ENZYMES AS PRIMARY TARGETS OF DRUGS[1]

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The concept of enzymes serving as primary targets for drugs evolved from the classical investigations on physostigmine and cholinesterase. Carbonic anhydrase and certain sulfonamides provide another example; the latter helped in gaining a better understanding of the function of carbonic anhydrase in various organs [Davenport (1)].

Approximately 10 years ago, when the antitubercular substance iproniazid (1-isonicotinyl-2-isopropylhydrazine; Marsilid) was found to block monoamine oxidase efficiently *in vitro* [Zeller, *et al.* (2)] and *in vivo* [Zeller & Barsky (3)], another group of enzyme-regulating drugs emerged. It was expected that "iproniazid may well become of the same importance for the study of the adrenergic system as has eserine in the analysis of the cholinergic system" (3). At the present time, intensive work is carried out on pharmaca which appear to act predominantly on amino acid decarboxylases.

In this review only two groups of target enzymes are discussed, cholinesterases and monoamine oxidases, which so far have been the most thoroughly studied. Even with this severe limitation relatively few papers can be presented. We favored the ones that stressed the enzymological aspects and thus contributed to a better insight into the mode of action of certain drugs.

DRUGS AS INHIBITORS OF THE CHOLINESTERASES

This review will not attempt to catalogue the vast number of compounds which can inhibit the various cholinesterases. Even if one were to limit the literature search to the last four years (1958 to 1961), as has been done, the simple listing of new anticholinesterases would be too great a task. This review has the specific goal of considering at least some of the compounds whose actions as cholinesterase inhibitiors can reasonably be the basis for their pharmacological effects in animals. To be included in this review, a research report must have made some effort to correlate cholinesterase inhibition *in vitro* or *in vivo* with the observed effects of drug administration to animals. This does not imply criticism of studies in which such correlations were not made—the reviewer has made a selection of which the reader should be aware.

As criteria for deciding whether cholinesterase inhibition can be the basis for a drug's pharmacological actions, most researchers knowingly or unknow-

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ingly use the statements on pages 90 to 91 of the review by Hunter & Lowry (4). It might be worth while to re-emphasize the importance of correlating enzyme inhibition and pharamacological actions in the same tissue. There are several tissues in the body which contain *e*-cholinesterase (true cholinesterase), yet subtle differences in these *e*-cholinesterases are probable. These enzymes may all have identical "active centers" although they may differ in the manner of folding of their peptide chains, or even in amino acid sequence or composition of these peptide chains. Such differences may not measurably change the physical or chemical properties of the enzyme, or these variations might affect things like enzyme-substrate, enzyme-cofactor, or enzyme-inhibitor interactions. It is this last possibility that seems to be pertinent. Such subtle differences in the "same" enzyme from different tissues might be more important with non-competitive inhibitors than with competitive inhibitors since the non-competitive type of inhibition assumes an enzyme-inhibitor interaction occurring at sites not a part of the active center of the enzyme.

One might also mention the occurrence of isozymes—where multiple forms of an enzyme exist in the final active enzyme complex. The ratios of these multiple enzyme moieties can vary from one tissue to another (5), and might well cause different reactions between enzyme and substrate, cofactors or inhibitor. Whether isozymes of the cholinesterases exist is not presently known, but may be worth investigating.

There are a number of recent reviews of the inhibitors of cholinesterases. Among them is the twenty-four chapter volume on cholinesterases and anticholinesterases edited by Koelle (6). Another general review is that by Koelle on the physiological and pharmacological significance of cholinesterases and anticholinesterases (7). The organo-phosphorous anticholinesterases are reviewed by Holmstedt (8) and Vandekar (9). Mention should also be made of a forthcoming symposium on the anticholinesterases to be given at the 1963 meeting of the Federation of American Societies for Experimental Biology in Atlantic City, New Jersey.

Problems in the Study of Anticholinesterases

One problem in the study of enzyme inhibitors is the determination of the actual substance responsible for enzyme inhibitions. This is true whether the study is made *in vitro* or *in vivo*. In other words, is the enzyme inhibited by the drug or by one of its metabolites? Obviously, metabolism will complicate a study of structure activity relationships (SAR) since the SAR studied may be that of the metabolizing system rather than, or in addition to, that of the target enzyme. Such problems have been encountered with the anticholinesterases, particularly some organophosphorous compounds such as schradan (10) and certain O,O-dialkyl-1-acyloxyethyl phosphonates (11). The thiophosphates are cholinesterase inhibitors whose action may also depend on their metabolism (12 to 14).

A lack of correlation between *in vitro* and *in vivo* actions of a cholinester-

ase inhibitor as well as species differences in enzyme effects may be related to the metabolic fate of the inhibitor. The same possibility might be considered if the onset of action of the enzyme inhibitor is delayed either *in vivo* or *in vitro*.

Cholinesterase Inhibitors

Reversible inhibitors.—Reversible and irreversible cholinesterase inhibitors have been distinguished by the type of bonding between enzyme and inhibitor—the irreversible inhibitor would react chemically with the enzyme, while the reversible inhibitor would bind to the enzyme by much weaker bonds (hydrogen bonds, electrostatic bonds, etc.). Competition for the enzyme between substrate and inhibitor can occur with either reversible or irreversible anticholinesterases, and reversible inhibitors can protect the enzyme from irreversible inhibitors (15, 16).

In a study of cholinesterase inhibitors the reversibility of the enzyme-inhibitor complex should be investigated. The terms "competitive" and "non-competitive" should probably be restricted to reversible inhibitors since the Michaelis-Menten analyses of kinetics (wherein these terms are used) assumes a dissociable enzyme-inhibitor complex. Thus the competiton between the substrate and an irreversible anticholinesterase might best not be termed competitive inhibition—whether or not competition is observed depends on which is added first, substrate or inhibitor, and this is therefore a different situation than seen with reversible inhibitors. To use the same adjective "competitive" for phenomena which are different may cause confusion.

Studies are still being reported correlating cholinesterase inhibition with the various pharmacological actions of the well-known anticholinesterases. These studies should be encouraged since they clarify the physiological role of cholinesterase as well as phenomena wherein the anticholinesterases may be acting other than as inhibitors of cholinesterase. Takaori *et al.* (17) reports the effects of physostigmine, neostigmine, and some well-known organophosphorous anticholinesterases on intracellular potentials of cardiac fibers. Their findings indicate some effects of these drugs other than as inhibitors of cholinesterase. Haase *et al.* (18) have studied the effects of neostigmine, physostigmine and certain organo-phosphorous cholinesterase inhibitors on some mono- and polysynaptic reflexes (stretch and bending) in the cat. As with the work of Takaori *et al.* the inhibitors used are well-known, but the correlation between enzyme inhibition and pharmacological action is studied in an unusual system. Actions of edrophonium at the neuromuscular junction were correlated with cholinesterase inhibition by Katz & Thesleff (19).

