postsynaptic receptors as extraneuronal uptake site may be more readily accessible than neuronal sites. This raises the possibility that extraneuronal uptake and subsequent metabolism in the adventitia may be physiologically more important than present experimental methods indicate.

The vas deferens has a rich atypical adrenergic innervation (327) consisting of short-postganglionic fibres. A proportion of the COMT activity in the rat and rabbit vas deferens is intraneuronal (239) and the total COMT activity is high (87, 239). In the rat vas deferens, however, when the noradrenaline stores are labelled with ^{3}H-noradrenaline there is no spontaneous or stimulated release of ^{3}H-normetanephrine though when MAO is inhibited there is a shift from deamination to O-methylation. In the rat O-methylation is probably intraneuronal as there is no apparent extraneuronal uptake of exogenous noradrenaline into the smooth muscle of the vas deferens though there is in the mouse (87). Field stimulation (2 and 16 Hz) of the isolated rabbit vas deferens increases the output of endogenous noradrenaline and this output is further enhanced by the addition of corticosterone (5 μg/ml) (216), an inhibitor of extraneuronal uptake (228). Furthermore, the increased output caused by the inhibition of COMT and MAO was seen after cocaine but not after corticosterone pretreatment indicating that in the rabbit vas deferens extraneuronal uptake occurs and this preceeds O-methylation while intraneuronal O-methylation of endogenous noradrenaline released by stimulation does not occur (*cf.* rat). The effect of corticosterone is enhanced in tissues pretreated with cocaine and *vice versa* and thus both neuronal and extraneuronal uptakes of endogenous noradrenaline released by stimulation operate simultaneously in the rabbit vas deferens. Whether the relative importance of the two processes alters during physiological changes in the firing rate of the neurons is not known.

Studies on the metabolism of noradrenaline in the vas deferens contain many conflicting results that are species based. There is a need for a new comparative study on species variations in both extraneuronal uptake, its occurrence or non-occurrence in relation to extra- and intraneuronal O-methylation and the effects of field stimulation at various frequencies on the overflow and metabolism of labelled and endogenous noradrenaline. If the absence of extraneuronal uptake in smooth muscle in the rat vas deferens (87) can be confirmed this would be an excellent organ for studying the control of neuronal uptake, retention, and metabolism.

The nictitating membrane is another tissue with a dense adrenergic innervation (327) in which some COMT is intraneuronal (240). O-Methylation appears to occur at both intra- and extraneuronal sites. Thus there is a good correlation, after nerve stimulation, between inhibition of neuronal uptake and a reduction in ^{3}H-O-methylated deaminated metabolites collected after labelling the noradrenaline stores with ^{3}H-noradrenaline indicating their intraneuronal formation subsequent to neuronal uptake of ^{3}H-noradrenaline (283). Field stimulation (10 shocks/sec) of the cat nictitating membrane also increases the spontaneous release of ^{3}H-normetanephrine (282) and the changes in amount released can be related to the degree of inhibition of extraneuronal uptake (283), although as extraneuronal uptake was inhibited with phenoxybenzamine the result needs to be confirmed with another inhibitor. The spontaneous release of ^{3}H-normetanephrine from the cat nictitating membrane is not influenced by either cocaine or phenoxybenzamine at concentrations of the drugs that influenced the output of the metabolite during stimulation (283). Similarly the recovery of O-methylated deaminated metabolites released spontaneously was unaffected by cocaine. The indication is that O-methylated metabolites released spontaneously are the products of intraneuronal metabolism of noradrenaline, while O-methylation is the primary inactivation mechanism extraneuronally.

Results with the cat nictitating membrane show that during stimulation intra- and extraneuronal uptake and metabolism occur. The outflow of labelled noradrenaline and its metabolites is increased when the membrane is stimulated at 3 shocks per

second and this effect is enhanced by increasing the shock frequency to 25 shocks per second. Cocaine (0.3 μg/ml) enhances the overflow produced by the lower frequency without modifying its metabolism but has no effect on the overflow at the higher frequency. The overflow is increased and metabolism is inhibited at both frequencies in the presence of phenoxybenzamine (282). One interpretation of this result is that at higher stimulation frequencies, when more transmitter is released, the release of noradrenaline is greater than can be dealt with by neuronal uptake so more is taken up and metabolized at extraneuronal sites. An alternative explanation is that when neuronal uptake is operative the concentration of released noradrenaline in the synaptic gap does not reach the threshold level that causes inhibition of the presynaptic *alpha*-receptors so triggering the negative feed-back mechanism for the control of transmitter release (147). Cocaine, on the other hand, by blocking neuronal uptake increases the concentration of transmitter at the presynaptic receptors and triggers the feed-back control without an increase in the amount of noradrenaline released. Phenoxybenzamine also inhibits uptake and so increases the concentration of transmitter in the synaptic gap. This increase, however, fails to trigger the negative feed-back as the drug is an antagonist of the presynaptic *alpha*-adrenergic receptors. The result of this is that the stimulated increase in transmitter is exaggerated. The effects of altering the amount of transmitter released by changing the stimulation frequency is another aspect of the relationship between neuronal and extraneuronal uptake that requires reassessment with inhibitors of extraneuronal uptake other than phenoxybenzamine.

