STUDIES ON RAT CARDIOVASCULAR MONOAMINE OXIDASE ACTIVITY

A Dissertation

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

Elizabeth Jane Dial

December 1978

STUDIES ON RAT CARDIOVASCULAR MONOAMINE OXIDASE ACTIVITY

An Abstract of

A Dissertation

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Elizabeth Jane Dial

December 1978

ABSTRACT

Studies were performed to determine the characteristics of rat cardiac monoamine oxidase (MAO) activity. Rat major blood vessels were also examined, since the coronary vasculature constitutes a significant portion of cardiac tissue.

MAO is known to exist in two types, A and B, as determined by the inhibitor clorgyline. However, rat heart MAO is claimed to differ radically from that found in other tissues and a multiplicity of MAO activities within the A and B types has been postulated. Thus, a comparison was made between rat heart MAO with that of the rat vas deferens and human cardiovascular tissues.

Rat cardiac and vas deferens MAO activity revealed no major differences regarding apparent Km values, inhibitor sensitivity (with the exception of pargyline), or mixed substrate interactions using kynuramine as the substrate. However, rat major blood vessels contained MAO activities which varied in substrate and inhibitor selectivity. While the rat heart had predominantly type A activity, the blood vessels contained type A and a clorgyline-resistant species. In contrast, the rat vas deferens had approximately equal amounts of A and B activity. The large A:B ratio in the rat heart explains why many MAO substrates are metabolized only by type A in that organ.

Human cardiovascular MAO differed from the rat only in the proportion of MAO types present. Human atria and saphenous vein

contained mostly type B activity, but overall the kinetic properties and inhibitor preferences were nearly identical to that found in the rat.

Studies with the preferential B substrate, phenylethylamine, revealed that its deamination could proceed through all three types of MAO (A, B and clorgyline-resistant activity). Experimentation showed that the type of MAO deaminating phenylethylamine depended upon the relative amounts of the MAO types present and the substrate concentration used in relation to the respective Km values. This concept extends to kynuramine and possibly other substrates as well.

Contrary to current opinion, the findings suggest a uniformity of properties within MAO types, both between tissues and between species.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation and deep gratitude to my major advisor, Dr. David E. Clarke, for the time and energy he expended in the supervision of this work. Special thanks are extended to Dr. Robert L. Hazelwood for the cooperation which made this degree possible. Acknowledgment is also due to Mr. David Hardy for his aid in obtaining human tissues.

Finally, I wish to thank my husband for his constant support and encouragement throughout this work.

TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

FIGURE PAGE

I. STATEMENT OF THE PROBLEM

The enzyme monoamine oxidase (MAO) is involved in the metabolism of numerous known or suspected neurotransmitters, including norepinephrine, serotonin, tryptamine, phenylethylamine and dopamine.

Two types of MAO have been defined (A and B). This division is based upon selective deamination of certain substrates and preferential inhibition with agents such as clorgyline. Rat liver, brain and vas deferens MAO have been shown to exhibit similar characteristics in regard to substrate and inhibitor preferences. However, evidence suggests that rat heart MAO activity may differ. While benzylamine is deaminated solely by type B activity in the brain, liver and vas deferens, it is metabolized by type A and B in the heart. Conversely dual substrates which normally detect A and B activity, show only deamination by the A type. Phenylethylamine, which like benzylamine is a highly preferential B substrate in other tissues, also differs in the rat heart. Unlike benzylamine, but like the dual substrates, it is deaminated solely by type A MAO. Also in the heart, but not the brain, liver or vas deferens, there is a reported MAO activity toward benzylamine that is resistant to inhibition by clorgyline. Finally, the MAO activity of the heart shows a large and progressive increase with age compared with other tissues indicating that the enzymatic activity, or the controls operating on it, differ significantly.

From the above account it is clear that rather large discrepancies appear to exist in the characteristics of rat heart MAO. Fowler, et al. (1977) have proposed that MAO may exist in sub-types within each of the A and B groups (e.g.: A_1, A_2, A_3 ---------etc. and B_1, B_2, B_3, -------etc.). Such multiplicity might explain the deviations from the usual route of substrate deamination in the rat heart but definitive proof for this contention is lacking. Thus, the objective of the present work was to further characterize the MAO activity of the rat heart and to determine whether human cardiac tissue displayed similar properties. Additionally, rat and human blood vessels were examined to evaluate whether any anomalies found in myocardial tissue extended to the vasculature.

II. LITERATURE SURVEY

1. An Introduction to Monoamine Oxidase

In 1928 Hare first identified a tyramine oxidase activity in the rabbit intestine. The oxidative deamination of numerous other amine substances was described subsequently. However, in 1951 Zeller first used the term monoamine oxidase (MAO) to distinguish this enzymatic activity from diamine oxidase. MAO (monoamine:O_2 oxidoreductase, deaminating, EC 1.4.3.4) catalyzes the oxidative deamination of tyramine, tryptamine, serotonin, norepinephrine, dopamine, and certain other monoamines. The basic reaction is as follows:

$$R\ CH_2\ NH_2 + H_2O + 1/2\ O_2 \rightarrow R\ CHO + H_2O_2 + NH_3$$

MAO is bound to the outer mitochondrial membrane (Schnaitman et al, 1967) and has a cofactor requirement for flavin adenine dinucleotide (FAD)(Erwin and Hellerman, 1967). The enzyme is reported to contain one mole of FAD per 120,000 grams of protein. Estimates of molecular weight for MAO vary widely depending on the tissue used, ranging from 1.2×10^5 to 1.25×10^6 (Tipton, 1965; Gomes et al., 1969).

Despite controversy, it is now fairly certain that MAO exhibits a requirement for iron (Symes et al., 1969) but not copper (Erwin and Hellerman, 1967). MAO activity is reduced in iron deficiency anemia (Youdim et al., 1975) but not in copper deficiency states (Symes et al., 1969).

2. Multiplicity of Monoamine Oxidase

The concept that MAO might exist in more than one activity (multiplicity of MAO types) actually originated at the beginning of the 1940's, although the idea was received with little impact at that time. Alles and Heegard (1943) studied the liver MAO activity derived from a number of different species. Using several substrates, they noted substantial differences in their relative rates of oxidation, suggesting that more than one activity might be involved. Later, Werle and Roewer (1952), using different species, identified enzymes which could oxidize either aliphatic or aromatic monoamines, but not both. However, it was not until 1955 that Satake verbalized the idea of multiplicity. He indicated that MAO might be a mixture of enzymes differing in substrate specificities. Furthermore, the distribution of enzyme types might vary from one tissue to another.

Subsequent research along several different lines accumulated much direct and indirect evidence to support the concept of a multiplicity of MAO types. Each of these lines of work is considered individually.

Perhaps the simplest and most widely used description of multiplicity involves the use of selective inhibitors. In 1968 Johnston introduced clorgyline, an irreversible inhibitor of MAO. Using rat brain tissue with tyramine as the substrate, he showed differential inhibition of MAO activity with increasing concentrations of the drug. The activity which was sensitive to low concentrations of clorgyline was termed the A type, while that requiring much higher concentrations

of clorgyline for full inhibition was called the B type. When this clorgyline-inhibition system was tested against other substrates, it was determined that serotonin was an A substrate while benzylamine was deaminated by the B type. Tyramine appeared as a dual substrate, being deaminated by both types. Subsequent workers were thus able to classify substrates for MAO as being either type A, B, or dual substrates.

Other irreversible inhibitors of type A were later synthesized (Fuller, 1968; Mantle et al., 1975). Additionally, specific B type inhibitors, deprenyl (Knoll and Magyar, 1972) and pargyline (Fuller et al., 1970) were defined. The following table contains both substrate and inhibitor classifications as listed recently by Houslay et al. (1976).

In addition to the above types of MAO, a "clorgyline-resistant" type has been defined (Coquil et al., 1973). This enzymatic species is not sensitive to inhibition by clorgyline and utilizes pyridoxal phosphate as its cofactor. Thus the activity is sensitive to inhibition by carbonyl reagents, such as semicarbazide.

The use of preferential substrates and selective inhibitors to classify MAO types may be an oversimplification (Houslay et al., 1976). A number of discrepancies in this system have been noted. For example, serotonin is described as a specific substrate for type A MAO (Johnston, 1968). Yet it has been shown to give biphasic responses with clorgyline (indicative of deamination by both types of MAO activity) in pig brain (Squires, 1972; Tipton and Spires, 1968), cat, dog, rabbit, ox and pig liver (Hall et al., 1969), and beef heart (Mantle, et al., 1976).

	Monoamine Oxidase	
	Type A	Type B
Preferred substrates	Serotonin Epinephrine m-O-Methylepinephrine Norepinephrine m-O-Methylnorepinephrine Normetanephrine Octopamine	Benzylamine 2-Phenylethylamine 4-Methoxy-2-phenyl-ethylamine 3,4-Dimethoxy-2-phenylethylamine Vanillylamine 5-Methoxytryptamine
Specific irreversible inhibitors	Clorgyline Lilly 51641 P.C.O.	Deprenyl Pargyline
Specific reversible inhibitors	Harmine Harmaline α-Ethyltryptamine	Tricyclic antide-pressants
Dual substrates	Dopamine, Tyramine, Tryptamine, 3-Methoxytyramine	
Non-specific inhibitors	Phenelzine, Iproniazid, Isocarboxazid, Tranylcypromine, Nialamide, Pheniprazine	

Conversely, the supposed specific B substrate, benzylamine, has been found to be a substrate for MAO A, B, and the clorgyline-resistant activity in rat heart (Lyles and Callingham, 1975). Likewise, the dual substrate dopamine is deaminated solely by type A in rat striatum (Waldmeier and

Maitre, 1975), but mainly by type B in human striatum (Glover, et al., 1977). In addition to the substrate variabilities mentioned, there are also differences in selectivity of inhibitor responses. Squires (1972) and others (Mantle, et al., 1976) have noted that deprenyl or pargyline do not always give complementary results to clorgyline in certain tissues.

Attempts to physically separate multiple forms of MAO have met with mixed success. MAO is tightly bound to mitochondria and separation and purification have proven extremely difficult. In 1963 Gorkin was able to partially separate two forms from rat liver mitochondria by sonication of mitochondrial membranes and adsorption on to modified calcium phosphate gel. These two types deaminated different substrates, but it was speculated that the mitochondrial membrane fragments acquired substrate specificities distinct from those of the parent organelle. This report was followed by the separation of solubilized forms of MAO by polyacrylamide gel electrophoresis (Youdim and Sandler, 1967) and shortly afterwards, Kim and D'Iorio (1968) reported the separation of enzyme forms with cellulose acetate electrophoresis. Both groups obtained several discrete bands of MAO activity with varying activities toward different substrates. As many as five bands of activity were distinguished from various tissue sources, three bands being anodic, one cathodic, and one band immobile. However, this latter band was shown later to be a procedural artefact (Houslay and Tipton, 1973). Because Kim and D'Iorio had used a variety of methods to solubilize MAO, it was inferred that the multiple activities obtained

were not artefacts. However, in spite of numerous reports of multiple molecular forms of MAO, it is still not clear as to the relationship between these forms and the various preferential substrates and inhibitors of the A and B types. The separated forms exhibit different Km values and inhibitor sensitivities to clorgyline compared with the crude enzyme prior to electrophoresis (Collins et al., 1970; 1972).

In a related series of experiments, Tipton (1972) found that the phospholipid content of the separated forms differed. He further proposed that the separable forms were artefacts of either the solubilization and purification procedures or the electrophoresis itself. Similarly, Jain and Sands (1974) have shown that the presence of multiple forms may depend on the method used to solubilize the enzyme.

An alternative method of separating forms of MAO is by molecular weight and the use of Sephadex gel filtration. Ragland (1968) used this technique with Sephadex G-200 to isolate soluble fractions of the beef liver enzyme that possessed varying activities toward different substrates. Oreland (1972) later showed that gel filtration without the use of a detergent would result in only a single type of activity. If, however, increasing amounts of sodium cholate were added to the enzyme preparation before filtration, two fractions could be obtained. Thus, variations in the method of gel filtration were partially attributable to the amount of solubilizing detergent in the preparation. Further, a common gel staining technique for MAO was shown to be non-specific (Inove et al., 1976).

These failures to isolate and purify the active enzyme form(s) have hampered attempts to determine the molecular weight of MAO. Estimates of molecular weight range from 55,000 obtained by Oreland et al. (1973) using Sepharose 4B filtration to 2,000,000 reported by Hashmoto and Okuyama (1970) with Sepharose 6B chromatography. More recently, Aoki et al. (1977) using ammonium sulfate-fractionated MAO subjected to both gel chromatography and electrophoresis have estimated the molecular weight of MAO to be about 44,000. They propose that higher weights can be obtained when the basic enzyme form aggregates in the presence of dilute concentrations of detergents. This finding might well explain the variability reported for the molecular weight of MAO.

In contrast to the previously mentioned artefact producing methods of separating multiple forms of MAO, a more physiologic method was described by Kroon and Veldstra (1972). They separated mitochondrial forms based on density gradient centrifugation. Gallagher (1972) performed such studies on mouse lung mitochondria and found a consistent separation of two types of activity toward phenylethylamine and serotonin, based upon the density of mitochondria. He was able to separate the A and B type activities, thereby inferring localization of the two types of MAO in different mitochondrial types. However, it still does not prove the existence of actual multiple forms of the enzyme.

In addition to these attempts to physically separate MAO forms, a number of studies have yielded indirect evidence of multiplicity of MAO types. One such method is the determination of pH optima. Numerous workers have reported variations in MAO activity with pH, depending on

the substrate used (Barbato and Aboad, 1963; Youdim and Sourkes, 1965; Achee et al., 1974). The differing pH optima for the various substrates was claimed as support for the idea of multiplicity. However, interpretation of these results is complicated by the fact that substrate binding and catalysis may also be affected by pH changes due to ionization of amino or hydroxyl groups on the substrate. Thus, it cannot be assumed that alterations in pH affect the enzyme only. Indeed it was shown that large differences in pH optima of MAO in beef brain mitochondria towards different substrates lessen considerably if it is assumed that only the unprotonated amine can act as substrate (Gabay et al., 1976).

Thermal stability of the enzyme is another indicator of multiplicity. A number of workers have shown differential heat denaturization with various substrates (Youdim and Sourkes, 1965; Jarrot, 1971; Squires, 1972; Yang and Neff, 1973). Yet the nature of the enzyme preparation itself seems to affect thermal stability, since the use of functionally competent mitochondria will cause differences (Gabay et al., 1976). Protection offered by mitochondrial lipids could explain differing thermal stabilities. In fact, Houslay and Tipton (1973) showed that reducing the lipid content of the enzyme eliminated these differences. Conversely, Oreland and Ekstedt (1972) showed that increased lipid binding could induce an increase in thermal stability. Regardless of these findings, differential heat denaturization of MAO activity is still considered by some to be an indicator of multiplicity (Fuentes and Neff, 1977; Arora and Meltzer, 1977).

Evidence for multiplicity of MAO types through kinetic analysis is far from clear. While it is possible to determine apparent Km values for various substrates, determinations are normally made in the presence of a preferential inhibitor (Ekstedt, 1976; White and Wu, 1977). Rather, it should be possible to detect high and low affinity components by Lineweaver-Burk plots in the presence of uninhibited enzyme. However, this phenomenon has been observed in only a few cases (Achee et al., 1974; Yang et al., 1972). More recently, the use of the preferential B substrate benzylamine or a derivative, has detected dual affinity components in rat heart and beef kidney (Lyles and Callingham, 1975; Dugal, 1977). The possibility thus remains open that with the use of an appropriate substrate, multiple affinity enzyme components can be detected.

Immunochemical techniques have also been used to study MAO multiplicity. By this means beef liver and brain enzyme were found to be homogeneous (Hartman et al., 1971) or heterogeneous (McCauley and Racker, 1973). Youdim (1974) found crossreactivity with antibody to rat liver MAO with the MAO in rat brain. But immunodiffusion studies could detect two bands with different diffusion rates. It is unclear whether these bands represent distinct proteins or modification of the diffusion rates by binding of lipid material. A new MAO purification procedure has enabled Dennick and Mayer (1977) to show a single immunogenic macromolecular species in rat and human liver with activity toward both serotonin and benzylamine. This preparation was also homogeneous by polyacrylamide-gel electrophoresis. Thus, the presence of multiple enzymes is still unproven.

Evidence has been reported that MAO types A and B exist *in vivo* as well as *in vitro*. A number of investigators have detected two types of activity in rat brain through selective inhibition with clorgyline or deprenyl (Fuller, 1972; Bevan-Jones *et al.*, 1972; Yang and Neff, 1974). In addition, experiments utilizing isolated, perfused rabbit or rat lungs, have demonstrated the presence of the two activities (Roth and Gillis, 1975; Bakhle and Youdim, 1976). In the studies of Bakhle and Youdim (1976), no competition between phenylethylamine (B substrate) and serotonin (an A substrate) was found *in vivo*. Yet such an interaction has been reported *in vitro* (Houslay and Tipton, 1974; White and Wu, 1975). The lack of competition *in vivo* is probably due to diffusional or transport barriers which are absent *in vitro*. Indeed, Gillis and Roth (1977) showed removal of amines in the perfused lung to be greatest for the more lipophilic compounds (phenylethylamine, tyramine) and least for the less lipophilic ones (norepinephrine).

While the evidence for separate molecular forms of MAO is not convincing, the evidence for multiple types of activity is almost overwhelming. Not only can at least two types of MAO activity be detected *in vitro* with a number of techniques, but they can be detected *in vivo* as well. Thus, even though the nature of the enzyme form is unclear, the concept of MAO multiplicity appears firm.

3. Localization and Distribution of Monoamine Oxidase

The relative proportions of type A and B MAO activity vary from organ to organ and from one species to another. Some workers have attempted to determine the proportions based on activities to

dual or preferential substrates. However, these can only be considered estimations until the MAO molecule(s) are actually purified. The following table is from Houslay, et al. (1976) and is based on activity toward various substrates.

	Percentage of Total Activity in the Rat	
	Type A	Type B
Liver	40	60
Liver parenchymal cells	50	50
Denervated liver	40	60
Kidney	70	30
Intestine	70	30
Intestinal mucosa	60-70	30-40
Spleen	95	5
Lung	50	50
Testis	90	10
Brain	55	45
Superior cervical ganglia	90	10
Pineal gland	15	85
Denervated pineal gland	5	95
Vas deferens	50	50
Denervated vas deferens	35	65

Following the definition of two types of MAO activity by Johnston (1968), several workers tried to determine if there was a differential tissue localization of the A and B types. Goridis and Neff (1971) showed a predominantly A activity in rat pineal gland which could be eliminated following bilateral superior cervical ganglionectomy. Coquil et al. (1973) also selectively reduced activity from mesenteric artery after 6-hydroxydopamine treatment. These experiments suggested an intraneuronal location for type A with type B being non-neuronal. However, supporting evidence for this distinction was not forthcoming. While Jarrott (1971) could show a greater fall in A activity in denervated rat vas deferens, there was still some loss of the B activity. Likewise, cultured glioma cells showed only A activity (Murphy et al., 1976) although they were shown to be of non-neuronal origin. From the previous table it can also be seen that the distribution of MAO type A occurs frequently in poorly innervated tissues. Thus, it may be concluded that this species is not solely confined to neuronal tissue. Indeed, data on the liver shows that both MAO types can coexist in the same cell type (parenchymal cells) and that 6-hydroxydopamine treatment exposes almost no contribution of neuronal MAO to the total activity (Tipton et al., 1976).

4. Nature of the Multiple Forms of Monoamine Oxidase

The concept of multiplicity of MAO types is well accepted in the literature. However, the nature of these forms is disputed. Explanations suggested include conformational differences (Collins et al., 1970; Youdim and Collins, 1971) or polymerization of enzymatically

active subunits (Gomes et al., 1969). The latter explanation can most probably be excluded since solubilized rat liver preparations of MAO generally behave in a homogeneous manner on gel filtration and ultracentrifugation (Youdim and Collins, 1971).

One early interpretation for the observed multiplicity of MAO was the suggestion of a single enzyme having varying amounts of membrane material bound to it (Veryovkina et al., 1964). Such a model, based on solubilization by ultrasonication, would predict an almost infinite number of subparticles after molecular fragmentation. However, remarkably consistent isoenzyme patterns were discovered, whether by the use of sonication or detergents.

Tipton et al. (1972) suggested that MAO isoenzymes may result from the binding of varying amounts of phospholipid phosphate to the enzyme protein. However, there is only a poor relationship between electrophoretic mobility and phospholipid phosphate content. The highest amounts of the lipid material were associated with the band remaining at the origin, while the lowest amounts were in both anodic and the cathodic bands of MAO. Yet Tipton et al. (1972) showed that the lipid moiety is a necessary requirement for enzyme activity. Treatment with the detergent Triton X-100 resulted in the loss of multiple forms on electrophoresis. This was confirmed by Houslay and Tipton (1973) who demonstrated that other chaotropic agents, such as sodium percholate and sodium thiocyanate, eliminate electrophoretic multiplicity as well as differential thermal stabilities and clorgyline sensitivity. More recently, Ekstedt and Oreland (1976) showed that in

pig and brain the A activity could be selectively removed by methyl ethyl ketone, leaving the B activity intact. These types of results have been interpreted by Houslay and Tipton (1973) to explain the multiplicity of MAO by the binding of a single enzyme species in different membrane (lipid) environments. The lipid attachments would then confer allotopic properties on the enzyme. This concept predicts one active site on the enzyme which is influenced by its lipid environment to assume A or B properties. However, if this is the case, it should be possible to show interconversion between A and B activities and this has not been successfully shown (Ekstedt and Oreland, 1976). Rather, it is more likely that there are actually two active sites, since mixed substrate competition studies and Km determinations have been carried out on tissue selectively inhibited for either the A or B activity (White and Wu, 1975; Ekstedt, 1976). Therefore, White and Glassman (1977) have proposed the concept of multiple active sites on a single molecular species of the enzyme. They further postulate that the A sites may be in a more lipid environment than those of the B. Also, the A sites may be more dependent upon the structural integrity of the MAO-mitochondrial complex. This could explain why procedures which remove lipid or cause conformational changes can selectively inactivate the A activity.

5. Functional Role of Monoamine Oxidase

Detailed knowledge as to the exact functional role of MAO still remains unknown. With the confirmation of multiplicity of enzyme types comes the possibility of control over particular amines in discrete

areas. This would allow the enzyme to maintain a finer control than was originally anticipated.

While MAO types are located both intraneuronally and extraneuronally, there is no reason to believe they have the same functions in either location. Intraneuronal MAO controls the concentrations of the transmitter monoamines (Kopin, 1964). This was determined through studies on MAO inhibition in which it was shown that neurotransmitter amines increase in the brain and peripheral tissues (Pletscher et al., 1966; Pletscher et al., 1960). Such findings have been used to support the catecholamine theory of affective disorders, since MAO inhibitors are used as antidepressant drugs. However, this does not mean that there is a defect in MAO in such disease states. Rather, studies on depressed geriatric patients have shown no difference at autopsy in brain MAO activities compared to non-depressed controls (Collins et al., 1970).

Houslay et al. (1976) summarized the role of intraneuronal MAO as a regulator of the dynamic balance between synthesis, storage, release and reuptake of transmitter amines in the nerve ending. In this regard, MAO has long been considered the primary inactivator of intraneuronal amines. It not only deaminates norepinephrine that enters the neuron following uptake 1 (Tarlov and Langer, 1971), but also metabolizes any norepinephrine that may leak from storage vesicles in the terminal region (Axelrod et al., 1961). Additionally, MAO has a protective function in preventing unwanted amines such as tyramine from interfering with neuronal activity (Kopin, 1971). Interference

with the reuptake process was shown by Trendelenburg et al. (1972) when MAO inhibition elevated the intraneuronal concentration of "free" amines. Still another possible role of intraneuronal MAO involves the regulation of dopamine levels in the nerve fiber. Since MAO has access to dopamine before it enters the storage granule for conversion to norepinephrine, MAO may additionally regulate norepinephrine levels by deamination of its precursor amine dopamine (Clarke and Sampath, 1973).

MAO activity in the sympathetic neuron is linked to neuronal activity (Clarke and Sampath, 1973). Nerve stimulation increases tyrosine hydroxylase activity (Gordon et al., 1966) which in turn increases norepinephrine synthesis. Along with these changes is an increase in intraneuronal deamination (Clarke and Sampath,1973). This type of control would tend to prevent exaggerated amounts of neurotransmitter from being released from the cell.

The function of extraneuronal MAO is much less well defined than that of intraneuronal MAO. Presumably it plays a protective role, dealing with metabolism of blood borne amines. The existence of a low affinity, high capacity amine uptake system in extraneuronal cells ($Uptake_2$: Clarke et al., 1969), argues for such a role. In addition, the high levels of MAO activity in the intestine, stomach, platelets and liver suggest a protective function for removal of dietary amines (Blaschko, 1952). In fact, the most severe side effect of MAO inhibitor drugs comes from the interference with metabolism of dietary tyramine (Blackwell, 1963). Patients eating cheese or other

foodstuffs with a high tyramine content are liable to a sudden and dangerous rise in blood pressure as a result of the norepinephrine-releasing property.

6. Some Physiological Factors Influencing Monoamine Oxidase.

An interaction between MAO and various hormones has been postulated for a number of years (Zile, 1960). In 1968 Avakian and Callingham reported an increased cardiac MAO activity in rats three days following bilateral adrenalectomy. No changes in cardiac catechol-O-methyltransferase activity occurred. In a subsequent study, Caesar et al. (1970) reported an increased mitochondrial MAO activity in heart, brain and vas deferens of adrenalectomized rats. Sampath and Clarke (1972) studied the vas deferens in adrenalectomized rats since this organ contains about fifty percent of its MAO activity within the sympathetic innervation. They found a specific rise in intraneuronal MAO since denervation completely prevented the increase. Furthermore, the rise in MAO activity was blocked by treatment with dexamethasone, but not by adrenocorticotropic hormone. Thus, the rise following adrenalectomy was due to steroid lack and was not related to enhanced adrenocorticotropic hormone secretion. Further work by Callingham and Laverty (1973) showed that the increased MAO activity was due to enhanced synthesis of enzyme. The mechanism was initiated by a steroid deficiency, but was not elucidated. Other organs were found in which the MAO activity was increased by adrenalectomy. These include the spleen, superior cervical ganglion and hypothalamus (Clarke and Sampath,

1975). Thus, organs with a high degree of sympathetic innervation seem to be the most affected by this surgical procedure.

The changes in MAO activity following adrenalectomy are large enough to alter monoamine metabolism. Increased urinary metabolites of catecholamines have been shown (Ceasar et al., 1970) and Clarke and Sampath (1973) reported enhanced deamination of norepinephrine in rat hearts.

The MAO activity in many animal tissues varies with age (Gripois, 1975). For example, MAO activity increases with age in the heart of the domestic fowl (Callingham and Fowler, 1977) and in the hippocampus of the domestic pig (Blatchford et al., 1976). In the rat there are also increases in MAO activity with age. These include the heart (Horita, 1968), thyroid (Knopp et al., 1976), vas deferens (Callingham and Lyles, 1975), adrenals, particular brain regions (Youdim and Holzbauer, 1976), liver and kidney (Parvez et al., 1976). The mesenteric artery and vein and the aorta do not change (Lai et al., 1975), while the lung decreases in activity (Parvez et al., 1976).

A limited number of studies have been performed to discover the types of MAO developing in these tissues. Callingham and Lyles (1975) showed an increase in type A activity with age in the rat vas deferens which reached a peak and then declined. Blatchford et al (1976) noted a difference in activity toward various substrates (notably dopamine and tyramine) in the pig hippocampus. However, they did not differentiate the activity into MAO types. Mouse brain also exhibits a difference in the rate of development of the A and B types (Diaz and Maderdrut, 1977).

Changes in MAO activity in the rat neonate have been shown to be affected by testosterone (Hoff, 1977), corticosteroids (Parvez et al., 1976) and thyroxine (Gripois and Fernandez, 1977). Indeed, MAO development may well be under the control of various hormonal factors and the types of MAO may even be differentially affected by hormones. Youdim and Holzbauer (1976) suggested the possibility of an independent development in the rat brain of an MAO which preferentially deaminates dopamine. This type of MAO would be localized in specific brain regions and its development might be linked to the maturation of a neuronal system utilizing dopamine as the transmitter. Consistent with this concept Youdim (1974) was able to partially separate brain mitochondria which have a high activity towards dopamine. This system was absent in the liver.

Other factors which could contribute to the increase in MAO activity with age are changes in the immediate environment of the enzyme which are required for full activity, such as cofactors or allosteric effectors. A part of the MAO cofactor, flavin, is in low tissue concentrations at birth (Kuzuya and Nagatsu, 1969), thereby possibly limiting the development of MAO activity. The synthesis of flavin also increases with age and is thought to be regulated by thyroid hormones (Rivlin et al., 1976). The phospholipids associated with MAO on the outer mitochondrial membrane are also important in the age-related effects of MAO activity. In rats brain total lipid and myelin concentrations are low at birth and increase gradually during the first three months of life (Crawford and Sinclair, 1972). These

increases are paralleled by an increase in dopamine deaminating activity in the hypothalamus (Youdim and Holzbauer, 1976).

In the human, MAO activity also changes with age. Robinson et al. (1971) were the first to show age-related increases in brain, platelet and plasma MAO activity. He also reported an age-related decrease in brain norepinephrine with no change in serotonin levels (Robinson, 1975). His studies suggest that the aging process affects monoamine mechanisms and may be a predisposing factor to the development of clinical diseases such as depression and Parkinsonism. However, in a major study involving 680 control subjects, Murphy et al. (1976) were unable to show any increase in either plasma or platelet MAO activity over a six decade span. However, sex-related changes were found. They noted a consistent 30% higher platelet MAO activity in females compared with males. Similar sex differences were found by Roth et al. (1976) in their study of platelet MAO in children. They found no change in male MAO activity after puberty, but a significant drop was found in the female at that time. However, the female levels of MAO activity were still greater than the male's, both before and after puberty. Thus, hormonal changes do not explain the sex difference seen in human platelet MAO activity.

7. Cardiovascular Monoamine Oxidase

Early studies on MAO activity dealt mainly with liver and brain tissue. As cardiovascular tissues were investigated, a number of anomalies arose. It appeared that cardiac and vascular MAO differed from those tissue activities described previously. Several

workers reported such discrepancies (Mantle et al., 1976; Lyles and Callingham, 1975; Fuentes and Neff, 1975).

For example, blood vessels have shown the presence of MAO activities with differing cofactor requirements. The rabbit aorta contains an amine oxidase nearly identical to rabbit plasma amine oxidase (Rucker and Goettlich-Riemann, 1972). Both enzymes utilize pyridoxal phosphate as their cofactor and are thus thought to be of a connective tissue origin. Additionally, vascular smooth muscle of the rabbit aorta contains "typical" MAO as well as catechol-O-methyl transferase (Levin and Wilson, 1977). The vascular tissue can be further divided to show that the adventitia (which contains the adrenergic innervation) has mostly type A activity, while the media contains predominantly type B. Rat arteries were also found to have two types of MAO activity, type A in the sympathetic neurons, and a clorgyline-resistant form located extraneuronally (Coquil et al., 1973). Rat cerebral microvessels also contain two types of MAO activity, A and B, both extraneuronal (Lai et al., 1976).

Compared with blood vessels, rat erythrocytes and platelets do not contain significant amounts of MAO activity (Blaschko, 1963; Edwards and Chang, 1975). Yet reticulocytes do have some activity which disappears as the red blood cell matures (Quiring et al., 1976; Quiring and Hubertus, 1977). The functional significance of this latter observation may be related to the adrenergic beta-receptor system of immature erythrocytes.

Human blood platelets contain considerable MAO activity of the B type only (Edwards and Chang, 1975; Donnelly and Murphy, 1977). Indeed, human platelet MAO has been used as a peripheral marker for activity of the enzyme in brain (Robinson et al., 1971; Murphy et al., 1974).

Cardiac tissue has been studied in a number of species with varying results. Dog heart was shown histologically to contain MAO only within the coronary vessels and not within the muscle tissue (Takeichi et al., 1975). Yet experimental myocardial infarction initiated the development of MAO activity along with fibrosis of muscle tissue. Similarly, human heart has been examined histologically, but differed from dog in that it was found to contain significant amounts of muscle enzyme activity (Partenen et al., 1976). There was a remarkably consistent amount of total activity present, in spite of the variety of disease states tested (ischemic heart disease, valvular heart disease, atrial septal defect and congestive heart failure).

In mouse (Squires, 1968) and beef heart (Mantle et al., 1976) two types of MAO activity have been found. In bovine tissue a large proportion of type B activity was seen with several substrates. However, the preferential type A substrate, serotonin, was metabolized by both types of activity. This finding prompted the authors to suggest a heterogeneity within the MAO types contained in this tissue.

A similar result was seen in rat cardiac tissue. Lyles and Callingham (1974) found that the preferential B substrate, benzylamine, exhibited multiple types of metabolism. Further, the percentage of

metabolism of benzylamine by the various MAO types varied with age (Callingham and Lyles, 1975). Benzylamine also revealed a clorgyline-resistant MAO species in the rat heart which decreased in proportion as the animal aged (Lyles and Callingham, 1975). Yet, the cardiac activity toward phenylethylamine, serotonin, tyramine and dopamine detected only a single species of MAO activity of the A type.

Thus, according to the literature, rat cardiac MAO activity may differ from other tissues in the properties of its enzyme. Indeed, Fowler et al. (1978) have recently used these cardiac anomalies to support the concept of multiplicity within MAO types. If this theory applies to rat heart MAO activity, it should be possible to systematically compare the rat heart to more defined tissues to determine where differences or similarities exist.

III. METHODS AND MATERIALS

1. Animals and Animal Housing

Experiments were made on male albino rats of the Wistar strain, obtained from Hilltop Laboratories, Inc., Scottsdale, Pennsylvania. The body weights ranged from 250 to 400 g, except in experiments where age was the experimental factor.

The rats were housed in gang cages in air-conditioned animal quarters maintained at 22-24^{o} C with a 12 hour light and dark cycle. Food and water were supplied ad libitum. Food pellets consisted of Purina Rat Chow obtained from the Ralston-Purina Company, St. Louis, Missouri.

2. Human Tissue

Specimens of human atria and saphenous vein were obtained during surgery for coronary by-pass operations at the Texas Heart Institute, Texas Medical Center, Houston, Texas. Tissues were from male patients between the ages of 39 and 62 years, except for two specimens obtained from infants. All tissues were rinsed well in normal saline immediately after removal and were placed over ice for immediate assay or frozen at -10^{o} C for later use. At this temperature MAO activity is stable for 5 to 7 days.

3. Monoamine Oxidase Determinations

Two different substrates, kynuramine and phenylethylamine, were used in the assay of monoamine oxidase (MAO).

a. Kynuramine assay

Since there are multiple types of MAO with differing substrate preferences, the choice of substrate used in a particular tissue is of utmost importance. Kynuramine has been reported to be a substrate for type A and B MAO (Squires, 1972). Furthermore, kynuramine is not an endogenous substrate, so tissue levels of interfering metabolites will not be encountered. Additionally, the kynuramine assay is rapid to perform and sensitive to at least 0.5 nanomoles of product.

The assay consists of measuring the fluorescence of 4-hydroxyquinoline formed after the oxidative deamination of kynuramine by MAO, as described by Krajl (1965). Weissbach et al (1960) showed earlier that kynuramine is deaminated by MAO to an unstable aldehyde, which spontaneously rearranges to 4-hydroxyquinoline by intramolecular condensation. Kynuramine is not known to be a substrate for any other enzymes.

Assay Procedure: Rats were sacrificed by cervical dislocation. Selected tissues were removed, rinsed in normal saline, blotted and used immediately or frozen at -10^{o} C until later use. Tissues were homogenized in glass distilled water for a total of two minutes (on for 10 seconds, off for 5 seconds) in a Tissumizer homogenizer obtained from Tekmar Company, Cincinnati, Ohio. Rehomogenization, after standing for 15 minutes at 4^{o} C, failed to increase product formation. Thus, maximal enzymatic activity was obtained under the prevailing environmental conditions. Homogenate concentrations made were: 10 mg/ml for rat atria, ventricles, vas deferens, blood vessels and human saphenous vein, 5 mg/ml for human atria and 1 mg/ml for rat liver. The composition

of the incubation mixture was as follows:

0.5 ml water

0.5 ml phosphate buffer, 0.5 M, pH 7.4

0.5 ml inhibitor or water

1.0 ml tissue homogenate

0.5 ml kynuramine (307 nanomoles)

The first four ingredients were added in that order to duplicate round-bottom centrifuge tubes and were incubated at 37^{o} C for 15 minutes in a Dubnoff Metabolic Shaking Incubator under air. Kynuramine was added and the incubation continued for 30 minutes. The reaction was stopped by the addition of 2 ml of 10% (w/v) trichloroacetic acid or 2 ml of 6% (w/v) perchloric acid. The protein thus precipitated was separated by low-speed centrifugation of the incubation tubes. A 1 ml sample of the supernatant was then mixed with 2 ml of 1 N NaOH in a Silica Cuvette. The fluorescence was read on an Aminco-Bowman Spectrophotofluorometer at uncorrected wavelengths of 315 nm (excitation) and 380 nm (emission). Reagent blanks, tissue blanks and 4-hydroxyquinoline standards were also run through the entire procedure. Standard curves to 4-hydroxyquinoline (4OHQ) were constructed so that tissue levels could be determined directly.

The specific activity (S.A.) of MAO was calculated per mg protein or per mg tissue, wet weight. The following formula was used:

$$\text{S.A.} = \text{4OHQ (nmoles)} \times \frac{1}{\text{mg tissue or mg protein used}} \times 2$$

where 2 is a factor to adjust the values for one hour.

The following investigations were conducted to determine the reliability and specificity of the assay in detecting MAO activity.

(i) The activation and emission spectra of 4-hydroxyquinoline were measured and found to occur maximally at 315 nm and 380 nm, respectively. These values were used in each assay.

(ii) The relationship between 4-hydroxyquinoline concentration and relative intensity of fluorescence was determined to be linear between 0.5 and 200 nanomoles.

(iii) Neither kynuramine nor any added inhibitor exhibited fluorescence at the wavelengths for 4-hydroxyquinoline.

(iv) The reaction was shown to be linear for both time and tissue concentration. These results are shown in figures 1 and 2.

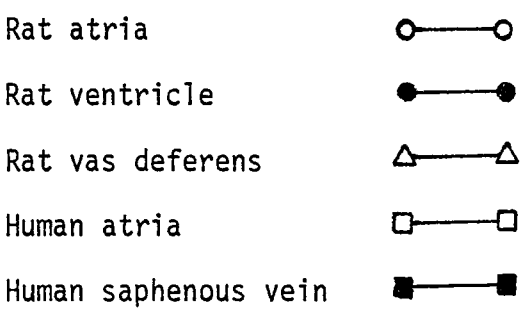
(v) Use of perchloric acid to stop the reaction increased fluorescence of 4-hydroxyquinoline, but not blank values, by about three-fold.

b. Phenylethylamine assay

Phenylethylamine is reported to be preferentially metabolized by MAO type B (Yang and Neff, 1974) and is an endogenously occurring substance. Measurable concentrations occur in the central nervous system (Durden et al., 1973), but peripheral levels are very low and do not present interference problems when using whole tissue homogenates. Several methods of assay are available, the most common being solvent or column separation of metabolites using the radio-labeled parent compound (Goridis and Neff, 1971; Jain et al., 1973). Solvent extraction techniques usually rely on separating the metabolites from the protonated parent amine by extraction into toluene. This method may give false results due to the differential partition coefficients of the aldehyde, alcoholic and acid metabolites (Tipton and Youdim, 1976).

Figure 1. Effect of tissue concentration on MAO activity in rat and human tissues using kynuramine.

The 4-hydroxyquinoline (4OHQ) formed is plotted versus the tissue concentration. Each point is the mean of at least two determinations. Preincubation time = 15 minutes; incubation time = 30 minutes.