Newer, apparently reversible, anticholinesterases have been described. Many of these are quaternary ammonium compounds (20 to 24) or amines (25). Interestingly, optical antipodes of one amine were found to have different combinations with cholinesterase. One antipode (+) inhibited the enzyme irreversibly while the other form (−) combined reversibly (26). Carbamoyl derivatives and carbamates are also being studied (27, 28).

Irreversible inhibitors.—Most of the irreversible cholinesterase inhibitors are organo-phosphorous or thiophosphorous derivatives. Much recent literature has appeared in abstracts of the Russian publications most of which are unavailable to many scientists. Fortunately, abstract services are performing valuable work in this area, though in some cases they fall 3 to 4 years behind. Some examples of studies by Russian workers correlating cholinesterase inhibition with biological effects of organophosphorous compounds are given in cited references (26 to 34).

The well-known organo-phosphorous inhibitors have been the subject of several recent reports. Effects of sarin on respiratory and cardiovascular systems are described in (35 to 39), while actions of diisopropyl fluorophosphate (DFP) on skin, and a vestibular syndrome induced by tabun are reported in (40 to 41) respectively. The relations between peripheral and central effects of a number of well-known anticholinesterases, including DFP and para-oxon, were studied by Schaumann (42). Barstad has studied actions of several organo-phosphorous anticholinesterases including DFP on the phrenic nerve diaphragm preparation (43). McIsaac & Koelle report an interesting use of both reversible and irreversible enzyme inhibitors (including DFP and newer quaternary organo-phosphorous compounds) to distinguish between external, internal, and total cholinesterase activity (16).

Some of the reports on newer organo-phosphorous inhibitors are essentially SAR studies including comparisons with other inhibitors such as sarin for estimating potency (44, 45). Comparisons of potency in drugs of all kinds are difficult and often misleading. *In vitro* and *in vivo* potencies can be different (46). One point often overlooked in *in vitro* work is the establishing of parallelism of the "dose-response" curves. It is questionable that one can say a certain inhibitor is several times more potent than another unless the plots of inhibition *vs*, inhibitor concentration of the two inhibitors are shown to be parallel.

Few irreversible inhibitors of cholinesterase which are not organophosphorous derivatives have been described. Whereas the mechanism of action of some of the irreversible organo-phosphorous anticholinesterases may be understood in terms of forming phosphates with essential hydroxyl groups of this enzyme, the mechanism by which nonphosphorous irreversible inhibitors act is largely unknown. The paper by Greenblat & Thoms (47) on the pharmacology of Duolite polyelectrolyte 402 and the report by Friess, *et al.* (25) are examples of studies on such irreversible enzyme inhibitors.

Conclusions

The importance of studies wherein cholinesterase inhibition cannot be related to pharmacological action of the drug should be emphasized. This lack of correlation may be an artifact and certainly calls for a study of the reasons for the finding. A compound which is a very active cholinesterase in-

hibitor *in vitro* or *in vivo* may fail to affect a given system *in vivo*. This does not allow one to say that cholinesterase has no role in the functioning of the system *in vivo*. The role of cholinesterase in the *in vivo* situation can be better assessed if the fate of the cholinesterase inhibitor *in vivo* is known—i.e., its absorption, distribution, excretion, and metabolism. This point was mentioned above (section III). The essential question: is the cholinesterase of the *in vivo* system inhibited by the inhibitor at the time when the system is still functioning normally? There are several ways to study this. One of the simplest is to remove parts of the *in vivo* system and assay for cholinesterase activity *in vitro*. This approach has many pitfalls since the process of isolating the system for a study of enzyme activity may allow an exposure of enzyme to inhibitor that would not occur *in vivo*. Histochemical techniques might therefore be more desirable if one could be certain that artifacts occurring during fixation and staining did not change the relation which existed *in vivo* between the cholinesterase and inhibitor. At present, the histochemical methods seem to be adequate for these purposes and might be used in deciding whether cholinesterase inhibition could or could not reasonably explain a given *in vivo* effect.

One other point of interest is the apparent excess of cholinesterase at most of its sites of function. The fact that inhibition of the enzyme can be proved to occur *in vivo* without affecting a given physiological function still may not answer the question of the role of cholinesterase in this function. If the enzyme is present in 100-fold excess, then any inhibition less than 100 per cent will fail to affect function. Histochemical techniques can seldom distinguish between 90 and 100 per cent inhibition of enzyme activity. It is to be hoped that newer techniques, including the use of radioisotopes, may finally permit differentiation between an inhibition that is related to drug action and one which is not. Certainly both alternatives are important.

MONOAMINE OXIDASE (MAO) AND MONOAMINE OXIDASE INHIBITORS

The stream of publications on MAO inhibitors is still swelling; the list of compounds synthesized and tested as potential MAO inhibitors is still lengthening [see Szmuszkovicz & Greig (48); Anderson, Kaminsky *et al.* (49); Green (50)], and meetings dedicated entirely to the discussion of this field are still being held. Fortunately, Pletscher, Gey & Zeller wrote a monograph on "Monoamine Oxidase Inhibitors" (51), henceforth referred to as Pletscher's monograph, which covers comprehensively the data collected between 1952 and the beginning of 1960, and includes 1389 references. In our review, primarily papers published between 1960 and the middle of 1962 are mentioned, and a few earlier ones from which new lines of thought and investigations have arisen.

A few recent data on MAO itself may serve as an introduction and basis for the subsequent discussion.

In Vitro INHIBITION

Qualitative and quantitative differences

amine oxidase [monoamine oxygen reductase (deaminating), E.C. 1.3.4] was given this name in order to separate it systematically from diamine oxidase [Zeller *et al.* (52)]. It occurs widely throughout the animal kingdom and in cells derived from all three germinal layers. While intracellularly most of the MAO activity is localized in mitochondria, it is also found in other particulate matter [de Duve, Wattiaux & Baudhuin (53)]. Since, in the course of many investigations on the substrate and inhibitor pattern, quantitative and qualitative differences appeared among oxidases of different origin, the symbol MAO does not represent a single entity, but an immensely large number of closely related catalysts [Zeller (54, 55)]. All members of this group of enzymes, called homologous (54), are characterized by the same classical equation of oxidative deamination, by their insensitivity toward unsubstituted acylhydrazides, e.g. 10^{-3} M semicarbazide, and by their predilection for certain alkylmonoamines and arylalkylamines.