The high frequency used in Langer's experiments (282) is above the physiological range but it is possible that at relatively high levels of physiological stimulation the amount of noradrenaline released results in increased removal by extraneuronal uptake relative to neuronal uptake especially in sparsely innervated tissues (*e.g.*, rabbit thoracic aorta).

As indicated, in the nictitating membrane both intra- and extraneuronal O-methylation occur and the O-methylation is of presynaptic origin during spontaneous release while normetanephrine is formed postsynaptically during stimulation (283). In the salivary gland, another tissue that contains intraneuronal COMT (81, 304), reserpine increases the formation of O-methylated noradrenaline metabolites. Although Jonasson (244) concluded that these were of extraneuronal origin they may be the result of the intraneuronal O-methylation of noradrenaline released by reserpine from vesicles. There is a reduction in the activity of the enzyme in the salivary gland 18 hours after reserpine treatment (5 mg/kg), which is after the depleting action of reserpine but before the restoration of vesicular retention. The degree of reduction is the same after atrophy of extraneuronal cells as after sympathectomy thus indicating that reserpine affects both extra- and intraneuronal COMT (81).

VIII. O-Methylation of Catecholamines in Central Nervous System

A. Dopamine

The deaminated metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), the deaminated O-methylated metabolite homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid, HVA) and the intermediate 3-methoxytyramine (3-MT) are the only endogenous metabolites of dopamine formed in appreciable amounts in the CNS both *in vivo* (8, 95, 351) and *in vitro* (359) (fig. 5). Homoisovanillic acid (3-hydroxy-4-methoxyphenylacetic acid) has been identified in human cerebrospinal fluid (CSF) (308) and the intermediate 4-methoxytyramine recovered from human urine after L-dopa treatment (329). 3-Methoxy-4-hydroxyphenylethanol (MHPE) has not been detected in human CSF (431) although Goldstein and Gerber (184) demonstrated the conversion of dopa to MHPE in

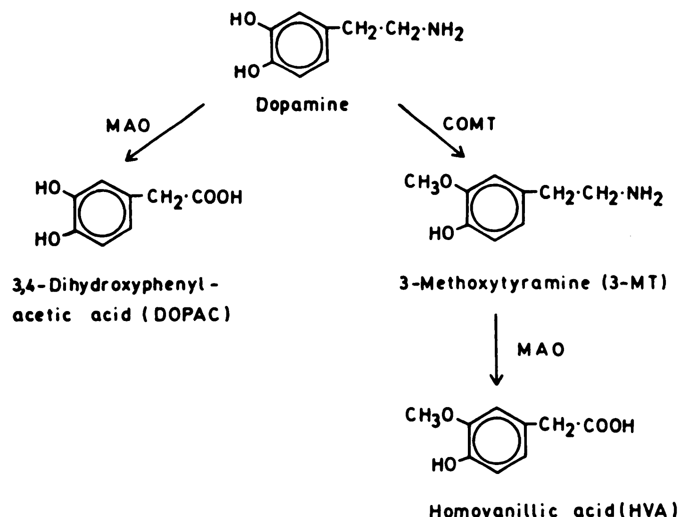

FIG. 5. The main metabolites from dopamine in brain. The abbreviations used are: MAO, monoamine oxidase; COMT, catechol-O-methyl transferase.

rat brain. In rabbit cerebral slices 81% of total metabolized ^{14}C-dopamine was recovered as DOPAC and HVA in the ratio of 2:1 while in the caudate nucleus, an area with high endogenous dopamine levels (214), the amount of HVA formed was only 7% that of DOPAC and the formation of 3-MT only 5% of that in the cerebral cortex (359). The endogenous levels of DOPAC and HVA in mouse striatum, however, are almost identical to one another (347). It is possible that *in vitro* both 3-MT and DOPAC act as precursors of HVA with 3-MT being the precursor at early time periods when levels were low and DOPAC when levels are higher (360).

B. Noradrenaline

Less is known about the endogenous metabolism of noradrenaline than dopamine in brain and neither 3-methoxy-4-hydroxymandelic acid (VMA) nor 3,4-dihydroxymandelic acid (DOMA) have been found *in vivo* in appreciable concentrations. The formation of normetanephrine (NM) in brain was demonstrated some time ago (197). ^{14}C-Noradrenaline injected into the lateral ventricle of the cat was metabolized to free and conjugated 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG) and 3,4-dihydroxyphenylethylene glycol (DOPEG) but there were only traces of the latter (299). By the use of gas chromatography, the endogenous occurrence of free MOPEG (368) and DOPEG (378) have been shown in CNS of several species (see fig. 6). When ^{3}H-NM was injected there is evidence that MOPEG was further metabolized to the sulphate conjugate (369) although there are species variation in the ratio of free to conjugated MOPEG. In man, rat, and guinea pig only the conjugate was found while in the cat and mouse the dominant form was free MOPEG (369). The levels of endogenous free DOPEG in the hypothalamus of the rabbit were similar to those of free MOPEG found in the cat and mouse (378).