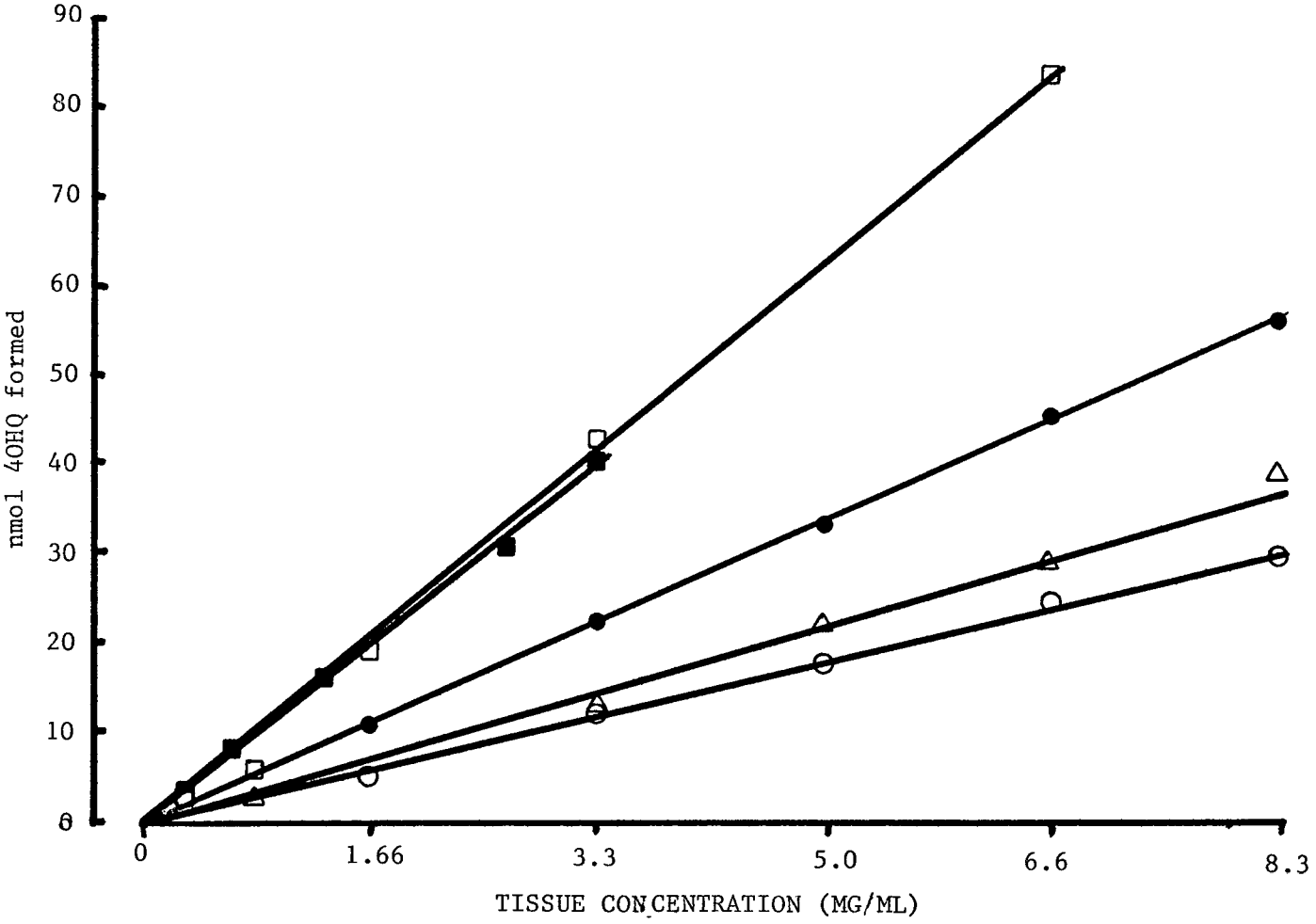
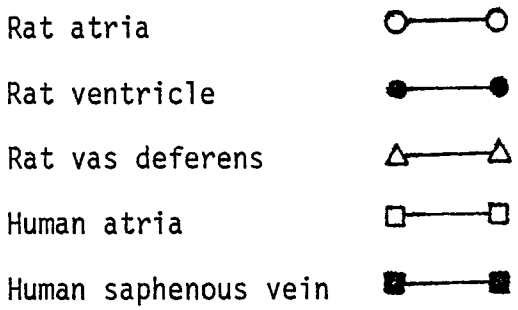
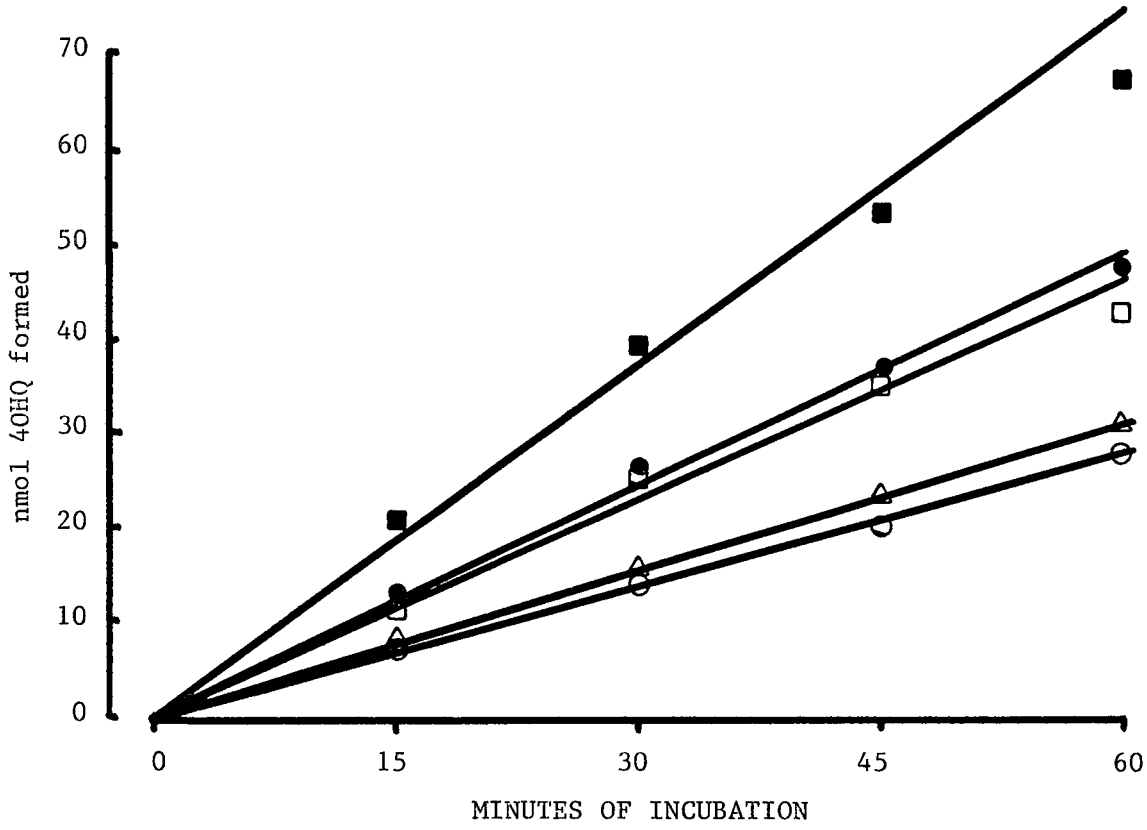

90
80
70
60
50
40
30
20
10
0
nmol 4OHQ formed
0
1.66
3.3
5.0
6.6
8.3
TISSUE CONCENTRATION (MG/ML)

Figure 2. Effect of incubation time on MAO activity in rat and human tissues using kynuramine.

The 4-hydroxyquinoline (4OHQ) formed is plotted versus the minutes of incubation. Each point is the mean of at least two determinations. Whole tissue homogenates of 3.3 mg/ml were used for the rat atria, ventricles, vas deferens and human saphenous vein, and 1.66 mg/ml for the human atria. Preincubation time = 15 minutes.




70
60
50
40
30
20
10
0
nmol 4OHQ formed
0
15
30
45
60
MINUTES OF INCUBATION

Metabolic separation using a weak cationic exchange resin is superior since it is devoid of these problems. Using prepared Bio-Rex 70 resin at pH 6.0, phenylethylamine is retained on the resin, while deaminated products appear in the effluent. A sample of the effluent can be counted by liquid scintillation spectrometry and the total deamination computed. The assay is extremely sensitive, detecting changes as low as 0.05 nmoles of deaminated products.

Assay procedure: Tissue homogenization was performed as described previously using glass distilled water. Tissue concentrations used were 16.5 mg/ml for rat ventricle, vasa deferentia, and human saphenous vein, 1.65 mg/ml for rat liver and 0.83 mg/ml for human atria. These concentrations yielded the same tissue:substrate ratio as the kynuramine assay. The contents of the incubation mixture were:

0.1 ml water

0.1 ml phosphate buffer, 0.15 M, pH 7.4

0.1 ml inhibitor or water

0.1 ml tissue homogenate or boiled tissue

0.1 ml phenylethylamine (50 nanomoles, 0.25 μCi)

The first four ingredients of the mixture were added consecutively to duplicate test tubes and incubated at 37^{o} C for 15 minutes in a Dubnoff Metabolic Shaking Incubator under air. The substrate was added, and incubation continued for 30 minutes. The reaction was stopped by plunging the tubes into an ice-salt mixture at -5^{o} C. The contents of each tube were poured over the top of a (0.75 x 1.5 cm) column of Bio-Rex 70 buffered to pH 6.0, and the eluate was collected. Columns were washed 4 times with 2 X 1 ml aliquots of distilled water and the individual washings were combined with the original eluate. A 2 ml sample of each

collection vial was mixed with 15 ml of the liquid scintillation counting solution, shaken, and counted on a Packard Tri-Carb Scintillation Spectrometer, Model 3330. Water blanks, boiled tissue blanks, and phenylethylamine standard were also carried through the entire procedure.

Calculations of specific activity (S.A.) of MAO in nanomoles phenylethylamine (PEA) deaminated/mg protein/hour were made using the following formula:

$$\text{S.A.} = \text{CPM} \times 4.25 \text{ (dilution factor)} \times \frac{1}{\text{CPM of PEA standard}} \times 50 \text{ (nmoles PEA in standard)} \times \frac{1}{\text{mg protein used}} \times 2 \text{ (time factor)}.$$

The following investigations were undertaken to determine the reliability and reproducibility of the assay system.

(i) C^{14}-phenylethylamine was purified before use by passing it over a Bio-Rex 70 column and eluting with 1 N hydrochloric acid. This ensured that the label was only on phenylethylamine and not any metabolites in the shipped sample. As a result, over 99.9% of the labeled amine was retained by the column.

(ii) The reaction in the incubation mixture was fully inhibited by cooling to -5^{o} C (Figure 3).

(iii) Elution of metabolites of PEA from the Bio-Rex column was over 98% complete after four washes.

(iv) The reaction was shown to be linear with both time and tissue concentration (Figures 5 and 6).

Figure 3. Effect of cold temperature on MAO activity in the rat ventricle using phenylethylamine (PEA).

The PEA deaminated is expressed in counts per minute (CPM). Each point is the mean of at least two determinations. Whole rat ventricle homogenate, 6.6 mg/ml, was used. The ice-salt mixture into which the incubation tubes were placed was -8^{o} to -5^{o}C.

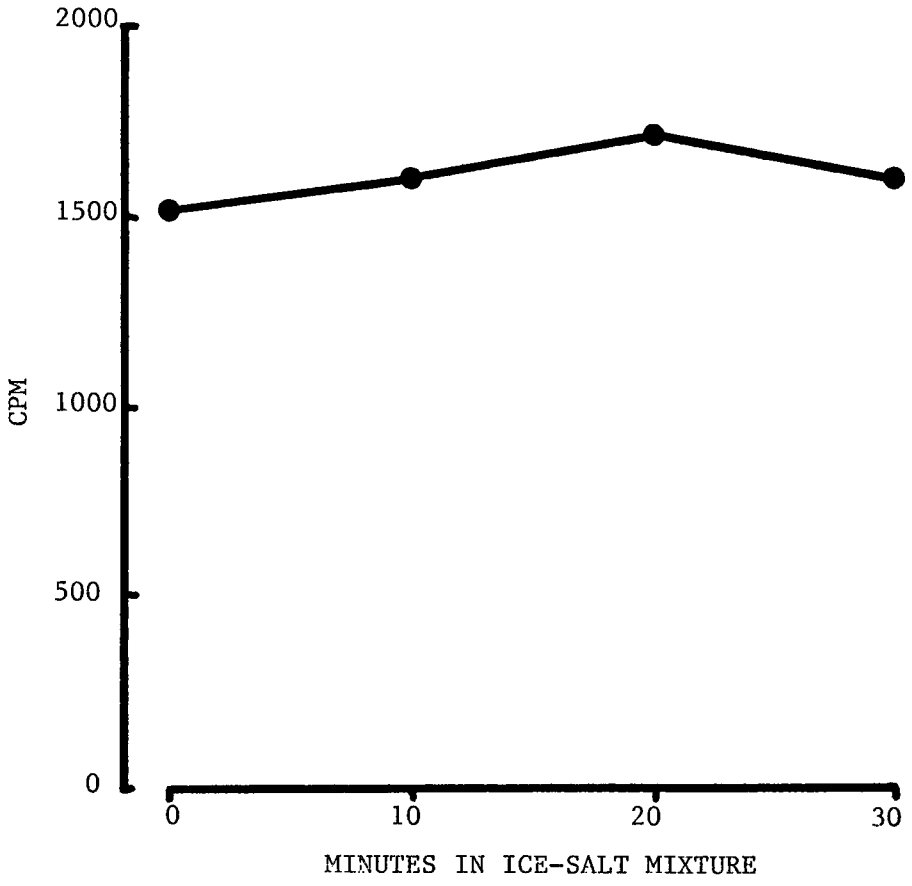

2000
1500
1000
500
0
CPM
0
10
20
30
MINUTES IN ICE-SALT MIXTURE

Figure 4. Effect of multiple washes on the elution of deaminated phenylethylamine (PEA) from a cation exchange column.

The PEA deaminated is expressed in counts per minute (CPM). Each point is the mean of at least two determinations. Whole rat ventricle homogenate, 6.6 mg/ml, was used. Washes consisted of 2 X 1 ml of distilled water. Percentages listed represent the proportion of the total activity eluted with that wash.

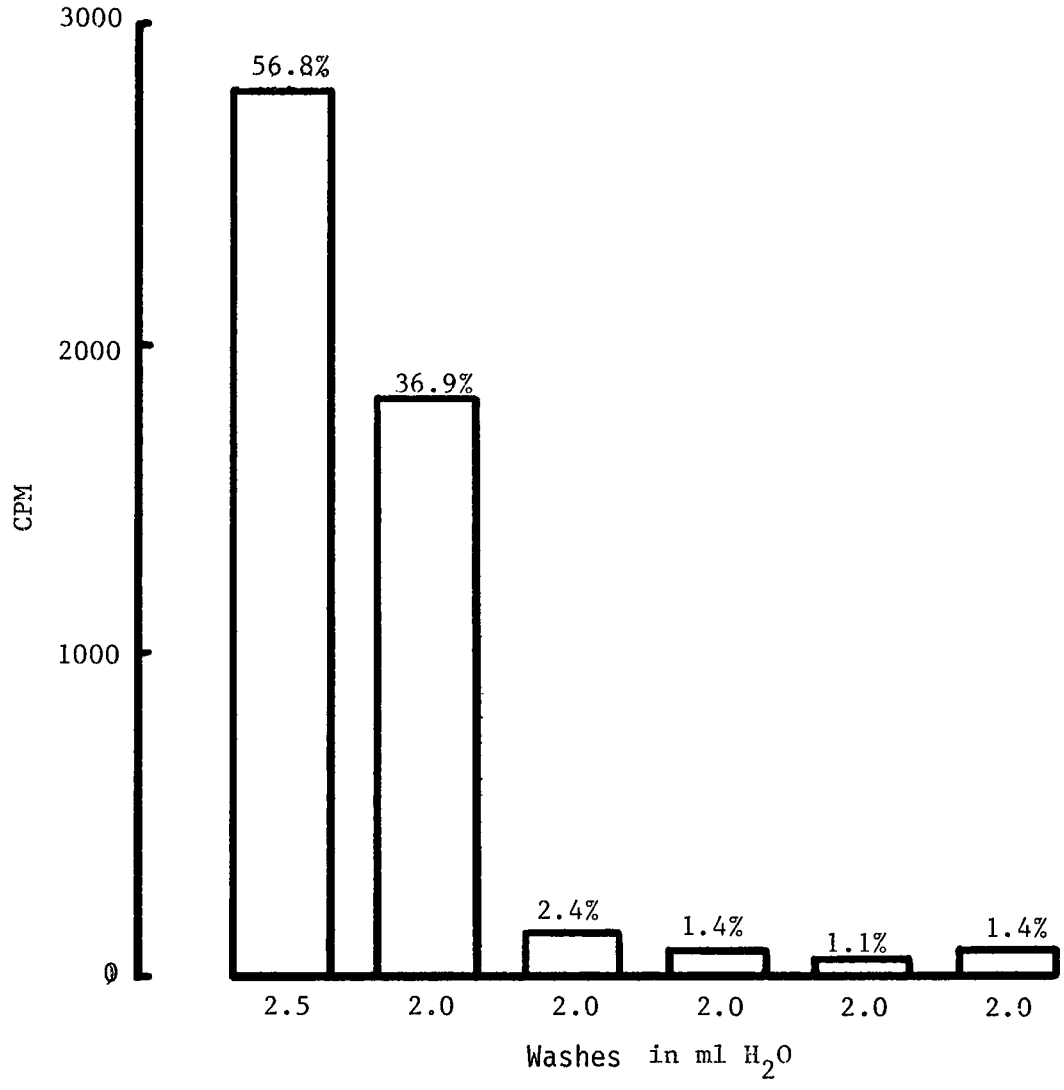

3000
2000
1000
0
CPM
56.8%
36.9%
2.4%
1.4%
1.1%
1.4%
2.5
2.0
2.0
2.0
2.0
2.0
Washes in ml H_2O

Figure 5. Effect of tissue concentration on MAO activity in the rat ventricle using phenylethylamine (PEA).

The PEA deaminated is expressed in counts per minute (CPM). Each point is the mean of at least two determinations. Preincubation time = 15 minutes; incubation time = 30 minutes.

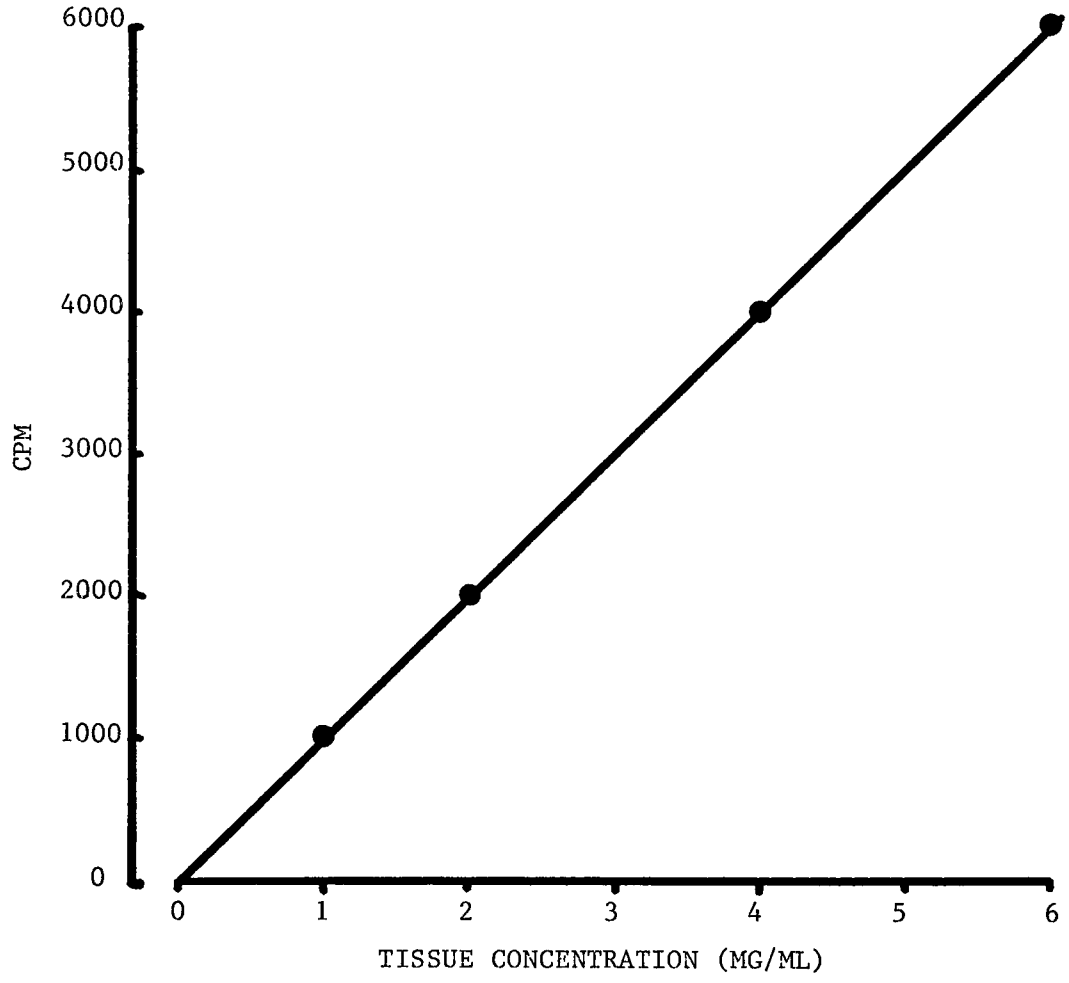
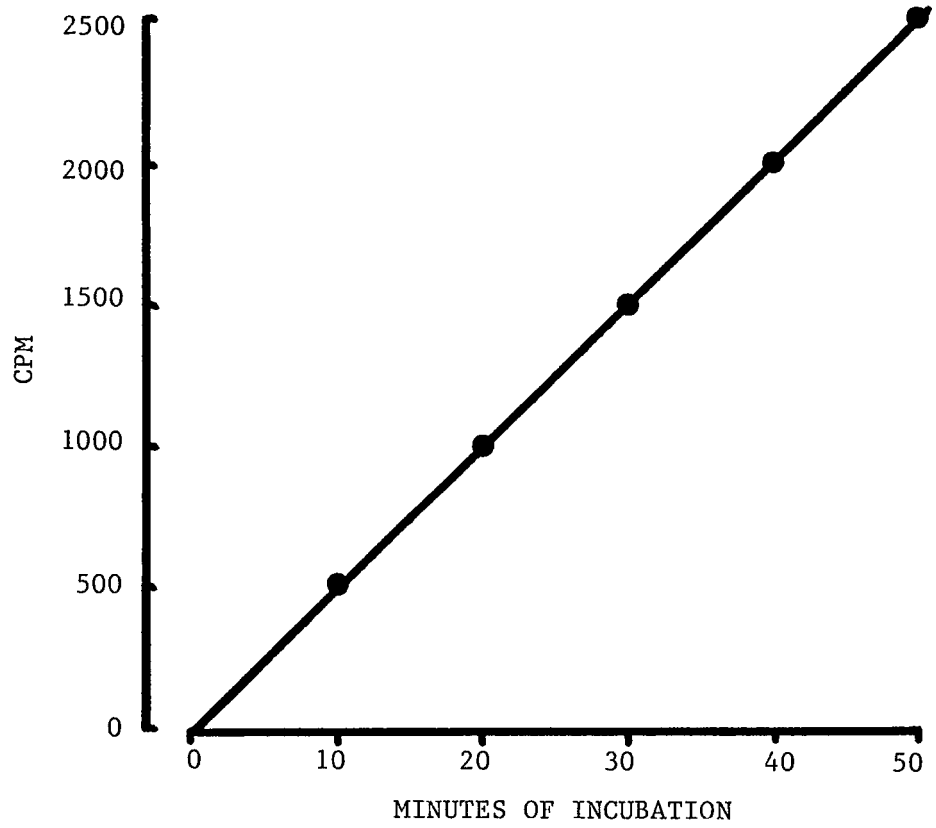

6000
5000
4000
3000
2000
1000
0
CPM
0
1
2
3
4
5
6
TISSUE CONCENTRATION (MG/ML)

Figure 6. Effect of incubation time on MAO activity in the rat ventricle using phenylethylamine (PEA).

The PEA deaminated is expressed in counts per minute (CPM). Each point is the mean of at least two determinations. Whole rat ventricle homogenate, 3.3 mg/ml, was used. Pre-incubation time = 15 minutes.


2500
2000
1500
1000
500
0
CPM
0
10
20
30
40
50
MINUTES OF INCUBATION

4. Inhibition Curves

MAO types A and B were defined by Johnston (1968) using tyramine in the rat brain with the inhibitor clorgyline in concentrations ranging from 10^{-11} M to 10^{-3} M. The activity most sensitive to inhibition by low concentrations of clorgyline was called the A type, while the less sensitive activity was called type B. Conversely, Knoll and Magyar (1972) introduced deprenyl, which was shown to inhibit type B MAO in low concentrations and type A MAO in higher concentrations. Another inhibitor, pargyline, is claimed to also be a preferential B inhibitor (Fuller, et al., 1970). Thus, by using a range of concentrations of various inhibitors, the type of MAO activity in a tissue can be defined.

Inhibition curves were obtained for a given drug by preincubating the tissue mixture for 15 minutes at 37^{o} C with the inhibitor in final concentrations of 10^{-11} M to 10^{-3} M. The substrate was then added and the assay proceeded as described previously.

The following criteria were established before the studies were made.

(i) None of the inhibitors, clorgyline, deprenyl, pargyline or semicarbazide, interfered with the fluorescence of 4-hydroxyquinoline in either the presence or absence of tissue or kynuramine, nor did they interfere with the blank values.

(ii) None of the inhibitors interfered with the extraction or determination of phenylethylamine deamination.

5. Kinetic Analysis

Lineweaver-Burk plots were made to determine the apparent Km and Vmax constants. These plots were constructed from measured values of initial velocity rates, 1/v (where v = initial velocity rates, nmoles product per mg tissue or protein per minute) vs. 1/s (where s = various substrate concentrations, μM). These were determined by incubating kynuramine (16.5, 20, 25, 33, 50, 67 and 100 μM) for the corresponding time intervals (4.5, 6, 8, 10, 15, 20 and 30 min). For phenylethylamine, combinations of the following concentrations and time intervals were employed (1, 1.34, 2, 3.4, 5, 10, 40, 50, 60 and 100 μM for 3, 4, 6, 10, 15, 30, 12, 15, 18 and 30 minutes). All other steps in the assay procedures remained unchanged. In most cases, the apparent Km values for the A and B types of MAO were obtained using clorgyline (10^{-7} M) to inhibit the A activity and deprenyl (10^{-7} M) to inhibit the B activity.

6. Mixed Substrate Experiments

Mixed substrate experiments were made using a combination of tryptamine or phenylethylamine with kynuramine. Tryptamine or phenylethylamine in concentrations of 12.5, 25 and 100 μM were mixed with 33 or 100 μM Kynuramine in the incubation mixture. The resulting 4-hydroxyquinoline produced was measured. Data was expressed as % activity with the added tryptamine or phenylethylamine.

7. Protein Determination

The method of Lowry et al. (1951) was used for protein determinations. Into duplicate test tubes 1 to 2 mg of tissue homogenate

was added and it was then diluted to 0.5 ml with water. A blank contained only water. Bovine serum albumin (BSA) standards ranged from 70 μg to 350 μg in 0.5 ml of water. To each tube was added 5 ml of Reagent C. The test tubes were mixed and incubated at room temperature for 10 minutes. Following this, 0.5 ml of Folin-Ciocalteu phenol reagent was added and the tubes were mixed and incubated 30 minutes longer. Absorbance at 700 nm was read on a Bausch and Lomb Spectronic 20 Spectrophotometer. The tissue values of protein were read directly from a standard curve of absorbance units versus μg BSA.

The following procedures were established before using the assay routinely.

(i) The optimum wavelength for measurement of absorbance by BSA was 700 nm.

(ii) A linear relationship existed between absorbance and concentration of BSA between 70 and 350 μg. Thus, all tissue concentrations were adjusted to read within that range.

(iii) A linear relationship existed between tissue concentration by weight and protein content in the range of 1 to 5 mg of tissue.

8. Preparation of Rat Heart Mitochondria

The method of Goridis and Neff (1971) was used in the preparation of rat heart mitochondria.

A rat heart from a freshly killed animal was weighed and then cut into thin slices over an ice-salt mixture in order to maintain a cold temperature. The tissue was transferred to a 2 ml ground glass hand homogenizer where it was homogenized in a small amount of 0.25 M

sucrose. The tissue was poured into a 15 ml Dounce glass tissue grinder (Wheaton Scientific, Millville, NJ) where it was ground first with a B pestle and then with a tighter-fitting A pestle. The volume of the homogenate was adjusted to give a tissue concentration of 100 mg/ml and was then centrifuged at 700 xg for 10 minutes at 4^{o} C. The resulting supernatant was decanted into another tube and spun at 12000 xg for 20 minutes. The pellet was resuspended in a small amount of sucrose solution and respun at 12000 xg for 20 minutes. The final pellet containing the washed mitochondrial fraction was resuspended in 100 ml of sucrose solution.

9. Adrenalectomy

Rats were anesthetized with ether and their backs shaved. A crosswise skin incision was made on the dorsal surface about one centimeter posterior to the twelfth rib. A sharp forceps was forced through the muscle layer on one side of the animal and into the abdominal cavity. The forceps were slowly spread to enlarge the opening without cutting the muscle. After locating the adrenal gland it was teased away from connecting tissue with the forceps and removed intact. The muscle opening was sutured with black silk thread. An identical procedure was used to remove the opposite adrenal gland. The skin was then clipped together with several 9 mm autoclips (Clay Adams, Parsippany, NJ) and the animal allowed to recover from the anesthetic.

Sham-operated rats were subjected to the same procedure, the adrenal glands being located but not removed. All sham and adrenalectomized animals were maintained on normal saline in place of drinking water.

Verification of completeness of adrenalectomy was obtained through post-mortem examination of the areas of excision of the glands and by sample determinations of corticosterone blood levels (Silber et al, 1958). The mean corticosterone level in adrenalectomized animals was 1.7 µg% compared to a control value of 12.8 µg%.

10. Intact Atria Experiments

In order to better evaluate results from in vitro experiments on whole tissue homogenates, a series of experiments was performed on whole, isolated atria. These were chosen because they remain viable in vitro. Additionally, the thin walls of the atria allow easy access of substances to the various cellular components.

Atria were removed from freshly killed rats. Left and right atria were separated and pooled together in beakers of Kreb's solution. Subsequently, individual atria were placed in 25 ml Erlenmeyer flasks containing 2.5 ml of Kreb's solution. The flasks were incubated at 37^{o} C for 15 minutes in a Dubnoff Metabolic Shaking Apparatus under 95% O_2 + 5% CO_2 atmosphere. Assays were performed in at least duplicate utilizing a left and a right atria at each point. The assay reaction was initiated by addition of 0.5 ml of kynuramine (307 nmol in Kreb's solution) to each flask. Incubation at 37^{o} C was continued for a further 30 minutes. The reaction was terminated by removing the atria from the flasks and freezing them in a vial contained in a dry ice-acetone mixture. To the remaining incubation fluid, 2 ml of 10% TCA was added and the entire mixture was then transferred to a test tube and centrifuged. The supernatant fluid was analyzed for 4-hydroxyquinoline

content as previously described. The frozen atria were weighed and then homogenized in 2 ml of 10% TCA. A 3 ml aliquot of Kreb's solution was pipetted into each homogenate and the mixture was centrifuged at 1500 rpm for 8 minutes. The supernatant was used for determination of 4-hydroxyquinoline production as described above.

In experiments where an inhibition curve was performed on the whole atria, 0.5 ml of clorgyline in Kreb's solution was placed into the flask for 15 minutes prior to substrate addition. Calculations of MAO activity in units of nanomoles 4-hydroxyquinoline (4OHQ) produced/mg tissue/hr were made by the following formula. Thus, values for total deamination by the tissue were obtained.

$$\text{S.A.} = \frac{\text{nmoles 4OHQ in incubation fluid + nmoles 4OHQ in tissue}}{\text{mg tissue in atria}} \times 2$$

The following series of experiments were run to determine the reliability of this assay procedure for intact atria.

(i) A linear relationship existed between the atrial weight and the amount of deamination as measured by 4OHQ production. Thus, S.A. was calculated based on mg of tissue rather than per atria.

(ii) Kreb's solution did not interfere with the fluorescence of 4-hydroxyquinoline, nor did it alter blank values.

11. Statistical Methods

The standard error (S.E.) of the mean was determined on a Monroe 1860 calculator using the following formula:

$$\text{S.E.} = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{N}}{N(N-1)}}$$

where x = data points; N = number of data points.

The probability level, P, was determined by the Student t-test with 95% confidence limits.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S.D. \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$$

where x_1 and x_2 = data points of experiment 1 and experiment 2; S.D. = standard error of the difference; N_1 and N_2 = numbers of data points x_1 and x_2. The degrees of freedom = $N_1 + N_2 - 2$.

All kinetic data were determined from a line calculated by the method of least squares utilizing a program on the Monroe calculator. The formula was:

$$m = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{N}}{\Sigma x^2 - \frac{(\Sigma x)^2}{N}} \quad \text{and}$$

$$i = \frac{\Sigma y - m\Sigma x}{N}$$

where m = slope and i = y intercept.

Km values were determined by calculating the point of interception of the line on the X axis ($= -\frac{i}{m}$) and finding the reciprocal.

12. Sources and Preparative Procedures for Drugs and Chemicals

(i) Bio-Rex 70 (100-200 mesh, sodium form, Bio-Rad Laboratories). To the resin was added enough distilled water to make a slurry. This was poured into a four liter Erlenmeyer flask and water was added up to two liters. The resin was mixed by gentle shaking, then allowed to settle for 1.5 minutes. The supernate was decanted, more water was

added, and the cycle was repeated a number of times until the fluid was clear after the resin had settled for 1.5 minutes. The resin was rinsed several times with 0.5 M phosphate buffer, pH 6.0, until the pH was constant. A final rinse was made in 0.1 M phosphate buffer, pH 6.0, and the diluted resin was stored under refrigeration.

(ii) Bovine Serum Albumin (fraction V powder, Sigma Chemical Co.). A 7 mg/ml aqueous solution was made and stored under refrigeration.

(iii) Calcium Chloride ($CaCl_2 \cdot 2H_2O$), Fisher Scientific Co.).

(iv) Clorgyline Hydrochloride (M & B 9302, May and Baker, Ltd.). Solutions of 6×10^{-11} M to 6×10^{-4} M were made in distilled water.

(v) Cupric Sulfate ($CuSO_4 \cdot 5H_2O$, fine crystal, J.T. Baker Chemical Co.).

(vi) Deprenyl Hydrochloride (a gift from Dr. M.B.H. Youdim). Solutions of 6×10^{-11} M to 6×10^{-4} M were made in distilled water.

(vii) Disodium Phosphate ($Na_2HPO_4 \cdot 7H_2O$, Fisher Scientific Co.).

(viii) Ether (anhydrous, Baker Chemical Co.).

(ix) Folin-Ciocalteu Phenol Reagent (2N, Fisher Scientific Co). A 1 N solution was prepared.

(x) D-Glucose (anhydrous, Fisher Scientific Co.).

(xi) Hydrochloric Acid (HCl, reagent ACS, Fisher Scientific Co.). A 0.001 N solution was made.

(xii) 4-Hydroxyquinoline Trihydrate (Aldritch Chemical Co., Inc.). A μMol/ml solution was stored in an opaque bottle under refrigeration.

(xiii) Kreb's Physiological Solution, isotonic, pH 7.40.

The following chemicals were dissolved in 1.5 liters of distilled water:

NaCl	13.8 grams
KCl	0.7
$NaHCO_3$	4.2
KH_2PO_4	0.32
$MgSO_4 \cdot 7H_2O$	0.58
Glucose	4.0

Then 0.76 grams $CaCl_2 \cdot 2H_2O$ were dissolved in 50 ml water. The two solutions were combined and the volume brought to two liters. The solution was bubbled for 10 minutes with 95% O_2:5%CO_2.

(xiv) Kynuramine Dihydrobromide (Regis Chemical Co.). A 200 µg/ml solution was stored in an opaque bottle under refrigeration.

(xv) Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$, Fisher Scientific Co.).

(xvi) Pargyline Hydrochloride (Regis Chemical Co.). Solutions of 6 X 10^{-11} M to 6 X 10^{-4} M were made in distilled water.

(xvii) Perchloric Acid ($HClO_4$, reagent ACS, Fisher Scientific Co.). A 6% w/v solution was prepared.

(xviii) Permafluor I (Packard).

(xix) β-Phenylethylamine (free base, Sigma Chemical Co.).

(xx) 2-C^{14}-Phenylethylamine Hydrochloride (52 mCi/mmole, New England Nuclear). A stock solution of labeled phenylethylamine (PEA) was purified prior to use. The contents of a vial were diluted to 10 ml of 0.5 mM PEA by adding water and cold PEA. The dilution was passed over a 0.75 X 4.5 cm column of Bio-Rex 70 buffered to pH 6.0. The column was washed with 16 X 1 ml portions of water. The C^{14}-PEA was then eluted from the column

with 8 X 1 ml aliquots of 1 N HCl. The pH was adjusted to 2.5 with 10 N NaOH. The volume was adjusted to a final concentration of 0.5 mM with 25 μCi/ml (50 μCi/μMol). A working solution of 0.05 mM, 2.5 μCi/ml (50 μCi/μMol) was prepared by dilution with 0.001 N HCl.

(xxi) Potassium Chloride (KCl, Fisher Scientific Co.).

(xxii) Potassium Phosphate (KH_2PO_4, primary monobasic, Fisher Scientific Co.).

(xxiii) Reagent A. 2% sodium carbonate in 0.1 N sodium hydroxide: 20 grams Na_2CO_3 and 4.0 grams NaOH were dissolved in one liter of water.

(xxiv) Reagent B. 0.5% cupric sulfate in 1% sodium potassium tartrate: 0.125 grams $CuSO_4 \cdot 5H_2O$ and 0.25 grams sodium potassium tartrate were dissolved in 25 ml water.

(xxv) Reagent C. Mix 50 ml Reagent A and 1 ml Reagent B.

(xxvi) Scintillation Counting Mixture. Mix 80 ml Permafluor I and 667 ml Triton X-100 with 1253 ml Toluene.

(xxvii) Semicarbazide Hydrochloride ($NH_2CONHNH_2 \cdot HCl$, crystal, Baker Chemical Co.). 6 X 10^{-3} M solution: dissolve 0.0335 grams in 50 ml water.

(xxviii) Sodium Bicarbonate ($NaHCO_3$, Baker Chemical Co.).

(xxix) Sodium Carbonate (Na_2CO_3, Fisher Scientific Co.).

(xxx) Sodium Chloride (NaCl, Fisher Scientific Co.). A 0.9% w/v solution was made.

(xxxi) Sodium Hydroxide (NaOH, pellets, Fisher Scientific Co.). A 1 N solution was prepared by dissolving 40 grams in one liter of distilled water.

(xxxii) Sodium Potassium Tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$, crystal, Fisher Scientific Co.).

(xxxiii) Sorensens Phosphate Buffer, 0.1 M, pH 6.0. Dissolved 13.8 grams KH_2PO_4 plus 14.2 grams $Na_2HPO_4 \cdot 7H_2O$ in one liter of water.

(xxxiv) Sorensens Phosphate Buffer, 0.15 M, pH 7.4. Dissolved 4.02 grams KH_2PO_4 plus 21.4 grams $Na_2HPO_4 \cdot 7H_2O$ in one liter of water.

(xxxv) Sorensens Phosphate Buffer, 0.5 M, pH 6.0. Dissolved 69.0 grams KH_2PO_4 plus 71.0 grams $Na_2HPO_4 \cdot 7H_2O$ in one liter of water.

(xxxvi) Sorensens Phosphate Buffer, 0.5 M, pH 7.4. Dissolved 13.5 grams KH_2PO_4 plus 107.6 grams $Na_2HPO_4 \cdot 7H_2O$ in one liter of water.

(xxxvii) Sucrose (Saccharose, Fisher Scientific Co.). A 0.25 M solution was made by dissolving 85.58 g in one liter of water. It was stored under refrigeration.

(xxxviii) Toluene ($C_6H_5CH_3$, spectranalyzed, Fisher Scientific Co.).

(xxxix) Trichloroacetic Acid (CCl_3COOH, reagent ACS, Fisher Scientific Co.). A 10% w/v wolution was made.

(xxxx) Triton-X 100 (Sigma Chemical Co.).

(xxxxi) Tryptamine Hydrochloride ($C_{10}H_{12}N_2 \cdot HCl$, Aldrich Chemical Co., Inc.).

IV. EXPERIMENTS AND RESULTS

Section A

General Rationale

The initial approach taken was to compare cardiac MAO of the rat to an MAO activity which had been previously well defined. Such an organ is the rat vas deferens. This tissue contains approximately equal proportions of A and B activity, about half of which is located intraneuronally. It is functionally quite different from the heart and, therefore, might be expected to reveal differences in MAO activity, should they exist. The primary substrate chosen for this study was kynuramine, a synthetic compound which is deaminated by both MAO types. Because kynuramine is not an endogenous compound, its use is not widespread. However, it provides a sensitive and reproducible assay system that is relatively quick and inexpensive to perform. Deamination of kynuramine by the heart and vas deferens was compared in three different ways: 1) through inhibition with preferential MAO inhibitor drugs, 2) by measurement of kinetic parameters (Km and Vmax), and 3) by the use of mixed substrate interactions.

Part 1. Inhibitor Characteristics

Rationale:

The inhibitor drugs chosen for this characterization were three irreversible acetylenic inhibitors, clorgyline, deprenyl and

pargyline. It was through the introduction of clorgyline by Johnston in 1968 that the A and B types of MAO were first described. Thus, clorgyline was defined as a preferential type A inhibitor. In contrast, deprenyl, and to a lesser extent pargyline, were preferential type B inhibitors. By means of progressive inhibition curves with these three compounds, the type or types of MAO in a tissue can be identified, and thereby partially characterized.

1. Inhibition characteristics of clorgyline, deprenyl and pargyline in rat ventricle homogenates:

Clorgyline, deprenyl and pargyline are irreversible inhibitors of MAO, binding co-valently to the enzyme cofactor (FAD). Effective use of these drugs to characterize MAO requires standardization of their use and knowledge as to how their inhibitory activity is affected by the amount of enzyme and substrate used. The following experiments were undertaken to define these variables.

Clorgyline, deprenyl and pargyline were preincubated for various times with rat ventricle homogenate using kynuramine as the substrate (Figure 7). While all clorgyline and deprenyl concentrations showed a relatively consistent degree of inhibition by 20 minutes of preincubation, pargyline did not. Subsequent studies revealed that 10^{-7} M pargyline reached a plateau of inhibition by 25 minutes of preincubation. Based upon these results, a 15 minute preincubation period was chosen for all further studies.

Figure 7. Effect of preincubation time on inhibition of MAO activity by clorgyline, deprenyl and pargyline in the rat ventricle.

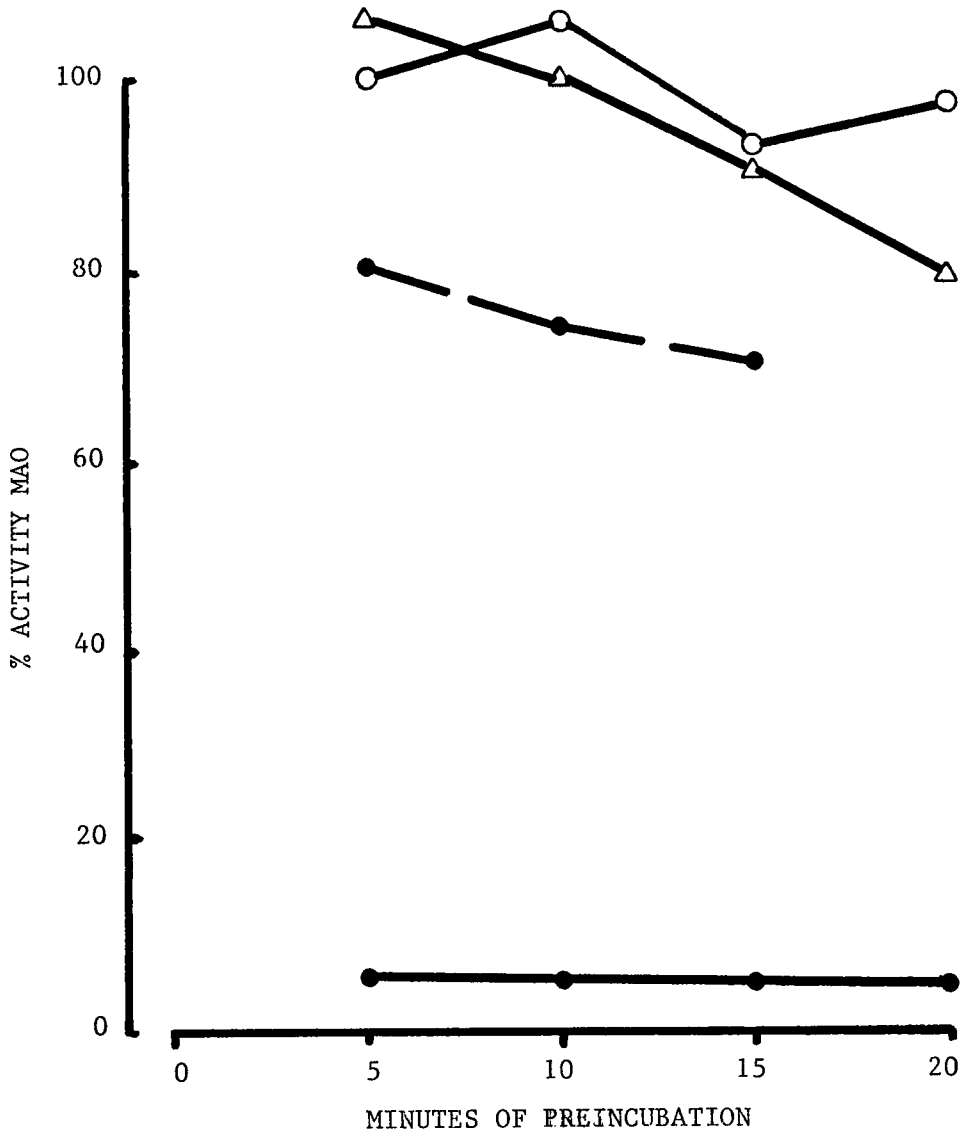
The 4-hydroxyquinoline formed is expressed as a percentage of the control activity (no inhibitor, 30 min preincubation = 100%). Each point is the mean of at least two determinations.