Weiner measured the rate of oxidation of 10 amines, mostly arylalkylamines, in the presence of brain mitochondria and observed marked quantitative differences in the relative velocities of amine degradation when various species, including man, were tested (56). A detailed analysis of the kinetics of enzymic oxidation of five consecutive members of the homologous series of phenyl-*n*-alkylamines revealed marked differences between rabbit and beef liver mitochondria. While phenylethylamine turned out to be the optimal substrate (of this series) for the bovine enzyme, phenylethylamine and phenylbutylamine were by far the best substrates for the rabbit MAO [Sarkar & Zeller (57)]. Since the ratio of Q-values for serotonin and kynuramine was 10 times higher in the liver than in the brain of rabbits, significant differences occur between the oxidases prepared from different organs of the *same* species [Weissbach *et al.* (58)].

Judging from statistical data, the MAO levels in a given organ and species fluctuate amazingly little. In the various parts of the human brain the standard deviation of the enzyme activities amounted to less than 10 per cent [Birkhäuser (59)] and in mouse liver to less than 13 per cent (60). This consistency, however, does not mean that the MAO levels are rigidly fixed throughout the whole lifespan. On the contrary, the enzyme activity changes markedly during ontogenesis and sexual maturation [Novick (61); Schweppe *et al.* (62)] and responds strongly to the administration of certain steroid hormones (60, 62).

Role of target differences on the inhibitory efficiency.—With the data presented in the preceding section in mind, we are not surprised when we see the degree of inhibition change substantially from one enzyme source to another. It was found that harmaline was 20 times more effective on the oxidation of serotonin by guinea pig liver than on the amine's degradation by rabbit liver Long (63), and Zeller & Sarkar observed that *cis*- and *trans*-2-phenylcyclo-

propylamines blocked bovine liver mitochondrial MAO 10 times more than the rabbit and mouse liver preparations (64). Iproniazid did not lead to analogous inhibitory variations with the same enzyme preparations. These differences are not only of a quantitative, but also of a qualitative nature: under otherwise identical conditions, MAO of beef liver mitochondria responded strongly to short-chained aliphatic hydrazines while the oxidase in rabbit liver was more effectively blocked by higher homologs of *n*-alkylhydrazines [Zeller (65)]. Differences in inhibitory intensity appear also when various organs of the same species are chosen. Rat brain MAO is more strongly influenced by tranylcypromine (*trans-d,l*-phenylcyclopropylamine; Parnate) than the fundus enzyme, while, isocarboxazid is equally effective on both enzyme preparations [Maxwell, Gray & Taylor (66)]. Similarly, rabbit brain MAO is six times more sensitive to tranylcypromine than the liver oxidase (65). Even enzymes occurring in the same organ may act differently toward the same inhibitor as the experiments of Green demonstrate (50). For guinea pig liver mitochondria and iproniazid the 50 per cent mark of inhibition is reached after 10 min, for microsomes after seven min. In following the course of inactivation of rat liver MAO by iproniazid, Hardegg & Heilbrunn observed a fast and a slow phase. The authors interpreted their data by assuming the presence of two different monoamine oxidases in their enzyme preparation (67).

Role of substrate structure on the efficiency of inhibitor.—When we carry out experiments with the same enzyme preparation, but with several substrates, the degree of inhibition varies often with the substrate used. This was seen with the reversible inhibitor harmaline which blocked the degradation of serotonin by rabbit (63), and by rat liver [Blanksma (68)] a hundred times more effectively than the oxidation of tyramine by the same enzyme preparations.

The majority of known MAO inhibitors act irreversibly. The enzyme is protected against these agents by its substrates, as shown first for iproniazid [Zeller, Barsky & Berman (69); Davison (70)]. Protection was also afforded by some substrates against *p*-methylphenylhydrazine [Arai (71)], benzylhydrazine, and a host of other arylalkylhydrazines [Green (50)], and against *cis*- and *trans*-2-phenylcyclopropylamines (64)

Another substrate effect was found when the action of *cis*- and *trans*-2-phenylcyclopropylamines on MAO was studied. In the absence of any substrate or in the presence of tyramine, the enzyme-inhibitor complex turned out to be fairly stable [Sarkar *et al.* (72)]. Upon addition of phenyl-*n*-butylamine it broke down and full MAO activity was rapidly restored (64). Almost certainly this phenomenon is not simply due to affinity differences of these two substrates for MAO, but rather to the special geometry of arrangements of these compounds on the active site of the enzyme (60). Phenylbutylamine does not reverse the action of nialamide or N-benzyl-N-methyl-2-propynylamine (Pargyline) (64).

In vitro *transformation of hydrazine derivatives.*—When simple alkyl-

hydrazines turned out to be better inhibitors than iproniazid (65); Barsky *et al.* (73)], it was suspected that this hydrazide might undergo conversion to isopropylhydrazine before acting on MAO. Actually, Seiden & Westly ascertained the production of isotopically labelled isonicotinic acid from iproniazid by solubilized guinea pig mitochondria (74). In a series of papers Schwartz reported the *in vitro* fate of another hydrazide, isocarboxazid [1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl, hydrazine; Marplan] which has a benzyl residue attached to the hydrazine moiety. With the help of paper chromatography and a reverse isotopic dilution effect, labelled benzylhydrazine was observed to be formed in homogenates of rat liver (75). The conversion of isocarboxazid to benzylhydrazine, which has been known for a long time to be one of the best inhibitors of MAO [Zeller (76)], is a heat-labile process requiring no oxygen (75). Recently, sodium 1,3-naphtoquinone-4-sulfate was introduced as a promising tool into this field. This compound is reduced to a fluorescent compound by hydrazine derivatives, but not by hydrazides, including isocarboxazid. Nevertheless, the quinone prevented the inactivation of MAO by the latter substance, apparently by interacting with the liberated benzylhydrazine and thus rendering it unavailable for inhibitory purposes [Schwartz (77)].

Binding of MAO inhibitors by cellular components.—In vitro systems are not only able to activate, but also to inactivate MAO inhibitors. Although it was demonstrated a long time ago that in homogenates the blocking power of iproniazid was drastically reduced as compared with its action on mitochondrial MAO (69), many authors proceed to use homogenates and neglect checking whether the inhibitor is prevented from reaching its targets. Our troubles, however, are not completely avoided when we replace homogenates by washed mitochondria. As shown by Green, the cessation of MAO inhibition after 30 min of contact between mitochondria and arylalkylhydrazines was due to a complete disappearance of free inhibitor molecules while iproniazid continued to affect MAO without such interference (50). This difference is compatible with the assumption that in cell particulates the isopropylhydrazine, after being set free from the hydrazide molecule, reached MAO before it was bound by extraneous matter.