The formation of DOPEG from either ^{3}H-noradrenaline or ^{3}H-dopamine *in vitro* in cerebral slices was twice that of MOPEG but sulphate conjugates were not measured (359). It is possible that when cerebral slices are used O-methylation may be limited by the availability of S-AMe and this could result in an abnormally high DOPEG/MOPEG ratio (397). Intraventricular injection of ^{14}C-noradrenaline in the rat produced the sulphate conjugates of DOPEG and MOPEG (397). The pool of the DOPEG-sulphate conjugate is suffi-

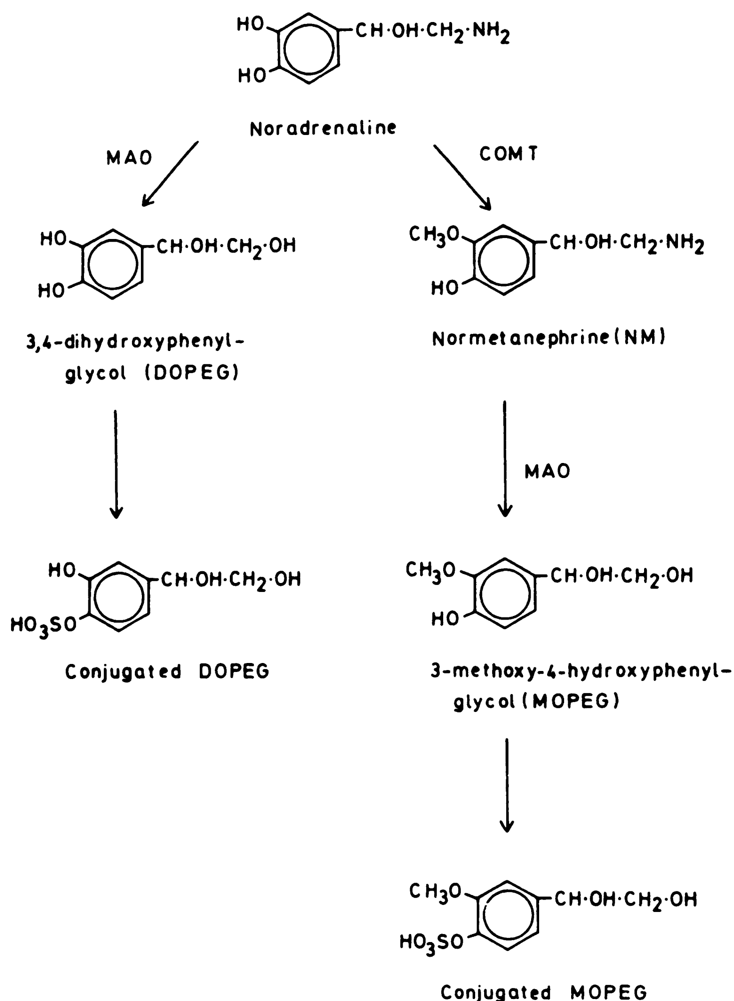

FIG. 6. The main metabolites from noradrenaline in brain.

cient to suggest deamination without subsequent O-methylation as a significant factor in the metabolism of exogenous noradrenaline in the CNS. The predominance of conjugated rather than free glycols is probably related to the high lipid solubility of the unconjugated form. MOPEG is the principle metabolite of brain noradrenaline recovered in the urine of the dog (294) indicating that metabolism of noradrenaline to free MOPEG and DOPEG may be more important than suggested by the brain levels of the free and conjugated forms. The levels of MOPEG in the CSF have been used as an index of human brain metabolism (99, 432).

C. Adrenaline

When ^{3}H-adrenaline is administered intravenously there is considerable uptake into pineal and pituitary glands but little into CNS (37, 392). The main metabolites are metanephrine and VMA with significant amounts of the dihydroxy-deaminated metabolite. ^{3}H-Adrenaline injected into the ventricles shows somewhat different metabolism with conjugated metabolites as the major fraction (392). There is recent evidence for an adrenergic fibre system in the rat brain based on the immunohistochemical localization of phenylethanolamine-N-methyltransferase

(213) and the metabolism of adrenaline in the brain warrants further investigation.

D. Function of COMT in Central Nervous System

Few studies have employed COMT inhibitors to investigate the function of the enzyme in CNS because of their relatively poor *in vivo* inhibition of COMT, their small effect on brain catecholamines although marked reductions in O-methylated metabolites have been reported, and the large dose required to produce an effect (see section VII for details).

Stimulation of the substantia nigra after the injection of ^{3}H-dopamine into the ventricle increased the efflux of both ^{3}H-dopamine and ^{3}H-3-methoxytyramine while stimulation of the caudate nucleus, *i.e.*, the area containing the terminals rather than the cell bodies, increased ^{3}H-dopamine but not ^{3}H-3-methoxytyramine (422). Thus stimulation of the dopamine-containing nerve cells released the amine from the terminals and implicates COMT in the inactivation of dopamine, while direct stimulation of the caudate nucleus caused a nonspecific release of dopamine. Tropolone infused together with ^{14}C-tyrosine into the caudate nucleus, using a push-pull cannula, transitorily increased the resting release of ^{14}C-dopamine, decreased that of ^{14}C-homovanillic acid and, after a 50-minute delay, decreased the output of ^{14}C-3,4-dihydroxyphenylacetic acid (343).