10^{-7} M Clorgyline ●——●

10^{-9} M Clorgyline ●— —●

10^{-7} M Deprenyl ○——○

10^{-7} M Pargyline △——△


100
80
60
40
20
0
% ACTIVITY MAO
0
5
10
15
20
MINUTES OF PREINCUBATION

The effect of varying the homogenate concentration on the inhibition of rat ventricle MAO by clorgyline is shown in Figure 8A. At low homogenate concentrations (0.33 to 3.33 mg/ml) little or no shift occurred in the resulting inhibition curves. However, the higher homogenate concentration (33.33 mg/ml) exhibited a 10-fold shift to the right. This shift could be simulated by artificially increasing the protein content of the homogenate with bovine serum albumin or heart tissue in which MAO activity had been completely inhibited (Figure 8B). It seems therefore that non-specific binding and/or other factors become a complicating influence at high concentrations of the homogenate. For instance, incomplete inhibition is seen with bovine serum albumin between 10^{-7} M and 10^{-4} M clorgyline. At 3.33 mg/ml and below, clorgyline would appear to be in excess, and the position of the curve more truely represents the sensitivity of MAO to the inhibitor.

Based upon the above findings, the 3.33 mg/ml homogenate was selected for further detailed study. This homogenate concentration exhibited a suitable activity and more than one inhibitory component to the clorgyline curve (a small secondary inhibitory component is seen between 10^{-8} M and 10^{-4} M). This secondary component was presumably "diluted out" at the lower homogenate concentrations.

The effect of increasing the kynuramine concentration by five-fold is shown in Figure 9. Virtually no differences resulted in the position of the inhibition curve to clorgyline. The 500 μM concentration exhibited a somewhat lower specific activity compared with the

Figure 8. Effect of tissue concentration on clorgyline inhibition of MAO activity in the rat ventricle.

(A) Effect of differing homogenate concentrations on clorgyline inhibition curves. The 4-hydroxyquinoline produced is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

0.33 mg/ml

0.66 mg/ml

3.33 mg/ml

33.33 mg/ml

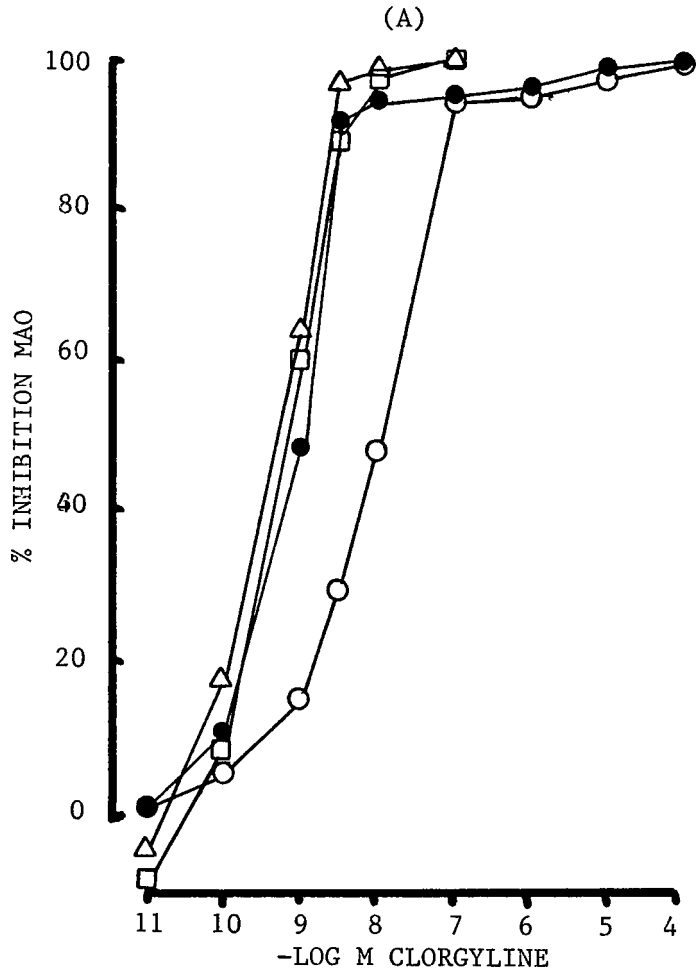
(B) Effect of MAO inhibited tissue or bovine serum albumin (BSA) on clorgyline inhibition curves. MAO activity was inhibited fully by treating rats with 75 mg/kg pargyline, i.p., 24 hours before sacrifice. Hearts were removed, rinsed well and homogenized as described in the Methods. BSA was added to the homogenate (3.33 mg/ml) to bring the total protein content to that found in 33.3 mg/ml of the homogenate.

The 4-hydroxyquinoline formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

3.33 mg/ml active tissue

3.33 mg/ml active tissue plus
29.97 mg/ml MAO inhibited tissue

3.33 mg/ml active tissue plus BSA


(A)
100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
-LOG M CLORGYLINE

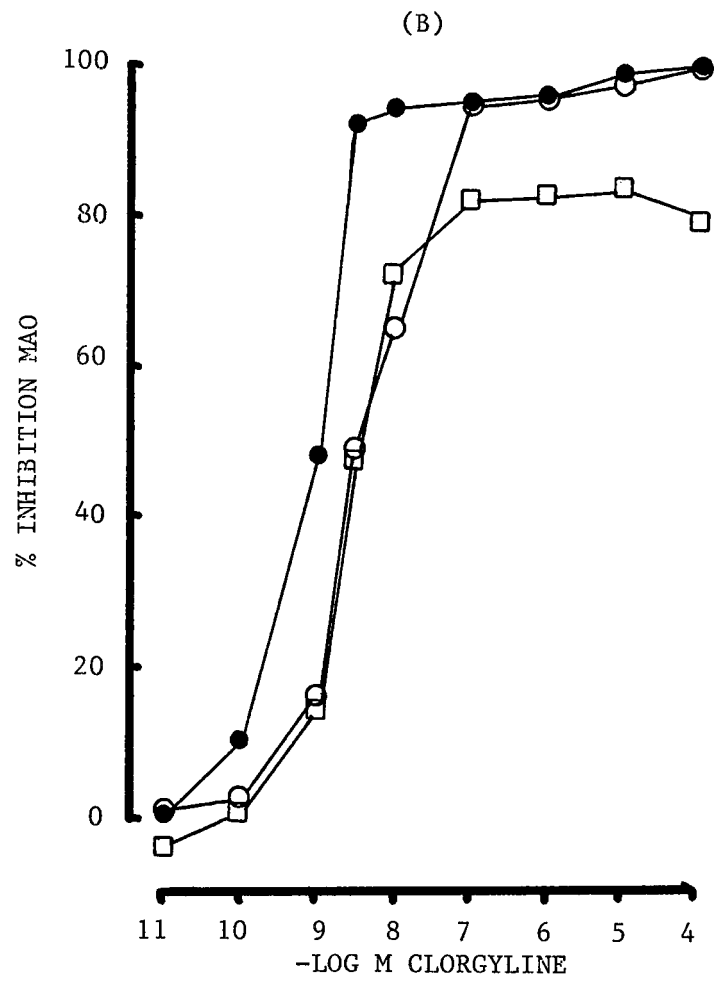
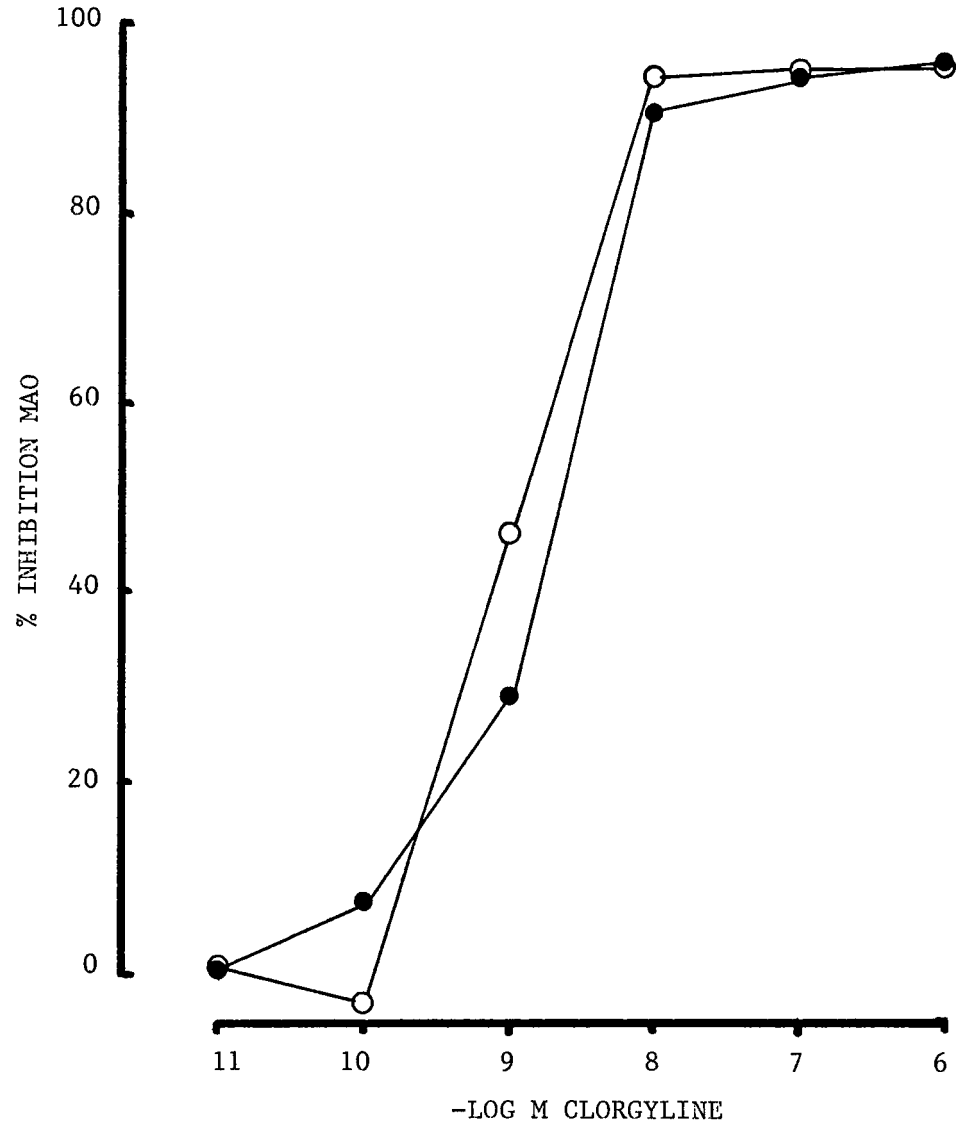

(B)
100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
-LOG M CLORGYLINE

Figure 9. Effect of differing kynuramine concentrations on clorgyline inhibition of MAO activity in the rat ventricle.

The 4-hydroxyquinoline (4OHQ) formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations. The specific activities were: 3.0 nmoles 4OHQ/mg tissue/hr (100 μM kynuramine) and 2.6 nmoles 4OHQ/mg tissue/hr (500 μM kynuramine).

100 μM Kynuramine ●—●

500 μM Kynuramine ○—○


100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
-LOG M CLORGYLINE

100 μM curve (2.6 vs. 3.0 nmoles 4OHQ/mg tissue/hr.). Kynuramine has been reported to exhibit substrate inhibition of MAO at high substrate concentrations (Youdim and Woods, 1975).

2. Comparison of inhibition curves to clorgyline, deprenyl and pargyline in rat vas deferens, ventricle and atria:

MAO types can be defined upon the basis of inhibitor sensitivity. Clorgyline preferentially inhibits type A MAO, whereas deprenyl and pargyline preferentially inhibit type B. The object of the experiment was to determine whether cardiac tissues were the same or different from the vas deferens.

In all experiments the inhibitor was preincubated with the homogenate for 15 minutes prior to the addition of kynuramine, 100 μM.

Figure 10 shows that the vas deferens contains both A and B activity. Clorgyline revealed a biphasic inhibition curve with a plateau at about 50% inhibition. The initial curve, between 10^{-11} M and 10^{-8} M, defines the presence of type A MAO, while the second curve defines type B activity.

When deprenyl was used as the inhibitor, kynuramine again revealed a biphasic curve. However, the relative percentage activity detected differed from that with clorgyline; about 68% of the total being classed as the B enzyme. The reason for this difference appears to be age related. The rats used for the clorgyline study had a mean body weight of 382 g; the deprenyl group weighed considerably

more (mean body weight, 800 g). In another experiment, vasa deferentia were pooled from four rats (mean body weight, 381 g) and separate inhibition curves to clorgyline and deprenyl were made from the common homogenate. The relative proportions of type A and B given by either inhibitor were virtually the same (55% type A).

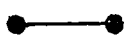
In contrast to clorgyline and deprenyl, pargyline failed to produce a biphasic curve, revealing its relative inability to distinguish between the two types of MAO.

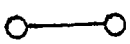
Figure 11 shows inhibition curves obtained in the ventricle. About 90 to 95% of the total activity defined by clorgyline is clearly type A MAO. The remaining 5 to 10% is much less sensitive to clorgyline and represents type B activity. A similar result was obtained with deprenyl, although the B component is less clearly evident. Pargyline gave an identical result to that obtained with deprenyl. Thus, pargyline discriminated between type A and type B in the ventricle while failing to do so in the vas deferens. Virtually identical results were obtained in atrial homogenates (Figure 12). About 90% of the total activity is defined as type A with the remainder being type B. Thus, little difference exists between atrial and ventricular tissue. Overall, the data shows that a small but significant proportion of type B activity exists in the rat heart using kynuramine as the substrate. Previously all other substrates, with the exception of benzylamine, have failed to reveal this component.

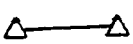
Table 1 shows the calculated IC_{50} concentrations for clorgyline and deprenyl on the type A and type B activities in the three tissues

Figure 10. Inhibition curves with clorgyline, deprenyl and pargyline on the rat vas deferens.

The 4-hydroxyquinoline formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least six determinations.

Clorgyline 

Deprenyl 

Pargyline 

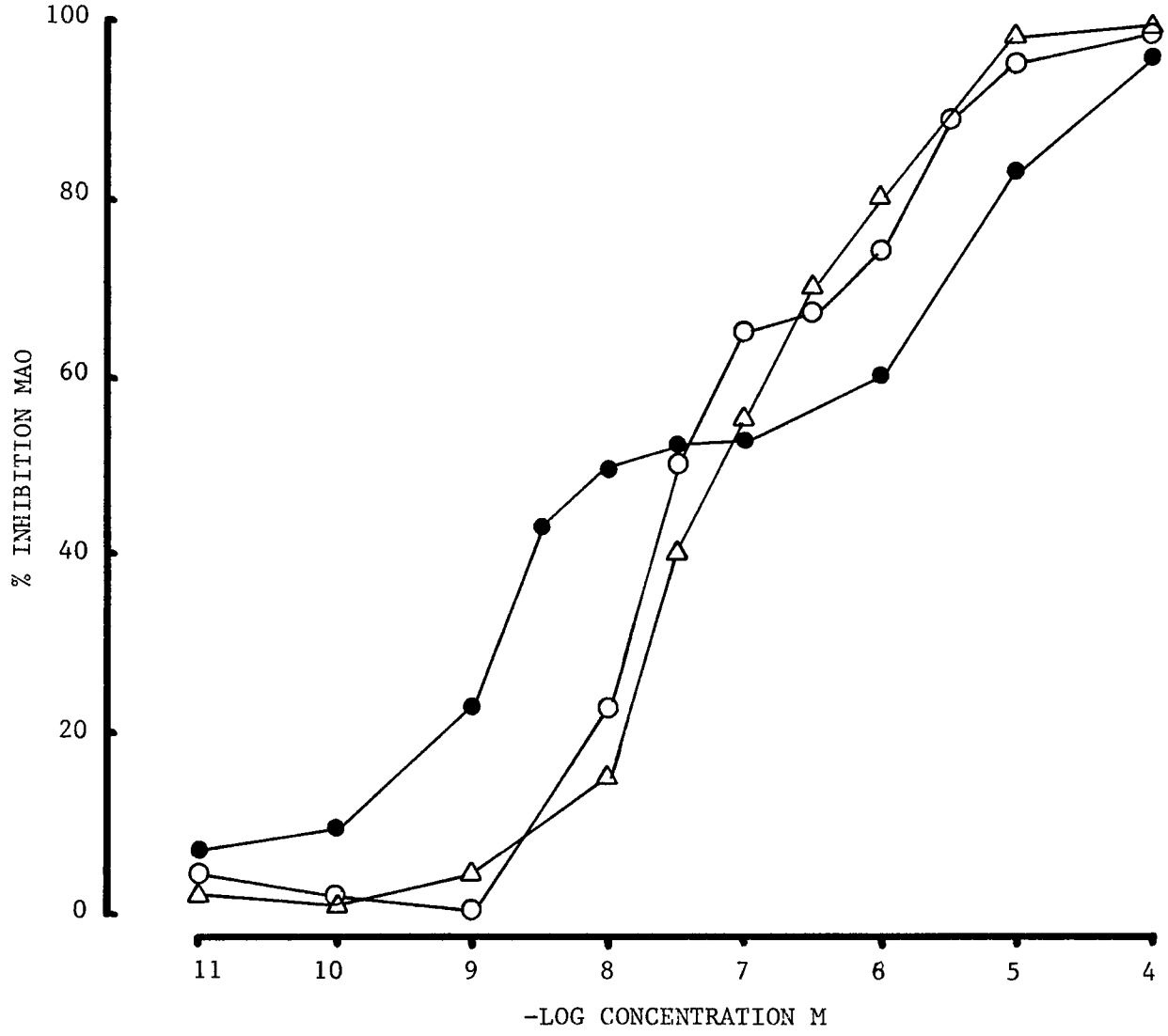

100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
-LOG CONCENTRATION M

Figure 11. Inhibition curves with clorgyline, deprenyl and pargyline on the rat ventricle.

The 4-hydroxyquinoline formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least six determinations.

Statistical significance: Clorgyline concentrations of 10^{-8} M to 10^{-6} M were not statistically different from each other ($p = 0.05$), but were statistically different from the higher clorgyline concentrations (10^{-5} M and 10^{-4} M) ($P < 0.01$).

Clorgyline ●——●

Deprenyl ○——○

Pargyline △——△

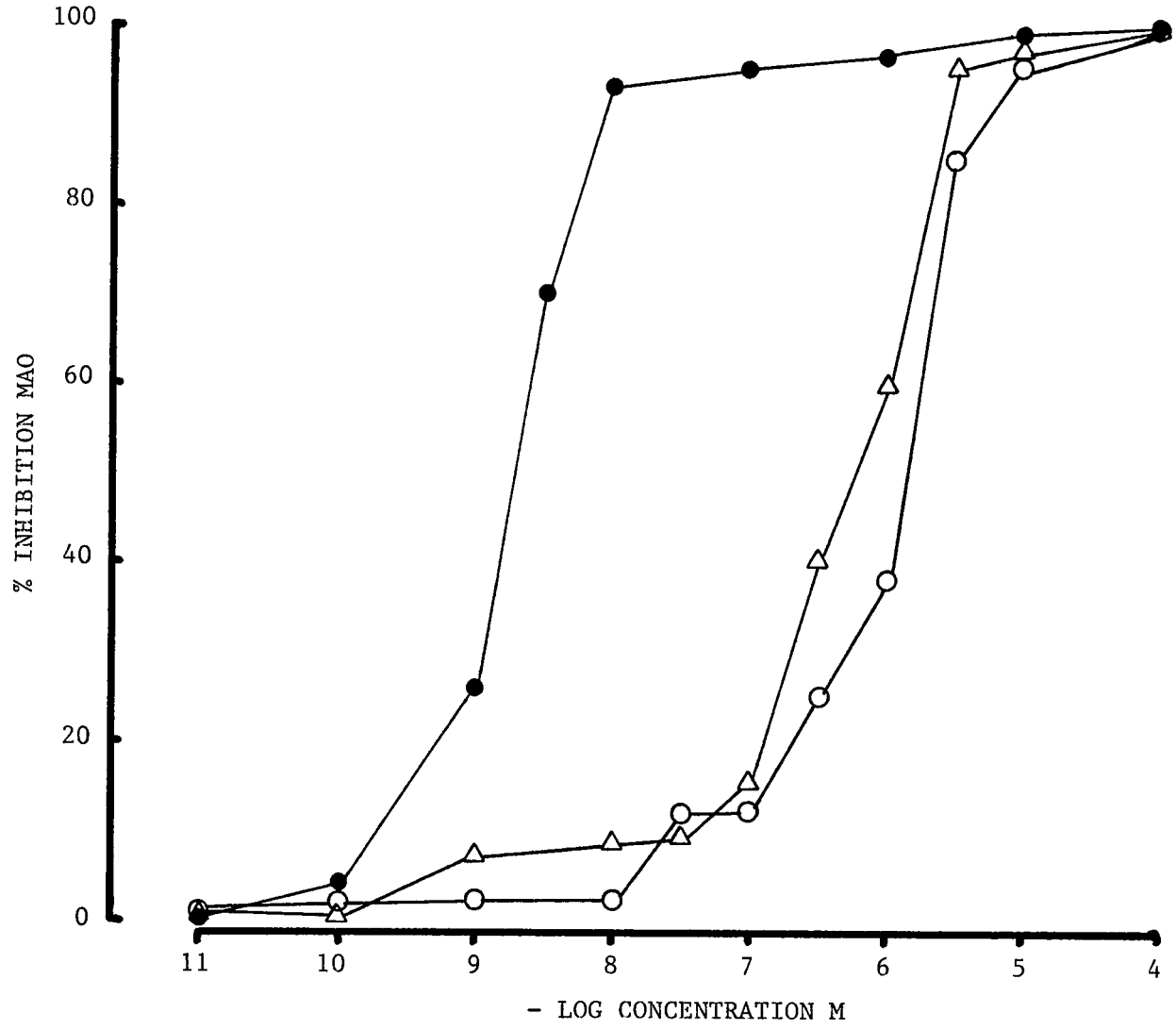

100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
- LOG CONCENTRATION M

Figure 12. Inhibition curves with clorgyline, deprenyl and pargyline on the rat atria.

The 4-hydroxyquinoline formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

Clorgyline ●——●

Deprenyl ○——○

Pargyline △——△

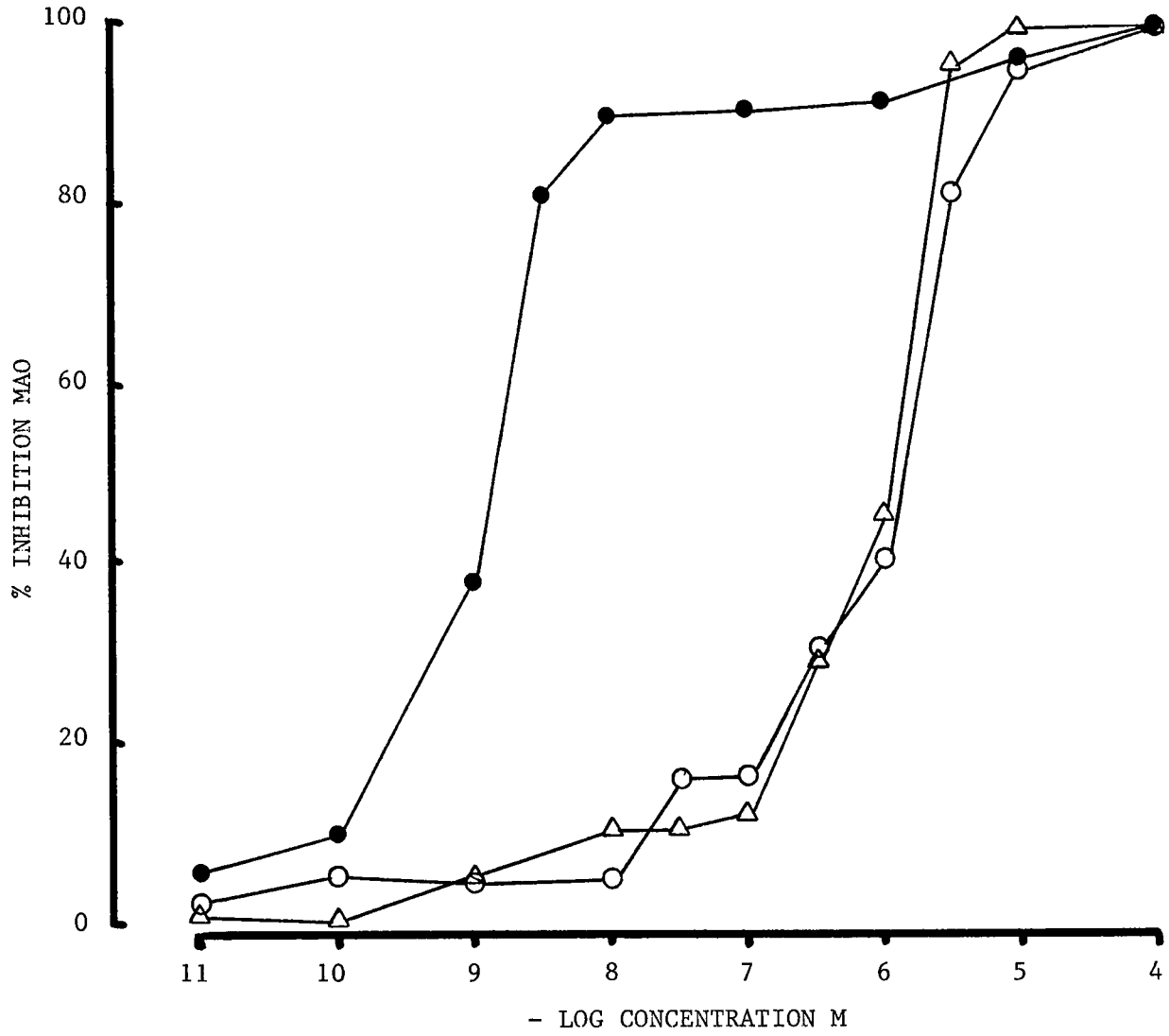

100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
- LOG CONCENTRATION M

TABLE 1. MAO activity and IC_{50} values for clorgyline and deprenyl in the rat vas deferens, ventricle and atria.

The specific activities are expressed as the mean ± S.E.M. for (n) experiments. The IC_{50} concentrations are mean values and are expressed as the molar concentration of the inhibitor producing 50% inhibition of either the A or B activity with kynuramine.

MAO Specific Activity (nmoles 4OHQ/mg tissue/hr)		IC_{50} (M)			
		Clorgyline		Deprenyl	
		A	B	A	B
Vas deferens	3.16±0.21 (10)	$1.1x10^{-9}$	$5.8x10^{-6}$	$2.6x10^{-6}$	$2.1x10^{-8}$
Ventricle	3.72±0.62 (11)	$1.6x10^{-9}$	$5.0x10^{-6}$	$2.1x10^{-6}$	$2.1x10^{-8}$
Atria	2.52±0.40 (3)	$1.2x10^{-9}$	$8.0x10^{-6}$	$2.5x10^{-6}$	$1.7x10^{-8}$

studied. In all cases the values are closely allied, indicating similar MAO activities between the various tissues. The MAO activity of the heart is seen to be lowest in the atria, while the vas deferens and ventricle exhibit comparable specific activities.

Finally, it is worthy of note that complete inhibition of MAO activity was always obtained with the three inhibitors used. Thus, evidence for clorgyline-resistant activity was not forthcoming.

3. Effect of clorgyline on MAO activity in rat abdominal aorta and inferior vena cava:

Cardiac tissue has been found to contain predominantly A activity. This phenomenon is somewhat unique since homogenates of other tissues have always revealed a sizable B component (vas deferens, liver, brain, etc.). Indeed, human platelets contain entirely MAO type B, but no whole organ has been described with solely A activity. It was of interest, therefore, to determine whether blood vessels were similar to cardiac tissue. Possibly, the B component of the heart could arise from blood vessel MAO.

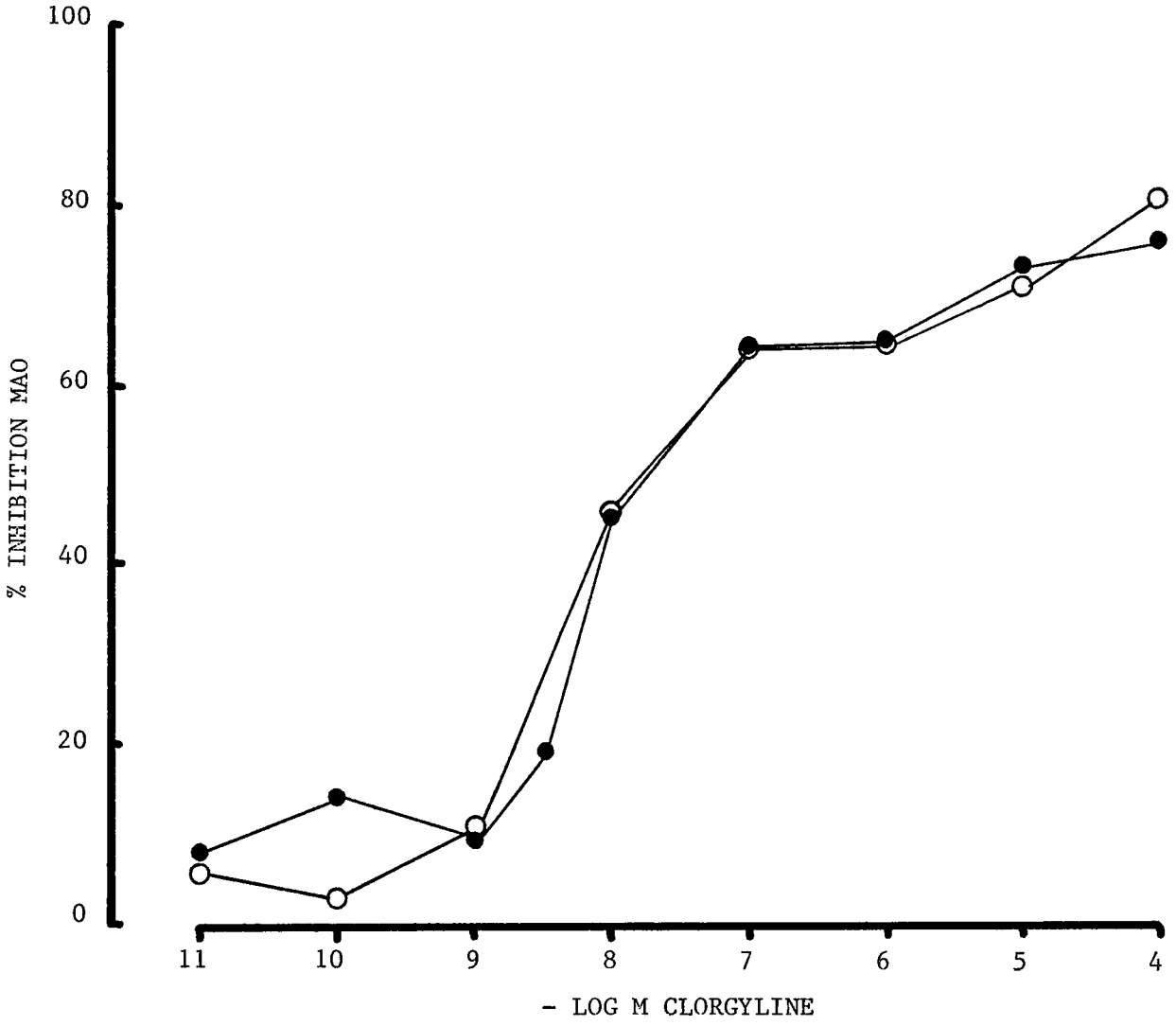
Figure 13 shows that about 65% of the total activity may be defined as MAO type A. Another 10% of the total activity appears to represent MAO type B. Clorgyline-resistant activity is seen to be present in both vessels since incomplete inhibition was obtained at 10^{-4} M clorgyline. Compared with the heart and vas deferens, the specific activities were much lower (see Table 1) and the IC_{50} values for clorgyline on the A activity somewhat higher (7.9×10^{-9} M, aorta and 5.8×10^{-9}M, vena cava.

Figure 13. Inhibition curves with clorgyline on the rat abdominal aorta and inferior vena cava.

The 4-hydroxyquinoline (4OHQ) formed is expressed as a percentage of the difference from the control activity (0.76 nmoles 4OHQ/mg tissue/hr = 0% inhibition of aorta; 0.53 nmoles 4OHQ/mg tissue/hr = 0% inhibition of vena cava). Each point is the mean of at least two determinations of pooled samples obtained from 10 rats (aorta) and 13 rats (vena cava). Whole rat abdominal aorta and inferior vena cava homogenates (3.33 mg/ml).

Abdominal aorta ●——●

Inferior vena cava ○——○


100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
- LOG M CLORGYLINE

4. Effect of semicarbazide on MAO activity of rat major blood vessels:

The previous experiment showed that clorgyline-resistant activity existed in the major blood vessels of the rat. Plasma and connective tissue amine oxidases are also very insensitive to clorgyline (Coquil et al., 1973). These amine oxidases utilize pyridoxal phosphate as their cofactor and as such are sensitive to inhibition by semicarbazide. Thus, it was of interest to determine whether the clorgyline-resistant activity of the major blood vessels behaved like connective tissue amine oxidases.

Semicarbazide (10^{-3} M) was preincubated for 15 minutes with the homogenates prior to the addition of kynuramine.

Figure 14 shows that semicarbazide inhibited about 30% (aorta) and 55% (vena cava) of the total activity. However, clorgyline and deprenyl alone only revealed about 15 to 20% of resistant activity. Thus, some overlap between semicarbazide and the acetylenic inhibitors is evident at the concentrations used. Complete inhibition was obtained when either clorgyline or deprenyl was used in combination with semicarbazide. These results show that at least two species of MAO, differing in cofactor requirements, exist in the major blood vessels of rats.

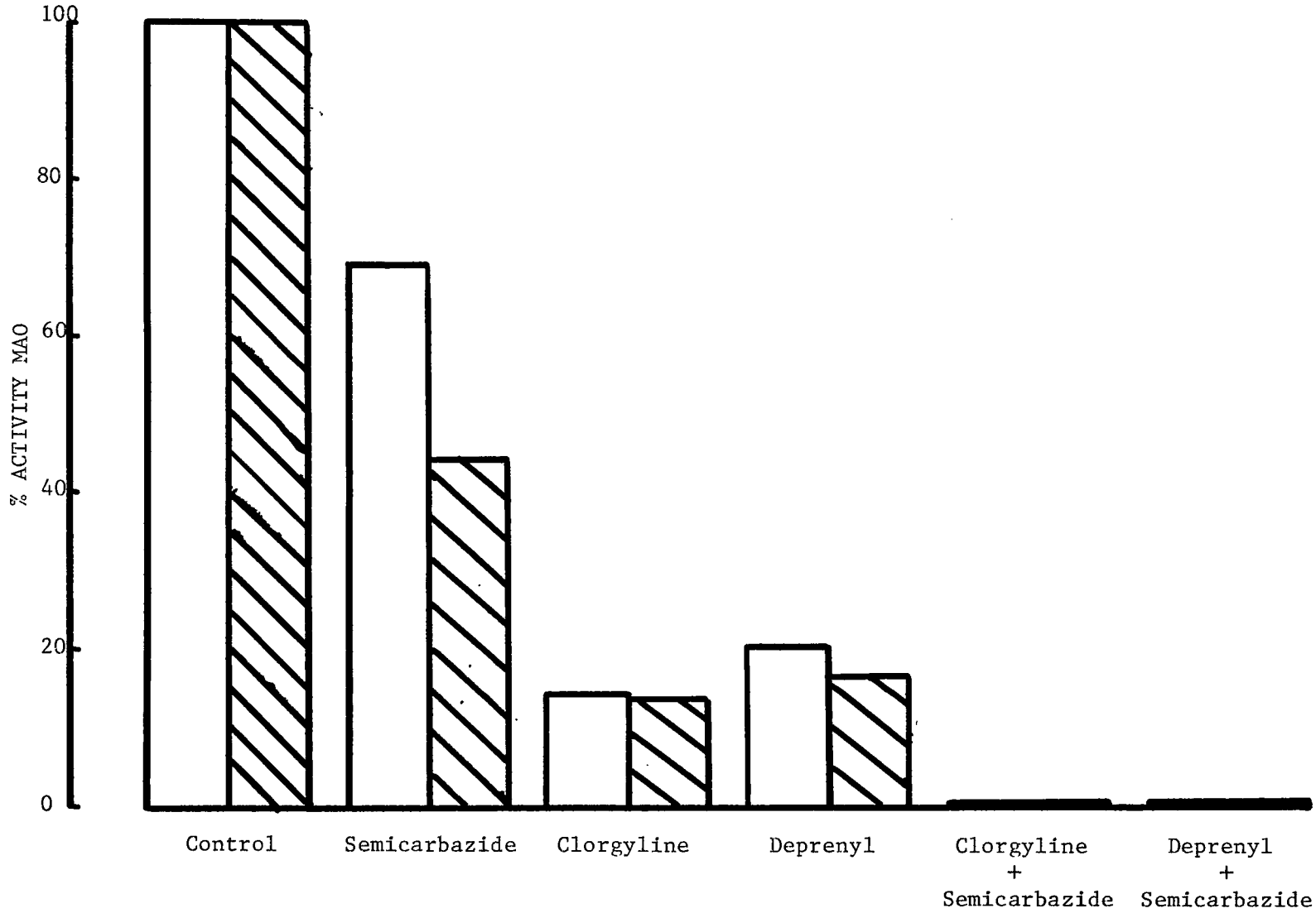
In contrast to the major blood vessels, semicarbazide (10^{-3} M) was found to inhibit only 6.8 $\pm$ 1.4% of the activity in the vas deferens and 8.5 $\pm$ 3.4% of the activity in the ventricle.

Figure 14. Inhibition with semicarbazide on the rat abdominal aorta and inferior vena cava.

The 4-hydroxyquinoline (4OHQ) formed is expressed as a percentage of the control activity (0.64 nmoles 4OHQ/mg tissue/hr = 100% activity of aorta; 0.32 nmoles 4OHQ/mg tissue/hr = 100% activity of vena cava). Each point is the mean of at least two determinations of pooled samples obtained from 4 rats (aorta) and 7 rats (vena cava).

Abdominal aorta 

Inferior vena cava 


% ACTIVITY MAO
100
80
60
40
20
0
Control
Semicarbazide
Clorgyline
Deprenyl
Clorgyline + Semicarbazide
Deprenyl + Semicarbazide

5. Subcellular characterization of MAO from perfused rat hearts:

The previous experiment had shown a small percentage of MAO type B activity in the major blood vessels. This MAO component might have derived from blood elements even though the tissues were washed well in saline. Correspondingly, the small B component of the rat heart might also derive from the same source. Thus, studies were made on isolated perfused hearts to determine this possibility. Additionally, crude subcellular fractions of the heart were prepared to ascertain whether cardiac MAO was of mitochondrial origin.

Mitochondria and other cellular fractions from the heart were prepared as described in the Methods section. Whole ventricular homogenates from perfused hearts showed the same percentage of A and B activity as that obtained previously (Figure 15). Only 23% of the total MAO activity was recovered in the mitochondrial fraction, the remainder being in the low speed pellet. However, both fractions showed 95% A and 5% B activity, indicating that both types of MAO were similarly distributed within the cell. Failure to liberate mitochondria most probably accounts for the high activity of the low speed pellet.