Mechanism of MAO inhibition.—As usual the study of the kinetics and specificity pattern was the most important tool in the elucidation of the mode of action of MAO inhibition. During the early period of investigation it was observed that, in the absence of substrate, the degree of inhibition increased with time of contact between enzyme preparation and iproniazid (69). This relationship between time and degree of inhibition was at first suspected to be due to the conversion of iproniazid into a more effective compound (see preceding section). When, however, similar results were obtained for isopropylhydrazine (70), benzylhydrazine (50), and 2-phenylcyclopropylamine (64) which, according to our present knowledge, act in the form in which they are added to the enzymic material, this idea had to be abandoned as a generally applicable principle. Since intact and "solubilized" mitochondria displayed

the course of inhibition for tranylcypromine, the transfer of the inhibitor into the mitochondria did not appear to be responsible for the observed time-inhibition relationship (64). Several authors found the rate of MAO inactivation to be of the first order (50, 67, 70) provided that no secondary processes led to binding and thus inactivation of the inhibitor molecule, e.g., benzylhydrazine (50) and 2-phenylcyclopropylamine. In view of the concentration range of active inhibitors, 10^{-5} to 10^{-7}M, and of the enzyme concentration, hardly ever more than 10^{-7}M, it is understandable that the inhibitor and substrate molecules do not interact instantaneously.

The analysis of the inhibitor pattern, which has told us much in the past, is still vigorously pursued. Recently a number of oxalic acid hydracides were investigated [Szmuszkovicz & Greig (48)]. In confirmation of previous studies unsubstituted hydrazides as well as N^2-disubstituted hydrazides were without action on MAO. Benzylhydrazine and phenylethylhydrazine, and some of their derivatives were investigated by Green (50). No extensive generalizations were discernible in the effect of structure on the inhibitory potency of either the arylalkylhydrazines or the hydrazides. The pyridylmethylhydrazines were all less active than their phenyl counterparts. Loading the phenyl ring with bulky substituents or with more than one substituent also tended to reduce the inhibitory power, as did substitution of one of the hydrogen atoms attached to the α-carbon. A single small substituent in the phenyl ring either has little effect or slightly increases the blocking activity. The electronic character of the substituent appeared to bear no direct relation to the degree of inhibition. Acylated compounds generally were all much less reactive with MAO than the corresponding nonacylated benzylhydrazines but, owing to their greater persistence in the presence of the crude enzyme preparation, the extent of inhibition achieved on long duration of contact between enzyme and inhibitor approached that found with similar concentration of the parent hydrazine (see preceding section).

While a common structural basis for the two classes of MAO inhibitors, phenylcyclopropylamines and hydrazines exists, some distinct differences between their blocking mechanisms are found [Zeller & Sarkar (64)]. They lead to the assumption that not only van der Waals' forces and hydrophobic bonds are involved in binding the inhibitor molecule to the active site but also one of the two α-hydrogens. This is formally reminiscent of Racker's mechanism of the degradation of glyceraldehyde-3-phosphate. By dehydrogenation a covalent bond may be established between the active site and the inhibitor (or substrate). Subsequently, the substrate moiety is released in deaminated form, but the hydrazine inhibitor remains attached to the active site, presumably because such an enzyme-inhibitor complex is stable. No experimental observations suggest a similar explanation for the action of cyclopropylamines; these substances apparently form a complex with the active site on the strength of secondary forces alone. Belleau & Moran's observation that the inhibitory power of tranylcypromine could be recovered unchanged after 24 hr of incubation with a MAO preparation, supports this concept (78).

METABOLIC FATE OF MAO INHIBITORS

Between the moment of *in vivo* administration of inhibitors and their arrival at the target area the inhibitors become involved in many reactions which affect their impact on MAO. Continuing prior work, Horita studied the influence of the route of administration of several hydrazines on rat brain and liver enzymes. Certain compounds, such as iproniazid and nialamide (1-[2-(benzylcarbamyl)-ethyl]-2-isonicotinylhydrazine; Niamid) exhibited a greater activity against the liver enzyme than on brain MAO. Other agents, such as pheniprazine (1-phenyl-2-hydrazinopropane; Catron), phenelzine (β-phenylethylhydrazine; Nardil), and to some extent, isocarboxazid, exerted markedly different patterns of inhibition with different modes of administration. Subcutaneous administration of these compounds resulted in greater brain than liver MAO inactivation. When, however, they were administered orally, reduction of liver MAO was predominant (79).

Hydrazides, while initially less effective than the corresponding arylalkylhydrazines, often display higher activity *in vivo* as would be expected from their *in vitro* potency (50). Apparently, the acyl residue affords some protection to the hydrazine moiety. Even so, iproniazid undergoes fairly rapid transformation in the animal organism. Within two to six hours 40 per cent of the C^{14}, attached to the N^2 of iproniazid, appears in form of $C^{14}O_2$ [Koechlin & Iliev (80)]. Free inhibitor molecules, therefore, are rapidly eliminated by oxidative processes. These data explain why reversible, fast acting inhibitors such as harmaline [Horita & McGrath (81)], and methylene blue [Ehringer, Hornykiewicz & Lechner (82)] protect the organism against irreversible MAO inhibitors. Similar oxidative processes may be responsible for the transformation of weak *in vitro* inhibitors, e.g., 1-benzyl-2-isopropylhydrazine, to agents highly effective *in vivo* [Weikel & Salmon (83)]. Removal of either the benzyl or the isopropyl residue would result in powerful MAO inhibitors.

In Vivo INHIBITION

Regularly the *consequences* of MAO inhibition, such as changes in amine metabolism and in pharmacological responses of the animal toward sympathicomimetic amines, are used as criteria to assess *in vivo* inactivation of MAO. While procedures of this type, from a pragmatical view point, may well be suitable for the screening of potential inhibitors, they produce only indirect and not entirely conclusive evidence. The term *in vivo* action, therefore, is here applied only when, in a given organ, the residual enzymic activity has been actually measured. Since the first so-defined experiments were carried out (63), astonishingly few additional determinations have been reported. The use of indirect methods, however, may become necessary when easily reversible inhibitors are investigated, because unavoidable dilution of the cellular preparations tends to reduce the shift of equilibrium of the system enzyme plus inhibitor.