3-Methoxytyramine is an intermediate metabolite but experiments indicate no major metabolism of 3-methoxytyramine other than by MAO, so the accumulation of 3-methoxytyramine after MAO inhibition can be taken as a measure of the rate at which dopamine is O-methylated (195). However, this approach suffers from a major drawback in that only after drugs which increase the rate at which dopamine is O-methylated will changes in 3-methoxytyramine levels be detected; for if a drug reduces this rate the slow rate of removal of 3-methoxytyramine from the brain will mask the effect. This is probably also true for normetanephrine formed from noradrenaline in brain. Inhibition of MAO elevated brain and striatal 3-methoxytyramine levels of several species (92, 195). Amphetamine, which releases catecholamines, markedly increased ^{3}H-normetanephrine levels in the brain (180) and endogenous normetanephrine and 3-methoxytyramine concentrations in nialamide pretreated rats (371). The D- and L-isomers are equally active on noradrenaline metabolism whereas D-amphetamine is more active on dopamine metabolism (371). Furthermore, neuroleptic drugs which block dopamine and/or noradrenaline receptors also increased 3-methoxytyramine and to a lesser degree normetanephrine levels—for example pimozide, a dopamine receptor blocker, increases 3-methoxytyramine only (372). Chlorpromazine, which blocks both dopamine and noradrenaline receptors, increases both methoxy amines (92, 195, 372). Drugs which inhibit neuronal uptake, however, have no significant effect on the O-methylated metabolites (372). These results indicate the role of O-methylation in the enzymatic inactivation of both noradrenaline and dopamine released from nerve terminals, although others have concluded that O-methylation is not a deactivating process (98).

Extraneuronal uptake of ^{3}H-normetanephrine and ^{3}H-noradrenaline occurs in rat cortex and striatum even though there were dissimilarities with peripheral extraneuronal uptake (205). Rabbit brain glial cell fractions accumulate noradrenaline and dopamine, in common with neuronal and synaptosomal fractions prepared from the same animal (206). It has been suggested that HVA and DOPAC are not formed at the same site in the striatum and that DOPAC is normally not converted to HVA to any great degree. Since reserpine increased DOPAC after the release of dopamine, the deaminated acid appears to be formed at an intraneuronal site (347). In

contrast, the onset of stereotyped behaviour in reserpine-treated rats coincided with a rise in HVA and preceded the rise in DOPAC (194), suggesting HVA formation is extraneuronal. However, it is suggested that HVA is formed at two sites. The site blocked by probenecid is extraneuronal while some other site is insensitive to probenecid (347). Probenecid-insensitive HVA may either be formed by deamination of 3-methoxytyramine following its uptake into neurones (347) or it could represent intraneuronal O-methylation followed by deamination. Similarly MOPEG may either be formed by deamination of normetanephrine or methylation of DOPEG. It is proposed that the latter glycol(free or conjugated) may be an index of intraneuronal metabolism of noradrenaline, and MOPEG in its free or conjugated form that of released amine (397). There is evidence for intraneuronal COMT in brain as one third of the enzyme activity is found in the synaptosomal fraction (section V). There may be two forms of COMT in rat brain (36). The K_m value of COMT found in the fraction precipitated with 40% ammonium sulphate differed from the K_m value of the enzyme precipitated at concentrations of ammonium sulphate between 40 and 60%. Furthermore the K_m value of the enzyme in the synaptosomal fraction differed from that in the supernatant (82). One can speculate that some of the supernatant enzyme may be the extraneuronal COMT and function in the inactivation of catecholamines released from nerve endings, while the synaptosomal COMT is found in nerve endings and collaborates with mitochondrial MAO in the metabolism of intraneuronal catecholamines. A diagram of a generalized dopaminergic synapse and the control of transmitter concentration is shown in figure 7.

IX. Clinical Aspects of COMT

Catecholamines have been implicated in several clinical conditions including hypertension (385), parkinsonism (for review see 214), and endogenous depression (264, 373). The following discussion will centre on three aspects of COMT in clinical conditions: 1) The possible involvement of catecholamine metabolism, with special reference to O-methylation, in the aetiology of disease; 2) the use of COMT activity as a diagnostic tool; and 3) COMT and the determination of drug efficacy with reference to the use of L-dopa in the treatment of parkinsonism.

A. Affective Disorders

COMT activity in human erythrocytes (27) is lower in women with primary affective disorders (history of mania, depression or both) than in controls, schizophrenics, men with primary affective disorders, and men showing antisocial behaviour (76, 107, 141). COMT activity is also lower in females with unipolar depression than in females with bipolar depressive states (depression and mania). The erythrocyte COMT activity in females with affective disorders was reduced regardless of phase of illness, degree of depression, medication and length of hospitalization, and activity was not related to age, though enzyme activity of human liver does decline after the age of 60 (2). The possibility that lowered COMT activity is sex-linked is suggested from animal studies (106), although a nutritional basis might explain these results (103).