Part 2. Kinetics

Rationale:

Enzyme activities can be defined by the determination of Michaelis-Menton constants. Kinetic parameters which can be determined

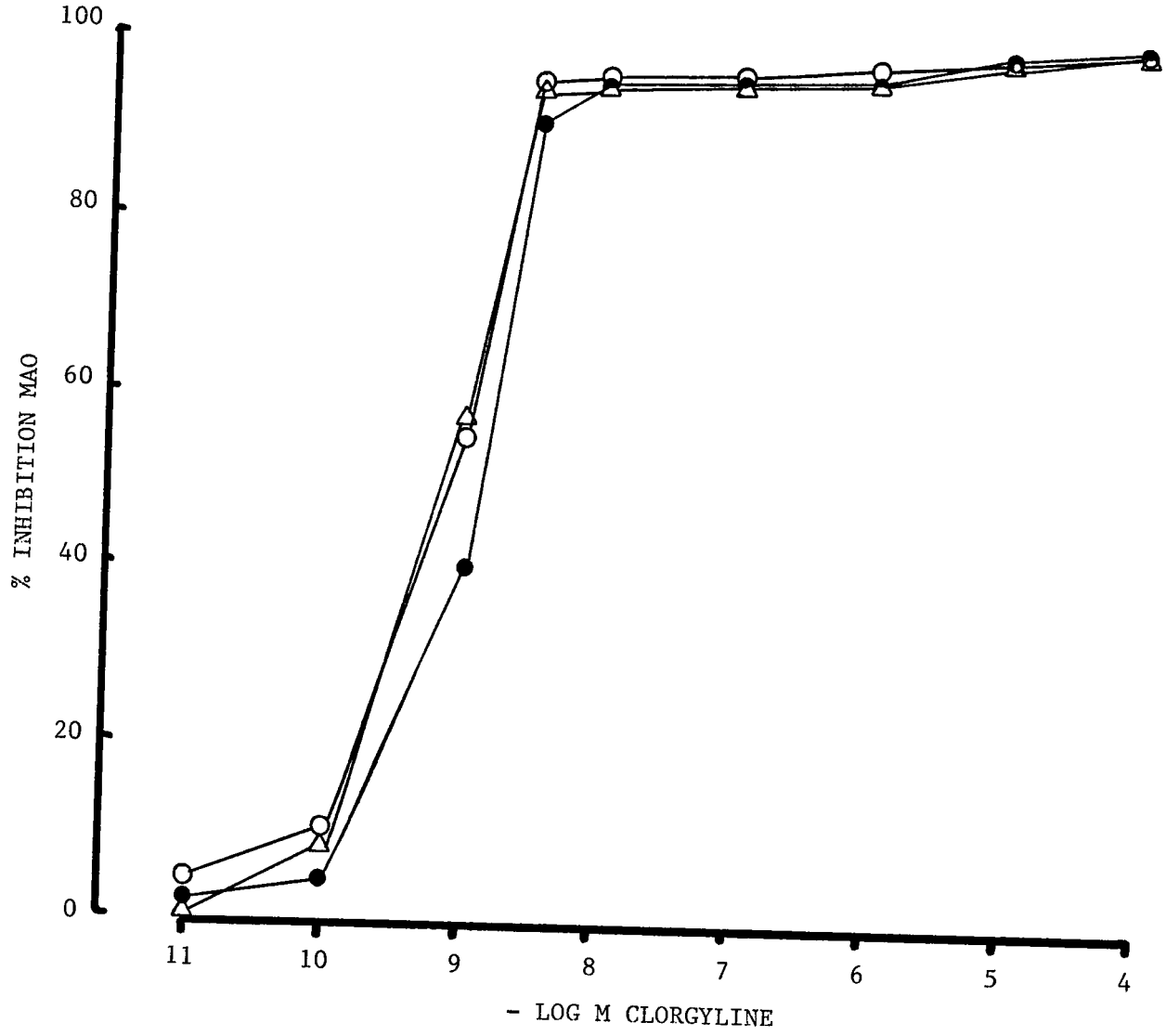
Figure 15. Inhibition curves with clorgyline of the whole homogenate, low-speed pellet and mitochondrial pellet of the perfused rat heart.

The 4-hydroxyquinoline (4OHQ) formed is expressed as a percentage of the difference from the control activity (1.96 nmoles 4OHQ/mg tissue/hr = 0% inhibition of whole ventricle homogenate; 1.44 nmoles 4OHQ/mg tissue/hr = 0% inhibition of low-speed pellet; 0.45 nmoles 4OHQ/mg tissue/hr = 0% inhibition of mitochondrial pellet). Each point is the mean of at least two determinations.

Whole homogenate ●——●

Low-speed pellet ○——○

Mitochondrial pellet △——△


100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
- LOG M CLORGYLINE

are the Km, or affinity constant for a particular substrate, and the Vmax, or maximum velocity of an enzymatic reaction with a given substrate. These determinations for MAO can be made on both the whole MAO activity in a given tissue and on the individual types of MAO, by the use of preferential inhibitors.

By classical definition, the Km is the substrate concentration that produces a half maximal-velocity of the reaction and is inversely related to the affinity of an enzyme for a particular substrate. That is, the greater the affinity, the lower the Km. Among several MAO substrates, the ones with the lowest Km values would thus have the highest affinity for the enzyme. By comparing the Km values for kynuramine on MAO types A and B of the heart and vas deferens, possible differences may be revealed. However, if the same types of enzyme activities are present in both tissues, similar Km values should be obtained. Previous workers have shown a Km for kynuramine on whole rat liver homogenates is 25 μM. In order to compare our technique and values with those of others, Km values for the liver were made in addition to those for the heart and vas deferens.

The Vmax value cannot be accurately measured for each of the MAO types since attempts to separate and purify the amount of A or B in a tissue have failed. (Vmax is expressed as nmoles 4OHQ produced/ mg tissue or protein/min). Therefore, Vmax values reflect the total activity in a tissue. A tissue such as the liver, with much MAO activity, would be expected to have a high Vmax.

1. Apparent Km determinations in rat vas deferens, ventricle and liver using kynuramine as the substrate:

In the following experiments the initial velocity rates were measured as described in the Methods. Clorgyline was used to inhibit type A activity and deprenyl to inhibit type B activity. Clorgyline, 10^{-7} M, was used for all tissues. Deprenyl, 10^{-7} M, was used in the ventricle and liver, and 5 X 10^{-7} M was used in the vas deferens. These concentrations were selected from the previous inhibition studies (Part 1). Similar inhibition curves were made in the liver, which was found to contain 20% type A and 80% type B MAO.

Figure 16 shows a Lineweaver-Burk plot obtained in the vas deferens. The apparent Km value for the whole homogenate is 15.1 μM. The values for MAO type A and B respectively, are 24.8 and 17.0 μM. Thus, the B activity has a slightly greater affinity for kynuramine than the A activity. Similar plots are shown in Figure 17 for the ventricle and Figure 18 for the liver. As with the vas deferens, linear plots were obtained and type B MAO exhibited the highest affinity for kynuramine. A summary of the data is given in Table 2. The apparent Km values for type A MAO are nearly identical in all tissues and the same situation applies for the type B activity. Thus, no evidence was obtained for differences between these two MAO activities across the three tissues studied. The velocity of deamination is more than eight times faster in the liver than the other tissues, consistent with the higher specific activity of the liver (161.5 nmoles 4OHQ/mg protein/hr. vs. 20.8 and 18.9 for the ventricle and vas deferens, respectively).

Figure 16. Kinetic analysis of rat vas deferens MAO using kynuramine.

The 4-hydroxyquinoline (4OHQ) formed is expressed as the reciprocal of the velocity in nmoles 4OHQ/mg tissue/min (1/v) plotted versus the reciprocal of the kynuramine concentration in μM (1/[S]). The Km value is obtained by calculating the negative reciprocal of the point of interception of the graph with the 1/[S] axis.

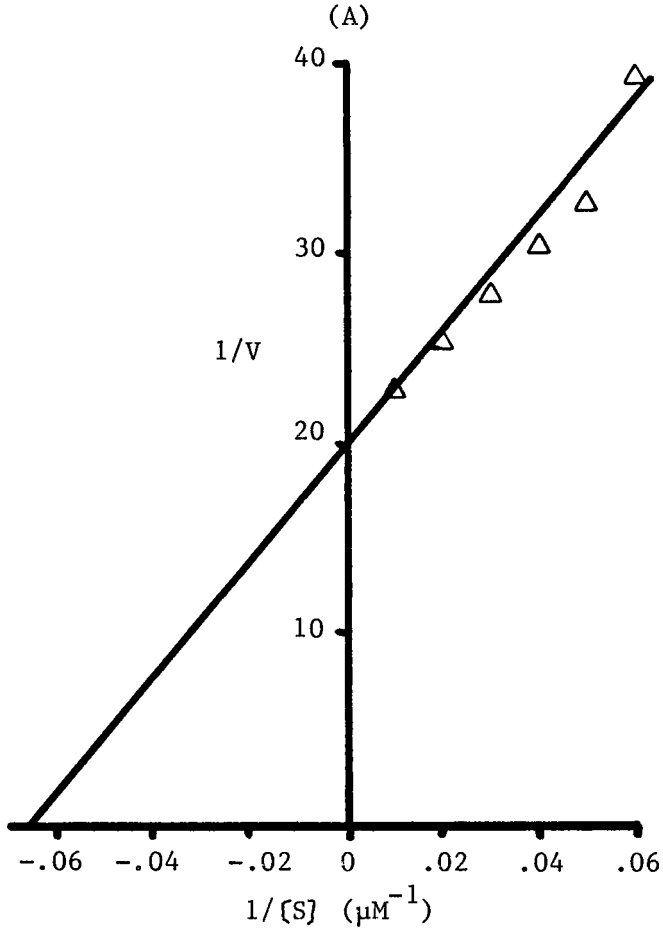
(A) Lineweaver-Burk plot of whole rat vas deferens homogenate. Each point is the mean of at least two determinations. The Km value obtained is 15.1 μM.

Whole vas deferens homogenate

(B) Lineweaver-Burk plot of type A and B activities in rat vas deferens. Each point is the mean of at least four determinations. The Km for type A (24.8 μM) was obtained in the presence of 5×10^{-7} M deprenyl, while the Km for type B (17.0 μM) was obtained in the presence of 10^{-7} M clorgyline.

Type A ●—●

Type B ○—○


(A)
40
30
20
10
1/V
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

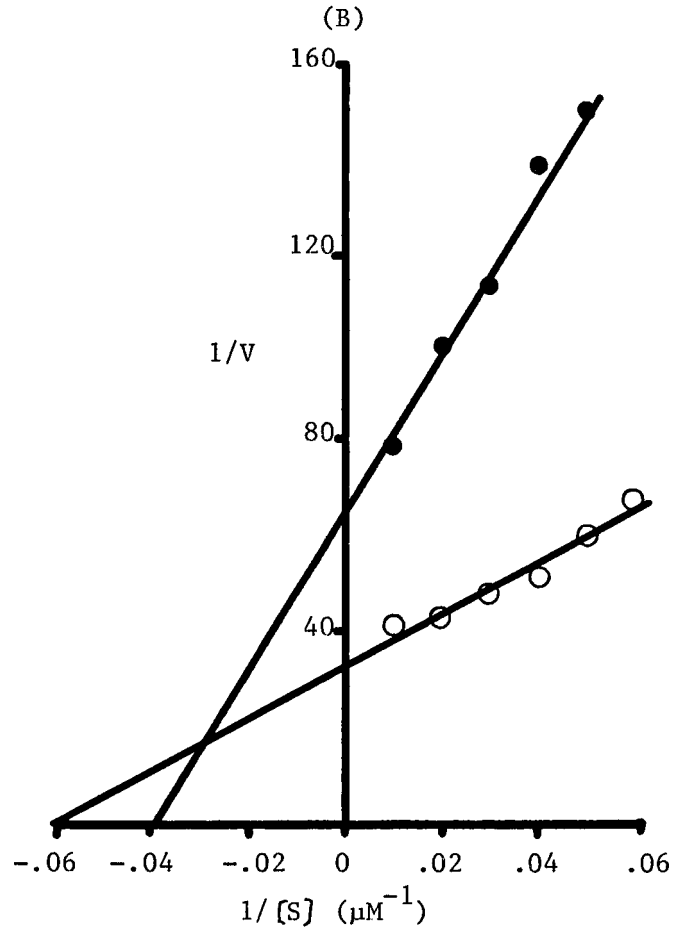

(B)
160
120
80
40
1/V
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

Figure 17. Kinetic analysis of rat ventricle MAO using kynuramine.

The 4-hydroxyquinoline (4OHQ) formed is expressed as the reciprocal of the velocity in nmoles 4OHQ/mg tissue/min (1/v) plotted versus the reciprocal of the kynuramine concentration in μM (1/[S]). The Km value is obtained by calculating the negative reciprocal of the point of interception of the graph with the 1/[S] axis.

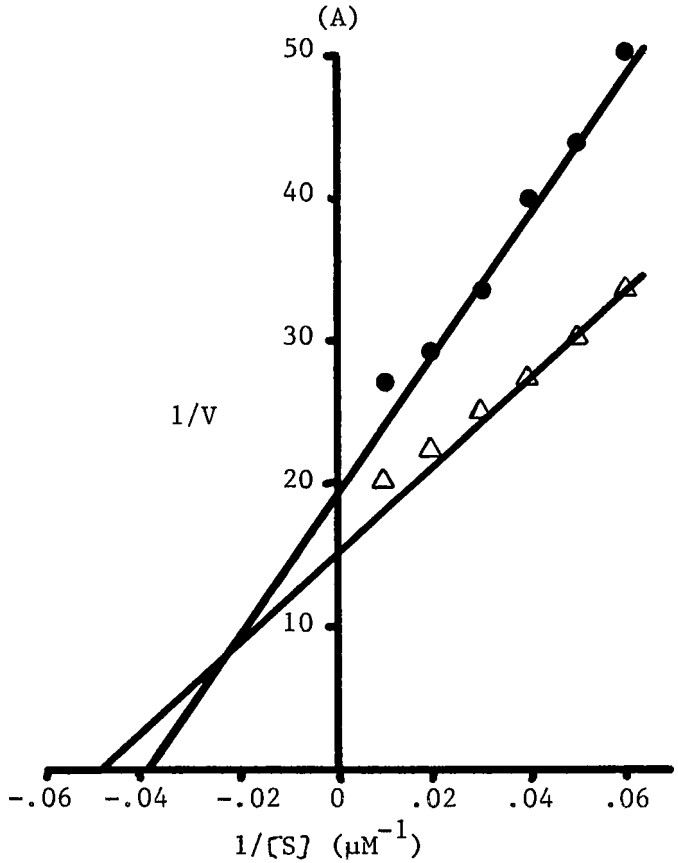
(A) Lineweaver-Burk plots of whole rat ventricle homogenate and type A activity. Each point is the mean of two to four determinations. The Km value for the whole homogenate is 21.6 μM. The Km for type A (26.0 μM) was obtained in the presence of 10^{-7} M deprenyl.

Whole ventricle homogenate △——△

Type A ●——●

(B) Lineweaver-Burk plot of type B activity in rat ventricle. Each point is the mean of at least four determinations. The Km for type B (15.0 μM) was obtained in the presence of 10^{-7} M clorgyline.

Type B ○——○


(A)
50
40
30
20
10
1/V
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

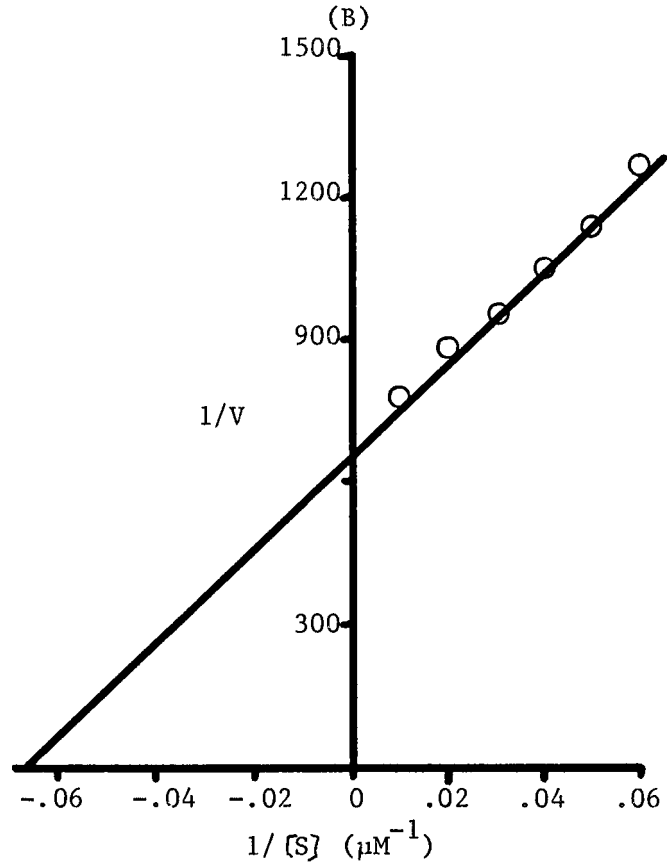

(B)
1500
1200
900
300
1/V
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

Figure 18. Kinetic analysis of rat liver MAO using kynuramine.

The 4-hydroxyquinoline (4OHQ) formed is expressed as the reciprocal of the velocity in nmoles 4OHQ/mg tissue/min (1/v) plotted versus the reciprocal of the kynuramine concentration in μM (1/[S]). The Km value is obtained by calculating the negative reciprocal of the point of interception of the graph with the 1/[S] axis.

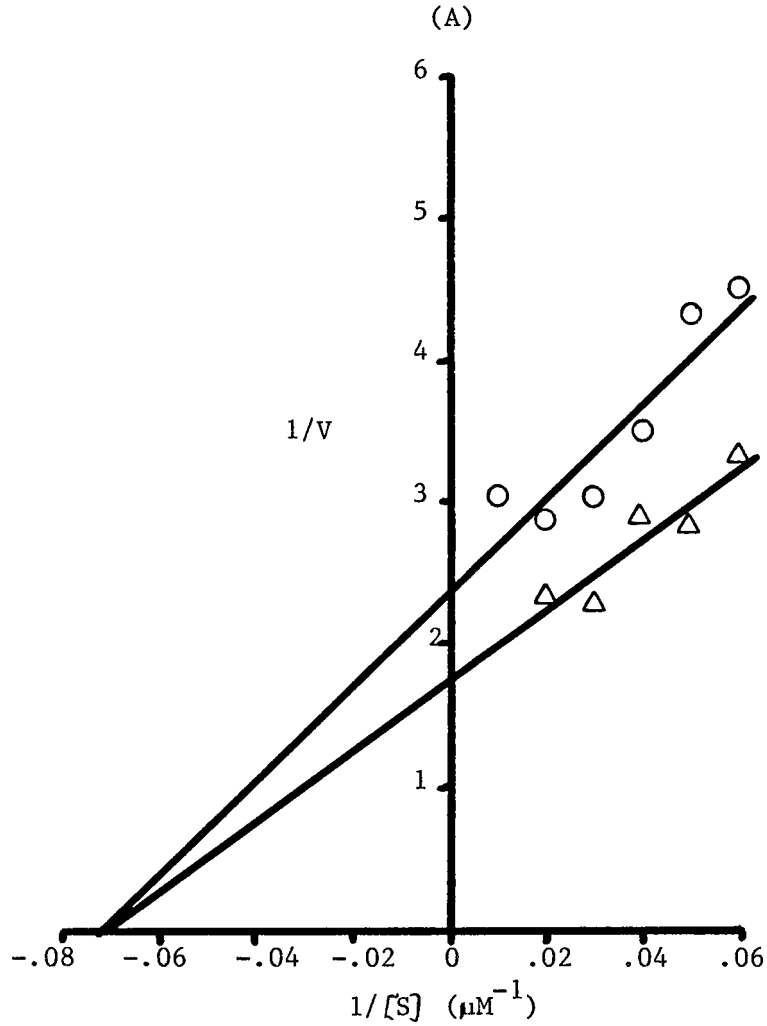
(A) Lineweaver-Burk plots of whole rat liver homogenate and type B activity. Each point is the mean of two to six determinations. The Km value for the whole homogenate is 14.3 μM. The Km for type B (13.9 μM) was obtained in the presence of 10^{-7} M clorgyline.

Whole liver homogenate △——△

Type B ○——○

(B) Lineweaver-Burk plot of type A activity in rat liver. Each point is the mean of at least six determinations. The Km value for type A (25.0 μM) was obtained in the presence of 10^{-7} M deprenyl.

Type A ●——●


(A)
6
5
4
3
2
1
1/V
-.08
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

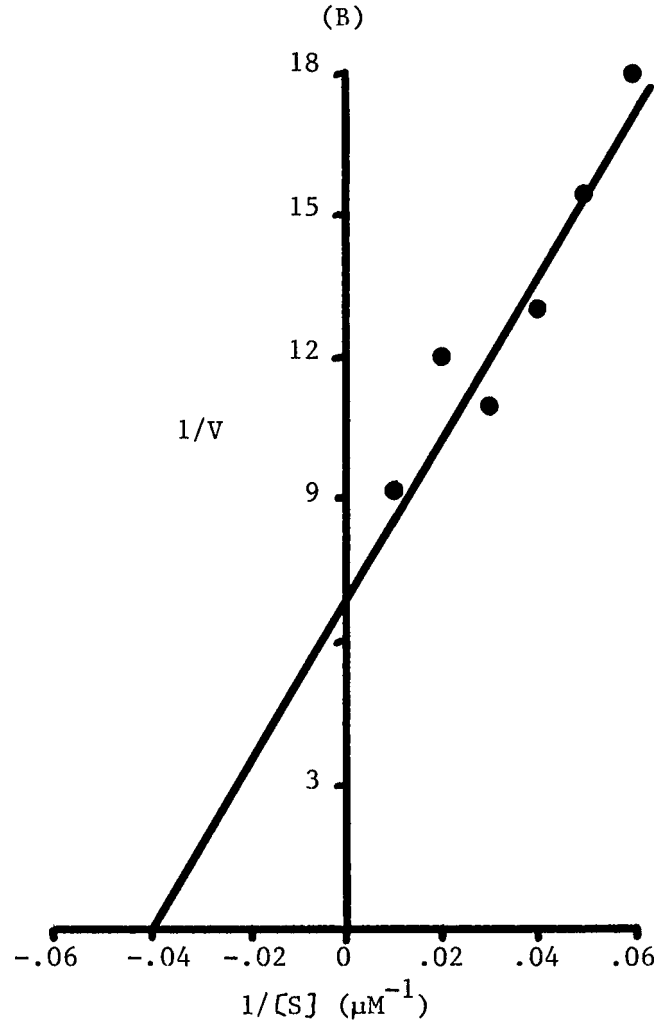

(B)
18
15
12
9
3
1/V
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

TABLE 2. Apparent Km and Vmax values of MAO activity in rat vas deferens, ventricle and liver using kynuramine as substrate.

The Km values for A and B activity were obtained from Lineweaver-Burk plots in figures 16-18 and are expressed as the mean ± standard error of the mean. The Vmax values were calculated from the reciprocal of the 1/v axis intercept of the whole tissue homogenate plots.

	Km (μM)		Vmax
	A	B	
Vas deferens	24.8 ± 9.2	17.0 ± 5.0	0.051
Ventricle	26.0 ± 5.8	15.0 ± 0.3	0.068
Liver	25.0 ± 4.8	13.9 ± 1.4	0.588

Part 3. Mixed Substrates

Rationale:

There are numerous endogenous substrates for MAO. Each has its own particular affinities for the MAO types. While determination of Km values will yield a measure of these affinities, it does not indicate whether the binding and/or catalytic sites on MAO are the same for all substrates. One method to test for such differences is by the use of mixed substrate experiments. In this type of study, two substrates are mixed with the enzyme simultaneously. Since kynuramine is the substrate being measured, an inhibition of its metabolism would be indicative of an interaction at a common site, either binding or catalytic.

The object of these studies was to determine whether two endogenous substrates, phenylethylamine and tryptamine, interacted at common sites with kynuramine. Further, these data could then be used to compare interactions of MAO types between the heart and vas deferens. Phenylethylamine was chosen for study because it is a preferential type B substrate, but is metabolized by type A MAO in the rat heart. Tryptamine however, is a dual substrate, yet is also metabolized only by A in rat cardiac tissue.

1. Effect of phenylethylamine or tryptamine on kynuramine deamination with time:

This initial study was conducted to determine whether interfering metabolic products of phenylethylamine and tryptamine were being formed in whole homogenates of the ventricle and vas deferens. Such product

formation would be expected to give rise to non-linear curves when studying kynuramine deamination with time. If this were to occur, mixed substrate interactions could not be attributed to the parent compound.

Phenylethylamine or tryptamine were added with kynuramine in equimolar concentrations (100 μM) to whole homogenates of rat ventricle and vas deferens. Enzyme activity was measured at various time intervals up to 60 min.

Figures 19A and B show linear reaction rates to kynuramine deamination. At the concentration used, tryptamine was much more active at inhibiting kynuramine deamination in the ventricle than phenylethylamine. However, in the vas deferens, both compounds were approximately equipotent.

2. Effect of mixed substrates on MAO types in rat vas deferens and ventricle:

In the following experiments kynuramine was used in a concentration of 33 μM; a concentration slightly above its Km values for both MAO types. This was done to allow better demonstration of second substrate inhibition. Phenylethylamine and tryptamine were studied at concentrations of 12.5 μM and 100 μM. This 8-fold range in concentration was chosen to try and allow for affinity differences of the two substrates for the A and B activities.

Figure 19. Effect of phenylethylamine (PEA) and tryptamine (TRY) on kynuramine (KYN) deamination with time and in the rat vas deferens and ventricle.

(A) The 4-hydroxyquinoline (4OHQ) formed in the rat vas deferens is plotted versus the time of incubation. Each point is the mean of at least two determinations. PEA and TRY were added in equimolar amounts with KYN (100 μM).

KYN alone ●——●

KYN plus PEA ○——○

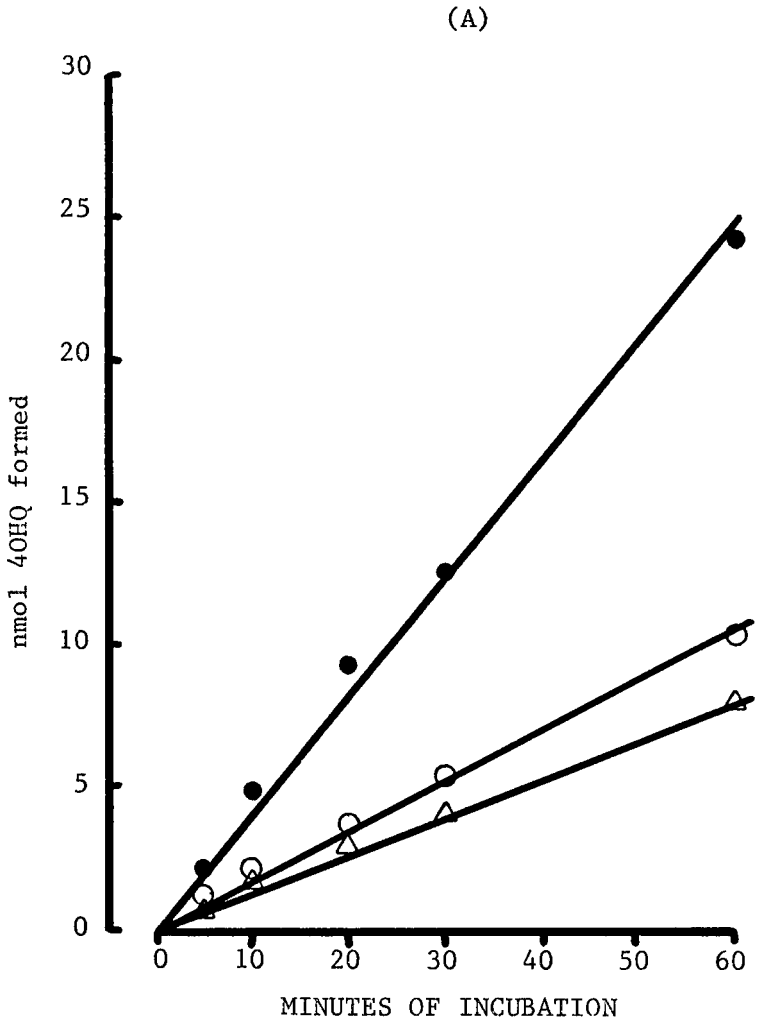
KYN plus TRY △——△

(B) The 4OHQ formed in the rat ventricle is plotted versus the time of incubation. Each point is the mean of at least two determinations. PEA and TRY were added in equimolar amounts with KYN (100 μM).

KYN alone ●——●

KYN plus PEA ○——○

KYN plus TRY △——△


(A)
nmol 4OHQ formed
30
25
20
15
10
5
0
0
10
20
30
40
50
60
MINUTES OF INCUBATION

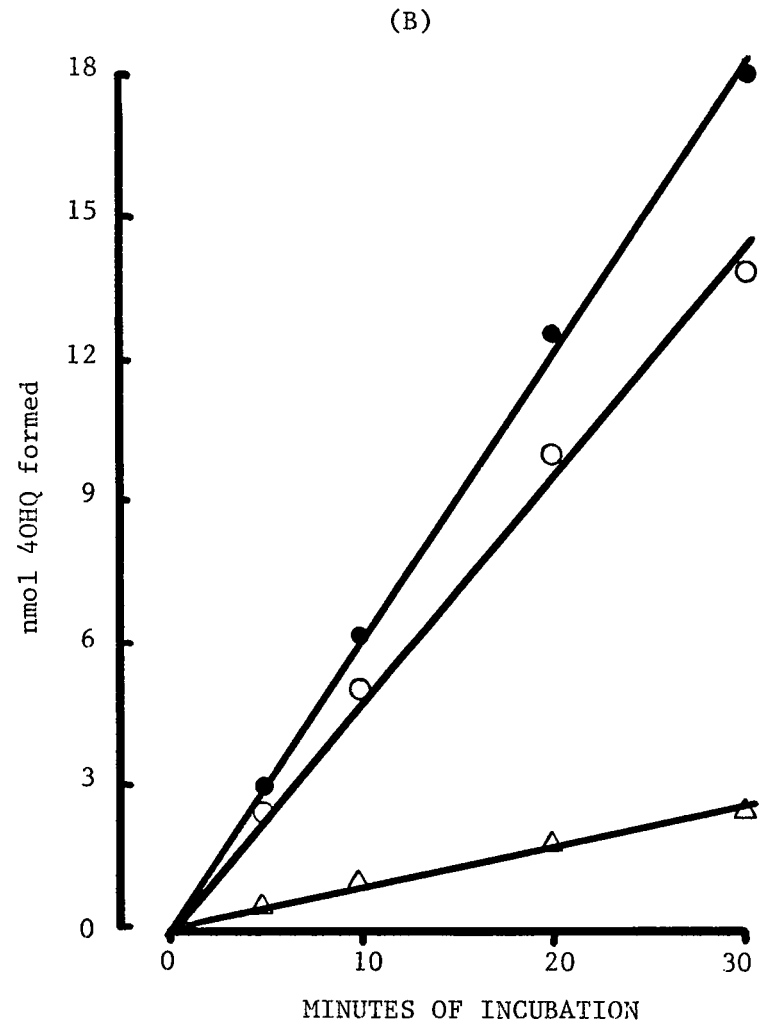

(B)
nmol 4OHQ formed
18
15
12
9
6
3
0
0
10
20
30
MINUTES OF INCUBATION

Figure 20 illustrates the results obtained. In the vas deferens phenylethylamine was a better inhibitor of kynuramine deamination on the B activity compared with the A type. The opposite situation prevailed for tryptamine. Similar results were obtained in the ventricle, although the small proportion of B activity makes quantitative comparison extremely difficult. The results do show that phenylethylamine possesses an affinity for both types of MAO and that its inhibitory activity in the vas deferens is not vastly dissimilar from that in the heart. In another experiment, phenylethylamine was studied vs. kynuramine (100 μM) in the ventricle. Phenylethylamine, 25 μM and 100 μM, showed reduced inhibitory activity on the A type, but still produced pronounced inhibition of MAO type B (Figure 21).

3. Determination of the type of inhibition caused by phenylethylamine and tryptamine on kynuramine deamination:

Even though phenylethylamine and tryptamine behaved similarly at inhibiting kynuramine deamination in the ventricle and vas deferens, the type of inhibition might vary between the two organs. Thus, kinetic analyses of these interactions were conducted.

Figures 22A and B show that tryptamine interacts as a mixed inhibitor on both MAO type A and B of the vas deferens, whereas phenylethylamine appears as a competitive inhibitor. However, a curvi-linear trend is seen in both plots with the latter substrate. Similar results were obtained in the rat ventricle (Figures 23A and B). Thus, once again differences between the ventricle and vas deferens were not obtained.

Figure 20. Effect of phenylethylamine (PEA) and tryptamine (TRY) on MAO activity in the rat vas deferens and ventricle with kynuramine (KYN, 33 μM).

The 4-hydroxyquinoline formed is expressed as a percentage of the control activity (control, no inhibitor = 100% activity). Type A activity was measured in the presence of 5 X 10^{-7} M deprenyl in the vas deferens and 10^{-7} M deprenyl in the ventricle, while type B activity was measured in the presence of 10^{-7} M clorgyline in both tissues. Each point is the mean of at least two determinations. Reaction time = 10 minutes.

(A) Effect of PEA on vas deferens MAO activity

KYN alone

KYN plus 12.5 μM PEA

KYN plus 100 μM PEA

(B) Effect of PEA on ventricle MAO activity

KYN alone

KYN plus 12.5 μM PEA

KYN plus 100 μM PEA

(C) Effect of TRY on vas deferens MAO activity

KYN alone

KYN plus 12.5 μM TRY

KYN plus 100 μM TRY

(D) Effect of TRY on ventricle MAO activity

KYN alone

KYN plus 12.5 μM TRY

KYN plus 100 μM TRY

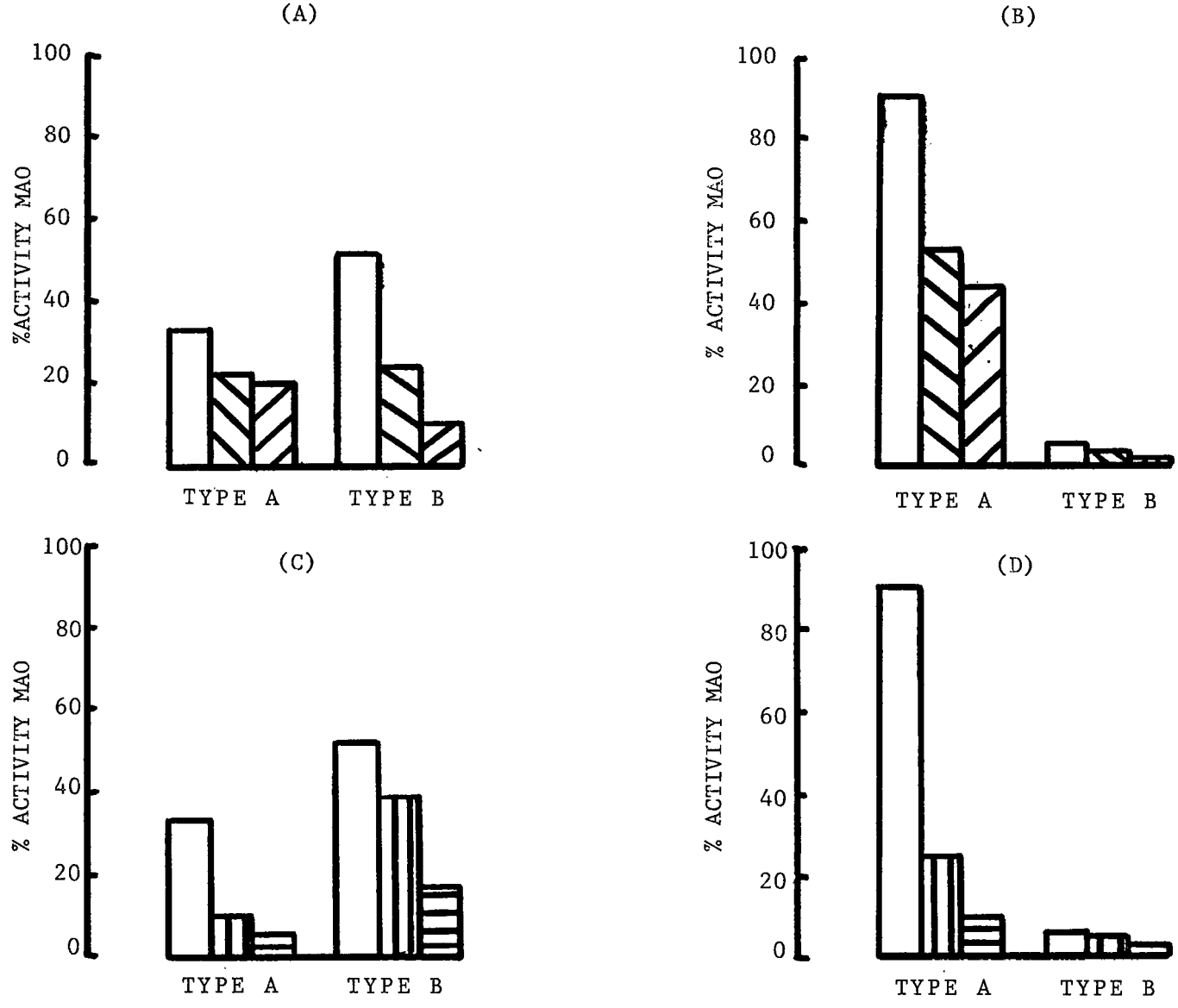

(A)
100
80
60
40
20
0
%ACTIVITY MAO
TYPE A
TYPE B
(B)
100
80
60
40
20
0
% ACTIVITY MAO
TYPE A
TYPE B
(C)
100
80
60
40
20
0
% ACTIVITY MAO
TYPE A
TYPE B
(D)
100
80
60
40
20
0
% ACTIVITY MAO
TYPE A
TYPE B

Figure 21. Effect of phenylethylamine (PEA) on MAO activity in rat ventricle with kynuramine (KYN, 100 μM).

The 4-hydroxyquinoline formed is expressed as a percentage of the control activity (control, no inhibitor = 100% activity). Type A activity was measured in the presence of 10^{-7} M deprenyl and type B in the presence of 10^{-7} M clorgyline. Each point is the mean of at least two determinations. Reaction time = 30 minutes.

KYN alone 

KYN plus 25 μM PEA 

KYN plus 100 μM PEA 

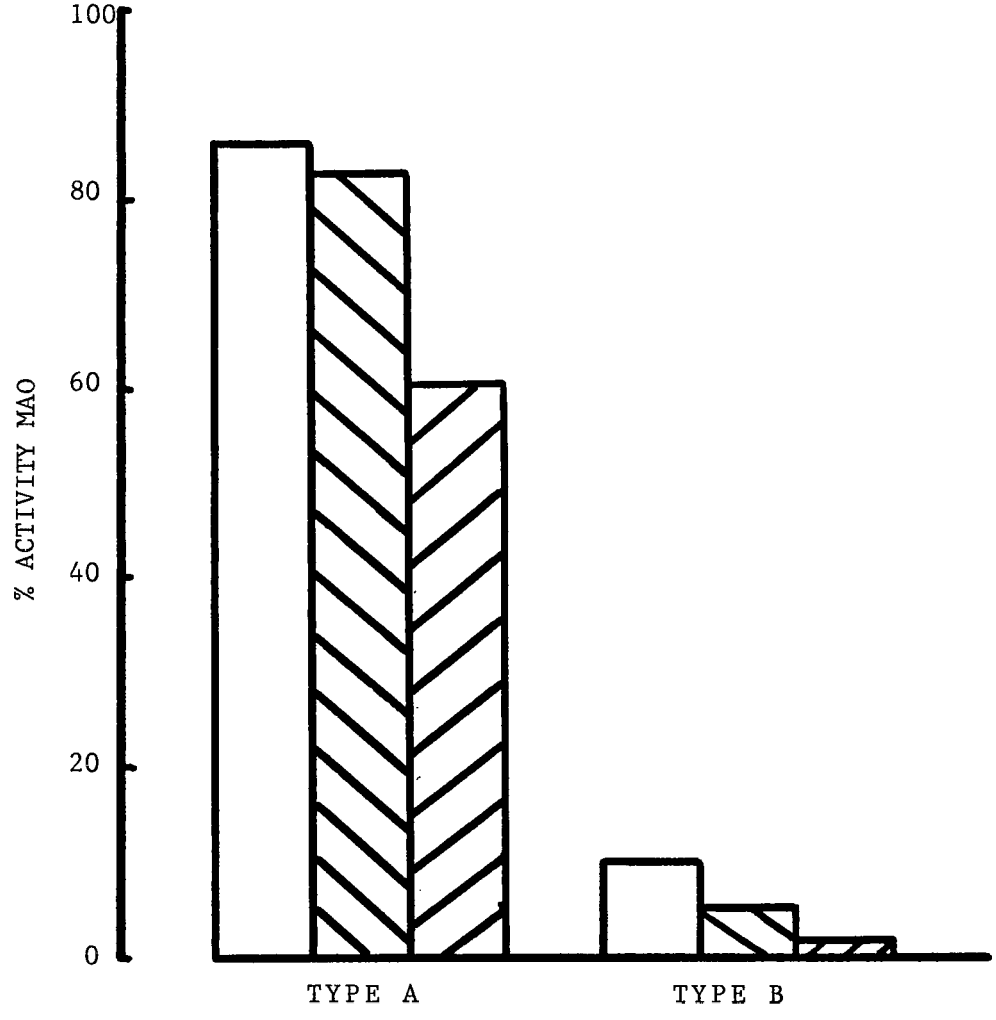

100
80
60
40
20
0
% ACTIVITY MAO
TYPE A
TYPE B

Figure 22. Effect of phenylethylamine (PEA) and tryptamine (TRY) on kinetic analysis of rat vas deferens MAO using kynuramine.

The 4-hydroxyquinoline (4OHQ) formed is expressed as the reciprocal of the velocity in nmoles 4OHQ/mg tissue/min (1/v) plotted versus the reciprocal of the kynuramine concentration in μM (1/[S]). PEA and TRY concentrations were 25 μM.

(A) Lineweaver-Burk plot of type A activity in rat vas deferens. Data was obtained in the presence of 5×10^{-7} M deprenyl. Each point is the mean of two to four determinations.

Type A ●——●

Type A plus PEA ■——■

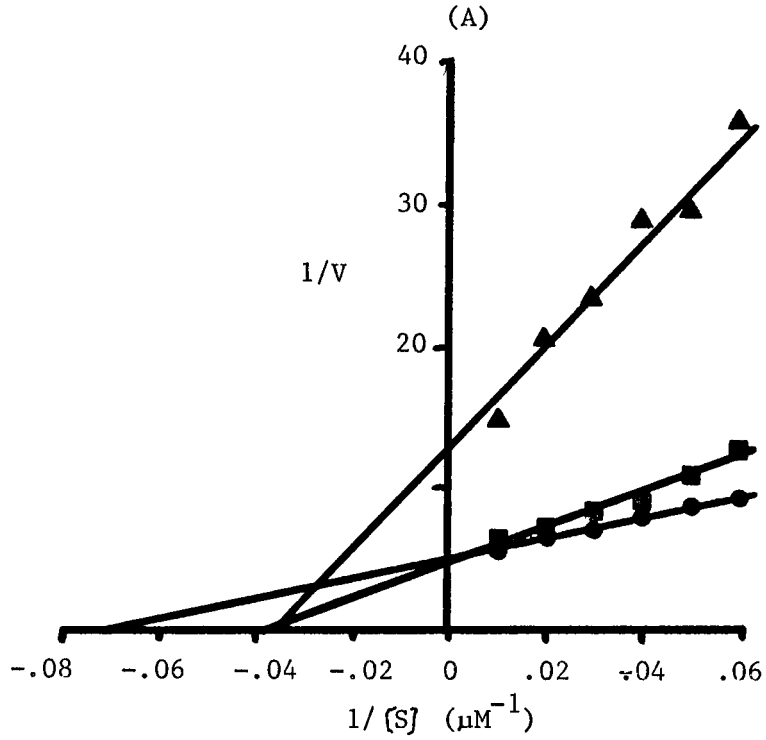
Type A plus TRY ▲——▲

(B) Lineweaver-Burk plot of type B activity in rat vas deferens. Data was obtained in the presence of 10^{-7} M clorgyline. Each point is the mean of two to four determinations.