In the course of their studies on the influence of psychotropic drugs on the web-weaving ability of spiders, Witt, Brettschneider & Boris followed MAO

activity for several days after treating the animals with iproniazid. They found that the enzyme needed 13 days for full recovery after the administration of a single dose of the inhibitor (84). In rabbit brain, methylene blue, a reversible MAO inhibitor caused a significant but only transient drop in the oxidase activity (82). Tranylcypromine produced a much longer reduction of MAO activity in the same organ which returned more slowly to the original level than the rabbit liver MAO (72). Analogous differences were observed in other species and organs also. Two more examples may illustrate this point: iproniazid blocked *in vivo* MAO of canine liver much more efficiently than that of spleen [Lotlikar & McCutcheon (85)] and the same drug as well as isopropylhydrazine lowered the oxidase activity of guinea pig liver more drastically than that of kidney [Heim & Diemer (86)]. A Finnish group of investigators determined *in vivo* inhibition with the help of histochemical methods. In the pheniprazine treated rat, the brain MAO was totally blocked except for the nucleus ceruleus which retained a moderate activity [Mustakallio, Levonen & Raekallio (87)]. Much needed studies were also carried out with human organs obtained from patients who had received iproniazid prior to death: the MAO levels in the brain and liver were sharply reduced as compared with control values [Ganrot, Rosengren & Gottfries (88)]. Extensive analyses of the inhibitory power of several compounds have been reported by Burkard, Gey & Pletscher (89). The inhibitors were injected into cats and the animals were sacrificed after various lengths of time. MAO of kidney mitochondria reached its lowest level after 4 to 16 hours and returned to its original activity after 21 days which is more time than previously recorded for other organs and species (69). The quantities, in 10^{-6} moles/kg, to produce 50 per cent inhibition after 16 hours, were: 2.6 for 1-pivalyl-2-benzylhydrazine, 3.6 for tranylcypromine, 6.9 for iproniazid, and 16.4 for benzyl-N-methyl-2-propynylamine. When the differential inhibition of rat liver and brain MAO by a series of arylalkylhydrazines was determined, the results were essentially the same as could be expected from *in vitro* experiments; the many factors influencing *in vivo* experiments, therefore, did not change the inhibitor pattern substantially [McGrath & Horita (90)].

Consequences of MAO Inhibition on Amine Metabolism

Soon after the *in vivo* inhibition of MAO was discovered this reaction began to be used for the study of the metabolism of labeled—exogenous, and endogenous amines [(51); Burns & Shore (91)].

Catecholamines and other phenylalkylamines.—Many papers deal with the level of catecholamines in the brain of mouse, rat, guinea pig, rabbit, cat, dog, monkey, and man (82, 88, 92 to 94) as a function of MAO blockade. As inhibitors, the authors used methylene blue (82), tranylcypromine (93 a, b, 95 b), N-benzyl-N-methyl-2-propynylamine (92 a, 93 b, 95 c, 96), phenelzine (96), pheniprazine (93 b, 94, 95 a, 96), iproniazid (88, 92 b, 93 b, 95 b, 96), and nialamide (95 d, 96). As a consequence of the *in vivo* administration of these reversible, "semireversible" (tranylcypromine), and irreversible inhib-

itors a significant rise of the level of one or several catecholamines was observed. In agreement with previous reports no increase of norepinephrine concentration was found in dogs with five different inhibitors, and a small elevation only in cats [Maling, Highman & Spector (96)]. Concomitant treatment of the animals with dopa (92 a, 94, 95 a, c) or phenylalanine (93 a) enhanced the response evoked by the inhibitors. The catecholamine content of the soluble fraction of rabbit brain displayed a higher increase than that of the cell particulate fraction [Weil-Malherbe, Posner & Bowles (95 a)].

Crout, Creveling & Udenfriend investigated the norepinephrine levels of rat hearts after treating the animals with iproniazid, pheniprazine, tranylcypromine, and N-benzyl-N-methyl-2-propynylamine. In agreement with the work of Pletscher *et al.* (51) the compounds caused a marked rise of the cardiac norepinephrine concentration which lasted for at least 24 hr after a single dose of iproniazid. Not only endogenous, but also intravenously injected norepinephrine accumulated in the heart (93 b). In some instances, however, no alterations in the catecholamine concentration of the heart were observed, e.g., in mice after the *in vivo* administration of pheniprazine [LeRoy & de Schaepdryver (92 b)]. Relatively few new data were collected recently with regard to catecholamine levels in other tissues, and some contradictions were noticed between papers published by the same authors (97).

Since catecholamines run through many metabolic channels, it is not surprising to find that by blocking one of the outlets these amines can be forced through others. Accordingly, in several tissues elevated levels of O-methylated catecholamines were observed after the administration of MAO inhibitors, e.g., in the heart and liver of the cat [de Schaepdryver & Kirshner (97); Goldstein, *et al.* (98)]. Pheniprazine, while having no effect on the tissue levels of H^3-norepinephrine in heart, spleen, adrenal gland and liver, elevated H^3-norepinephrine in skeletal muscle and H^3-normetanephrine in all tissues mentioned [Hertting, Axelrod & Whitby (99)]. The rise of normetanephrine in various organs of the rabbit was, however, rather small (100).

A number of aromatic compounds, some not identified as yet, normally were so rapidly oxidized by MAO that they escaped detection until this enzyme could be blocked by inhibitors. As an example, we mention octopamine (*p*-hydroxymandelamine) which Erspamer discovered in the salivary glands of octopus, but which was never found in mammalian material. In iproniazid treated rabbits, however, sizable amounts of it occurred, and it became the most prominent phenolic amine in kidney extracts [Kakimoto & Armstrong (100)]. Metabolic shifts due to MAO blockage are also revealed in the urinary excretion of arylalkylamines and their degradation products. According to Rosen & Goodall, administration of iproniazid to human subjects increased the excretion of normetanephrine and decreased the excretion of 3-methoxy-4-hydroxymandelic acid during the infusion of *d,l*-norepinephrine-1-C^{14} (101). No alteration was found, however, in the chromatographic pattern of blood and urinary catecholamines when patients were treated with iso-

carboxazid. This compound, however, caused prolongation of catecholamine excretion after the oral application of dopamine [Friend (102)]. Similarly, Kopin, Axelrod & Gordon could not detect any change in the metabolic rate of H^3-labeled epinephrine after treating mice with iproniazid, while the excretion of metanephrine-C^{14} was enhanced by this drug (103). An extensive analysis of the urinary excretion products of *d,l*-epinephrine-2-C^{14}, after infusing this substance to cats, was carried out by Kirshner, Terry & Pollard (104). After the intraperitoneal administration of iproniazid more of the total radioactivity was recovered in form of metanephrine as compared with controls, while the excretion of labeled epinephrine remained the same (approximately 5 per cent of the total radioactivity).