The COMT activity in the erythrocytes of women with primary affective disorder, while not indicating the degree of severity of the illness, may reflect an underlying genetic classification. To confirm this more families with a history of endogenous depression need to be studied. Such studies will be complicated by the fact that there is some overlap between COMT activity in primary affective disorder and the control group (141).

Erythrocyte COMT was also significantly lowered in women taking oral contraceptives (oral oestrogen and progestogen) but not those taking depot progestogen or oral corticosteroids, and there was a significant fall in COMT during second

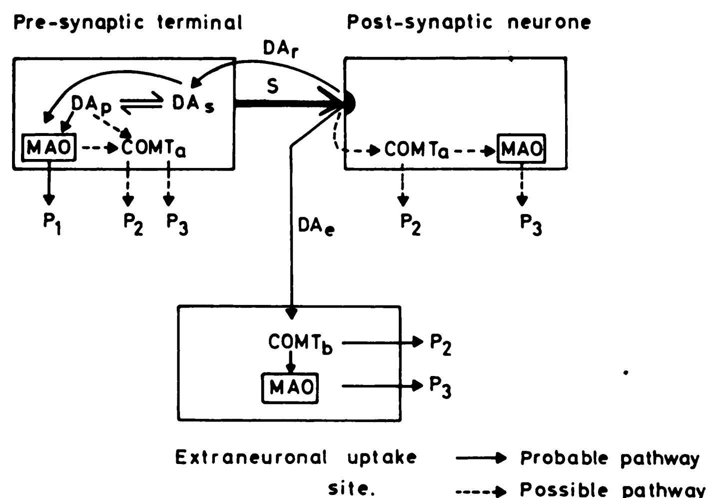

FIG. 7. A generalized dopaminergic synapse. The abbreviations and symbols used are: DA_p, dopamine (DA) in the terminal that is not found within synaptic vesicles, regulated by MAO, and DOPAC represents intraneuronal metabolism. There is probably some intraneuronal COMT. DA_s, DA that is found within synaptic vesicles and available for release on stimulation or by drugs. S, DA released from synaptic vesicles across the synaptic gap and onto the postsynaptic receptor. DA_r, re-uptake of DA by the presynaptic neuron where it may be re-used or metabolized by MAO and/or COMT. The importance of re-uptake in DA transmission has not been evaluated fully. DA_e, extraneuronal uptake (probably glial) of DA followed by O-methylation. This is probably the most important means of inactivating DA released at nerve endings. There may be subsequent deamination by MAO. Alternatively 3-methoxytyramine may leave the extraneuronal site and be taken up by the pre-synaptic neurone where it is deaminated (347). It is possible that uptake and deamination of released DA could occur within the postsynaptic neuron as MAO and COMT have a very wide distribution. P_1, DOPAC, 3,4-dihydroxyphenylacetic acid. P_2, 3-MT, 3-methoxytyramine. P_3, HVA, homovanillic acid. MAO, Monoamine oxidase. COMT, catechol-O-methyl transferase. $COMT_a$, and $COMT_b$, Possible different forms of the enzyme found at intraneuronal and extraneuronal sites (see text).

and third trimester of pregnancy and during the 7-day postpartum period. This suppression in COMT activity may be related to the depression noted in some women taking oral contraceptives and during the immediate postpartum period (76). In another limited study (189) an increase, rather than a decrease, in erythrocyte COMT was found in some women taking high doses of oral oestrogen and the authors suggested that the raised COMT activity was a consequence of its involvement in the methylation of oestradiol.

N-Butyl-gallate, an inhibitor of COMT, had no clinical efficacy nor significant side-effects in six endogenous depressives; the sex of the subjects was not given (13).

The other question that arises is whether the COMT activity reflects altered O-methylation of catecholamines in depression. It is proposed that depression is associated with a defect in catecholamine metabolism resulting in a lack of functional noradrenaline at receptor sites in the CNS (373). Measurements of cerebrospinal fluid amine metabolites as an index of brain catecholamine metabolism have, however, produced conflicting results (312, 432). The catecholamine hypothesis, though attractive, is hard to verify directly in man.

An increased plasma noradrenaline concentration was reported in 1971 in depressed patients (10 females and 3 males, 2 of whom showed only very small elevation in plasma noradrenaline) and in reviewing the literature the authors concluded that "noradrenaline excretion, noradrenaline content in plasma, spinal fluid, and brain specimens are not reduced in patients with depression, and in fact may be elevated" (439). Others found elevated metabolic products, after infusion of tritiated ^{3}H-

noradrenaline, in the urine of bipolar depressives compared with the values from unipolar depressives (350). The occurrence of elevated plasma noradrealine but normal blood pressure in patients with affective disorders has led to the suggestion that depression is associated with decreased catecholamine receptor sensitivity (439).