Type B ○——○

Type B plus PEA □——□

Type B plus TRY △——△


(A)
40
30
20
1/V
-.08
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

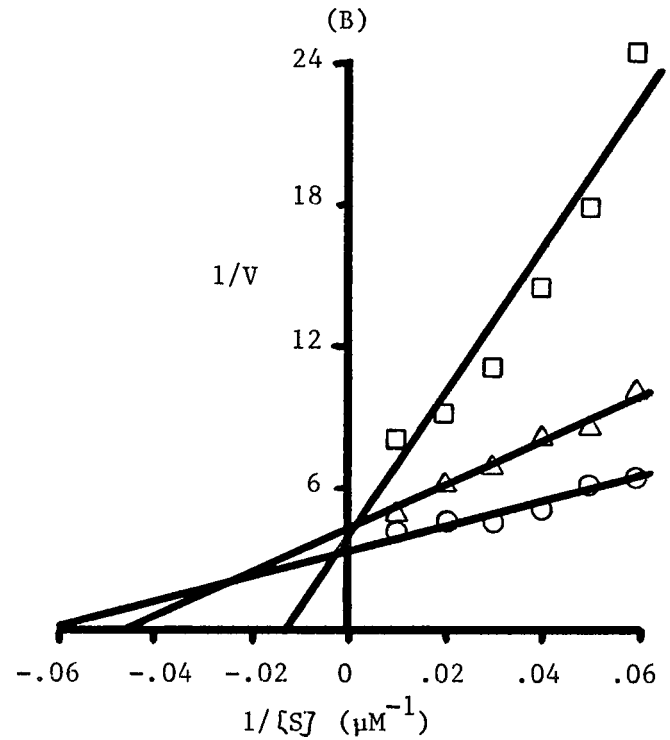

(B)
24
18
12
6
1/V
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

Figure 23. Effect of phenylethylamine (PEA) and tryptamine (TRY) on kinetic analysis of rat ventricle MAO using kynuramine.

The 4-hydroxyquinoline (4OHQ) formed is expressed as the reciprocal of the velocity in nmoles 4OHQ/mg tissue/min (1/v) plotted versus the reciprocal of the kynuramine concentration in μM (1/[S]). PEA and TRY concentrations were 25 μM.

(A) Lineweaver-Burk plot of type A activity in rat ventricle. Data was obtained in the presence of 10^{-7} M deprenyl. Each point is the mean of two to four determinations.

Type A

Type A plus PEA

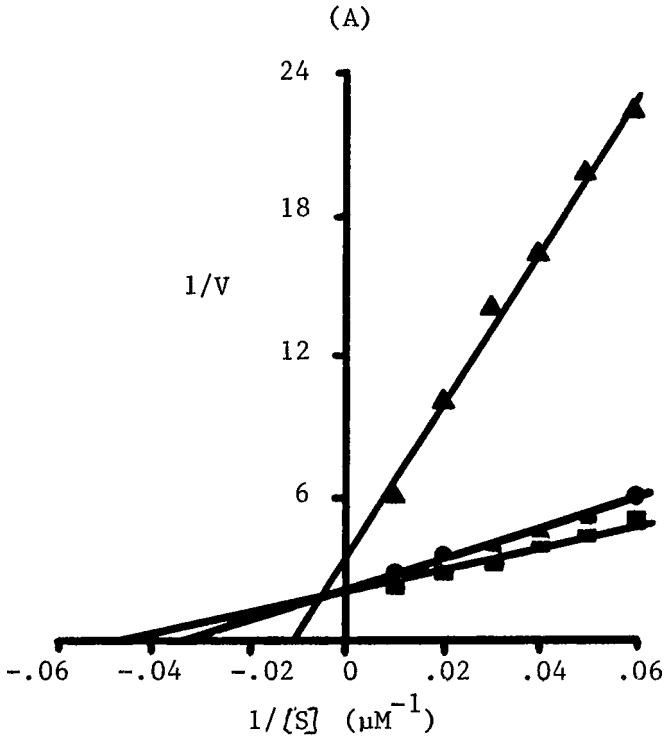
Type A plus TRY

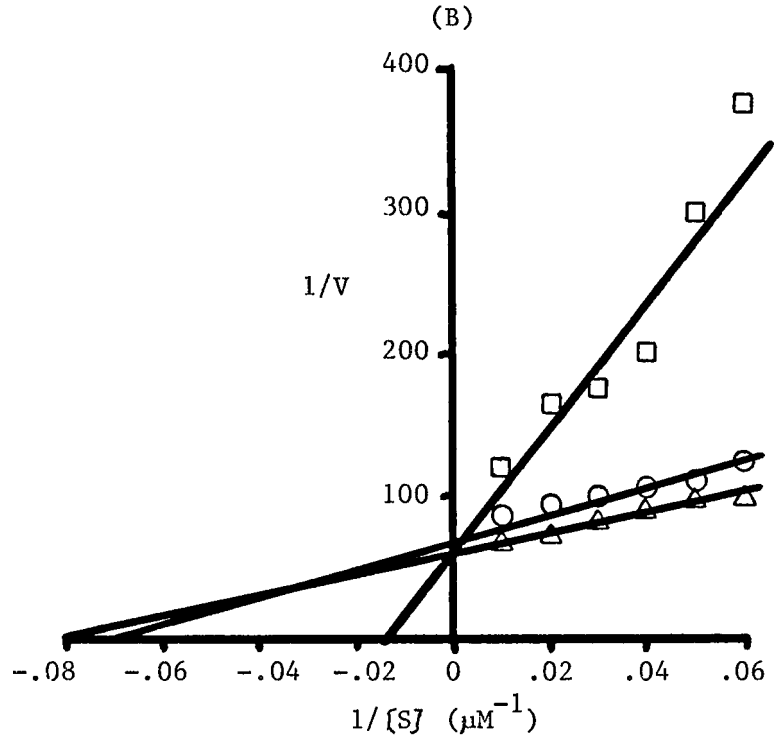
(B) Lineweaver-Burk plot of type B activity in rat ventricle. Data was obtained in the presence of 10^{-7} M clorgyline. Each point is the mean of two to four determinations.

Type B

Type B plus PEA

Type B plus TRY


(A)
24
18
12
6
1/V
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})


(B)
400
300
200
100
1/V
-.08
-.06
-.04
-.02
0
.02
.04
.06
1/[S] (μM^{-1})

Part 4. Physiological Factors Affecting MAO Activity

Rationale:

Examination of cardiac MAO had revealed little to distinguish it from the activity in other tissues. There are, however, a number of physiological factors which are known to affect cardiac MAO preferentially over other organs *in vivo*. These include aging and adrenalectomy. Total MAO activity increases with age and following adrenalectomy in the rat heart but the effect of these processes on the proportion of A and B types is unknown. These factors were investigated utilizing clorgyline inhibition curves. In addition, only a few studies have been reported on MAO activity at the whole organ level. Most workers have utilized broken-cell whole homogenates or mitochondrial fractions for enzyme analysis. Thus studies were undertaken to evaluate cardiac MAO at a high organizational level, one involving intact, isolated atria.

1. Types of MAO in the developing rat heart:

Clorgyline inhibition curves were performed on the whole heart homogenates of rats at 10 grams (2-3 days), 36 grams (16 days), 69 grams (24 days) and 300 grams (about 60 days).

The proportion of A:B activity varied with age (Figure 24). The youngest and oldest rats had a similar ratio of activities, while the ages in between revealed a greater type B content. The total specific activity generally increased with age in the order 4.5, 3.85, 4.6 and 9.98 nmoles 4OHQ/mg protein/hour in the youngest to oldest rats, respectively.

Figure 24. Effect of age on inhibition curves with clorgyline in the rat heart.

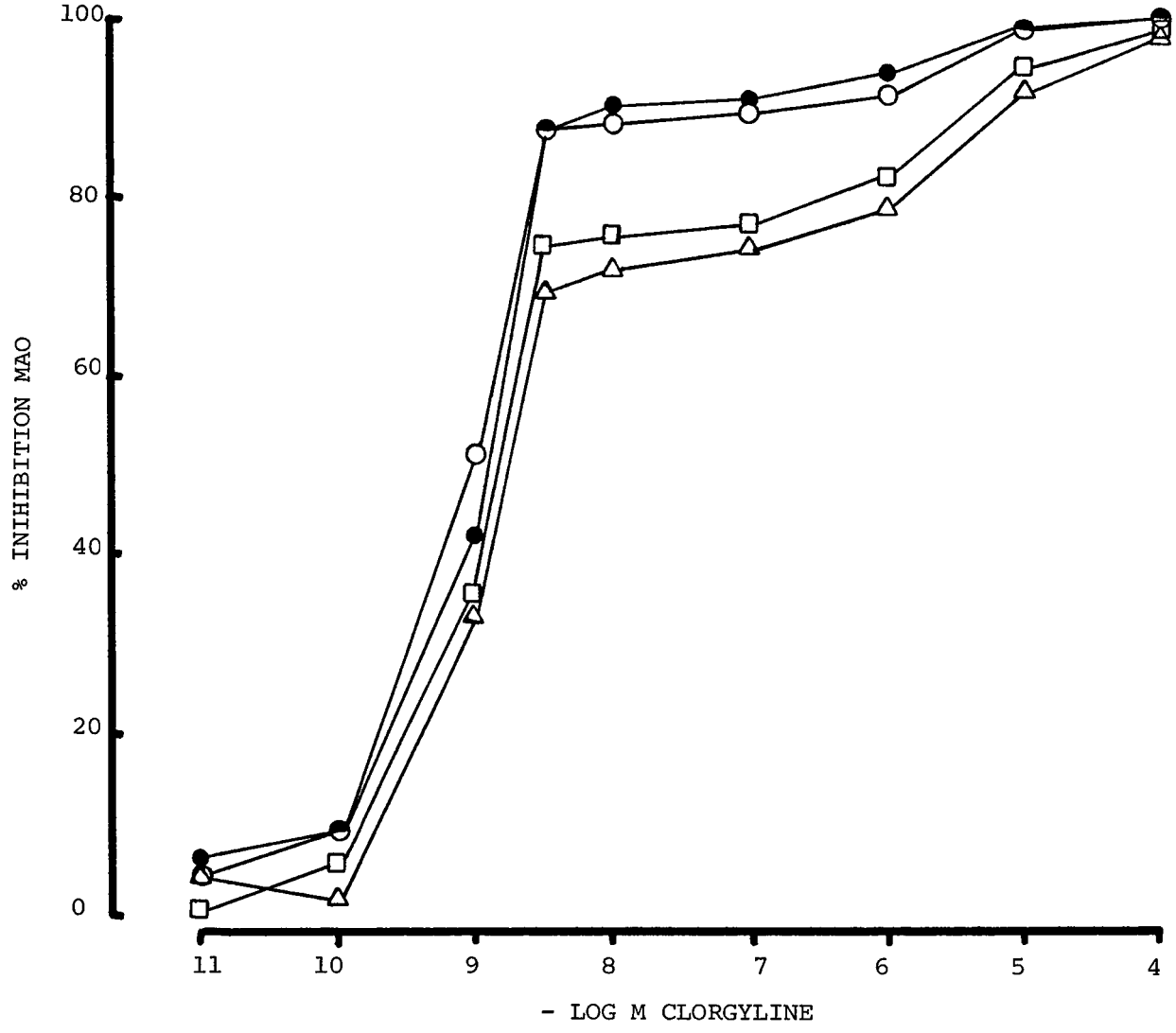
The 4-hydroxyquinoline formed is expressed as a percentage of the difference from the control activity (control, no inhibition = 0% inhibition). Each point is the mean of at least two determinations. Whole rat heart homogenate (3.33 mg/ml).

10 grams

36 grams

69 grams

300 grams


100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
- LOG M CLORGYLINE

2. Effect of adrenalectomy on MAO types and activity in rat tissues:

Rats were adrenalectomized under ether anesthesia and maintained for 10 days following surgery on regular normal feed plus saline (sham and adrenalectomized) or tap water (control). At that time the animals were sacrificed and the appropriate tissues were taken for assay.

From the combined data of three separate experiments (Table 3) there was no change in MAO specific activity of the vas deferens, atria, abdominal aorta, or inferior vena cava, although the latter two tissues tended to increase. The activity of the ventricle did show a significant increase over the sham and control following adrenalectomy ($p < 0.05$). However, clorgyline inhibition curves (Figure 25) did not reveal any difference in proportions of A and B activity in any tissue.

3. Types of MAO in isolated, intact rat atria:

MAO activity was investigated at the level of a whole, isolated tissue using the spontaneously beating rat atria. These were chosen because they had previously been studied as homogenates and had been shown to be representative of cardiac tissue as a whole. Further, they could be kept "alive" during the incubation period, and since they were relatively thin-walled, they would present little in the way of diffusional barriers to the cellular components.

Preliminary experiments were necessary to determine whether the reaction product was linear with time and tissue weight. Then

TABLE 3. Effect of adrenalectomy on MAO activity in the rat vas deferens, atria, ventricle, abdominal aorta and inferior vena cava.

Tissue samples were assayed for total MAO activity with kynuramine at 10 days following adrenalectomy. The 4-hydroxyquinoline formed is expressed as the mean specific activity (nmoles 4OHQ/mg tissue/hr) ± S.E.M. for (n) animals. For assay of the blood vessels it was necessary to pool the tissue from four to five rats.

*Statistical significance: The activity of the ventricle following adrenalectomy was significantly different from the activity in both the sham and control groups ($P < 0.05$).

	Tissue Specific Activity		
	Control	Sham	Adrenalectomy
Vas deferens		3.17±0.60 (6)	3.17±0.17 (7)
Atria	2.23±0.11 (4)	2.51±0.18 (8)	2.90±0.29 (10)
Ventricle	2.88±0.08 (4)	2.61±0.40 (10)	*4.03±0.50 (12)
Abdominal aorta		0.73	1.14
Inferior vena cava		0.67	0.81

Figure 25. Effect of adrenalectomy on inhibition curves with clorgyline in the rat vas deferens, atria, ventricle, abdominal aorta and inferior vena cava.

Tissue samples were collected at 10 days following adrenalectomy. The 4-hydroxyquinoline formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition).

(A) Inhibition curves with clorgyline in the atria of sham and adrenalectomized rats. Each point is the mean of four determinations.

(B) Inhibition curves with clorgyline in the ventricle of sham and adrenalectomized rats. Each point is the mean of eight determinations.

(C) Inhibition curves with clorgyline in the vas deferens of sham and adrenalectomized rats. Each point is the mean of two determinations.

(D) Inhibition curves with clorgyline in the abdominal aorta of sham and adrenalectomized rats. Each point is the mean of two determinations of pooled samples obtained from 4 to 5 rats.

(E) Inhibition curves with clorgyline in the inferior vena cava of sham and adrenalectomized rats. Each point is the mean of two determinations of pooled samples obtained from 4 to 5 rats.

Sham 

Adrenalectomy 

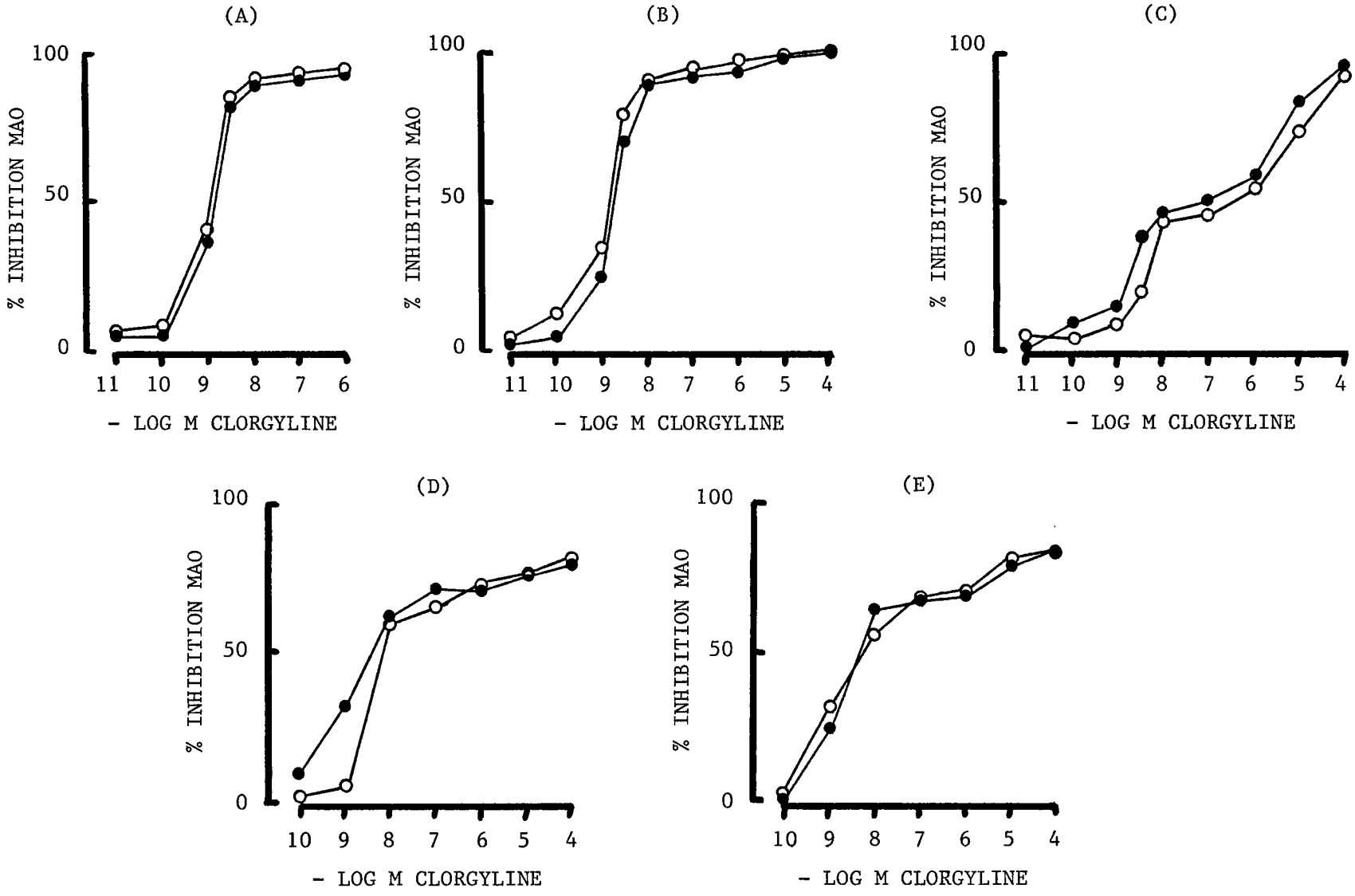

(A)
(B)
(C)
(D)
(E)
% INHIBITION MAO
100
50
0
11 10 9 8 7 6
10 9 8 7 6 5 4
- LOG M CLORGYLINE

Figure 26. Effect of incubation time on kynuramine deamination by intact, isolated atria.

The 4-hydroxyquinoline (4OHQ) formed by whole atria is expressed as the nmoles of 4OHQ formed per mg of atrial tissue. The time of incubation varied from 5 to 30 minutes. Each point is the mean of at least two determinations.

(A) Kynuramine deamination in separated right and left atria. The 4OHQ content of the tissue plus that in the incubation fluid was combined.

Left atria ●——●

Right atria ○——○

(B) Kynuramine deamination in combined right and left atria. The 4OHQ contents of a left and a right atria and their incubation fluids were combined at each point.

Left and right atria ●——●

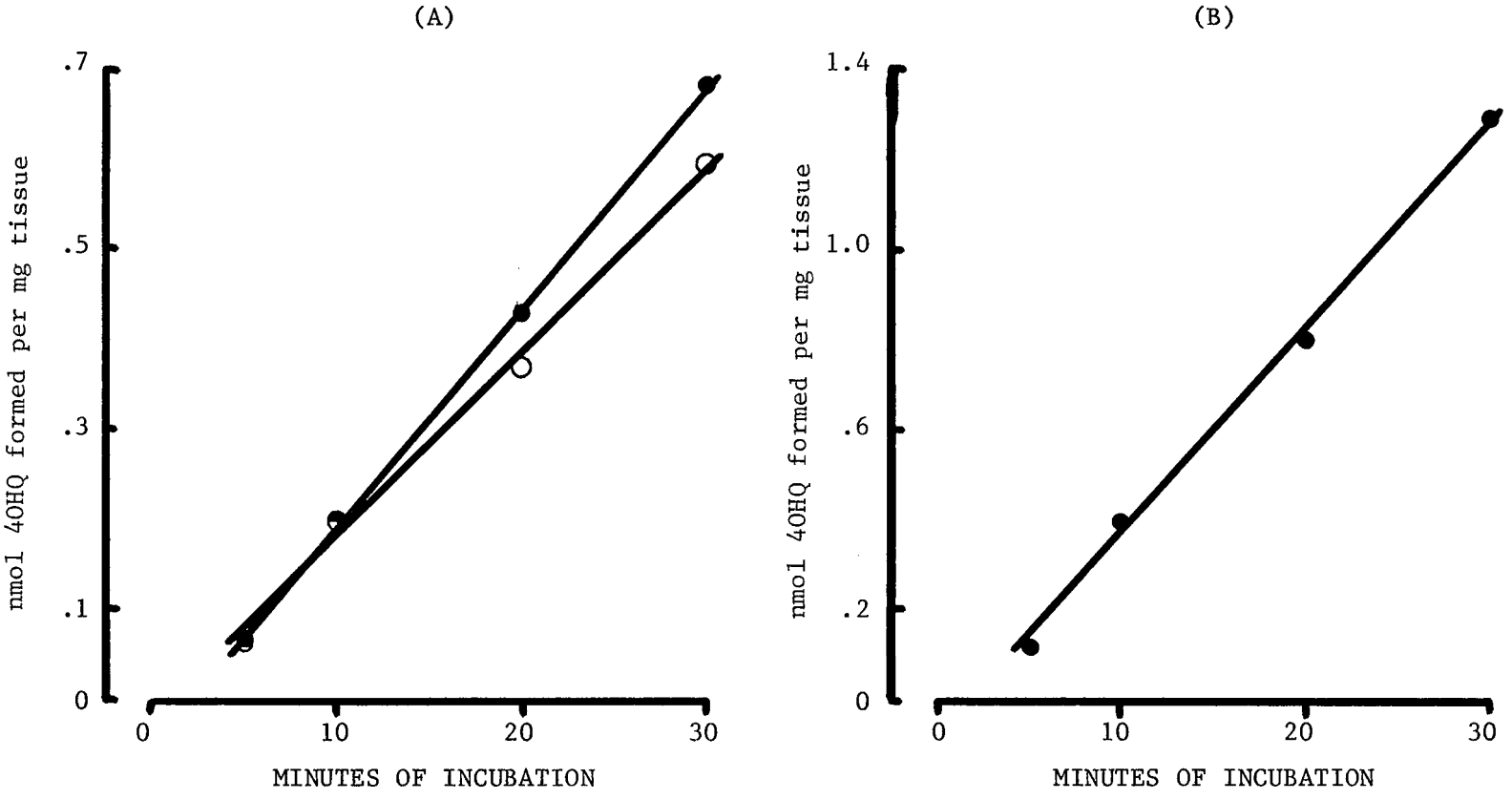
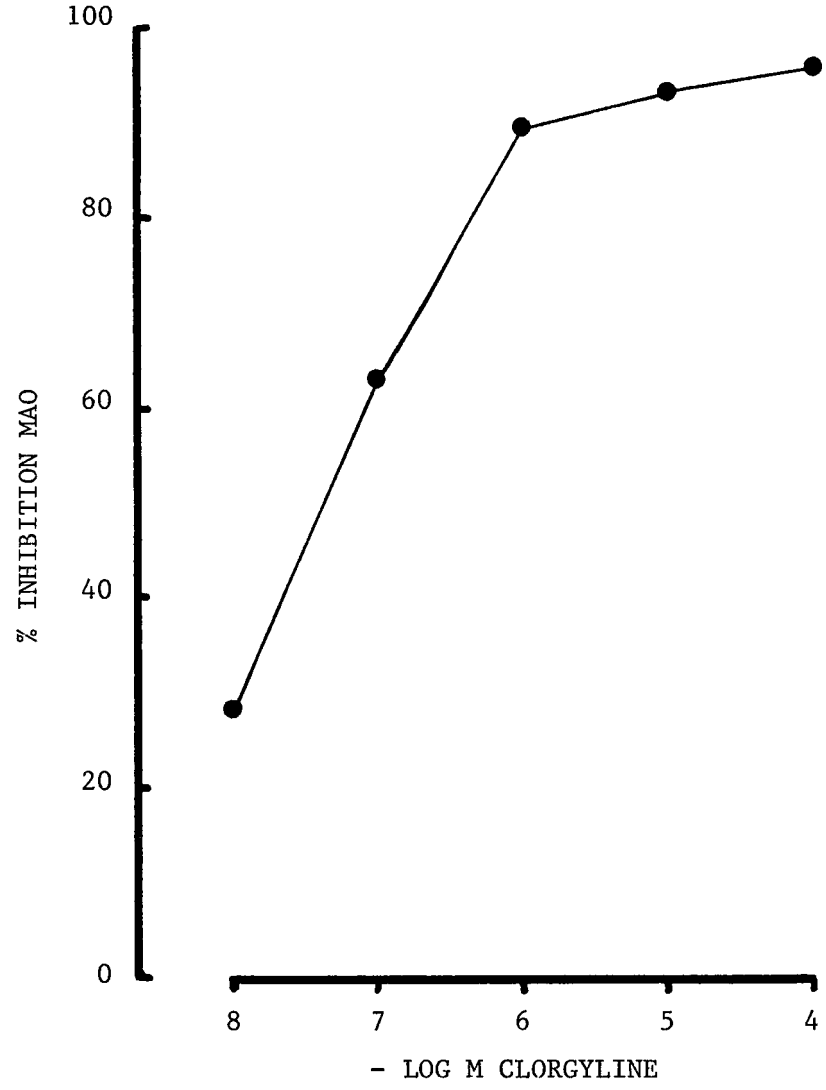

(A)
.7
.5
.3
.1
0
nmol 4OHQ formed per mg tissue
0
10
20
30
MINUTES OF INCUBATION
(B)
1.4
1.0
.6
.2
0
nmol 4OHQ formed per mg tissue
0
10
20
30
MINUTES OF INCUBATION

Figure 27. Inhibition curve with clorgyline in the intact, isolated rat atria.

The 4-hydroxyquinoline (4OHQ) formed by isolated atria is expressed as a percentage of the difference from the control activity (1.10 nmoles 4OHQ/mg tissue/hr = 0% inhibition). Each point is the mean of two to four determinations from equal numbers of left and right atria. Reaction time = 30 minutes.


100
80
60
40
20
0
% INHIBITION MAO
8
7
6
5
4
- LOG M CLORGYLINE

whole, isolated atria were inhibited with clorgyline to detect the type of MAO with which they deaminated kynuramine.

Although left atria formed more 4-hydroxyquinoline than right atria, the combination of left and right atria plus their incubation fluids resulted in linear product formation with time (Figure 26). Thereafter, a left and right atria were pooled at each experimental point. Clorgyline inhibition of the whole, isolated atria revealed a single type of MAO activity. The IC_{50} of the curve (6×10^{-8} M, see Figure 27) was approximately midway between those seen in atrial homogenates for type A and B activity.

Section B

General Rationale

The characteristics of MAO have been defined in only a few human tissues. These include the brain, liver and platelets. Thus, it was of interest to determine whether human cardiovascular MAO resembles or differs from that defined in the rat. A close similarity between rat and human cardiac and vascular tissue might allow the use of the rat as a valid model for human cardiovascular MAO.

1. Clorgyline inhibition curves in human atria with kynuramine as the substrate:

Inhibition curves using clorgyline were made on three separate samples of human atria. All specimens were from males over 48 years of age undergoing open heart surgery. The samples were washed well in

normal saline and frozen immediately for later assay. While Figure 28 shows some individual variation, all samples showed a majority of type B activity with 20% to 30% as type A. No clorgyline-resistant activity was present. In another experiment a separate sample was studied vs. clorgyline, deprenyl and pargyline (Figure 29). This sample showed a lower A activity, but all three inhibitors gave complementary results. Thus, human atria appears to differ in its relative proportions of type A and type B MAO from rat cardiac tissue.

Figure 30 shows clorgyline inhibition curves in samples of atria obtained from infants. The five-month old infant atria showed considerably more A activity than was obtained in adults. However, this relationship did not hold true for the 14-month sample. Nevertheless, the specific activities increased progressively with age (see legend to Figure 30).

2. Kinetic analysis of human atrial MAO:

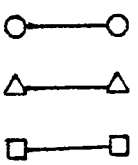
Lineweaver-Burk plots for kynuramine on the A and B activities of human atria are shown in Figure 31. The derived apparent Km values are 37.7 $\pm$ 2.9 μM and 33.0 $\pm$ 6.4 μM for the A and B activities, respectively. Both values are somewhat higher than that obtained in the rat, particularly with regard to the B type.

3. Effect of differential MAO inhibitors on human saphenous vein:

Figure 32 shows that human saphenous vein differs appreciably from the characteristics of MAO found in rat major blood vessels. The major activity is predominantly MAO type B. The inset shows that one

Figure 28. Inhibition curves with clorgyline on three human atria.

The 4-hydroxyquinoline formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of two determinations on individual human atria from males over 48 years of age.

Individual human atria 

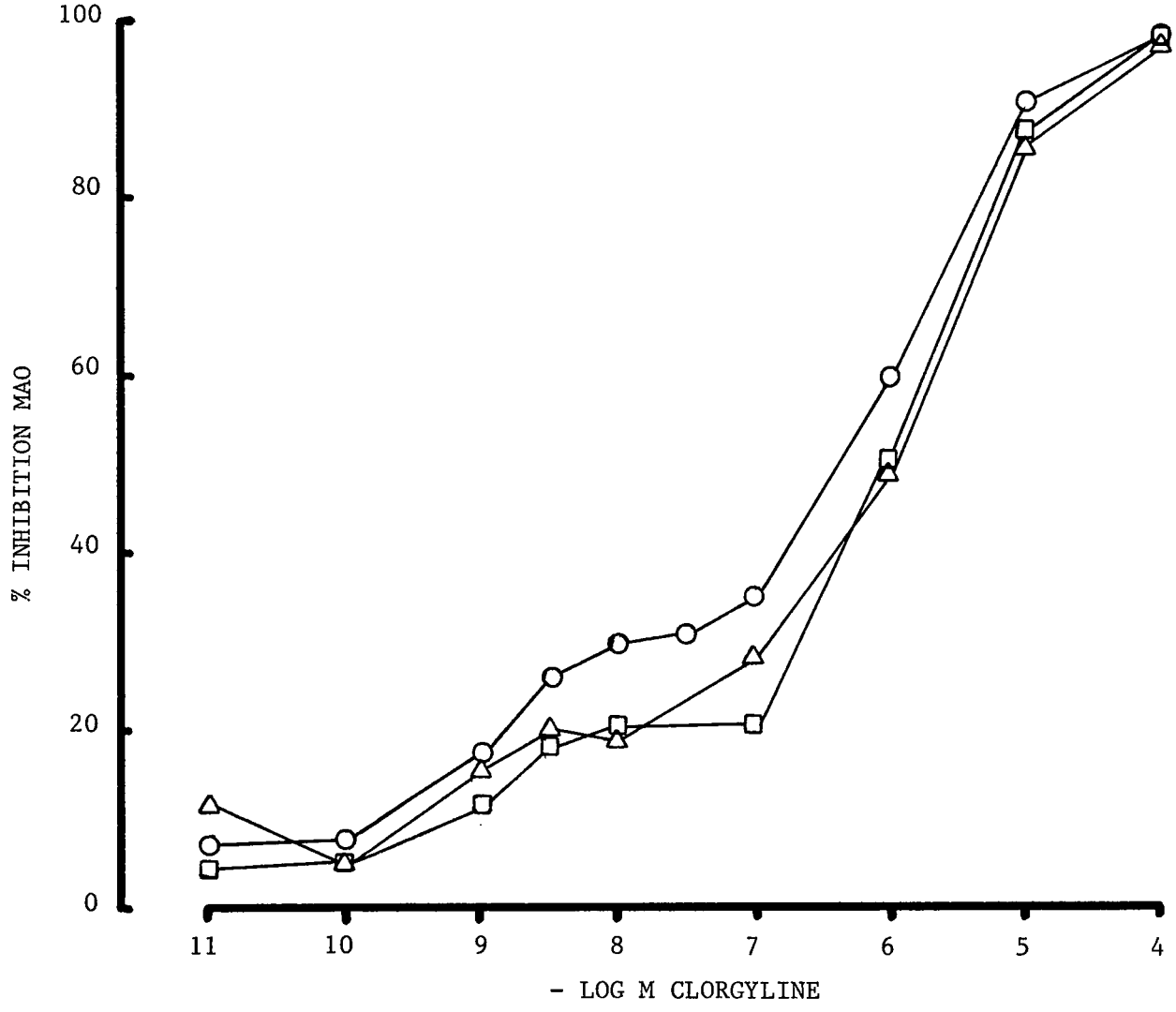

100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
- LOG M CLORGYLINE

Figure 29. Inhibition curves with clorgyline, deprenyl and pargyline on the human atria.

The 4-hydroxyquinoline formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

Clorgyline ●——●

Deprenyl ○——○

Pargyline △——△

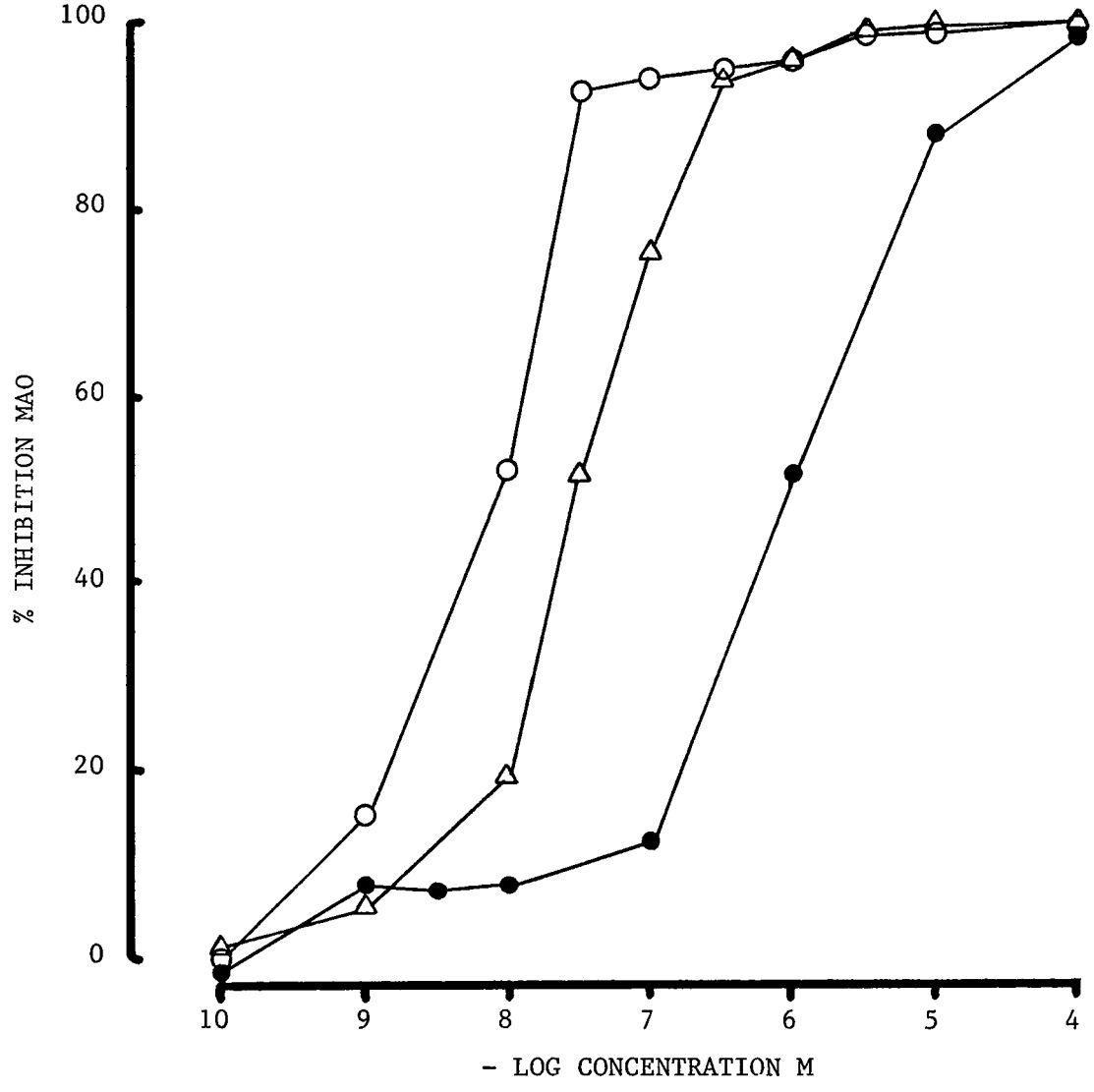
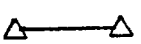
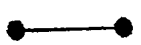

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
- LOG CONCENTRATION M

Figure 30. Effect of age on inhibition curves with clorgyline in the human atria.

The 4-hydroxyquinoline (4OHQ) formed is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations. The specific activities were: 5 mo. infant, 60.9 nmoles 4OHQ/mg protein/hr; 14 mo. infant, 138.6; and adult, 170.4. The adult curve is marked with the range of values found at each point from Figure 28.

Infant, 5 mo. 

Infant, 14 mo. 

Adult 

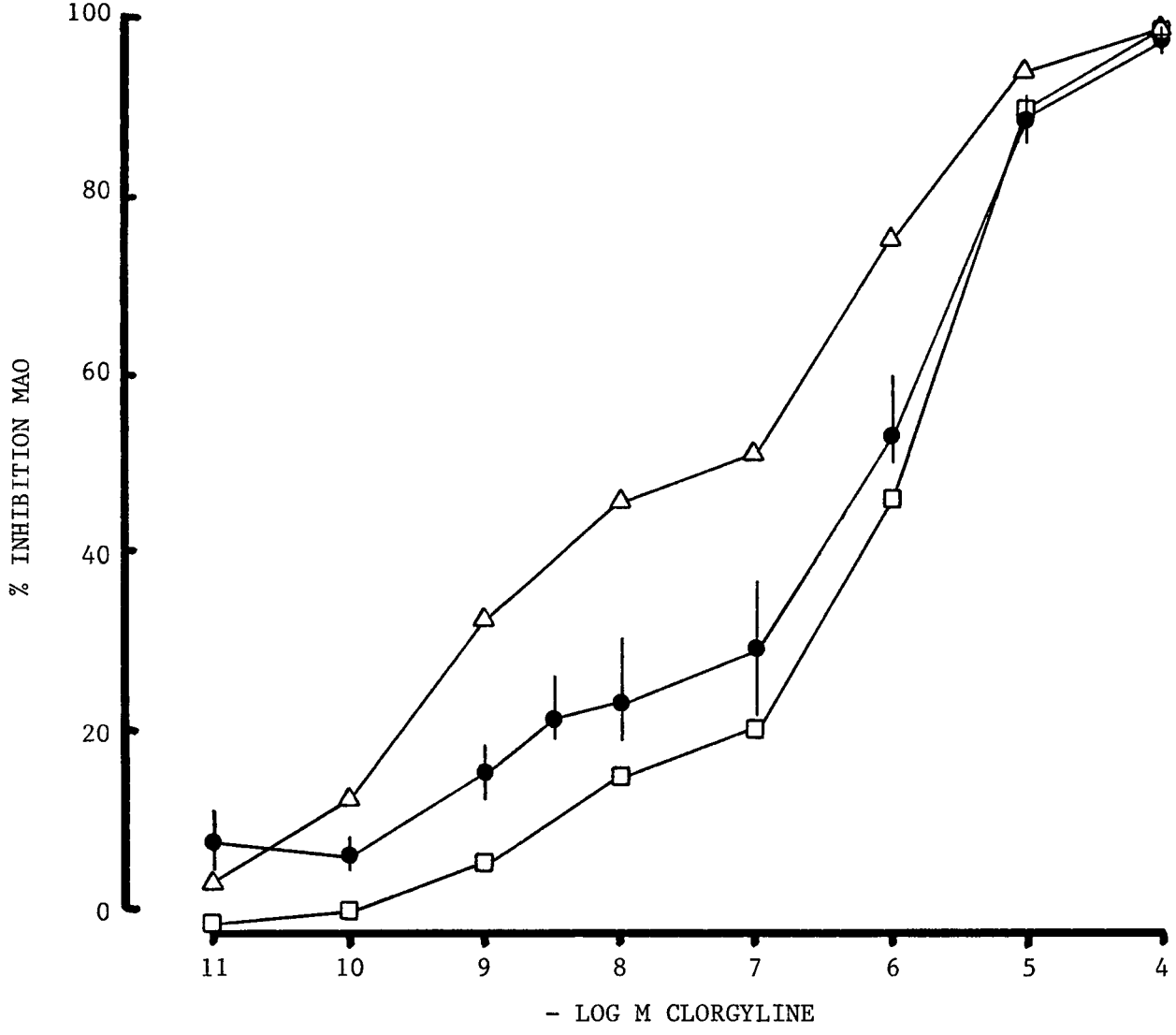

100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
- LOG M CLORGYLINE

Figure 31. Kinetic analysis of human atrial MAO using kynuramine.

The 4-hydroxyquinoline (4OHQ) formed is expressed as the reciprocal of the velocity in nmoles 4OHQ/mg tissue/min (1/v) plotted versus the reciprocal of the kynuramine concentration in μM (1/[S]). The Km value is obtained by calculating the negative reciprocal of the point of interception of the graph with the 1/[S] axis. Each point is the mean of four to six determinations.

The Km for type A (37.7 μM) was obtained in the presence of 10^{-7} M deprenyl, while the Km for type B (33.0 μM) was obtained in the presence of 10^{-7} M clorgyline.