The presence of octopamine in mammals was suggested when *p*-hydroxymandelic acid was found in human urine. By blocking MAO with iproniazid or pheniprazine, this acid was replaced by octopamine in the urine of rats, rabbits, and man (100). A switch from one pathway to another was also indicated by the enhanced excretion of N-acetyl-3-methyldopamine by treating rats with iproniazid and dopamine, thus revealing a novel metabolic pathway of dopamine [Goldstein & Musacchio (105)].

Serotonin and other indoleethylamines.—The past years saw the publication of extensive studies on the influence of MAO inhibitors on the serotonin metabolism in the brain of various species (93 a, 95 b, 106, 107). Mice, rats, guinea pigs, rabbits, dogs, and human patients were treated with α-ethyltryptamine (107 g, h), tranylcypromine (93 a, 95 b, 106 a, 107 b, c), pheniprazine (97, 106 c, 107 a, b), other alkylhydrazines (106 c, 107 d), iproniazid (95 b, 106 a, 106 b, e, f), and isocarboxacid (106 a). In all instances, marked elevations of the serotonin levels were reported. Daily oral administration of tranylcypromine and of iproniazid to rats for several days resulted in cumulative increases in brain serotonin concentrations of two to four times that produced by a single dose of the drugs [Green *et al.* (107 i)]. Particularly extensive investigations were carried out by Goeschke who determined the amount of four different inhibitors capable of producing a 50 per cent increase of the serotonin concentration in the brain of four species. The response to a given inhibitor changed up to eightfold when one species was replaced by another (106 a). The gain in serotonin concentration in general was not evenly distributed over various cell fractions of rat brain homogenates. In some instances the granular fraction was favored [pheniprazine: Schanberg & Giarman (107 e)], in other cases the soluble components [iproniazid and tranylcypromine: Green & Sawyer (107 c)], and in still other experiments both cellular fractions increased their serotonin stores to the same degree [iproniazid (107 e)]. It remains to be explained why these inhibitors evoke different responses within the same species.

Relatively few new data were collected for organs other than the brain. In rats an increase in serotonin levels in the thyroid and intestinal mucosa was observed when the animals were treated with iproniazid and 5-hydroxytryptophan [Paasonen, Karki & Molkka (108)].

The counterpart of the rise of serotonin levels, a drop of the concentration of 5-hydroxyindoleacetic acid as a consequence of MAO inhibition, was also reported, e.g., for rabbit brain and nialamide [Roos (109)]. Urinary excretion of 5-hydroxyindoleacetic acid decreased also, as found on many occasions (51). Wiseman & Sourkes observed it again after having administered iproniazid, tranylcypromine and harmala alkaloids to rats (110). As mentioned for the catecholamines, the blockade of one metabolic channel opens other ones for serotonin. While iproniazid did not affect the overall rate of serotonin disappearance in mice, it caused a marked elevation of the serotonin-*O*-glucuronide excretion in the urine [Weissbach *et al.* (111)].

Both serotonin and its isomer 4-hydroxytryptamine appear to be degraded *in vivo* by MAO. This conclusion can be drawn from experiments carried out by Erspamer & Bertaccini who treated rats with 4-hydroxy-d,l-tryptophan and iproniazid, and observed that 4-hydroxytryptamine accumulated in several organs. The animals excrete less 4-hydroxyindoleacetic acid in the urine as a consequence of inhibitor application (112).

Finally, the metabolism of the tryptamine itself is profoundly affected by MAO blockade as demonstrated by rats pretreated with iproniazid, nialamide or pheniprazine. They show an increase in brain tryptamine and serotonin levels after having received tryptophan [Hess & Doepfner (113)]. Man and animals respond so regularly to the administration of MAO inhibitors with a sharp increase in the excretion of tryptamine, that this reaction can be used as a sensitive test for the *in vivo* inhibition of MAO [Sjoerdsma *et al.* (114)].

Imidazoleethylamines.—Iproniazid influences not only MAO but also diamine oxidase [diamine oxygen reductase (deaminating), E.C. 1.3.6]. The old data regarding the *in vitro* blockade of diamine oxidase (DAO) by iproniazid [(3, 115, 116)] were recently supplemented by the results of *in vivo* experiments. DAO of cat kidney [Burkard *et al.* (89)] and rat intestine [Shore & Cohn (117)] responded strongly to this drug. Other hydrazine derivatives, e.g., phenelzine and 1-pivaloyl-2-benzylhydrazine, were less active than iproniazid (89). Since histamine and N^1-methylhistamine appear to be physiological substrates of DAO [Zeller (118)], the blockade of DAO by iproniazid should disturb the metabolism of imidazoleethylamines. When iproniazid was administered to human subjects they excreted less imidazoleacetic acid and methylimidazoleacetic acid; histamine appeared in the urine primarily in its ring-methylated form [Lindell, Nilsson & Roos (119)]. The effect of iproniazid on the excretion of N-methylated imidazoles may be due not only to the blockade of DAO, since the product of the biological methylation of histamine, N^1-methylhistamine, is a much better substrate of MAO than histamine (120, 121).

Effect of MAO inhibitors on biosynthesis of amines.—Besides DAO, many enzymes such as amino acid decarboxylases and other pyridoxal phosphate enzymes are sensitive to carbonyl reagents. Therefore, those inhibitors of MAO which incidentally are also carbonyl reagents [Zeller *et al.* (115)], e.g.,

simple phenylalkylhydrazines, or are metabolically convertible into carbonyl reagents (N^2-substituted acylhydrazines) should prevent amine formation by blocking amino acid decarboxylases. In the preceding sections, however, it has been made abundantly clear that the administration of these very inhibitors does not prevent the accumulation of several biogenic amines in various tissues. Either the degree of *in vivo* inhibition is insignificant or a small fraction of the cellular decarboxylases are able to produce sufficient amounts of the amines. According to a recent evaluation, 95 or even 99 per cent of the amino acid decarboxylase activity of mouse brain has to be eliminated by phenelzine before the serotonin augmentation is reduced (Dubnick *et al.* (106 b)]. But when the inhibition reaches 100 per cent, as shown for N-(3-hydroxybenzyl)-N-methylhydrazine, a very efficient MAO inhibitor N-benzyl-N-methyl-2-propynylamine does not cause the usual increase in the concentration of cerebral serotonin and dopamine (Brodie *et al.* (122)]. In less extreme cases, the partial inhibition of amino acid decarboxylases by MAO inhibitors may at least reduce the rate of amine accumulation after onset of the MAO blockade.