COMT in the peripheral system is closely associated with the effector cell (see section VII), the extent to which this applies to the CNS needs further study. If erythrocyte COMT, which is membrane bound *in vivo* (20), reflects the situation at CNS receptors, the decline in erythrocyte COMT activity of females with primary affective disorders may further indicate receptor inactivity rather than defective catecholamine metabolism as a biochemical basis of depression. Conversely, reduced COMT activity may be compensation for reduced receptor activity so increasing the availability of active noradrenaline (107).

There is a similarity between the cyclic oxidative noradrenaline product, noradnamine, and the iminodibenzyl antidepressant derivatives except that the former contain catechol groups and produce hypoactivity (245). O-Methylated intermediates will not undergo cyclisation and Roberts and Broadley (344) suggested that a malfunction of COMT is the main cause of psychotic depression with a concomitant excessive production of noradnamine. There is, however, no evidence that noradnamine formation occurs *in vivo*, though both noradnamine and adnamine inhibit COMT activity *in vitro* (1).

Lithium chloride, used in the treatment of mania, *in vitro* has no effect on either COMT or MAO activity but the intracisternal administration of ^{3}H-noradrenaline to lithium chloride-treated rats shifts metabolism form O-methylation to deamination; this suggests increased intraneuronal noradrenaline metabolism thus reducing noradrenaline available at the receptors (370, 432).

B. Dimethoxy Derivatives in Mental Illness

3,4-Dimethoxyphenylethylamine. The evidence for a biochemical malfunction in schizophrenia was reviewed by Boulton (72) and only particular aspects will be discussed here.

Since the original report in 1963 on the occurrence of 3,4-dimethoxyphenylethylamine (DMPEA) in the urine of schizophrenics and the *in vitro* conversion of dopamine to DMPEA in the liver of schizophrenics (73, 167, 168), the uniqueness of DMPEA to schizophrenics has been challenged (326, 398). Boulton (72) concluded that the occurrence of DMPEA in urine was not unique to schizophrenics and further stressed the need for sensitive identification methods and the importance of diet and medication. Another factor could be the development of tolerance in man to psychotomimetic compounds necessitating the use of 10- to 80-day studies on the output of urinary DMPEA (or other hallucinogens) as they will be produced at varying rates (247). COMT can O-methylate in both the para- and meta-position (275, 376) but will not demethylate dopamine *in vitro* (274) although the formation of dimethylated dopamine metabolites *in vitro* has been reported (166). COMT shows no significant change in schizophrenics (141, 275), and the COMT inhibitor, N-butyl-gallate, had no beneficial effect on 11 patients with schizophrenia when given for 12 weeks at doses of 250 to 5000 mg daily (384). A more rewarding approach to the study of possible aberrations in O-methylation in schizophrenia may be the measurement of S-AMe. In brain S-AMe values are raised by methionine administration (102), and decreased by methyl acceptors, certain MAO inhibitors and imipramine, all of which favour O-methylation (43, 44). Another approach is to study the methionine-activating enzyme (108).

DMPEA produces akinesia, tremor, and

other behavioural abnormalities in animals (77, 381, 389) but there is no evidence for the involvement of abnormal DMPEA production in the aetiology of parkinsonism. In 1972 aberrant 4-O-methylation of dopamine to 3-hydroxy-4-methoxyphenylacetic acid (HMPEA), which also produces hypokinesia and tremor (77), has been suggested to be involved in parkinsonism (55). HMPEA is a normal but minor metabolite of dopamine as it is found in CSF (308) and in the urine after L-dopa treatment (329). In Parkinson's disease the urine levels of HMPEA are reported to be consistantly higher than in controls (110). Excessive HMPEA may accelerate caeruloplasmin-catalysed oxidations of dopamine so reducing striatal dopamine (55). The fundamental lesion could be in the activity of COMT although at present there is no evidence that COMT inhibition causes any striking improvement in Parkinson patients. It has been suggested that the O-methylated derivatives, DMPEA and HMPEA, are involved in the pathogenesis of both parkinsonism and schizophrenia (388).

One of the side effects of L-dopa treatment is depression and, in a few cases, delirium which could be related to excessive O-methylation in 4-position (364). 3-Hydroxy-4-methoxyphenylacetic acid is found in the urine after ingestion of L-dopa at about 10% of the value for 4-hydroxy-3-methoxyphenylacetic acid (329).

C. COMT and the Clinical Use of L-Dopa

The advent of L-dopa in the treatment of parkinsonism has had an important impact on its clinical treatment and biochemical study (89, 214). The use of L-dopa, however, has been somewhat hindered by its side-effects and the high doses needed to obtain adequate brain levels.

Large doses of L-dopa could radically alter catecholamine metabolism by an effect on turnover or product inhibition, and it is interesting that tyrosine hydroxylase and dopa decarboxylase show a decline in activity during prolonged L-dopa treatment (428). L-Dopa is degraded by decarboxylation or O-methylation and peripheral decarboxylase inhibitors have been used successfully to increase the efficacy of L-dopa (89).