Type A ●——●

Type B ○——○

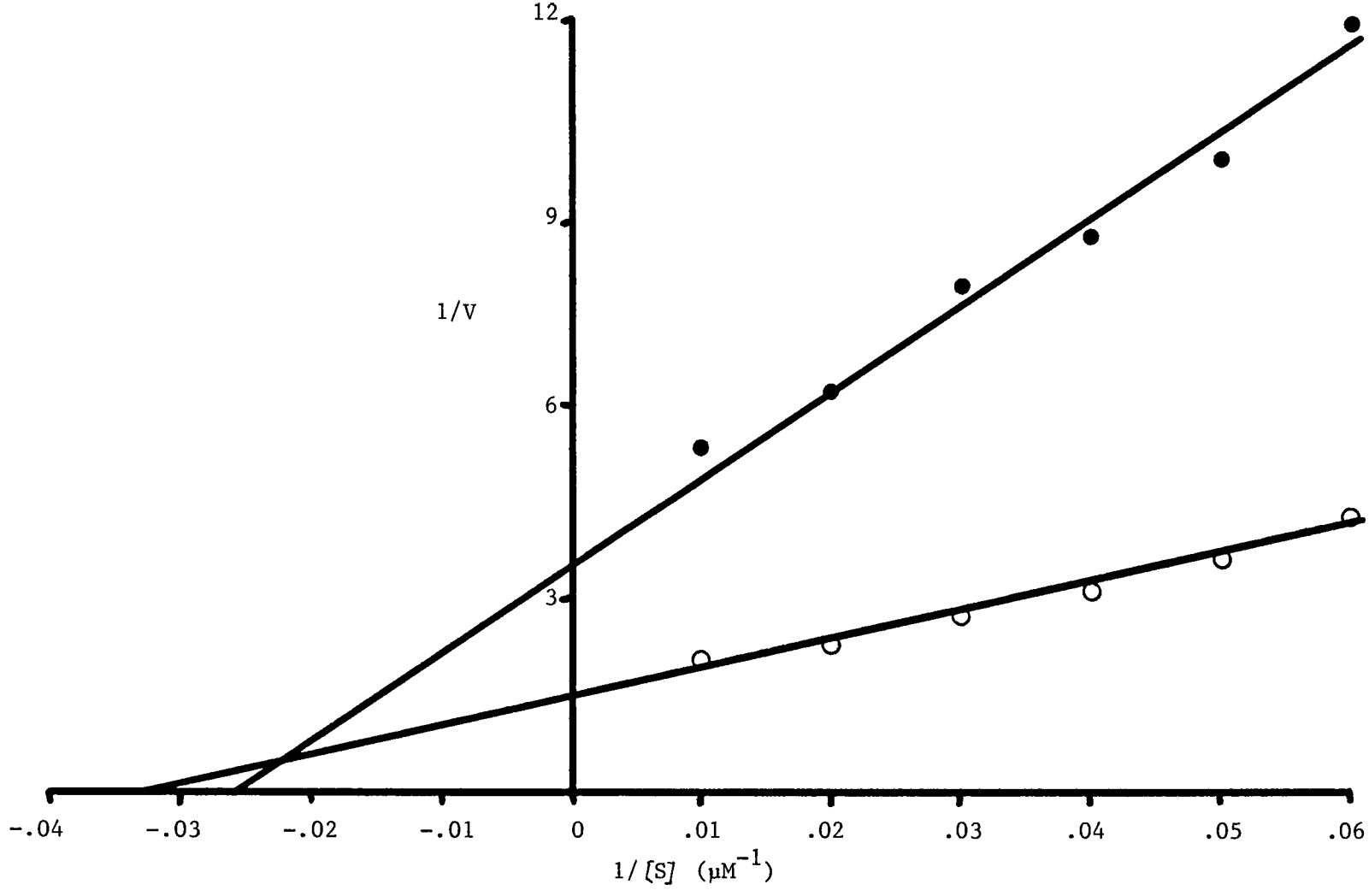
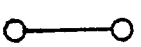

12
9
6
3
1/V
-.04
-.03
-.02
-.01
0
.01
.02
.03
.04
.05
.06
1/[S] (μM^{-1})

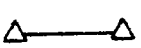
Figure 32. Inhibition curves with clorgyline, deprenyl and pargyline on the human saphenous vein.

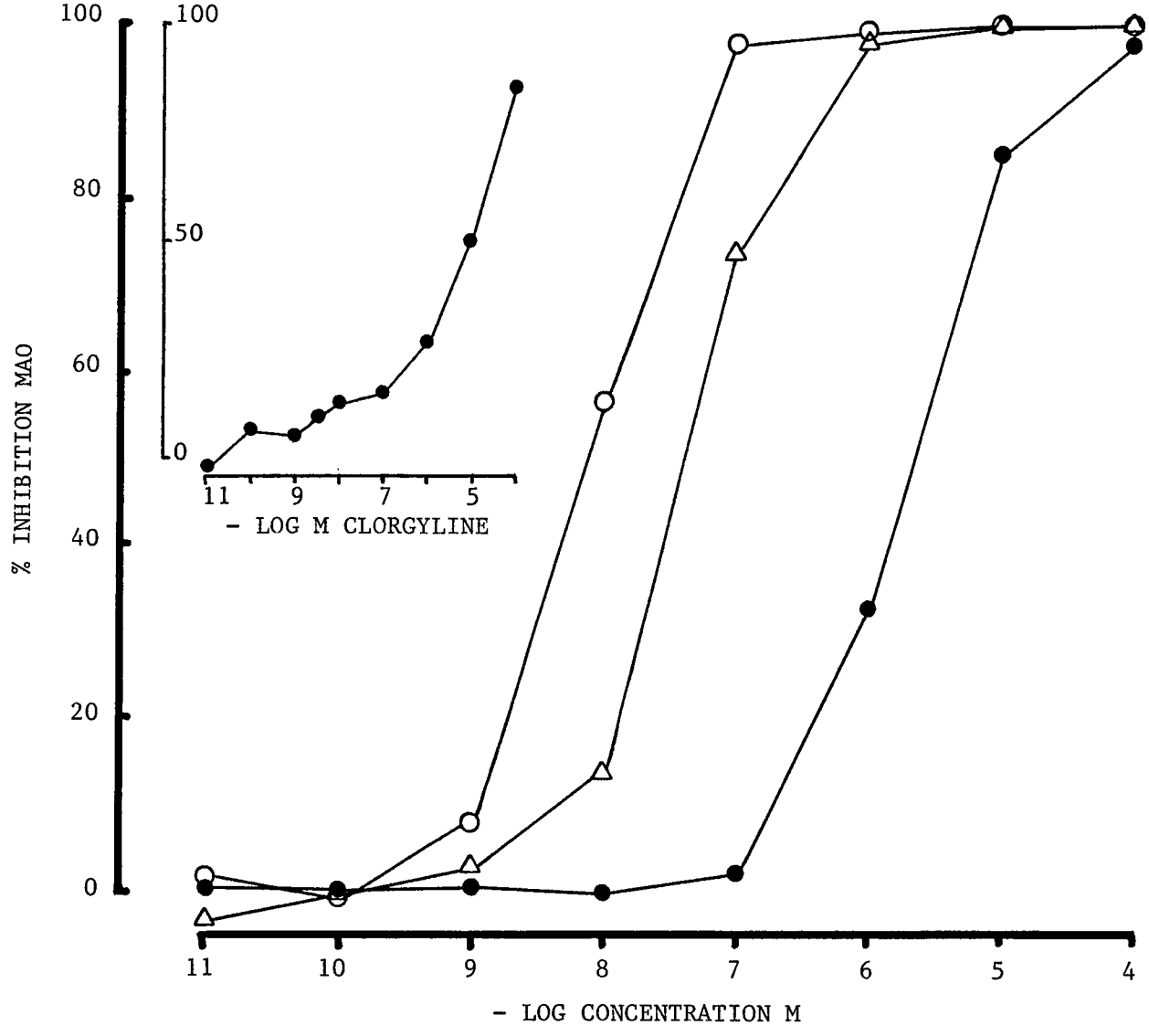
The 4-hydroxyquinoline (4OHQ) formed is expressed as a percentage of the difference from the control activity (6.10 nmoles 4OHQ/mg tissue/hr = 0% inhibition). Each point is the mean of at least two determinations.

The inset contains a clorgyline inhibition curve on another human saphenous vein.

Clorgyline 

Deprenyl 

Pargyline 


100
80
60
40
20
0
% INHIBITION MAO
100
50
0
11
9
7
5
- LOG M CLORGYLINE
11
10
9
8
7
6
5
4
- LOG CONCENTRATION M

sample contained a low percentage of the A type, but this was obtained from a varicosed condition. No inhibition of MAO activity could be obtained with semicarbazide, 10^{-3} M. Thus, unlike the rat major blood vessels, clorgyline-resistant activity was absent.

Section C

General Rationale

In the preceding sections the MAO of cardiac tissue was found to be closely similar to that of the vas deferens and liver. Major differences also seem to be absent between human atrial MAO and that of the rat. These findings were somewhat surprising in view of the fact that certain other substrates, such as phenylethylamine, showed "anomolous deamination" in rat cardiac tissue. Phenylethylamine, a highly selective B substrate in brain and liver, had been shown to be deaminated by the A type in rat heart (Lyles and Callingham, 1975). However, experiments made in Section 1 showed phenylethylamine to be an effective inhibitor of kynuramine metabolism on both the A and B types of rat cardiac tissue. It was important, therefore, to determine whether phenylethylamine was an actual substrate for these two types. Phenylethylamine might be acting as a substrate for the A type and an inhibitor of the B type in the rat heart. Also essential, was to compare the deamination characteristics of phenylethylamine in other rat organs. The vas deferens and liver were selected. Comparison was also made with human atria.

1. Clorgyline inhibition curves using phenylethylamine as the substrate: A comparison of tissues:

Although phenylethylamine is a highly preferential B substrate it is not devoid of affinity for the A type. In rat liver (Ekstedt, 1976) and human cortex (White and Glassman, 1977), phenylethylamine has been shown to exhibit 15 to 30 times higher affinity for MAO type B than MAO type A. The Km value on the B type is about 3 μM. Thus, it was hypothesized that the concentration of phenylethylamine used for assay would materially affect which type of MAO was involved in its deamination. Additionally, it was argued that at high concentrations of phenylethylamine (above the Km values of both the A and B types) the relative amounts of these two activities in a particular tissue would greatly influence the amount of product formed from each type. Section 1 of this dissertation indicates that radical differences in the relative amounts of A and B activity do exist between the heart, vas deferens and liver of rats. Similarly, differences between rat and human heart were exposed with regard to the relative percentages of the A and B types.

To test the above hypotheses, the following experiments were made using a range of phenylethylamine concentrations. Enzymatic activity having a high affinity for phenylethylamine would be predicted to be preferentially exposed at low concentrations of the substrate.

Figure 33 of the rat vas deferens shows phenylethylamine can be a substrate for three different MAO types. At 10 and 1 μM

phenylethylamine, single sigmoidal curves were obtained, indicative of type B activity. With 100 μM phenylethylamine both type A and B activity was revealed. Clorgyline-resistant activity was also observed at all substrate concentrations. More resistance was seen as the substrate concentration was increased. The clorgyline-resistance could be selectively inhibited with 10^{-3} M semicarbazide.

The rat ventricle (Figure 34) showed A and B activity at 10 and 1 μM phenylethylamine. At 100 μM the A activity predominated and the B accounted for only a small percentage of the total activity. A slight amount of clorgyline-resistance (5%) was present at all concentrations and was sensitive to 10^{-3} M semicarbazide.

The liver (Figure 38) showed solely B activity at 10 and 1 μM phenylethylamine. Type A activity was observed only at 1000 μM phenylethylamine. As in the ventricle, a small percentage of clorgyline-resistant activity was present.

The human atria (Figure 36) also depicted types A and B at 100 μM phenylethylamine. The lower substrate concentration (10 μM) revealed only B activity. Likewise, human saphenous vein (Figure 37) showed dual metabolism at 100 μM phenylethylamine and type B alone at the 10 μM concentration. Some indication of a slight amount of clorgyline-resistance was seen in both tissues.

Two different concentrations of kynuramine in the rat vas deferens (Figure 38) exposed results similar to those with phenylethylamine. At 100 μM kynuramine, both A and B types were evident in approximately equal proportions. However, at 10 μM, kynuramine revealed a greater percentage of B activity. No clorgyline-resistance was seen.

Figure 33. Effect of substrate concentration on clorgyline inhibition curves in the rat vas deferens using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

1 μM PEA △——△

10 μM PEA ○——○

100 μM PEA ●——●

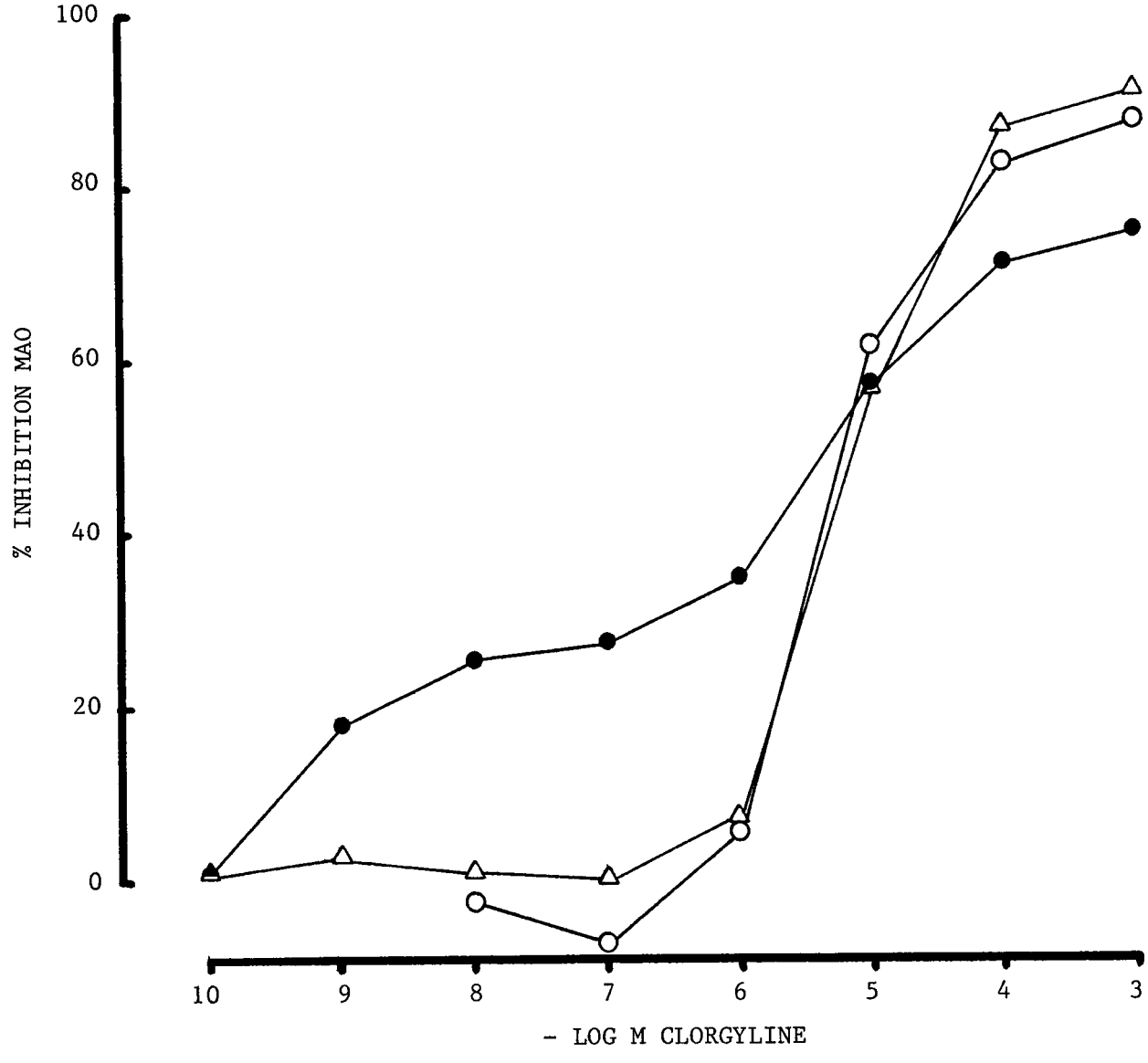
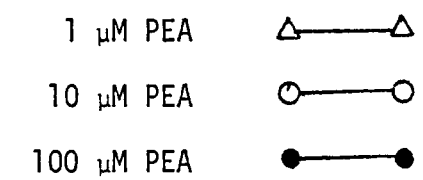

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M CLORGYLINE

Figure 34. Effect of substrate concentration on clorgyline inhibition curves in the rat ventricle using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.



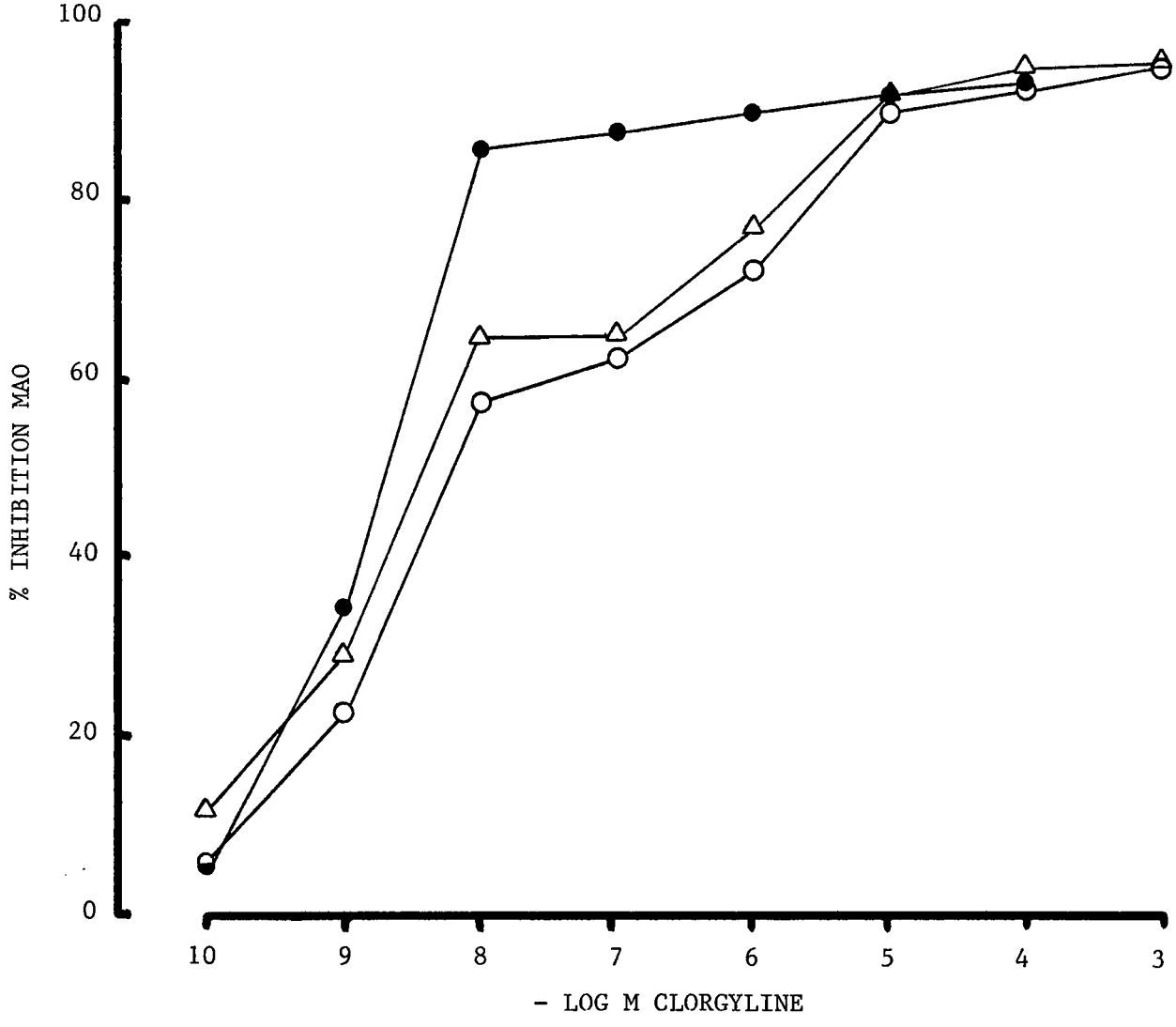

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M CLORGYLINE

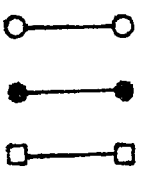
Figure 35. Effect of substrate concentration on clorgyline inhibition curves in the rat liver using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

10 μM PEA

100 μM PEA

1000 μM PEA



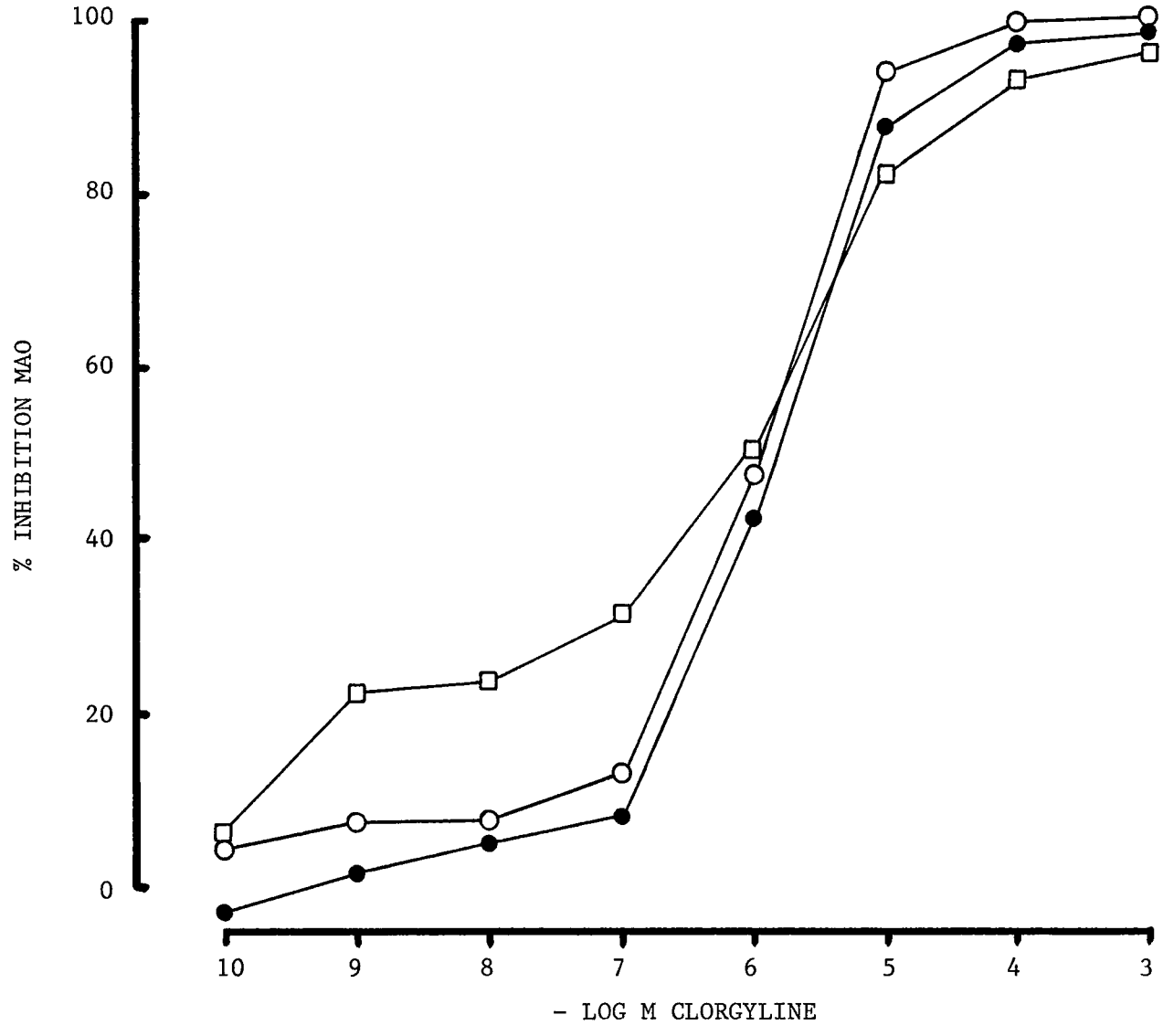
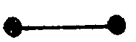

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M CLORGYLINE

Figure 36. Effect of substrate concentration on clorgyline inhibition curves in the human atria using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

10 μM PEA 

100 μM PEA 

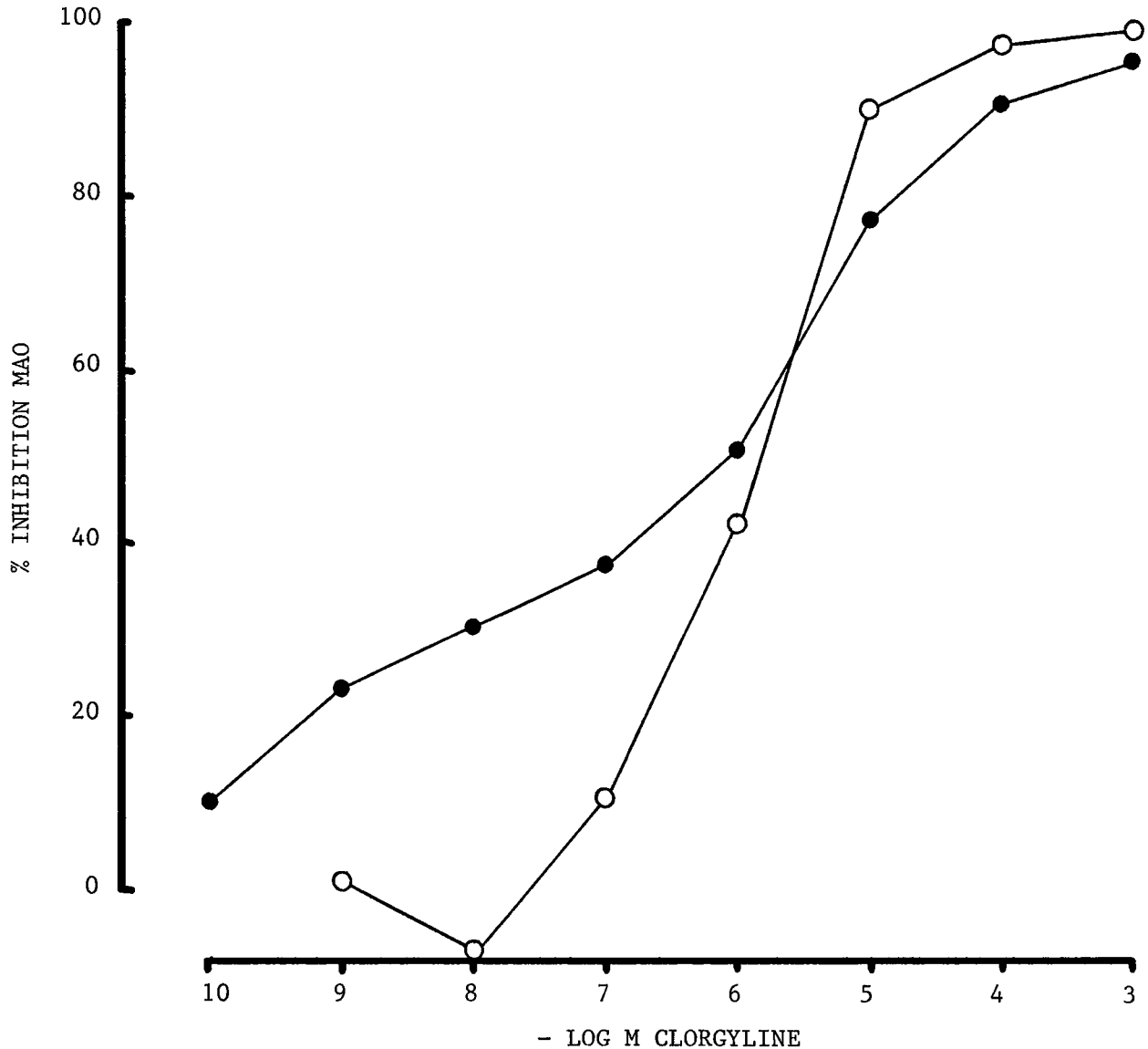
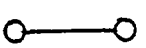
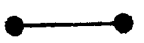

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M CLORGYLINE

Figure 37. Effect of substrate concentration on clorgyline inhibition curves in the human saphenous vein using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

10 μM PEA 

100 μM PEA 

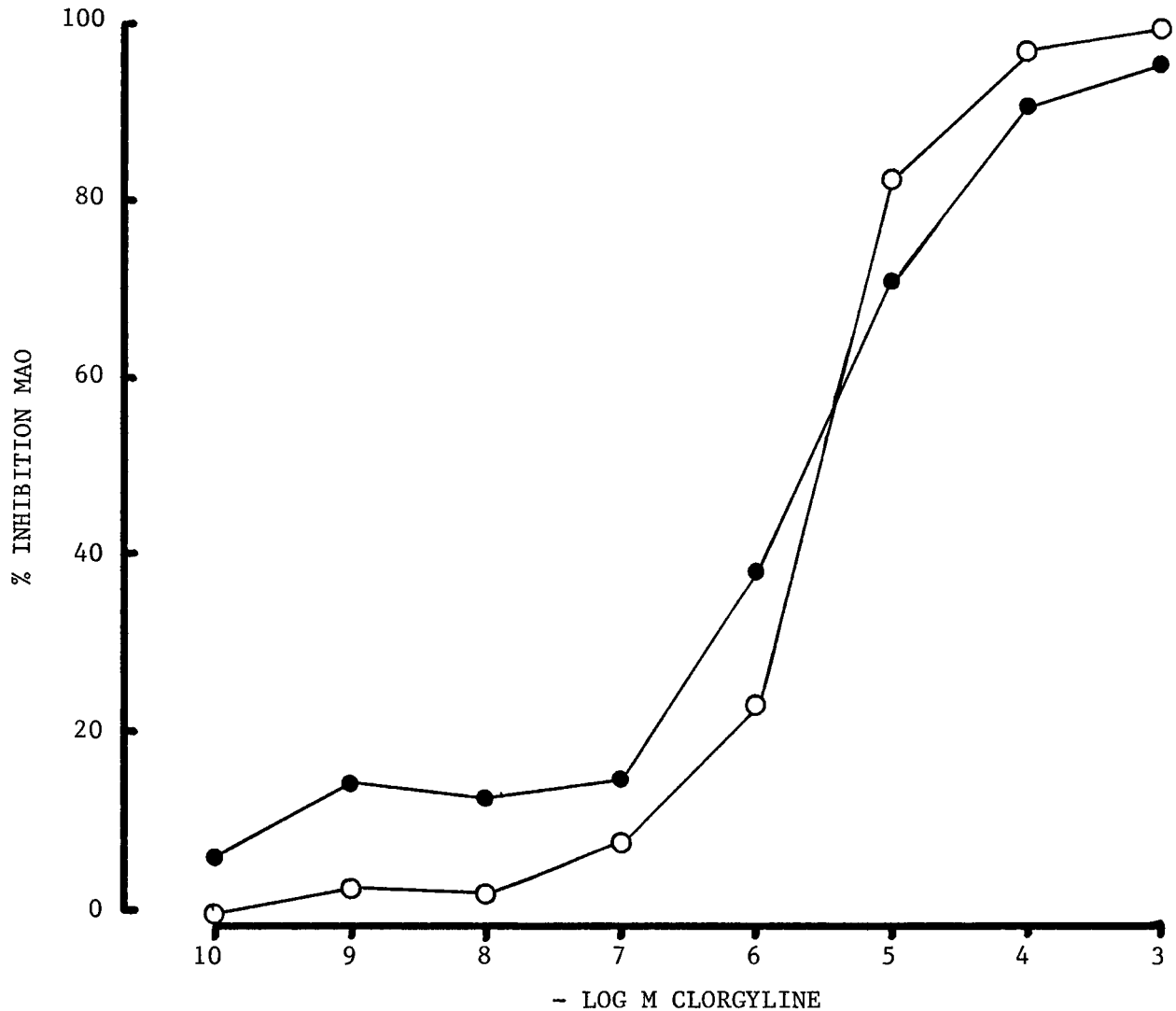
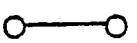
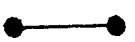
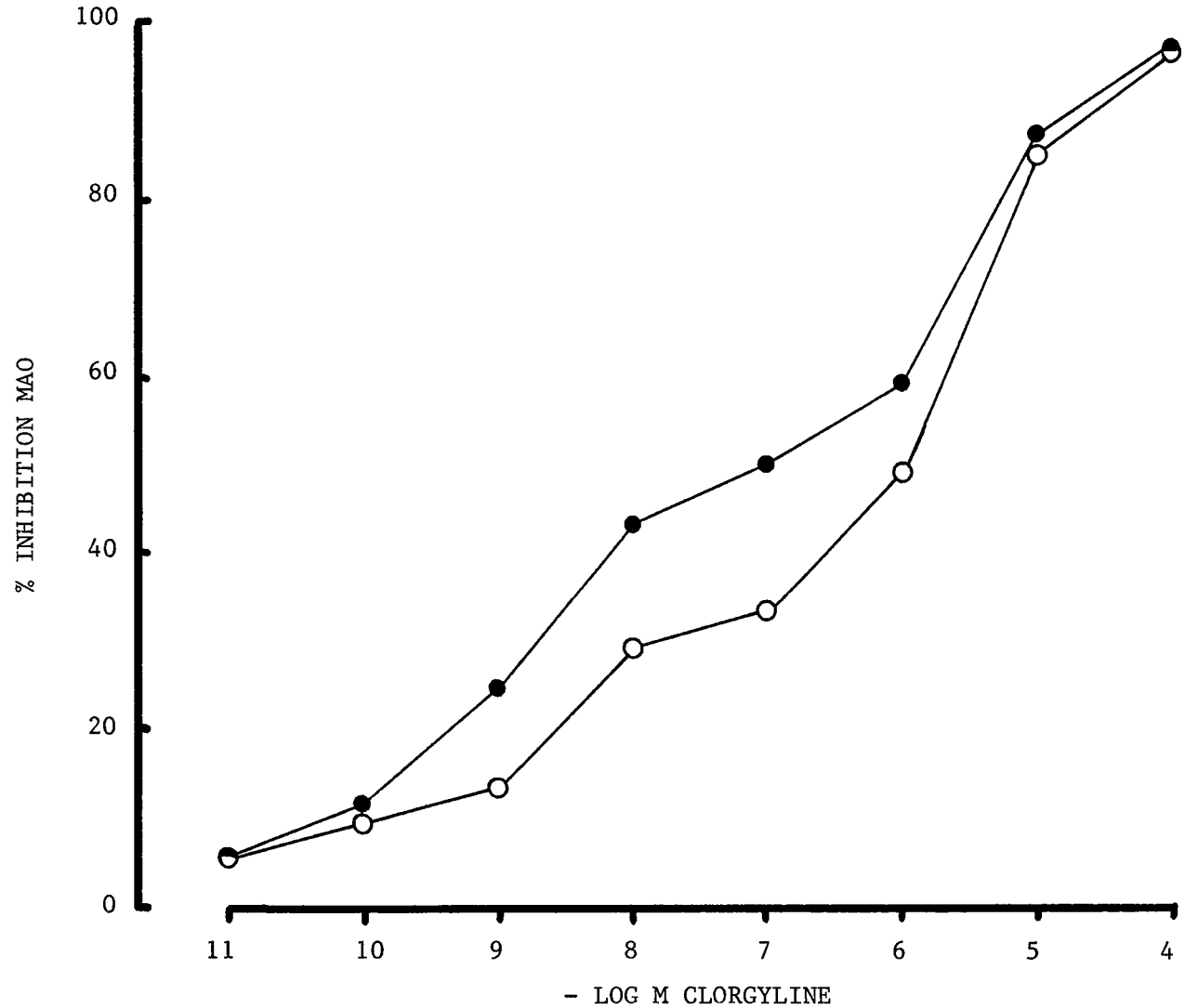

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M CLORGYLINE

Figure 38. Effect of substrate concentration on clorgyline inhibition curves in the rat vas deferens using kynuramine (KYN).

The 4-hydroxyquinoline produced is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of six determinations.

10 μM KYN 

100 μM KYN 


100
80
60
40
20
0
% INHIBITION MAO
11
10
9
8
7
6
5
4
- LOG M CLORGYLINE

2. Deprenyl inhibition curves using phenylethylamine as the substrate: A comparison of tissues:

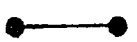
With kynuramine as the substrate, deprenyl and clorgyline revealed similar results (Section 1). The following experiments were made to determine whether this complementary relationship holds true when using phenylethylamine.

Phenylethylamine concentrations of 10 μM and 100 μM were selected for use in the vas deferens, since the former shows only type B activity with clorgyline while the latter shows A and B activity. Figure 39 shows that the 10 μM concentration is compatible with the demonstration of MAO type B and resistant MAO activity. However, deprenyl failed to reveal clear A and B deamination with phenylethylamine at the higher concentration. As with clorgyline, a significant percentage of resistant activity was detected. Thus, in the vas deferens, deprenyl is not complementary with clorgyline when dual metabolism of phenylethylamine is involved. Similar results were obtained in the ventricle (Figure 40) and liver (Figure 41). In the ventricle, the 10 μM concentration of phenylethylamine exhibited dual metabolism with clorgyline (Section 3, #1) but with deprenyl, no clear discrimination between type A and type B activity was obtained. In the liver, deprenyl again failed to define the A and B types as seen with clorgyline (1000 μM), but showed clearly pure B metabolism at 10 μM phenylethylamine. Resistant MAO activity was present in the ventricle but not in the liver.

Figure 39. Effect of substrate concentration on deprenyl inhibition curves in the rat vas deferens using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

10 μM PEA 

100 μM PEA 

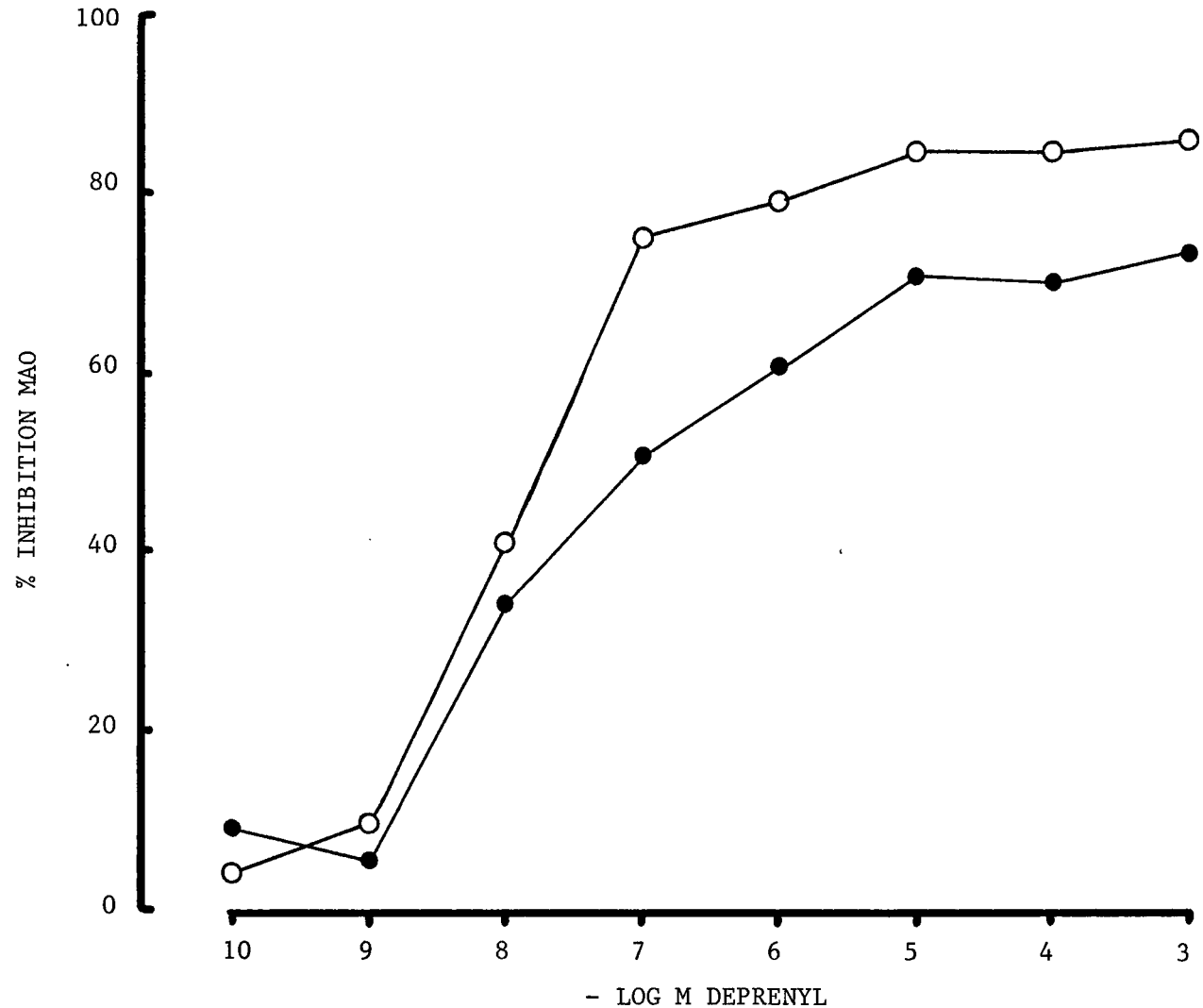
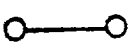

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M DEPRENYL

Figure 40. Effect of substrate concentration on a deprenyl inhibition curve in the rat ventricle using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of four determinations.