Indirect effects of MAO inhibitors on amine metabolism.—MAO inhibitors seem to interfere with amine metabolism by affecting enzymes immediately involved in amine formation and degradation. In view of the intricacies of the metabolic network they may also influence the amine levels of some organs in an indirect way. Out of many examples we selected the catecholamine concentration of the adrenal medulla as an illustration.

There was some reason to assume that the release of catecholamines from the adrenals by reserpine was mediated through serotonin. Rats, therefore, were dosed for five days with large amounts of serotonin, but only an insignificant amine depletion was obtained. When, however, the animals received serotonin and phenelzine, they responded with a marked drop of the catecholamines in the medulla; supposedly the exogenous serotonin was protected against rapid oxidative deamination by phenelzine [Mirkin (123)].

MAO inhibitors possibly could also change the epinephrine content of the adrenals if they were able to influence substantially the carbohydrate metabolism. Actually, the plasma level of lactic acid was increased by a factor of two to three by treating rats with tranylcypromine or iproniazid [Gey & Pletscher (124); (51)]. This observation may be related to the outcome of some *in vitro* experiments: it was noticed that iproniazid enhanced the glycogen mobilizing power of epinephrine on guinea pig liver slices [Meyer (125)]. Iproniazid, although not altering the blood glucose levels in rat, did potentiate the hypoglycemic action of insulin [Soulairac, *et al.* (126)]. This phenomenon may be responsible for de Schaepdryver's statement which was made without giving experimental details—that pretreatment of dogs with iproniazid "rather enhances the discharge of catecholamines" from the adrenals after insulin injections (127).

Effect of MAO inhibitors on cellular amine storage of amines.—As shown above, many interpretative data connect MAO blockade causally with amine

accumulation in certain tissues. In order to explain some storage phenomena, several authors considered other targets for MAO inhibitors [(51); Giarman & Schanberg (128)]. Pepeu *et al.*, in comparing the action of iproniazid and pheniprazine, found both compounds to be capable of completely blocking MAO of isolated guinea pig heart atria. Iproniazid, however, was much more effective in preventing the spontaneous loss of endogenous catecholamines than pheniprazine (129). Two long-acting MAO inhibitors, pheniprazine and N-benzyl-N-methyl-2-propynylamine, blocked the "slow" release of H^3-norepinephrine from the rat heart; the same inhibitors and the short-acting harmaline also opposed the releasing action of reserpine on H^3-norepinephrine [Axelrod, Hertting & Patrick (130)]. The authors suggested that these agents elevated the catecholamine concentration in certain tissues not by inhibiting MAO, but by somehow preventing the release of the hormone from its binding site. Since no other information pertaining to the fate of the released catecholamine was presented, other mechanisms might have been considered. (*a*) It seems possible that the slowly disappearing H^3-norepinephrine, prior to liberation, was "diluted" by the endogenously formed amine, since the latter is known to be increased in rat heart by the two inhibitors [Crout *et al.* (936 b)]. Consequently, the probability of a given labeled amine molecule escaping from its storage site will be reduced after the application of the MAO inhibitor. (*b*) Many biogenic amines are known to pass the lipoidal layers of the cell membrane with difficulty, presumably because of their distinctly ionic nature. The corresponding aldehydes, their immediate degradation product by MAO, should have a better chance to escape, provided that they are not immediately oxidized by one of the aldehyde attacking enzymes. Inhibitors of MAO, therefore, may curb the slow liberation of amines by keeping them in ionized form. (*c*) Whenever isoniazid (isonicotinylhydrazine) was tested it exerted little influence on the catecholamine content of hearts [Gertner (131); Pepeu *et al.* (129)]. The inefficiency of isoniazid which chemically is so closely related to iproniazid and yet is devoid of any action on MAO (2, 115, 117) needs to be explained before MAO is ruled out as a target of "MAO inhibitors."

By comparing the response of cat and rat hearts to the *in vivo* administration of MAO inhibitors, Goldstein & Shideman obtained definite evidence of the amine-releasing power of these agents (132). In the cat heart, tranylcypromine produced a rapidly developing reduction in the catecholamine concentration; in the rat heart, however, a marked rise of the amine level. These metabolic differences were related by the authors to the occurrence of MAO in heart homogenates: in the heart of adult rats MAO activity is fairly high [Novick (61)] whereas in the cat heart little enzymic activity appears to be present [Bernheim & Bernheim (133)]. Some other observations may serve as a basis for the interpretation of these results. The 2-phenylcyclopropylamines have a chemical structure very similar to that of amphetamine; in fact, Burger & Yost synthesized them with the expectation of obtaining substances with amphetamine-like properties (134). Iproniazid is isosterically so closely

related to epinephrine that it is capable of competing with the latter for the rabbit aorta receptor [Griesemer *et al* (135)]. Due to these chemical relationships, iproniazid and tranylcypromine may share with tyramine, amphetamine, ephedrine, and other sympathomimetic amines the power to release norepinephrine from its binding sites. When we assume that in some cells MAO is a part of the catecholamine releasing machinery (for one of the several conceivable mechanisms see preceding paragraph), we come to the following hypothesis: the two inhibitors, in spite of their amine releasing power, keep the catecholamines within those cells where MAO forms a major part of the catecholamine releasing mechanism. Since MAO is widely distributed, accumulation of biogenic amines, as in rat heart, is observed more often than depletion. When MAO is absent, or does not play a substantial role in the elimination of catecholamines, the releasing effect of the "MAO inhibitors" predominates. The pharmacological responses to amphetamine and tranylcypromine in general are quite different possibly because the amphetamine-like nature of the latter is overshadowed by its MAO binding action. When iproniazid and a few alkylhydrazines, e.g., phenelzine, were tested they produced elevated serotonin levels in the brain of mice. Although the quantities of the administered inhibitors were so chosen that practically a complete inhibition of brain MAO was secured, phenelzine caused a higher serotonin accumulation than iproniazid. In the course of 24 hr, the phenelzine induced serotonin concentration went half-way down to the original level while MAO still was completely inhibited. A "booster" dose of the inhibitor, given during this time, resulted in the elevation of the serotonin concentration to the previously attained high level. Apparently, phenelzine—but not iproniazid—favored the serotonin augmentation beyond its MAO blocking ability [Dubnick *et al.* (106 c)].