The effective dose of L-dopa can sometimes be reduced during a period of treatment and this could be related to a decrease in the O-methylation of L-dopa or an increase in dopamine formed from L-dopa. Hence the inhibition of COMT and the depletion of the S-AMe by L-dopa may increase the effectiveness of L-dopa therapy. The COMT activity is reduced 28 to 40% in erythrocytes of patients and rats undergoing chronic but not acute L-dopa treatment (164, 278, 428). There is, however, no fall in rat erythrocyte COMT activity after a 52-week diet containing 1% L-dopa, and only a small decrease in the liver and kidney occurred (379). The effect on COMT activity is the same whether L-dopa is given alone or in combination with a dopa decarboxylase inhibitor, thus excluding the suggestion that the effect on COMT reflects the weak inhibition of COMT by the dopa decarboxylase inhibitor, L-α-methyldopa-α-hydrazine (MK 486) (45). COMT is not inhibited directly by L-dopa (428) but possibly indirectly by depleting tissue concentrations of the methyl donor S-AMe (97, 438) and inhibiting methionine uptake, which may reduce the synthesis of S-AMe (49) and hence reduce COMT activity. In 1971 Ericsson (149) gave the COMT inhibitor GPA-1714 (N-butyl-gallate) to patients on L-dopa therapy and was able to reduce the dose of L-dopa neceseary to alleviate the primary symptoms of parkinsonism. The effect was not as striking as that of L-dopa given with a dopa decarboxylase inhibitor. The problem, however, is to find suitable inhibitors of COMT without unacceptable side-effects or high toxicity. All competitive inhibitors of COMT also deplete tissue S-AMe levels (43) which is an additional problem as this produces general inhibition of tissue methylation reactions.

The possible use of COMT inhibitors to increase the efficiency of L-dopa treatment requires further studies on the long term biochemical effects of COMT inhibition especially whether inhibitors effective under acute conditions are also suitable for chronic administration.

D. COMT and Hypertensive Disease

An early suggestion that essential hypertension was caused by COMT deficiency (313) was not substantiated by studies on the excretion of catecholamines and their methoxy-metabolites (385, 395). Further it was shown that the metabolism of infused isoprenaline was unchanged in essential hypertension (385).

There are several animal models of human essential hypertension including: 1) the renal hypertensive rat; 2) the salt loaded deeoxycorticosterone (DOCA)-treated rat; and 3) the genetically determined, spontaneously hypertensive rat. It is essential to find which of the models most closely resemble, biochemically and pathologically, the human essential hypertensive state. Noradrenaline turnover is increased in the renal hypertensive rat (423) and indicates increased activity of the sympathetic nerves. In contrast the genetic hypertensive rat, in common with human essential hypertension, shows an increase in blood pressure with age, normal endogenous noradrenaline concentration in the heart but increased retention of ^{3}H-D,L-noradrenaline (i.v.) 24 hours but not 5 minutes after injection of this catecholamine (292). The increased retention may represent decreased release of noradrenaline from sympathetic nerve endings or decreased metabolism, though COMT activity of the heart, liver, kidney, and brain of the spontaneously hypertensive rat is markedly increased (118). The increase was found in the prehypertensive state and during hypertension, implying there is either increased inactivation of catecholamines by COMT and/or reduced neuronal uptake.

In both the renal and the genetic hypertensive rat the sensitivity of the mesenteric arteries to noradrenaline is increased, in proportion to the elevation in blood pressure. There was no further increase after cocaine or denervation indicating that the hypertensive effects are prejunctional and involve impaired neuronal uptake with resulting increased receptor activity (196). The increased COMT (118) activity in the spontaneously hypertensive rat may be a compensatory mechanism for the decreased neuronal uptake. The apparent prejunctional action, however, could reflect a reduction in the diffusion of exogenous noradrenaline through the thickened arterial wall which is a pathological feature of hypertension.

When using animal models, however, any interpretation of results must consider species and intraspecies variation in the structure and adrenergic innervation of different vascular smooth muscle (391). These differences may determine the relative importance of O-methylation and neuronal uptake in the inactivation of catecholamines (see section VII).

Hydrocortisone potentiates the response of the rabbit aortic strip to catecholamines and the potentiation is not seen after giving inhibitors of COMT (U-0521, tropolone, pyrogallol) (248). Kalsner (248) concluded that the potentiating action of hydrocortisone was due to inhibition of O-methylation though a more likely explanation is the inhibition of extraneuronal uptake (228) and thus subsequent O-methylation. This may contribute to the enhanced sensitivity of smooth muscle to catecholamines during prolonged hypercorticism. Other steroids, such as corticosterone and aldosterone, may also interfere with the inactivation of catecholamines by O-methylation (249) by inhibiting extraneuronal uptake of neuronally released noradrenaline (228).