10 μM PEA 

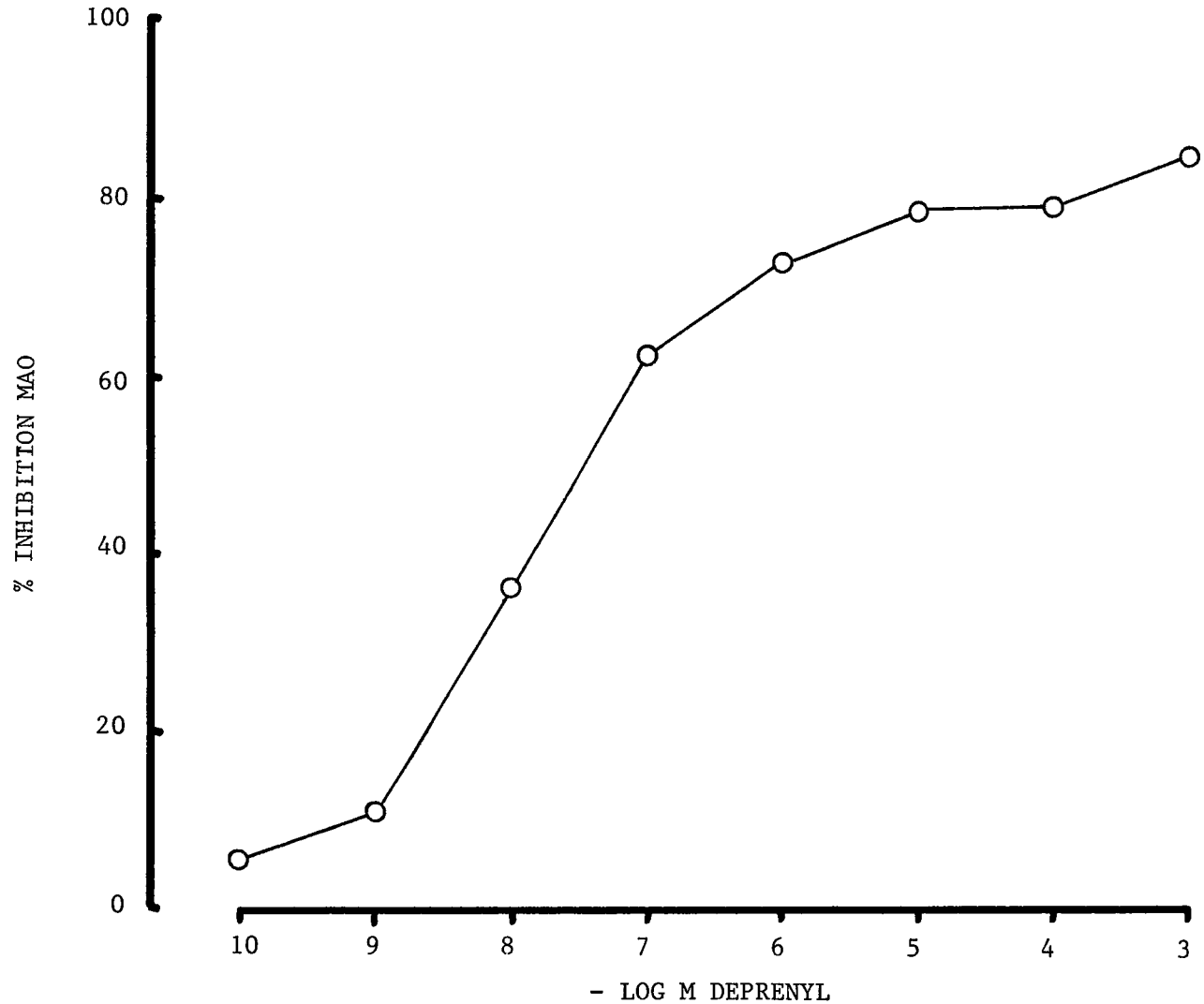

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M DEPRENYL

Figure 41. Effect of substrate concentration on deprenyl inhibition curves in the rat liver using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

10 μM PEA ○——○

1000 μM PEA □——□

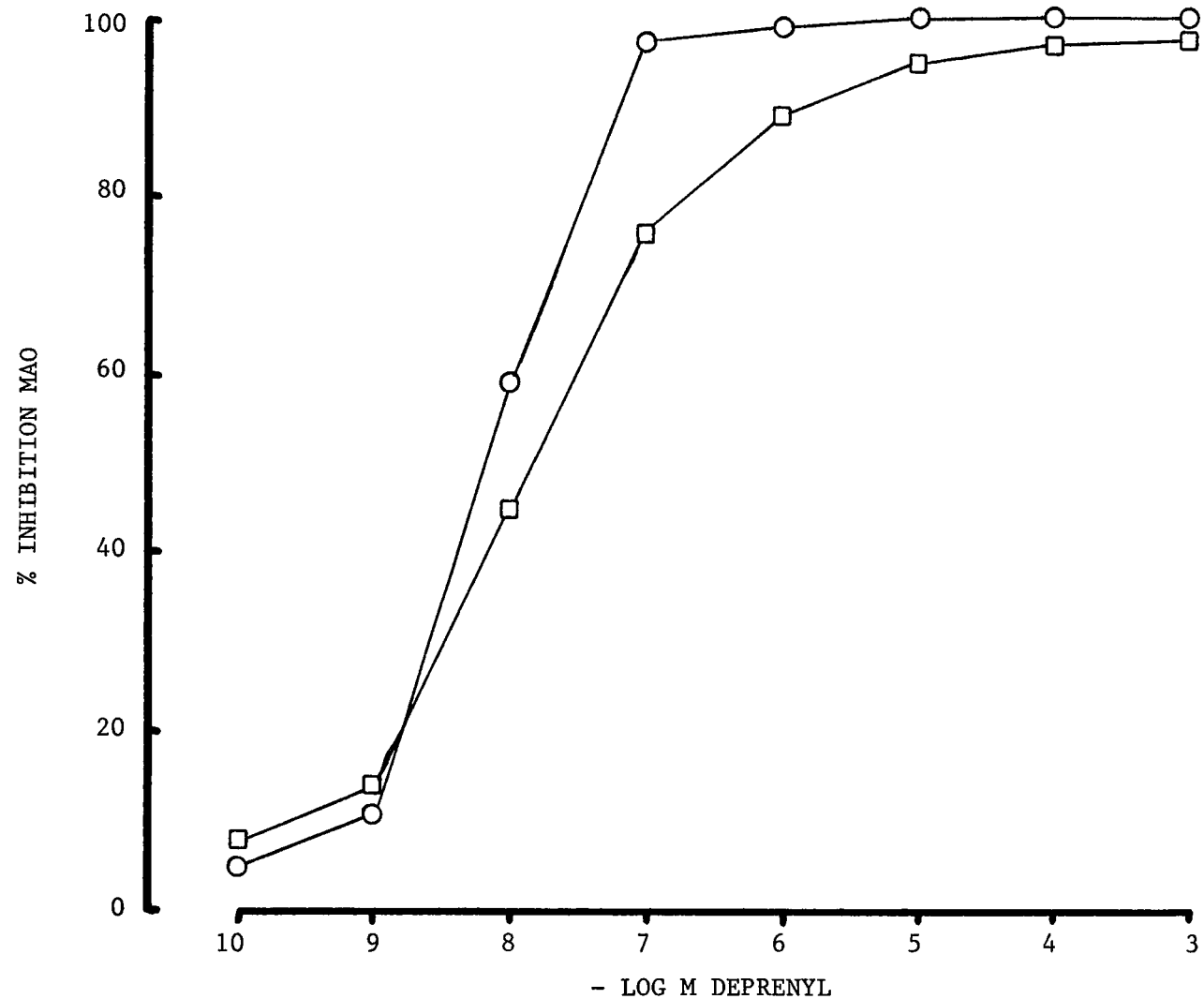
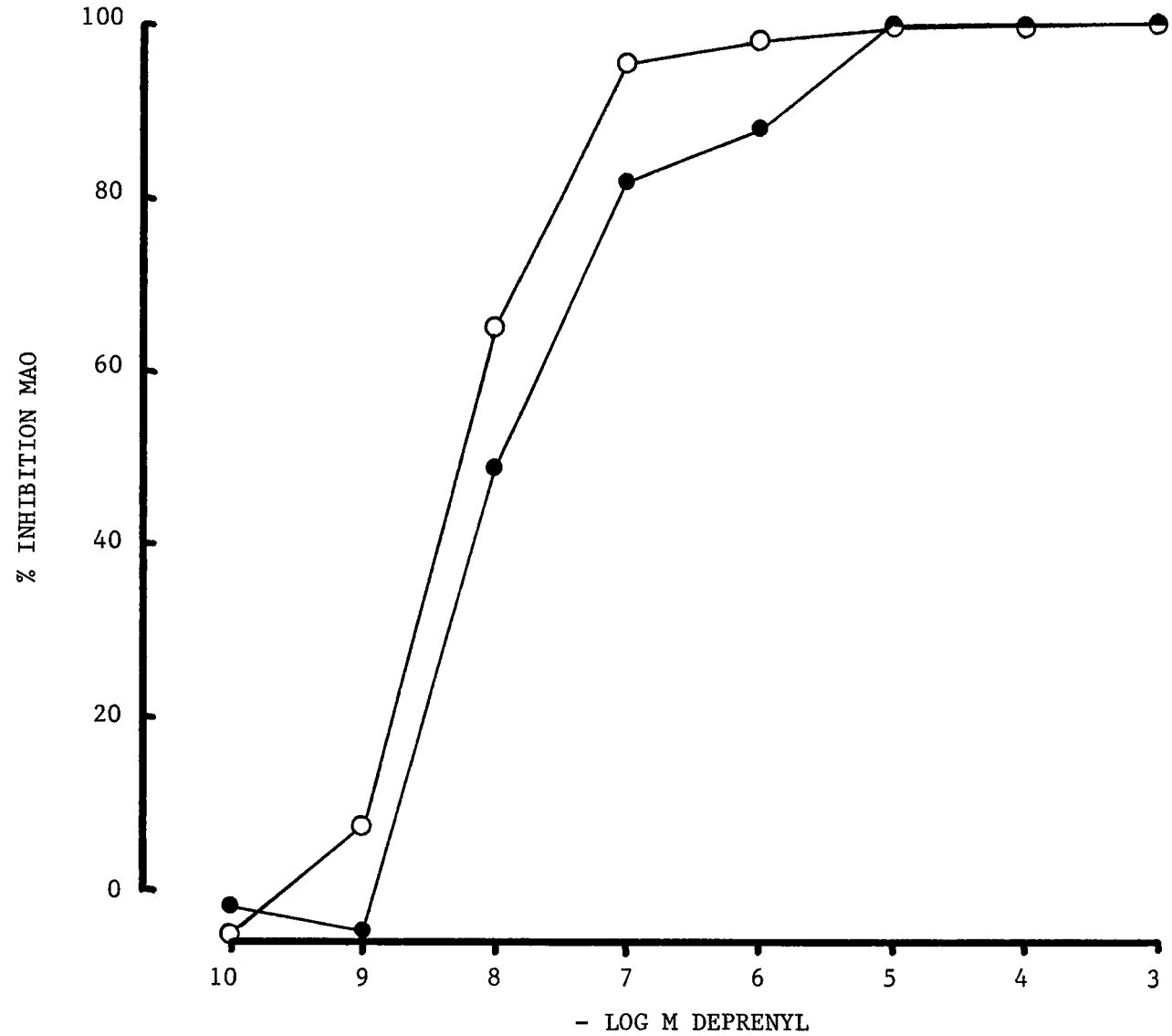

100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M DEPRENYL

Figure 42. Effect of substrate concentration on deprenyl inhibition curves in the human atria using phenylethylamine (PEA).

The PEA deaminated is expressed as a percentage of the difference from the control activity (control, no inhibitor = 0% inhibition). Each point is the mean of at least two determinations.

10 μM PEA

100 μM PEA


100
80
60
40
20
0
% INHIBITION MAO
10
9
8
7
6
5
4
3
- LOG M DEPRENYL

The results obtained with deprenyl on human atria also show the same phenomenon (Figure 42). MAO type B activity is defined (10 μM), but A and B activity as seen with clorgyline (100 μM) was not so well described.

3. Apparent Km determinations in rat liver, ventricle and human atria using phenylethylamine as the substrate:

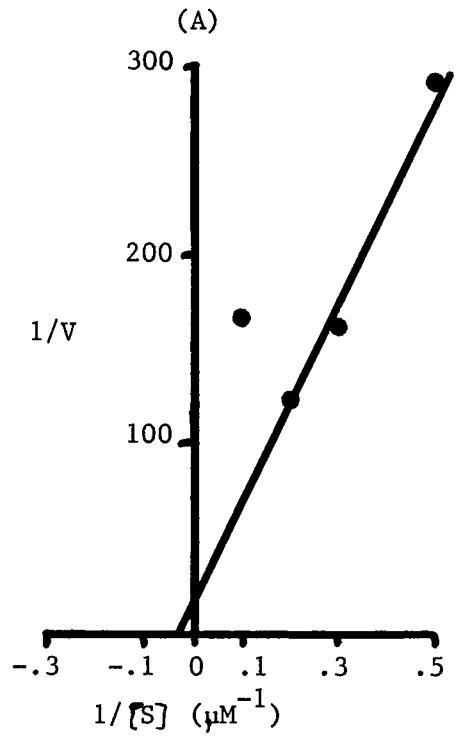
In the following experiments the Michaelis-Menton constants for the type B MAO were determined in the presence of 10^{-7} M clorgyline. Km values ranging from 1.2 μM to 2.98 μM were obtained in rat liver, rat ventricle and human atria (Figures 43, 44 and 45). The liver was chosen, rather than the vas deferens, since it allowed comparison with the previous work of Ekstedt (1976). The A type Km values were more difficult to determine accurately, since deprenyl had failed to clearly distinguish between the A and B types of MAO (Section C, #2). However, in the rat liver and human atria the discrepancy between deprenyl and clorgyline was least marked, and 10^{-7} M deprenyl was utilized to provide the A activity. Figures 43 and 45 show that Km values of 28.6 μM and 25.0 μM were obtained. One point in Figure 43A shows evidence of substrate inhibition. In the ventricle the discrepancy between deprenyl and clorgyline was most apparent (Section C, # 1 and 2). The Km value was determined by raising the substrate concentrations, thereby forcing deamination through the A type. A value of 32.8 μM was obtained (Figure 44). As shown in Table 4, about a 10 to 28-fold difference exists in the affinity of

Figure 43. Kinetic analysis of rat liver MAO using phenylethylamine (PEA).

The PEA deaminated is expressed as the reciprocal of the velocity in nmoles PEA metabolized/mg tissue/min (1/v) plotted versus the reciprocal of the PEA concentration in μM (1/[S]). The Km value is obtained by calculating the negative reciprocal of the point of interception of the graph with the 1/[S] axis.

(A) Lineweaver-Burk plot of type A activity. Each point is the mean of at least two determinations. The Km value (28.6 μM) was obtained in the presence of 10^{-7} M deprenyl.

(B) Lineweaver-Burk plot of type B activity. Each point is the mean of at least two determinations. The Km value (2.1 μM) was obtained in the presence of 10^{-7} M clorgyline.


(A)
300
200
100
1/V
-.3
-.1
0
.1
.3
.5
1/[S] (μM^{-1})

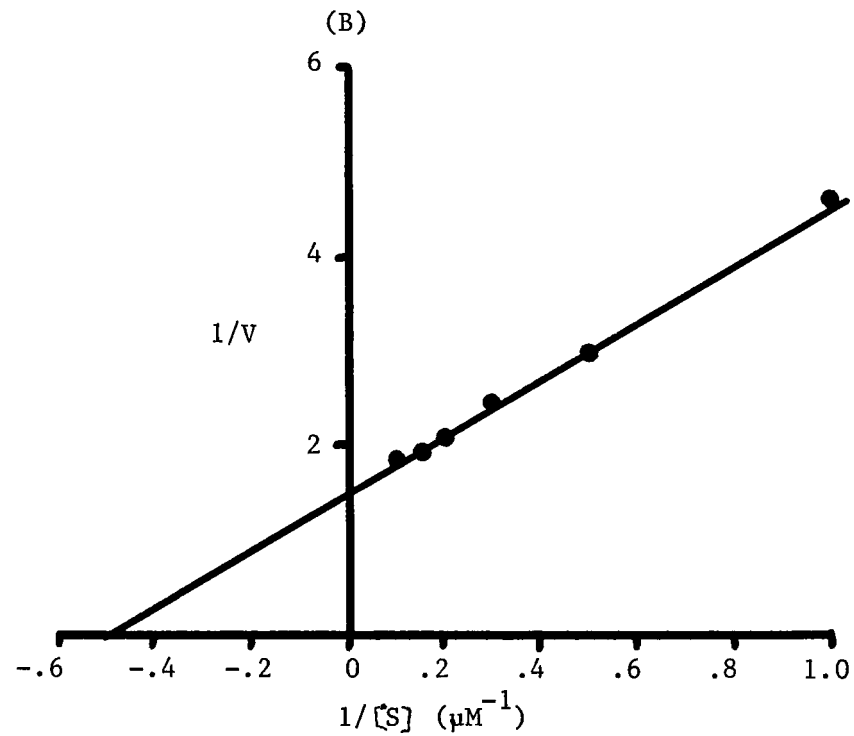
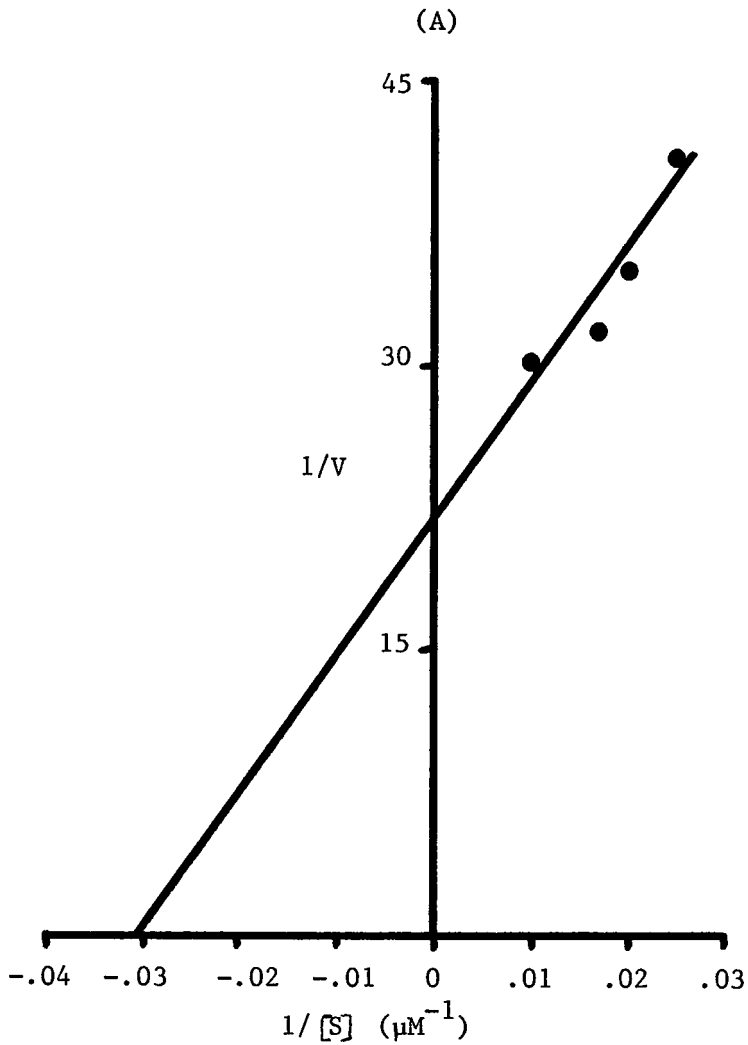

(B)
6
4
2
1/V
-.6
-.4
-.2
0
.2
.4
.6
.8
1.0
1/[S] (μM^{-1})

Figure 44. Kinetic analysis of rat ventricle MAO using phenylethylamine (PEA).

The PEA deaminated is expressed as the reciprocal of the velocity in nmoles PEA metabolized/mg tissue/min (1/v) plotted versus the reciprocal of the PEA concentration in μM (1/[S]). The Km value is obtained by calculating the negative reciprocal of the point of interception of the graph with the 1/[S] axis.

(A) Lineweaver-Burk plot of type A activity. Each point is the mean of at least four determinations. The Km value (32.8 μM) was obtained in the absence of any inhibitor.

(B) Lineweaver-Burk plot of type B activity. Each point is the mean of at least two determinations. The Km value (2.98 μM) was obtained in the presence of 10^{-7} M clorgyline.


(A)
45
30
15
1/V
-.04
-.03
-.02
-.01
0
.01
.02
.03
1/[S] (μM^{-1})

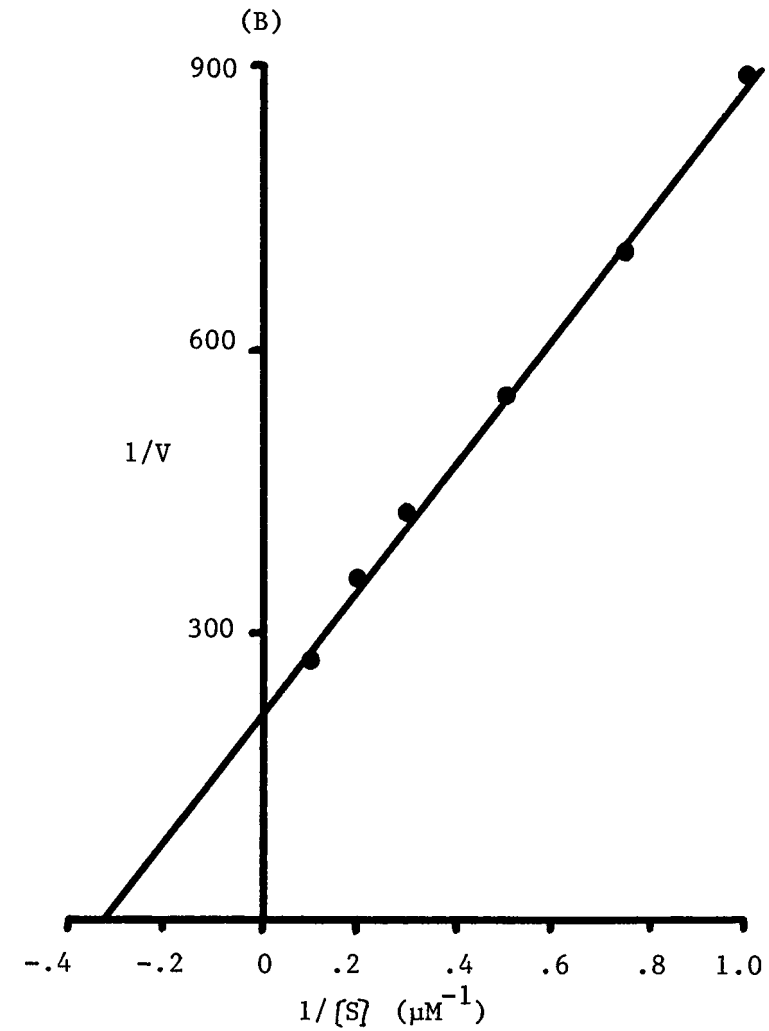

(B)
900
600
300
1/V
-.4
-.2
0
.2
.4
.6
.8
1.0
1/[S] (μM^{-1})

Figure 45. Kinetic analysis of human atrial MAO using phenylethylamine (PEA).

The PEA deaminated is expressed as the reciprocal of the velocity in nmoles PEA metabolized/mg tissue/min (1/v) plotted versus the reciprocal of the PEA concentration in μM (1/[S]). The Km value is obtained by calculating the negative reciprocal of the point of interception of the graph with the 1/[S] axis.

(A) Lineweaver-Burk plot of type A activity. Each point is the mean of at least two determinations. The Km value (25.0 μM) was obtained in the presence of 10^{-7} M deprenyl.

(B) Lineweaver-Burk plot of type B activity. Each point is the mean of at least two determinations. The Km value (1.2 μM) was obtained in the presence of 5×10^{-7} M clorgyline.

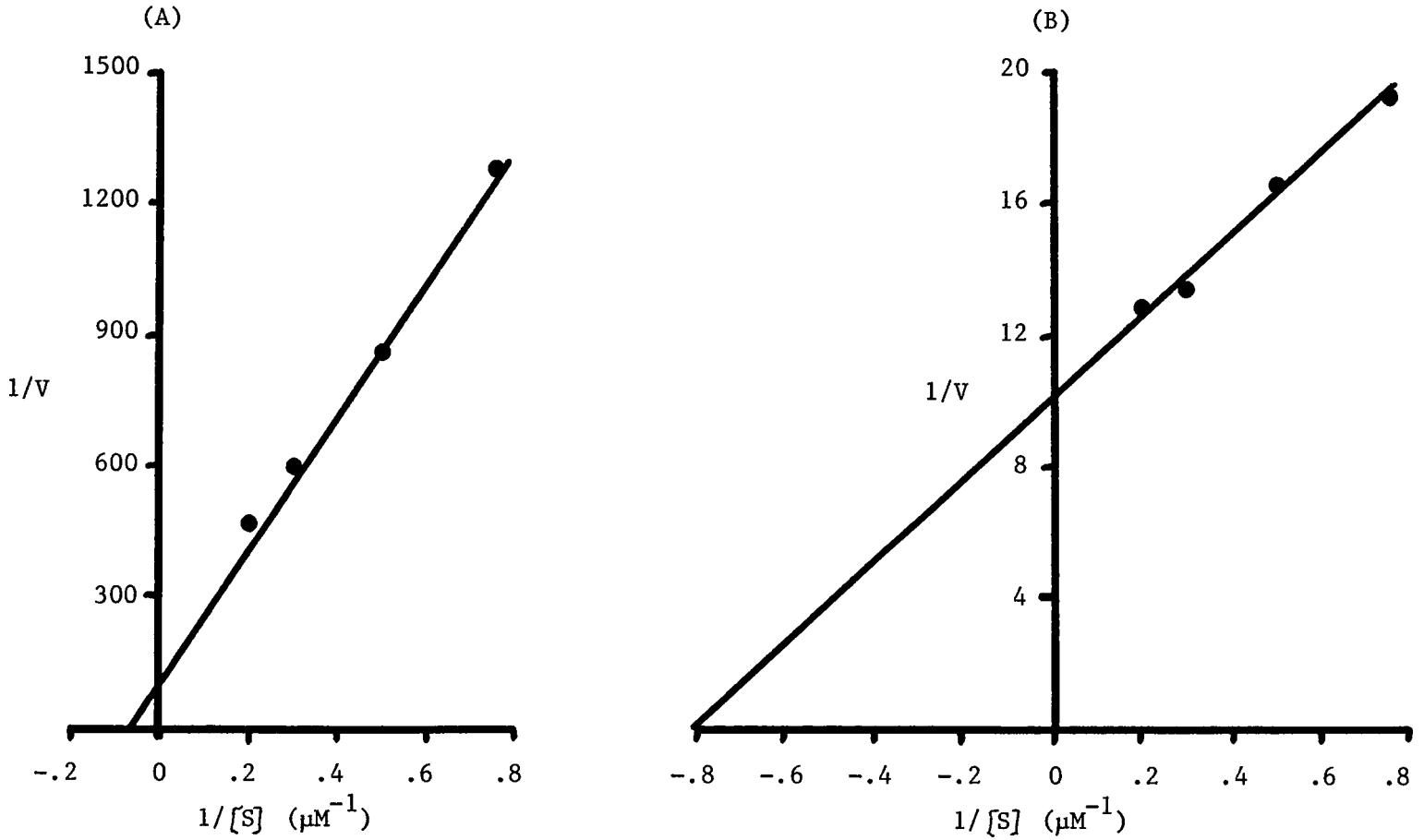

(A)
1500
1200
900
600
300
1/V
-.2
0
.2
.4
.6
.8
1/[S] (μM^{-1})
(B)
20
16
12
8
4
1/V
-.8
-.6
-.4
-.2
0
.2
.4
.6
.8
1/[S] (μM^{-1})

TABLE 4. Apparent Km and Vmax values of MAO activity in rat liver, ventricle and human atria using phenylethylamine as substrate.

The Km values for A and B activity were obtained from Lineweaver-Burk plots in Figures 43-45. The Vmax values were calculated from the reciprocal of the 1/v axis intercept of a whole tissue homogenate plot.

	Km (μM)		Vmax
	A	B	(nmoles PEA deaminated/ mg tissue/min)
Rat liver	28.6	2.1	0.68
Rat ventricle	32.6	3.0	0.05
Human Atria	25.0	1.2	0.10

phenylethylamine for the two MAO types. The velocity of deamination was fastest in the rat liver and slowest in the rat ventricle.

Despite the problems involved in determining the Km values for the A type, the difference in affinity of phenylethylamine on the A and B activities is closely similar to that found by Ekstedt (1976) in rat liver. He obtained a 15-fold difference, with Km values of 4 μM (B type) and 62 μM on the A type.

V. DISCUSSION

The work presented in this dissertation has attempted to classify and characterize the monoamine oxidase activities present in cardiovascular tissues. Despite evidence that cardiac MAO may differ radically from that found in other tissues (Mantle et al., 1976; Lyles and Callingham, 1975; Fuentes and Neff, 1977), the present work disclosed many more similarities than differential characteristics.

A major tool for the classification of MAO activities are the recently developed preferential inhibitors of either the A or B types, namely clorgyline (Johnston, 1968) and deprenyl (Knoll and Magyar, 1972). Pargyline is also claimed to preferentially inhibit MAO type B (Fuller et al., 1970) and has been used widely for this purpose (e.g. Fuentes and Neff, 1977). However, the information gained by using these agents depends upon their intrinsic properties and the conditions under which they are used. The degree of inhibition obtained with these compounds was shown to vary with time, although patterns consistent with progressive binding due to irreversible inhibition deviated somewhat from predicted curves. Similarly, Lyles and Greenawalt (1978) have noted an initial inhibition with clorgyline that is a combination of reversible and irreversible, depending on the substrate used. Each inhibitor appeared to inactivate the enzyme at a slightly different rate. This is consistent with the findings of Lyles and Greenawalt (1977) who showed that type B was inhibited at a slower rate than type A with clorgyline. Since these workers found inhibition to be complete by 10 minutes and virtually

all other workers have used these compounds with preincubation times ranging from 10 to 30 minutes, it was decided, for comparison's sake, to utilize 15 minutes in the present experiments. Under these circumstances it was shown that the homogenate concentration could influence the position of the resulting inhibition plots. Thus, interpretations regarding the sensitivity of tissue MAO to clorgyline may be erroneous due to the complication of presumed non-specific binding (Figure 8). Below 3.33 mg/ml the position of the inhibition curve more truely represents the sensitivity of MAO to the inhibitor drug. An interesting observation resulted from the use of bovine serum albumin. An artificial situation appearing like "clorgyline-resistance" was created. It seems likely that this represents more than non-specific binding and suggests that the characteristics of MAO itself may have become altered. The concentration of the substrate was shown not to alter the position of the inhibition curve. This is consistent with irreversible inhibition and also reveals the absence of any low affinity process over the five-fold concentration range studied.

The present study has shown conclusively that kynuramine is a substrate for both MAO type A and type B. At the initiation of the work little documentation existed with regard to the MAO preferences of this substrate, except for work by Squires (1968, 1972). Indeed, Lowe et al. (1975) stated that this problem remains for elucidation and several review articles which classified the substrate preferences for the various types of MAO failed to include information regarding

kynuramine (Neff and Yang, 1974; Houslay et al., 1976; Neff and Fuentes, 1976). Thus, experiments on the rat vas deferens served not only for comparison with the heart, but also to delineate the types of MAO deaminating kynuramine. Figure 10 shows that kynuramine deamination is revealed as a biphasic curve versus clorgyline. By definition, the initial portion of the curve corresponds to type A and the second to type B MAO (after Johnston, 1968). The approximately equal percentages of the two types agree with studies made by Jarrott (1971) using tyramine as the substrate. However, deprenyl failed to give a complementary result. This difference was shown to be age-related (see Results: Section A, Part 1) and not to discrepancies between the two inhibitors with regard to their ability to clearly discern the A and B types. It seems that the B:A ratio is higher in older rats, an observation similar to that made by Callingham and Lyles (1975) using clorgyline and tyramine. They showed that the percentage of A activity increased and then decreased with age, although they always detected a greater percentage of the A type. The failure of pargyline to distinguish both types of activity is somewhat surprising in view of claims that it functions as a preferential B inhibitor (Houslay et al., 1976; Neff and Fuentes, 1976). A continuum of inhibition from the B to A type seems the most likely explanation. This contention would agree with in vitro and in vivo data showing that low concentrations of pargyline preferentially inhibit the deamination of substances which favor the B activity (Fuller, 1972; Neff and Fuentes, 1976).

By comparison the rat ventricle was described by clorgyline as 95% type A and 5% type B MAO. This result, as that found in other inhibition experiments, should not be interpreted as amounts of A and B activity. The relative percentage activities obtained depend upon the kinetic constants of the substrate for the MAO types present. Deprenyl and pargyline both gave closely complementary percentage activities to those obtained with clorgyline. Similar results were obtained in atrial homogenates, although the B activity was slightly greater (about 10% of the total activity).

Thus, the initial experiments revealed clear evidence of some B activity in rat hearts. Previously, only benzylamine had been shown to undergo deamination by this type in rat cardiac tissue (Lyles and Callingham, 1975). Many other substrates, including preferential B substrates, had been shown to be metabolized by type A MAO in the rat heart (Lyles and Callingham, 1975). Because of this discrepancy, checks were made to determine whether the B activity resulted from contamination with blood elements. Perfusion experiments and the mitochondrial localization of the activity provided convincing evidence that the B form was of cardiac origin. It seems therefore, that previous workers had missed the small percentage of B activity due to experimental errors, strain differences (Fuentes and Neff, 1977, used Sprauge-Dawley rats) or to the choice of substrate. Because of the fact that preferential B substrates (e.g. phenylethylamine) and dual substrates (e.g. tyramine) showed deamination by MAO type A in rat hearts (Lyles and Callingham, 1975; Fuentes and Neff, 1977), the opinion evolved that

rat cardiac tissue must differ in MAO characteristics from that defined in other organs (Fowler et al., 1978). However, comparison of the IC_{50} values for clorgyline versus kynuramine in the vas deferens and cardiac tissue (Table 1) revealed strong evidence that the sensitivity of the MAO types to this inhibitor was virtually identical. Only very minor differences were obtained. Conversely, pargyline failed to discern the types of MAO present in the vas deferens while clearly revealing the small percentage of B activity in the heart. Thus, the possibility of differences between the two tissues remained open. However, further experimentation (kinetic analysis, mixed substrate experiments) failed to provide any additional supporting evidence. Taken overall, the MAO characteristics of the rat heart seem so close to that of the vas deferens that grounds to support radical differences are very minor. No satisfactory explanation can be provided for the discrepancy seen with pargyline, although it can be speculated that subtle environmental differences altered its inhibitory activity in the vas deferens from that in the heart. Such changes have been noted on MAO (Youdim and Woods, 1975) and could possibly extend to the inhibitor itself.

Kinetic analysis was utilized with kynuramine to define further the types of MAO in the heart and to compare them with that in the vas deferens and liver. Similar Km values were obtained for the A and B types in all three tissues. The Km values found in the liver are in close agreement with those reported by Blatchford et al. (1976) for both rat liver and brain, providing good support for the homogeneity

of MAO types between organs. It is important to note that the Km values for kynuramine on the A and B types were very close. Less than a two-fold difference was evident. Thus, it is possible that kynuramine deamination may provide a very approximate means of estimating the actual relative amounts of the A and B types. Currently, no reliable method exists, since attempts to separate and purify the two forms have failed (White and Glassman, 1977). Binding studies, using radioactive inhibitors, are also complicated by non-specific attachments to mitochondrial components (Magyar and Knoll, 1977). Thus, assuming equal velocities of the A and B types for kynuramine (see below), the following speculative interpretations can be made.

From the studies with clorgyline (the inhibitor which shows the greatest selectivity between A and B types) the vas deferens may be said to contain equal amounts of A and B while the ventricle contains 95 parts of A to 5 parts of B. (The slightly lower Km of kynuramine for the B type will lead to an overestimation of this form.) Correlating these assumed amounts with the published Km values for tryptamine and tyramine might explain more fully why these particular dual substrates failed to reveal B activity in rat cardiac tissue. The Km values for tryptamine on the A and B types are 8 μM and 25 μM, respectively (White and Glassman, 1977), while those for tyramine are 57 μM and 102 μM (Ekstedt, 1976). Thus, both substrates favor the A activity. As such, the small percentage of B activity in the heart could easily be missed due to preferential deamination by the A form. Kynuramine may be one of a limited number of dual substrates with a

greater potential for deamination by type B, than type A MAO. Although the deductions made above are speculative, they do provide one reasonable explanation for differences between kynuramine and two other dual substrates in detecting the cardiac forms of MAO.

The calculation of Vmax values in Table 2 is derived from total tissue MAO activity. Vmax values are dependent upon the amount of enzyme present, whereas the Km is independent of this parameter. Thus, it is not possible to compute the real velocity constant for a particular substrate unless the actual amount of enzyme protein is known.

Just as previous studies with kynuramine had failed to reveal differences between the heart and vas deferens, mixed substrate experiments also detected no differences. Tryptamine acted as a better A inhibitor of kynuramine deamination than B inhibitor. The reverse was seen with phenylethylamine. These results are consistent with the respective Km values of the two amines for the A and B types. At this stage in the study, it was somewhat surprising to find phenylethylamine interacting with the B form in the ventricle, since only A metabolism had been reported (Lyles and Callingham, 1975). However, mixed substrate experiments only prove that the second substrate binds to the enzymatic form, they do not give information regarding deaminating capabilities. The kinetic studies showed that whereas tryptamine acted essentially in a mixed fashion, phenylethylamine appeared as a competitive inhibitor of kynuramine. Again the vas deferens and ventricle acted in like fashion. However, the results

of the kinetic analysis does open up the possibility that additional binding sites and/or catalytic sites exist on each of the A and B forms. Similar postulates have been made by others (White and Wu, 1975; Severina, 1976) and an inhibitory binding site sensitive to oxygen has been reported by Roth (1976). Additionally, the distinct tendency to non-linear Lineweaver-Burk plots in the presence of phenylethylamine is suggestive of more than one interaction. None of these differences appear to be related to the production of interfering metabolites (see Figure 19). Thus, they can be taken as real events rather than anomalous findings.

Whereas the heart and vas deferens showed closely similar MAO characteristics, definite differences were found in the limited work conducted on the major blood vessels of the rat. (The low specific activity of MAO in this tissue necessitated the pooling of samples from 10 to 12 rats, thus limiting experimentation due to expense.) Arterial and venous tissue was found to contain A activity versus clorgyline, although the IC_{50} values were somewhat higher than those derived for the vas deferens and cardiac tissue. A small percentage of B activity may be deduced to be present from the inhibition curves and it remains possible that the B activity of the heart derives, at least in part, from blood vessel MAO. The main finding was the presence of a considerable percentage of clorgyline-resistant activity. This species of MAO was shown to be quite different from types A and B since it was inhibited by semicarbazide. This inhibitor failed to produce marked inhibition of rat ventricle and vas deferens MAO activity using

kynuramine. Thus, the major blood vessels of the rat contain at least two species of MAO, differing in cofactor requirements. These observations are in line with those made by Coquil et al. (1973) using samples of rat mesenteric arteries and tyramine as the substrate. The significance of the clorgyline-resistant activity is not known, but its similarity to connective tissue amine oxidases raises the possibility that it is concerned with cross-linking of elastin and collagen fibrils. In this respect it might play a similar role to lysyl oxidase (Page and Benditt, 1967; Pinnell and Martin, 1968; Siegel et al., 1970) and be of importance with regard to blood vessel integrity.

One general method of approach to studying enzyme characteristics and properties is to perturb the enzyme system by varying physiological factors which influence its activity. In this respect the effect of aging and bilateral adrenalectomy were utilized. Both factors have been reported to markedly alter the specific activity of MAO in the rat, particularly the MAO of cardiac tissue (Horita, 1968; Avakian and Callingham, 1968).

The experiments on aging confirmed the previously stated findings that MAO type B is present in rat cardiac tissue. The A:B ratio initially declined with age, being lowest in 36 g rats. Subsequently, increases occurred, up to a body weight of 300 g. The increase in total specific activity with age was due to an increase in A activity, the B component remaining fairly constant from 36 g body weight upwards. Additionally, the sensitivity of the A and B types to

clorgyline showed no marked changes with progressive aging. Thus, no evidence was obtained that the MAO of the rat heart showed different inhibitor sensitivities with growth and development. Similarly, the increased ventricular MAO activity obtained after bilateral adrenalectomy showed the same characteristics to clorgyline. However, in this instance both A and B MAO activities appear to have been increased. Thus, aging and adrenalectomy are not exactly parallel in their effects on cardiac MAO. Adrenalectomy is reported to involve the synthesis of new enzyme (Della Corte and Callingham, 1977) while aging results in a gradual decrease in the degradation rate of MAO (Callingham and Della Corte, 1972).

Bilateral adrenalectomy increased the specific activity of MAO only in the ventricle of the heart. This appears to be a novel finding. It seems that ventricular MAO is more sensitive to adrenal steroid lack than that of the atria, although it does not preclude the possibility of atrial MAO increasing at later time points. The possibility of an increase in the major blood vessels was also obtained. However, in all tissues the sensitivity to clorgyline did not change. Therefore, the differential effect of adrenalectomy upon ventricular MAO is best related to the synthesis of new MAO (Della Corte and Callingham, 1977) which exhibits the same inhibitor sensitivity and catalytic properties toward kynuramine.

The lack of any increase in the MAO activity of the vas deferens after adrenalectomy was surprising in view of previous reports (Ceasar et al., 1970; Sampath and Clarke, 1972). These studies used tryptamine

as the substrate. The greater affinity of this amine for type A MAO may explain the discrepancy, since only intraneuronal MAO of the vas deferens is affected (Sampath and Clarke, 1972). Intraneuronal MAO is usually considered to be the A type (Jarrott, 1971; Goridis and Neff, 1971). Youdim, Holzbauer and Woods (1974) also reported that effects of adrenalectomy on organ MAO can sometimes completely fail to become exposed, but no explanation for this occasional failure was offered. However, inadequate adrenalectomy is one obvious solution.

Overall, the experiments on aging and bilateral adrenalectomy provided no evidence for real differences in the intrinsic properties of cardiac MAO.

Using intact atria, the ten-fold difference obtained in the position of the clorgyline inhibition curve, compared with whole homogenates, is difficult to interpret. The most likely explanation resides in the problem of accessability of both the substrate and particularly clorgyline, to the enzyme. Nevertheless, the studies do point out that kynuramine can penetrate cell membranes to reach the intracellular locus (Schnaitman et al., 1967) of MAO. Further development of this system might provide a useful approach for studying MAO in an intact cellular environment.

A comparison of rat heart MAO with that derived from human atria revealed more similarities than differences. Human atria showed both type A and type B activity, however with kynuramine the latter type predominated. The Km values for kynuramine on the A and B types were somewhat higher than those obtained in the rat, but overall, the

differences were insufficient to suggest different activities. Considering the fact that whole cell homogenates were used and that the patients received a variety of drugs prior to surgery, the agreement is remarkably close. Like rat hearts, the specific activities appeared to increase with age, although dependable changes in the relative percentages of the A versus B activities were not obtained from the limited number of infant samples. As with the atria, human saphenous vein showed predominantly B activity. This result differs markedly from rat major blood vessels. Additionally, no clorgyline-resistant activity was detected.

The finding of MAO types A and B in human cardiovascular tissues is consistent with other studies showing the presence of these two types of MAO in the human brain and liver (White and Wu, 1975; White and Glassman, 1977). Many workers have shown that human blood platelets contain only MAO type B (Edwards and Chang, 1975; Donnelly and Murphy, 1977). In this respect the human saphenous vein is very similar. Type B MAO in blood elements and vascular tissue might allow the transport of preferential A substrates such as epinephrine (Houslay and Tipton, 1974) to its target organs, while plasma and vascular tissue levels of preferential B substrates would be curtailed by metabolism. This same logic however, cannot be applied easily to the rat, since the majority of vascular MAO appears to be of the A type. Clearly, the physiological significance of the A and B types of this enzyme requires detailed investigation. Marked differences in cellular localization and availability to various substrates obviously

exists. In vivo membrane transport mechanisms must play both a discriminative and rate-limiting role in the deamination of biogenic amines.