Analysis of drug action: Rauwolfia alkaloids and chlorpromazine.—Almost as soon as Rauwolfia alkaloids became known to deplete brain and other organs of serotonin and catecholamines, the research groups of Brodie and of Pletscher began to study these effects with the help of iproniazid and later with other MAO inhibitors. It was found that iproniazid not only counteracted the reserpine-induced sedation and elicited marked central stimulation, but also prevented the loss of various biogenic amines from several tissues [Burns *et al.* (91); Pletscher *et al.* (51)]. Since that time —1956—the pharmacological studies on Rauwolfia alkaloids and on MAO-inhibitors have become intimately interwoven to the mutual benefit of both fields and the anti-reserpine effects have been used for large scale *in vivo* screening of potential MAO inhibitors [Anderson *et al.* (49)]. The paper of Bickel, Carpi & Bovet is given as an example of a recent study (136). These authors showed that the single injection of two chemically fairly different substances, nialamide and N^2-(1,4-benzodioxane-2-methyl)-N^2-benzylhydrazine, prevented the reserpine-caused elevation of the urinary catecholamines. This action lasted for one week, exactly as long as their MAO-inactivating effect. In many other publications the interplay between Rauwolfia alkaloids (and similarly acting

synthetic compounds) continues to be analyzed throughout the animal kingdom (130, 137).

Inhibitors of MAO were instrumental in the discovery of a new and important facet of the mechanism of action of chlorpromazine. It was observed that high doses of chlorpromazine prevented the protection afforded by iproniazid against the reserpine-caused release of catecholamines in the adrenal medulla and in the brain [Camanni, Molinatti & Olivetti (138); Ehringer, Hornykiewicz & Lechner (139); Pletscher & Gey (140)]. A further analysis of this phenomenon was recently published by Gey & Pletscher (141). Chlorpromazine alone did not affect the levels of serotonin and norepinephrine in the rat brain, but it counteracted the iproniazid-produced increase in the concentrations of these two amines. The authors suggest that chlorpromazine may act by decreasing the permeability of the storage organelles for these amines. In this field of investigation, the usefulness of MAO inhibitors stems from their ability to raise the amine level far above the low concentration induced by the Rauwolfia alkaloids. Within the wide range of these two levels the chlorpromazine effect becomes easily discernible. The mechanism of the interaction between MAO inhibitor and chlorpromazine remains to be elucidated.

Consequence of MAO Inhibition on the Activity of the Nervous System

If the amine metabolism is of any importance to the function of the nervous system, and if MAO in some situations represents a limiting component in the cerebral amine metabolism, the blockade of MAO should influence a number of brain activities. The first observations were made by Brodie, Spector & Shore who noticed parallel changes between the norepinephrine level in the rabbit brain and central stimulation (142). In many instances, alterations were found in the EEG as a result of inhibitor administration (51). Pheniprazine and tranylcypromine produced persistent desynchronization of the EEG and evoked a rapid rise in brain serotonin [Costa *et al.* (143)]. In dosing untrained rats with a series of arylalkylhydrazines, a correlation was noticed between the MAO inhibitory power and behavioral changes. The latter lasted for at least 24 hr, while for the short-acting inhibitor harmaline the effect disappeared in less than three hr [Brimblecombe & Green (144)]. Easier for interpreting, however, are the results of experiments carried out with exogenous amines as shown in the following examples. Pheniprazine prolonged and intensified the action of intraventricularly injected serotonin [Schain (145)]. The epileto action of 5-hydroxytryptophan could be produced by much smaller quantities when the rats were pretreated with iproniazid than with the amino acid alone [Wada (146)]. Since Parkinson's disease appears to be characterized by the loss of dopamine in the urine [Barbeau, Murphy & Sourkes (147)] and in the N. caudatus and putamen [Bernheimer, Birkmayer & Hornykiewicz (148)], attempts were made to correct this deficiency. When large doses of L-dopa were given to

the patients, akinesis disappeared in part or *in toto* for a few hours. This period of improvement could be prolonged by treating the subjects with L-dopa and isocarboxazid [Birkmayer & Hornykiewicz (149)].

Influence of MAO Inhibitors on the Availability of Intracellular Amines

In the preceding section of this review the manifold influence of MAO inhibitors on amine metabolism has been described. The MAO blocking effect of these agents is a function (*a*) of the chemical nature of the enzyme which apparently differs from one species, organ, and cell compartment to another, (*b*) of the molecular structure and concentration of substrates, (*c*) of the molecular structure and reversibility of inhibitors, and (*d*) of the inhibitor concentration at the enzymic *locus* and, in some instances, of the metabolic conversion of inactive to active compounds. We also have to consider whether the inhibitor molecule acts on enzymes other than MAO, e.g., diamine oxidase and amino acid decarboxylases, or whether at a given moment, MAO is a rate limiting catalyst in the metabolism of a substrate. Since some biogenic amines (e.g., catecholamines) are transformed metabolically in many more ways than others (e.g., tryptamine), we suspect that the oxidase is at the same time rate-limiting for the degradation of one substrate but not for the next one. Furthermore, some inhibitors seem to be able to release amines from their storage organelles and to protect the liberated substrate against the oxidase. The MAO inhibitors may also shield biogenic amines liberated by other agents, e.g., amphetamine [Witt *et al* (84)]. Occasionally, the inhibitor competes with the biologically active amines for the receptors of the latter [Griesemer *et al.* (135)]. A recent example of this type of competition pertains to N-methyl-α-methyltryptamine. At relatively low doses this compound enhanced the effects of tryptamine by virtue of its blocking of MAO. This action was reversed at relatively high doses, presumably because it competed with tryptamine for the activation of receptor sites [Tedeschi, Tedeschi, Fowler, Green & Fellows (150)].

All these effects of MAO inhibitors on the concentration and intracellular distribution of amines will alter differentially the availability of various biologically active amines for their physiological receptors. This situation is complicated by the appearance of "new" compounds as a consequence of MAO blockade. Some of the amines are increasingly forced into other metabolic channels and are converted into substances such as the O-methylated catecholamines and octopamine. At the observed concentrations these compounds may well cause marked pharmacological responses. While little is known about the physiology and pharmacology of octopamine, some data regarding the catecholamine derivatives have been secured. According to Bacq & Renson, metanephrine and normetanephrine sensitize various organs to the action of epinephrine and norepinephrine (151). From this viewpoint, the *O*-methylation appears to be not so much a process of inactivation but that of functional transformation.

The concept of MAO emerging from the work reported here is quite different from the conventional idea that MAO performs its function by a simple overflow action [Zeller (152)]. The results rather suggest that this enzyme changes the relative concentration of a whole set of biogenic amines, and thus the competitive power of each amine for its physiological receptor. This model of the biological function of MAO may prove its pragmatic value by helping to further elucidate the mechanism of action of MAO inhibitors.

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