E. COMT and Tumors

There was a marked rise in urinary catecholamines and 3-O-methylated and 4-O-methylated metabolites in neuroblastoma (110, 395) and phaeochromocytoma

(125). This elevated urinary excretion fell during clinical improvement (424). In neuroblastoma both *p*- and *m*-O-methylation occurred (329) as both methoxy forms were found in urine with about 5% of the total metabolism being in the para form. The nature of the metabolites found with different tumours may be of diagnostic value in distinguishing types of tumour and indicate whether a particular tumour is benign or malignant. There is evidence of COMT activity in neuroblastomas (276, 277).

X. Conclusions

Catechol-O-methyl transferase (COMT) research has moved from strict enzymological examinations through pharmacological investigations on inhibitors of the enzyme to a limited number of applied studies in man. This review has dealt with COMT distribution, assay properties and reaction kinetics, inhibitors and their pharmacology, the function of COMT in adrenergic transmission, and, finally, certain clinical aspects.

Although the different methods of COMT determination have utilized the same principle, namely the transfer of methyl groups to catechols in the presence of co-substrates, and most frequently have centered on a radiochemical mode of end product detection, we often have found it difficult to compare data obtained in different laboratories prinicpally because of lack of standardization of methods; this is linked with the problem of defining the ideal conditions for the enzyme reaction in a given experimental situation.

In future work steps towards standardizing the method must be guided by such factors as: the most suitable type of tissue preparation, limiting the choice of substrate, the use of a known substrate concentration of S-AMe and an optimal concentration of Mg^{2+}, and strictly defining the incubation medium with its ionic strength and composition of ions, pH, temperature, *etc.* To date, methods used for studying COMT have been based primarily on obtaining information inherent in its *in vitro* biochemical properties, but methods may have to be modified significantly in order to obtain adequate *in vivo* information on the enzyme as, for instance, in studies where one is concerned with its role in physiological or pathological states.

Despite the broad substrate specificity of COMT, all known substrates have a catechol configuration. The factors which determine *m*- and *p*-O-methylation have been elucidated and future work may provide information on the mode of substrate/enzyme interaction at receptor sites and the relation between aberrant O-methylation and abnormal function.

The kinetic data on COMT reported in the literature show considerable variation. An extreme example illustrating this is the K_m values for noradrenaline reported from different laboratories of 6×10^{-6} M and 1.6×10^{-3} M. Often in the analyses of kinetic data it has not been possible to disentangle the host of factors which might explain the varied results, and for the reviewer the lack of methodological standardization has been frustrating. Most commonly the variations could not be ascribed to innate biological properties of the enzyme.

Because of the relatively low COMT activity and instability during purification procedures, there are few studies on properties of purified enzyme. Fractionation with partial purification of the enzyme has provided some evidence for the occurrence of isoenzymes or at least different functional states of COMT in some tissue preparations. The heterogenity of COMT in relation to its biological significance should provide a challenge for future research.

The wide distribution of COMT in animals and plants and its highest activities in organs which possess detoxicating mechanisms probably reflects its role in biotransformation of certain biologically active substances. In relation to neuronal tissues, the most recent evidence indicates intraneuronal as well as extraneuronal COMT. Furthermore, subcellular distribu-

tion studies indicate the presence in addition to the well established "soluble" fraction of COMT, a membrane bound fraction in red blood corpuscles and a microsomal fraction in the liver. COMT is probably best described as a "cytoplasmic" enzyme which can be membrane bound.

In assessing the relative importance of uptake mechanisms and enzymatic breakdown of catecholamines in their inactivation in the peripheral and central nervous system, a number of factors such as the species, type of tissue, the type of sympathetic innervation, the morphology of the adrenergic innervation and dissimilarities between peripheral and central synapses must be assessed carefully. In the past, most schemes denoting the physiological function of COMT in the metabolism of catecholamines have placed the enzyme merely at an extraneuronal site whereas recent evidence indicates that there is intraneuronal COMT in some tissue. Whether intraneuronal COMT has any specialized function is not known at present. However, the close connection between extraneuronal uptake and O-methylation has been demonstrated repeatedly and their causal relation stresses the importance of not undermining the one when the other primarily is under study.

A real problem in the study of the function of COMT in adrenergic mechanisms is the lack of suitable inhibitors of the enzyme. A number of classes of COMT inhibitors have been listed in this review and many of them adequately inhibit the enzyme *in vitro* but apparently are poor inhibitors *in vivo*. After an initial search for COMT inhibitors there has been no systematic program for the development of COMT inhibitors with improved pharmacological properties in the past decade.

The high toxicity of most inhibitors of COMT make them quite unsuitable for clinical use. Recently, a COMT inhibitor, gallic acid which occurs naturally in foods, was administered to patients. This was in two clinical situations in which there was a rationale for the uses of this kind of drug, namely, patients with Parkinson's disease on L-dopa therapy and patients with certain forms of mental depression, although the latter group did not receive conventional antidepressive drug therapy. The future use of COMT inhibitors in man must await either the more thorough preclinical testing of existing inhibitors or, more likely, the development of entirely new drugs. In order to avoid the toxicity arising from the general inhibition of this widely distributed detoxicating enzyme in the body, selective inhibition of, for instance, particular forms of the enzyme, with different physicochemical properties, might lead to COMT inhibitors with acceptable pharmacological properties for clinical uses.

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