The results obtained at this stage in the research had depended entirely upon the use of kynuramine as the substrate. Thus, the lack of discernable differences in rat heart MAO might be attributable to the singularity of approach with regard to the substrate. If multiple activities and properites of MAO exist, they may only become evident with certain substrates but not others. Phenylethylamine was chosen for study since this amine appeared to pose the greatest problems with regard to the characteristics of rat heart MAO. As a highly preferential B substrate, its metabolism by MAO type A in the heart (Lyles and Callingham, 1975) appeared highly anomolous. Indeed, information such as this, prompted Fowler et al. (1978) to propose that MAOs in different tissues exhibit differential properties. They and others (Mantle et al., 1976) suggested that the A and B forms may each represent families of MAO activities. In this dissertation an alternative possibility was entertained. Although phenylethylamine is widely recognized as a highly preferential B substrate (Yang and Neff, 1973) it is not devoid of activity for the A type (Ekstedt, 1976; White and Glassman, 1977). Thus, it was hypothesized that differential tissue deamination might be consequential to (a) the concentration of phenylethylamine used, and (b) the relative amounts of the MAO forms present. The results obtained using phenylethylamine and clorgyline in tissues

containing widely differing proportions of type A and type B MAO support this contention.

Examination of Figures 33, 34 and 35 (vas deferens, ventricle and liver) shows clearly that phenylethylamine can be deaminated by both MAO types. Thus, deamination of phenylethylamine in the rat heart by the A type is not singular to that tissue. Also common to all tissues is the fact that most A activity became exposed at the highest concentrations of phenylethylamine. For instance, in the vas deferens 100 μM phenylethylamine revealed A as well as B activity, but only the B activity was evident with lower concentrations. These observations are fully consistent with high and low affinity processes for deamination. Indeed, phenylethylamine has been reported to exhibit 15 to 30 times the affinity for MAO type B than type A (Ekstedt, 1976; White and Glassman, 1977). Determination of Km values made in this study supports these data. A ten to fifteen-fold difference was obtained between the A and B types in rat tissues. The very low Km values for the B type (2.1 μM, liver and 3.0 μM, ventricle) agree well with those determined by Ekstedt (1976) and White and Glassman (1977) (4 μM rat liver and 3.3 μM human brain, respectively). The Km values obtained on the A type must be considered only as an approximation. Problems encountered with deprenyl (see below) precluded precise determination. The Km values derived by Ekstedt (1976) and White and Glassman (1977) for the A type were 62 μM and 100 μM, respectively. Thus, it is clear that a wide difference in the affinity of phenylethylamine for the A and B types does exist, even if the precise value on the A activity appears somewhat variable.

It is important to note that the demonstration of A activity with phenylethylamine does not depend entirely upon substrate concentration. In the liver, 100 μM phenylethylamine failed to reveal A activity, whereas in the vas deferens, and particularly the ventricle, significant product formation still resulted through deamination by the A type. Conversely, low concentrations of phenylethylamine did not always exhibit solely B activity. Although this was the case in the vas deferens and liver, the ventricle still revealed 60% of the total activity as type A MAO. This occurred even with concentrations of phenylethylamine as low as 1 μM. The reason for these differences appears to reside in the relative amounts of the two MAO types present. Interpretations made earlier with kynuramine argued that the ventricle contained almost all type A activity, the vas deferens about equal amounts of A and B, while the liver predominately the B type. Utilizing these deductions it is now possible to offer a logical explanation for the differential tissue deamination of phenylethylamine that does not require recourse to postulates of multiple activities within the A and B types (Fowler et al., 1978).

In the ventricle the amount of B activity is so small compared to the A type that its contribution to product formation is only minor at 100 μM phenylethylamine. At this concentration the Km of the A type is exceeded by about four-fold. Type B activity is only revealed distinctly at concentrations below the Km of A (10 and 1 μM). However, because of the vast amount of A present, product formation from A still forms a significant percentage of the total even though the 1 μM concentration of phenylethylamine falls below the Km of the B type. Thus, due to the

disproportionate amounts of A versus B, deamination solely via the B activity is not possible in the ventricle. The converse is seen in the liver, where type B activity predominates. The same concentration of phenylethylamine (100 μM) which showed 85% A activity in the ventricle shows only B activity in the liver. Due to the low amount of A in this tissue, very high concentrations of phenylethylamine are required to expose its affinity for this substrate. Compared with the liver, the vas deferens shows a shift from B to A and B deamination at a lower substrate concentration of phenylethylamine (100 μM). This is consistent with the presence of approximately equal amounts of the A and B types.

The results obtained in human atria and saphenous vein also conform to the same analysis. The Km values on the A and B types for phenylethylamine in human atria are closely allied to those derived for rat tissues. The 100 μM concentration of phenylethylamine was metabolized in a dual fashion, whereas at 10 μM phenylethylamine (below the Km of A) the B activity was favored. This result is consistent with the relative percentages of A and B activities as detected by kynuramine (Figure 28).

The experiment made with kynuramine in the vas deferens of the rat (Figure 38) lends further credence to the interpretations drawn for phenylethylamine deamination. When the concentration of kynuramine was reduced from 100 μM to 10 μM, the percentage deamination by type B MAO was increased. The latter concentration falls below the Km values for kynuramine on both the A and B types (Table 2), thus favoring the lower Km activity (type B).

The interpretations made above concerning phenylethylamine metabolism were based upon inhibition experiments using clorgyline. When deprenyl was used as the inhibitor no such clear cut results were obtained. Low concentrations of phenylethylamine which showed only B activity with clorgyline were likewise identified with deprenyl (Figures 39, 41 and 42). However, wherever phenylethylamine metabolism proceeded via the A and B types with clorgyline, deprenyl failed to distinguish clearly the two activities. This was most evident in the rat ventricle (Figure 40) where only type B and about 15% of resistant activity was seen. Thus, when A and B deamination of phenylethylamine occurred with clorgyline, deprenyl failed to act in a complementary manner. This is in contrast to the results obtained using kynuramine as the substrate. Under these conditions clorgyline and deprenyl always acted as complementary indicators of dual metabolism.

Several other workers have noted discrepancies between inhibitors in relation to the classification of A and B types. Squires (1972) found that clorgyline and harmaline acted as selective inhibitors of MAO in several rabbit organs, whereas deprenyl and pargyline did not. Mantle et al. (1976), using beef heart, found that serotonin, tyramine, dopamine and tryptamine were all inhibited in a biphasic manner by clorgyline and deprenyl. However, the apparent proportions of the two enzyme types on dopamine and tryptamine depended upon the inhibitor used. Tranylcypromine has been shown to act as a selective inhibitor of the MAO activity in human blood platelets towards tryptamine and phenylethylamine (Honecker et al., 1976) despite the

fact that studies with clorgyline and deprenyl have shown this source to contain the B form of MAO alone (Donnelly and Murphy, 1977). Differential inhibition of rat liver MAO activity towards benzylamine and phenylethylamine by Tris buffer has been demonstrated (Fowler et al., 1977). In view of the above data, Fowler et al. (1978) have suggested that the A and B types of MAO are an oversimplification. They conclude that a multiplicity of MAOs exist between tissues within the same species and between tissues from different species.

The present work does not negate this possibility. However, the data with phenylethylamine and clorgyline demonstrates that the type of MAO detected depends upon the substrate concentration used, in relation to affinity constants, and the actual amounts of the MAO types present. Unless this important concept is taken into account, false interpretations will result, irrespective of the inhibitor used. The information gained from using an inhibitor of MAO depends entirely upon understanding fully the characteristics of the substrate, under the experimental conditions used for assay. Furthermore, differences in response to various inhibitors may well reside in the inhibitors themselves, rather than in the properties of the enzyme. More than one binding site appears to exist on MAO (Severina, 1976) and differential attachments by the various inhibitors and substrates could lead to differential findings. To avoid this complexity it would be possible to define MAO forms in terms of sensitivity to clorgyline alone. This inhibitor shows the greatest selectivity between the two types and as shown by Squires (1972) and in this work, clorgyline reveals selectivity even when deprenyl does not.

The discrepancy between clorgyline and deprenyl with phenylethylamine resided solely in demonstrating clear distinctions between concomitant A and B metabolism, suggesting that the difference occurs only at the level of the A activity. This would explain why the discrepancy was most apparent in the ventricle, being less noticeable in the vas deferens, liver and human atria. As postulated by Fowler et al (1978), the difference is consistent with their concept of multiplicity within the A type. Two forms, A_1 and A_2 can be postulated, with phenylethylamine being a substrate for both types. Clorgyline would inhibit both equally well, but deprenyl would be preferential for one form over the other. The result would be a "slurring" of clear cut inhibition on the A types with deprenyl but not with clorgyline. Kynuramine would be postulated to interact only with one of the A types. Thus, deprenyl would clearly distinguish kynuramine and be complementary to clorgyline. Conversely, the concept of homogeneity of the A type could still be retained if differences in the binding sites for clorgyline, deprenyl and the two substrates on the A type were evoked. This model would be in line with the concept presented by Severina (1976) in which different binding sites are postulated, and serve to orientate substrate and inhibitor molecules onto the active site. As mentioned previously, the mixed substrate experiments do imply the presence of more than one interaction. Irrespective of whether homogeneity or heterogeneity of the A form actually exists, it is clear that the rat ventricle is no exception compared to the other tissues studied.

Phenylethylamine deamination in rat tissues also exposed clorgyline-resistant activity. This was especially evident in the vas deferens (Figure 33). From the figure it can be deduced that phenylethylamine has a lower affinity for this species of MAO than for the B type. When the concentration of phenylethylamine was reduced, the percentage of clorgyline-resistant activity also declined. At this juncture it should be recalled that kynuramine failed to detect clorgyline-resistant activity in this tissue, but did reveal this species of MAO in the major blood vessels of the rat. It is possible that the two species differ between tissues. Conversely, the relative amounts may differ and the affinity constant for kynuramine (at 100 μM) only reveals its presence in vascular tissue. That phenylethylamine exhibits a good affinity for clorgyline-resistant activity is supported by recent data of Fuentes and Neff (1977). They showed phenylethylamine to detect about 80% of the total activity in rat mesenteric blood vessels as the resistant type using pargyline as the inhibitor. As shown in the present work, the resistant activity was sensitive to inhibition by semicarbazide. Clorgyline-resistant activity has also been shown to exist in the rat heart, particularly with benzylamine as the substrate (Lyles and Callingham, 1975). However, the present work failed to detect significant cardiac resistant activity with either kynuramine or phenyelthylamine using clorgyline as the inhibitor. Deprenyl, when used against phenylethylamine, did reveal about 15% of the total activity as the resistant type. Even though the resistant activity has been shown to decline

markedly in rat hearts with age (Lyles and Callingham, 1975) no evidence of clorgyline-resistance was obtained in the present aging studies using kynuramine as the substrate. Thus again, the possibility is raised that blood vessel resistant activity differs from that present in the ventricle and vas deferens. However, proof for such differences remains for further study.

VI. GENERAL CONCLUSIONS

The work contained in this dissertation shows that cardiac MAO of the rat is not dissimilar from that found in other tissues, such as the vas deferens. This conclusion is based upon several different lines of approach, employing two different substrates, selective inhibitors and kinetic studies. It can now be seen why false interpretations have arisen from observations made previously in the literature. The deamination of substrates by cardiac MAO has not been considered in terms of relative amounts of the MAO types in relation to the substrate concentration and its affinity constants. When these considerations are taken into account, the characteristics of cardiac MAO appear very closely allied to those of the vas deferens and liver. Although these conclusions are based upon studies made with kynuramine and phenylethylamine, it seems that the same principle would apply to other substrates. Thus, the dual metabolism of benzylamine in the rat heart (Lyles and Callingham, 1975) and serotonin in the beef heart (Mantle et al., 1976) might well be explained by their affinity differences for the A and B types.

Whether a multiplicity of MAOs within the A and B types actually exists has not been resolved conclusively. One major piece of evidence for this concept is the differential metabolism of substrates such as phenylethylamine, serotonin and benzylamine, between tissues. In light of the present work, this factor can be explained adequately

utilizing the interpretations outlined above. Additionally, using clorgyline and deprenyl no evidence for multiplicity between tissues was obtained. However, clorgyline and deprenyl were not complementary with regard to defining the A and B metabolism of phenylethylamine. The discrepancy may lie more with the properties of the inhibitor than with the enzyme. However, the differential effect of pargyline in the rat heart and its lack of selectivity in the vas deferens is more supportative of enzymatic differences between tissues. It seems that definition of MAO types would best be made with clorgyline as the accepted standard. Two facts support this proposition. First, clorgyline appears to be the most selective of the differential inhibitors, showing about a 100 to 1000-fold sensitivity range between MAO types A and B. It denotes resistant activity and is selective when deprenyl and pargyline are not. Secondly, clorgyline also reveals clear distinctions between the A and B types in vivo (Bakhle and Youdim, 1976) thus allowing in vitro and in vivo comparisons to be drawn.

Clorgyline-resistant activity was defined with both kynuramine and phenylethylamine. However, phenylethylamine appears to be the better substrate of the two. It is clear that this MAO activity is not solely localized to cardiovascular tissues, since a significant percentage was detected by phenylethylamine in the rat vas deferens. Little is known with regard to substrate affinity constants for this activity but its presence in tissue could well complicate interpretations concerning the multiplicity of MAO types A and B.

Several workers have used the deamination of phenylethylamine as a measure of the B activity in tissues. For instance, Fuentes et al.

(1977) used phenylethylamine in a concentration of 200 μM to measure the type B activity of rat tissues. This procedure is no longer acceptable. The work in this dissertation shows that erroneous values will be obtained in the presence of A activity. Even low concentrations of phenylethylamine, at or below the Km of the B type, will involve A activity if sufficient amounts of this enzymatic type are present. Before phenylethylamine or other preferential substrates can be utilized for estimating the selective activity of either the A or B types, it would be advisable to construct clorgyline inhibition curves to define the type actually being measured.

Human cardiac MAO was examined and found to be similar in characteristics to MAO activities in rat tissues. Although the percentage of B activity was much greater in the human atria than in the rat heart, no significant differences were noted in their respective sensitivities to inhibitors or Km values with two substrates. Human saphenous vein contained predominantly type B activity while rat venous tissue revealed more than half of its activity as type A, a small portion as type B and the remainder as a clorgyline-resistant species. Thus, the rat does not appear to be a good model for the study of vascular deaminating properties in man. However, the similarity in cardiac MAO characteristics may allow the rat to serve as a reasonable model for that tissue in man.

BIBLIOGRAPHY

Achee, F.M., Togulya, G. and Gabay, S.: Studies of monoamine oxidases: properties of the enzyme in bovine and rabbit brain mitochondria. J. Neurochem. 22:651-661, 1974.

Alles, G.A. and Heegard, E.V.: Substrate specificity of amine oxidase. J. Biol. Chem. 147:487-494, 1943.

Aoki, S., Manabe, T. and Okuyama, T.: Molecular weight estimation of bovine brain mitochondrial monoamine oxidase. J. Biochem. 82:1533-1539, 1977.

Arora, R.C. and Meltzer, H.Y.: Characterization of rat skeletal muscle monoamine oxidase. Biochem. Pharmacol. 26:45-49, 1977.

Avakian, A.V. and Callingham, B.A.: An effect of adrenalectomy upon catecholamine metabolism. Br. J. Pharmacol. 33:211P-212P, 1968.

Axelrod, J., Hertting, G. and Patrick, R.W.: Inhibition of H^3-norepinephrine release by monoamine oxidase inhibitors. J. Pharmacol. Exp. Ther. 134:325-328, 1961.

Bakhle, Y.S. and Youdim, M.B.H.: Metabolism of phenylethylamine in rat isolated perfused lung: evidence for monoamine oxidase 'type B' in lung. Br. J. Pharmacol. 56:125-127, 1976.

Barbato, L.M. and Abood, L.G.: Purification and properties of monoamine oxidase. Biochem. Biophys. Acta. 67:531-541, 1963.

Bevan-Jones, A.B., Pare, L.M.B., Nicholson, W.J., Price, K. and Stacey, R.S.: Brain amine concentrations after monoamine oxidase inhibitor administration. Brit. Med. J. 1:17-19, 1972.

Blackwell, B.: Hypertensive crisis due to monoamine oxidase inhibitors. Lancet ii:849-851, 1963.

Blaschko, H.: Amine oxidase and amine metabolism. Pharmacol. Rev. 4:415-458, 1952.

Blaschko, H.: Amine oxidase. In: The Enzymes, Ed. by P.D. Boyer, H. Lardy and K. Myrabaeck, Vol. 8. Academic Press, New York-London, 1963, pp. 337-351.

Blatchford, D., Holzbauer, M., Grahame-Smith, D.G. and Youdim, M.B.H.: Ontogenesis of enzyme systems deaminating different monoamines. Br. J. Pharmacol. 57:279-293, 1976.

Caesar, P.M., Collins, G.G.S. and Sandler, M.: Catecholamine metabolism and monoamine oxidase activity in adrenalectomized rats. Biochem. Pharmacol. 19:921-926, 1970.

Callingham, B.A. and Della Corte, L.: Effect of adrenalectomy upon some rat heart enzymes. Br. J. Pharmacol. 41:392P, 1971.

Callingham, B.A. and Della Corte, L.: The influence of growth and adrenalectomy upon some rat heart enzymes. Br. J. Pharmacol. 46:530-531P, 1972.

Callingham, B.A. and Fowler, C.J.: Monoamine oxidation in tissues of the developing chick. Brit. J. Pharmacol. 60:306P, 1977.

Callingham, B.A. and Laverty, R.: Studies on the nature of the increased monoamine oxidase activity in the rat heart after adrenalectomy. J. Pharm. Pharmacol. 25:940-947, 1973.

Callingham, B.A. and Lyles, G.A.: Some effects of age upon irreversible inhibition of cardiac MAO. Br. J. Pharmacol. 53:458-459P, 1975.

Callingham, B.A. and Lyles, G.A.: Changes in the MAO activity with age in the rat vas deferens. Br. J. Pharmacol. 55:305P, 1975a.

Clarke, D.E., Jones, C.J. and Linley, P.A.: Histochemical fluorescence studies on noradrenaline accumulation by uptake 2 in the isolated rat heart. Br. J. Pharmacol. 37:1-9, 1969.

Clarke, D.E. and Sampath, S.S.: Studies on the functional role of intraneuronal monoamine oxidase. J. Pharmacol. Exp. Ther. 187:539-549, 1973.

Clarke, D.E. and Sampath, S.S.: Effects of hypophysectomy, bilateral adrenalectomy and hormone replacement therapy upon organ monoamine oxidase activity. Experientia 31:1098-1100, 1975.

Collins, G.G.S., Sandler, M., Williams, E.D. and Youdim, M.B.H.: Multiple forms of human brain mitochondrial monoamine oxidase. Nature (London) 255:817-820, 1970.

Collins, G.G.S., Youdim, M.B.H. and Sandler, M.: Multiple forms of monoamine oxidase. Comparison of *in vitro* and *in vivo* inhibition patterns. Biochem. Pharmacol. 21:1995-1998, 1972.

Coquil, J.F., Goridis, C., Mack, G. and Neff, N.H.: Monoamine oxidase in rat arteries: evidence for different forms and selective localization. Br. J. Pharmacol. Chemother. 48:590-599, 1973.

Crawford, M. and Sinclair, A.J.: Nutritional influences in the evolution of mammallian brain, In: Lipids, Malnutrition and the Developing Brain (Ciba Found. Symp. 3), Associated Scientific Publishers, Amsterdam, 1972, pp. 267-292.

Della Corte, L. and Callingham, B.A.: The influence of age and adrenalectomy on rat heart monoamine oxidase. Biochem. Pharmacol. 26:407-415, 1977.

Dennick, R.G. and Mayer, R.J.: Purification and immunochemical characterization of monoamine oxidase from rat and human liver. Biochem. J. 161:167-174, 1977.

Diez, T.A. and Maderdrut, J.L.: Development of multiple forms of mouse brain monoamine oxidase in vivo and in vitro. Brain Res. 128:187-192, 1977.

Donnelly, C.H. and Murphy, D.L.: Substrate- and inhibitor-related characteristics of human platelet monoamine oxidase. Biochem. Pharmacol. 26:853-858, 1977.

Dugal, B.S.: Localization, purification and substrate specificity of monoamine oxidase. Biochem. Biophys. Acta 480:56-69, 1977.

Durden, D.A., Philips, S.R. and Boulton, A.A.: Identification and distribution of β-phenylethylamine in the rat. Can. J. Biochem. 51:995-1002, 1973.

Edwards, D.J. and Chang, S.S.: Multiple forms of monoamine oxidase in rabbit platelets. Life Sci. 17:1127-1134, 1975.

Egashira, T., Ekstedt, B. and Oreland, L.: Inhibition by clorgyline and deprenyl of the different forms of monoamine oxidase in rat liver mitochondria. Biochem. Pharmacol. 25:2583-2586, 1976.

Edstedt, B.: Substrate specificity of the different forms of monoamine oxidase in rat liver mitochondria. Biochem. Pharmacol. 25:1133-1138, 1976.

Edstedt, B. and Oreland, L.: Heterogeneity of pig liver and pig brain mitochondrial monoamine oxidase. Archs. Int. Pharmacodyn. Ther. 222:157-165, 1976.

Ekstedt, B. and Oreland, L.: Effect of lipid-depletion on the different forms of monoamine oxidase in rat liver mitochondria. Biochem. Pharmacol. 25:119-124, 1976.

Erwin, V.G. and Hellerman, L.: Mitochondrial monoamine oxidase: purification and characterization of bovine kidney enzyme. J. Biochem. 242:4230-4238, 1967.

Fowler, C.J., Callingham, B.A. and Houslay, M.D.: The effect of tris buffers on rat liver mitochondrial monoamine oxidase. J. Pharm. Pharmacol. 29:411-415, 1977.

Fowler, L.J., Callingham, B.A., Mantle, T.J. and Tipton, K.F.: MAO A and B: A useful concept? Biochem. Pharmacol. 27:97-101, 1978.

Fuentes, J.A. and Neff, N.H.: Monoamine oxidases of the rat cardiovascular system. The Pharmacologist 17:291, 1975.

Fuentes, J.A. and Neff, N.H.: Inhibition by pargyline of cardiovascular amine oxidase activity. Biochem. Pharmacol. 26:2107-2112, 1977.

Fuentes, J.A., Trepel, J.B. and Neff, N.H.: Monoamine oxidase activity in the cardiovascular system of young and aged rats. Exp. Geront. 12:113-115, 1977.

Fuller, R.W.: Kinetic studies and effects in vivo of a new monoamine oxidase inhibitor, N-[2-(O-chlorophenoxy)-ethyl]-cyclopropylamine. Biochem. Pharmacol. 17:2097-2106, 1968.

Fuller, R.W.: Selective inhibition of monoamine oxidase. Adv. Biochem. Psychopharmacol. 5:339-354, 1972.

Fuller, R.W., Warren, B.J. and Molloy, B.B.: Selective inhibition of monoamine oxidase in rat brain mitochondria. Biochem. Pharmacol. 19:2934-2936, 1970.

Gabay, S. and Achee, F.M. and Mentes, G.: Some parameters affecting the activity of monoamine oxidase in purified bovine brain mitochondria. J. Neurochem. 27:415-424, 1976.

Gallagher, B.M.: Multiple monoamine oxidase activities in heterogeneous populations of mouse lung mitochondria. Biochem. Pharmacol. 26:935-938, 1977.

Gillis, C.N. and Roth, J.A.: The fate of biogenic monoamines in perfused rabbit lung. Br. J. Pharmacol. 59:585-590, 1977.

Glover, V., Sandler, M., Owen, F. and Riley, G.J.: Dopamine is a monoamine oxidase B substrate in man. Nature 265:80-81, 1977.

Gomes, B., Igaue, I., Kloepfer, H.G. and Yasunobu, K.T.: Amine oxidase XIV. Isolation and characterization of the multiple amine beef oxidase components. Arch. Biochem. Biophys. 132:16-27, 1969.

Gordon, R., Spector, S., Sjoerdsma, A. and Udenfriend, S.: Increased synthesis of norepinephrine and epinephrine during exercise and exposure to cold. J. Pharmacol. Exp. Ther. 153:440-447, 1966.

Goridis, L. and Neff, N.H.: Monoamine oxidase in sympathetic nerves: a transmitter specific enzyme type. Br. J. Pharmacol., 43:814-818, 1971.

Goridis, C. and Neff, N.H.: Evidence for a specific monoamine oxidase associated with sympathetic nerves. Neuropharmacol. 10:557-564, 1971a.

Gorkin, V.A.: Partial separation of rat liver mitochondrial amine oxidase. Nature (London) 200:77-78, 1963.

Gripois, D.: Review: developmental characteristics of monoamine oxidase. Comp. Biochem. Physiol. 51C:143-151, 1975.

Gripois, D. and Fernandez, C.: Thyroxine and propylthiouracil-induced changes in the activity of monoamine oxidase in the fetal rat. Mech. Aging Develop. 6:407-412, 1977.

Hall, D.W.R., Logan, B.W. and Parsons, G.H.: Further studies on the inhibition of monoamine oxidase by M and B9302 (clorgyline). Substrate specificity in various mammalian species. Biochem. Pharmacol. 18:1447-1454, 1969.

Hare, M.L.C.: Tyramine oxidase, a new enzyme system in liver. Biochem. J. 22:968-979, 1928.

Hartman, B.K., Yasunobu, K.T. and Udenfriend, S.: Immunological identity of the multiple forms of beef liver mitochondrial monoamine oxidase. Arch. Biochem. Biophys. 147:797-804, 1971.

Hashimoto, Y. and Okuyama, T.: Chromatographical purification of monoamine oxidase from chicken brain and its properties. Seikaguku 42:350-360, 1970.

Hoff, K.M.: Interaction of testosterone with monoamine oxidase in mouse brain maturation. Gen. Pharmacol. 8:55-57, 1977.

Honecker, H., Christ, W., Muller-Oerlinhausen, B. and Coper, H.: Critical evaluation of measurement of platelet monoamine oxidase in man. J. Clin. Biochem. 14:453-458, 1976.

Horita, A.: Cardiac monoamine oxidase in rat. Nature (London) 215: 411-412, 1967.

Horita, A.: The influence of age on the recovery of cardiac monoamine oxidase after irreversible inhibition. Biochem. Pharmacol. 17:2091-2096, 1968.

Houslay, M.D. and Tipton, K.F.: The nature of the electrophoretically separable multiple forms of rat liver monoamine oxidase. Biochem. J. 135:173-186, 1973.

Houslay, M.D. and Tipton, K.F.: A kinetic evaluation of monoamine oxidase activity in rat liver mitochondrial outer membrane. Biochem. J. 139:645-652, 1974.

Houslay, M.D., Tipton, K.F. and Youdim, M.B.H.: Minireview: Multiple forms of monoamine oxidase: fact and artefact. Life Sci. 19: 467-478, 1976.

Inoue, F., Robinson, J.B. and Dost, K.A.: Some observations on the attempted separation of isoenzymes of monoamine oxidase. J. Pharm. Pharmacol. 28:521-522, 1976.

Jain, M.L. and Sands, F.L.: Electrophoretic homogeneity of solubilized human brain monoamine oxidase. J. Neurochem. 23:1291-1293, 1974.

Jain, M., Sands, F. and van Korff, R.W.: Monoamine oxidase activity measurements using radioactive substrates. Analyt. Biochem. 52:542-554, 1973.

Jarrott, B.: Occurrence and properties of monoamine oxidase in adrenergic neurons. J. Neurochem. 18:7-16, 1971.

Johnston, J.P.: Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmacol. 17:1285-1297, 1968.

Kim, H.C. and D'Iorio, A.: Possible isoenzymes of monoamine oxidase in rat tissues. Can. J. Biochem. 46:295-297, 1968.

Knoll, J. and Magyar, K.: Some puzzling pharmacological effects of monoamine oxidase inhibitors. Adv. Biochem. Psychopharmacol. 5:393-408, 1972.

Knopp, J., Saleh, N.A. and Kvetnansky, R.: Ontogenesis of monoamine oxidase in the thyroid gland of rats. Experientia 32:1113-1114, 1976.

Kopin, I.J.: Storage and metabolism of catecholamines: role of monoamine oxidase. Pharmacol. Rev. 16:179-191, 1964.

Kopin, I.J.: False neurotransmitters in the mechanism of action of central nervous system and cardiovascular drugs. In: Biogenic Amines and Physiological Membranes in Drug Therapy, Part B, ed. by J.H. Biel and L.G. Abood, p. 329-353, Marcel Dekker, Inc., New York, 1971.

Krajl, M.: A rapid microfluorimetric determination of monoamine oxidase. Biochem. Pharmacol. 14:1683-1685, 1965.

Kroon, M.C. and Veldstra, H.: Multiple forms of rat brain mitochondrial monoamine oxidase: subcellular localization. FEBS Lett. 24:173-176, 1972.

Kuzuya, H. and Nagatsu, T.: Flavins and monoamine oxidase activity in the brain, liver and kidney of the developing rat. J. Neurochem. 16:123-126, 1969.

Lai, F.M. Berkowitz, B.A. and Spector, S.: Influence of age on the monoamine oxidase in the cardiovascular tissues of the rat. Fedn. Proc. 34:3267, 1975.

Lai, F.M., Berkowitz, B.A. and Spector, S.: Studies on the catecholamine degradative enzymes in the rat cerebral microvessels. Fedn. Proc. 35:445, 1976.

Levin, J.A. and Wilson, S.E.: The effect of monoamine oxidase and catechol O-methyltransferase inhibitors on the accumulation and metabolism of [1-3H] norepinephrine by the adventitia and media of rabbit aorta. J. Pharmacol. Exp. Ther. 203:598-609, 1977.

Lowe, M., Reichenbach, D. and Horita, A.: Extraneuronal monoamine oxidase in rat heart: biochemical characterization and electron microscopic localization. J. Pharmacol. Exp. Ther. 194:522-536, 1975.

Lyles, G.A. and Callingham, B.A.: The effects of thyroid hormones on monoamine oxidase in the rat heart. J. Pharm. Pharmacol. 26:921-930, 1974.

Lyles, G.A. and Callingham, B.A.: Evidence for a clorgyline-resistant monoamine metabolizing activity in the rat heart. J. Pharm. Pharmacol. 27:682-691, 1975.

Lyles, G.A. and Greenawalt, J.W.: Observations on the inhibition of rat liver monoamine oxidase by clorgyline. Biochem. Pharmacol. 26:2269-2274, 1977.

Magyar, K. and Knoll, J.: Selective inhibition of the B form of monoamine oxidase. Pol. J. Pharmacol. Pharm. 29:233-246, 1977.

Mantle, T.J., Houslay, M.D., Garrett, N.J. and Tipton, K.F.: 5-Hydroxytryptamine is a substrate for both species of monoamine oxidase in beef heart mitochondria. J. Pharm. Pharmacol. 28:667-671, 1976.

Mantle, T.J., Wilson, K. and Long, R.F.: Studies on the selective inhibition of membrane-bound rat liver. Biochem. Pharmacol. 24:2031-2038, 1975.

McCauley, M.R. and Racker, E.: Separation of two monoamine oxidases from bovine brain. Mol. Cell Biochem. 1:73-81, 1973.

Lyles, G.A. and Greenawalt, J.W.: Possible heterogeneity of type B monoamine oxidase in pig heart mitochondria. Biochem. Pharmacol. 27:923-935, 1978.

Murphy, D.L., Belmaker, R. and Wyatt, R.J.: Monoamine oxidase in schizophrenia and other behavioral disorders. J. Psychiat. Res. 11:221-247, 1974.

Murphy, D.L., Donnelly, C.H. and Richelson, E.: Substrate and inhibitor-related characteristics of monoamine oxidase in C6 rat glial cells. J. Neurochem. 26:1231-1235, 1976.

Murphy, D.L., Wright, C., Buchsbaum, M., Nichols, A., Costa, J.L. and Wyatt, R.J.: Platelet and plasma amine oxidase activity in 680 normals: sex and age differences and stability over time. Biochem. Med. 16:254-265, 1976.

Neff, N.H. and Fuentes, J.A.: The use of selective monoamine oxidase inhibitor drugs for evaluating pharmacological and physiological mechanisms. In: Monoamine Oxidase and its Inhibition, Ciba Found. Sym. 39, Elsevier, Amsterdam, 1976, pp. 163-173.

Neff, N.H. and Yang, H-Y.T.: Another look at the monoamine oxidases and the monoamine oxidase inhibitor drugs. Life Sci. 14: 2061-2074, 1974.

Oreland, L. and Ekstedt, B.: Soluble and membrane-bound pig liver mitochondrial monoamine oxidase: thermostability, tryptic digestibility and kinetic properties. Biochem. Pharmacol. 21:2479-2488, 1972.

Oreland, L., Kinemuchi, H. and Stigbrand, T.: Pig liver monoamine oxidase: studies on the subunit structures. Arch. Biochem. Biophys. 159:854-860, 1973.

Page, R.C. and Benditt, E.P.: Molecular diseases of connective and vascular tissue. II. Amine oxidase inhibition by the lathrogen, B-amino-propionitrile. Biochemistry 6:1142-1148, 1967.

Partanen, S., Penttila, O., Kyosola, K., Merikallio, E. and Siltanen, P.: Histochemically demonstrable monoamine oxidase activity in the adult human heart in various cardiac diseases. Virchows Arch. A Path. Anat. Histol. 370:291-296, 1976.

Parvez, H., Gripois, D. and Parvez, S.: Corticosteroid influence in the postnatal development of monoamine oxidase activity in the young rats. Biol. Neonate 28:326-335, 1976.

Pinnell, S.R. and Martin, G.R.: The cross-linking of collagen and elastin: Enzymatic conversion of lysine in peptide linkage to α-aminoadipic-δ-semialdehyde (allylsine) by an extract from bone. Proc. Natl. Acad. Sci. USA. 61:708-716, 1968.

Pletscher, A., Gey, K.F. and Burkhard, W.P.: Inhibitors of monoamine oxidase and decarboxylase of aromatic amino acids. In: Hdb d. exp. Pharmakologie, Vol. 19. Spinger-Verlag, Heidelberg, 1966, pp. 593-735.

Pletscher, A., Gey, K.F. and Zeller, P.: Monoamineoxydase-Hemmer. In: Progress in Drug Research, ed. by E. Jucker, Vol. II, Birkhauser-Verlag, Basel/Stuttgart, 1960, p. 417.

Quiring, K. and Hubertus, S.: Monoamine oxidase in rat reticulocytes: subcellular localization and identification of isoenzymes. Arch. Pharmacol. 300:273-279, 1977.

Quiring, K., Kauser, G. and Gauger, D.: Monoamine oxidase activity in rat erythrocytes: evidence for its localization in reticulocyte mitochondria. Experentia 32:1132-1133, 1976.

Regland, J.B.: Multiplicity of mitochondrial monoamine oxidases. Biochem. Biophys. Res. Comm. 31:203-208, 1968.

Rivlin, R.S., Fazekas, A.G., Huang, Y.P. and Chaudhuri, R.: Flavin metabolism and its control by thyroid hormones, In: Flavins and Flavoproteins, ed. by T.P. Singer, pp. 747-753, Elsevier, Amsterdam, 1976.

Robinson, D.S.: Changes in monoamine oxidase and monoamines with human development and aging. Fedn. Proc. 34:103-107, 1975.

Robinson, D.S., Davis, J.M., Nies, A., Ravaris, C.L. and Sylvester, D. Relation of sex and aging to monoamine oxidase activity of human brain, plasma and platelets. Arch. Gen. Psychiat. 24:536-539, 1971.

Roth, J.A.: Evidence for a single catalytic binding site on human brain type B monoamine oxidase. J. Neurochem. 27:1107-1112, 1976.

Roth, J.A. and Gillis, C.N.: Multiple forms of amine oxidase in perfused rabbit lung. J. Pharmac. Exp. Ther. 194:537-544, 1975.

Roth, J.A., Young, J.G. and Cohen, D.J.: Platelet monoamine oxidase activity in children and adolescents. Life Sci. 18:919-924, 1976.

Rucker, R.B. and Goettlich-Riemann, W.: Properties of rabbit aorta amine oxidase. Proc. Soc. Exp. Biol. Med. 139:286-289, 1972.

Sampath, S.S. and Clarke, D.E.: A specific increase in intraneuronal monoamine oxidase activity in the rat vas deferens following adrenalectomy. Life Sci. 11:1037-1048, 1972.

Satake, K.: Sakinno Kosokazaku, 4:39, 155; cited by H. Okumoro, Bull. Osaka Med. Sch. 6:58, 1960.

Schnaitman, G., Erwin, V.G. and Greenwalt, J.W.: Submitochondrial localization of monoamine oxidase. An enzymatic marker for the outer membrane of rat liver mitochondria. J. Cell Biol. 32:719-735, 1967.

Severina, I.S.: Multiplicity of mitochondrial monoamine oxidases and methods of selective blocking of enzyme activity. Biokhimiya 41:955-967, 1976.

Siegel, R.C., Pinnell, S.R. and Martin, G.R.: Cross-linking of collagen and elastin. Properties of lysyl oxidase. Biochemistry 9: 4486-4492, 1970.

Squires, R.F.: Additional evidence for the existence of several forms of mitochondrial monoamine oxidase in the mouse. Biochem. Pharmacol. 17:1401-1409, 1968.

Squires, R.F.: Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability: A comparison of eight mammalian species. Adv. Biochem. Psychopharmacol. 5:355-370, 1972.

Symes, A.L., Sourkes, T.L., Youdim, M.B.H., Grigoriadis, G. and Birnbaum, H.: Decreased monoamine oxidase activity in liver of iron deficient rats. Can. J. Biochem. 47:999-1002, 1969.

Takeichi, O., Nagao, T., Mizunuma, Y., Mikawa, K., Aihara, T., Tsuyuguchi, N., Kondo, C., Taniguchi, T., Mori, H. and Hotchi, T.: Histochemical studies of monoamine oxidase in experimental myocardial infarctions. Jap. Circ. J. 40:29-34, 1975.

Tarlov, S.R. and Langer, S.Z.: The fate of ^{3}H-norepinephrine released from isolated atria and vas deferens: effect of field stimulation. J. Pharmacol. Exp. Ther. 179:186-197, 1971.

Tipton, K.F.: The purification of pig brain mitochondrial monoamine oxidase. Eur. J. Biochem. 4:103-104, 1968.

Tipton, K.F.: Some properties of monoamine oxidase. Adv. Biochem. Psychopharmacol. 5:11-24, 1972.

Tipton, K.F., Houslay, M.D. and Mantle, T.J.: The nature and locations of the multiple forms of monoamine oxidase. In: Monoamine oxidase and its inhibition, Ciba Found. Sym. 39, Elsevier, Amsterdam, 1976, pp. 5-31.

Tipton, K.F. and Spires, I.P.C.: The homogeneity of pig brain mitochondrial monoamine oxidase. Biochem. Pharmacol. 17: 2137-2141, 1968.

Tipton, K.F. and Youdim, M.B.H.: Assay of monoamine oxidase. In: Monoamine Oxidase and its Inhibition, Ciba Found. Sym. 39, Elsevier, Amsterdam, 1976, pp. 393-403.

Tipton, K.F., Youdim, M.B.H. and Spires, I.P.C.: Beef adrenal medulla monoamine oxidase. Biochem. Pharmacol. 21:2197-2204, 1972.

Trendelenburg, V., Draskoczy, P.R. and Graefe, K.F.: The influence of intraneuronal monoamine oxidase on neuronal net uptake of noradrenaline and on sensitivity to noradernaline. Adv. Biochem. Psychopharmacol. 5:371-377, 1972.

Veryokina, N., Gorkin, V.A., Mityushin, V.M. and Elpiner, I.E.: On the effect of ultrasonic waves on monoamine oxidase connected with the submicroscopical structure of mitochondria. Biophysics 9:503-506, 1964.

Waldmeier, P.C. and Maitre, L.: Lack of significance of MAO-B for in vivo deamination of dopamine. Arch. Pharmakol. 287:R2, 1975.

Weissbach, H., Smith, T.E., Daly, J.W., Witkop, B. and Udenfriend, S. A rapid spectrophotometric assay of monoamine oxidase based on the rate of disappearance of kynuramine. J. Biol. Chem. 235:1160-1163, 1960.

Werle, E. and Roewer, F.: Uber tierische und pflanzliche monoaminoxydasen. Biochem. Z. 322:320-326, 1952.

White, H. and Glassman, A.T.: Multiple binding sites of human brain and liver MAO: substrate specificities, selective inhibitors, and attempts to separate enzyme forms. J. Neurochem. 29: 987-997, 1977.

White, H.C. and Wu, J.C.: Multiple binding sites of human brain monoamine oxidase as indicated by substrate competition. J. Neurochem. 25:21-26, 1975.

Yang, H-Y.T., Goridis, C. and Neff, N.H.: Properties of monoamine oxidases in sympathetic nerve and pineal gland. J. Neurochem. 19:1241-1250, 1972.

Yang, H-Y.T. and Neff, N.H.: β-phenylethylamine: a specific substrate for type B monoamine oxidase. J. Pharmacol. Exp. Ther. 187: 365-371, 1973.

Yang, H-Y.T. and Neff, N.H.: The monoamine oxidases of brain: selective inhibition with drugs and the consequences for the metabolism of the biogenic amines. J. Pharmacol. Exp. Ther. 189:733-740, 1974.

Youdim, M.B.H.: Heterogeneity of rat brain mitochondrial monoamine oxidase. Adv. Biochem. Psychopharmacol. 11:59-63, 1974.

Youdim, M.B.H. and Collins, G.G.S.: The dissociation and reassociation of rat liver mitochondrial monoamine oxidase. Eur. J. Biochem. 18:73-79, 1971.

Youdim, M.B.H. and Holzbauer, M.: Physiological aspects of the oxidative deamination of monoamines. In: Monoamine Oxidase and its Inhibition, Ciba Found. Sym. 39, pp. 105-133, Elsevier, Amsterdam, 1976.

Youdim, M.B.H., Holzbauer, M. and Woods, H.F.: Physico-chemical properties development and regulation of central peripheral monoamine oxidase activity. Adv. Biochem. Psychopharmacol. 12:11-28, 1974.

Youdim, M.B.H. and Sandler, M.: Isoenzymes of soluble monoamine oxidase from human placental and rat liver mitochondria. Biochem. J. 105:43P, 1967.

Youdim, M.B.H. and Sourkes, T.L.: The effects of heat, inhibitors and riboflavin deficiency on monoamine oxidase. Canad. J. Biochem. 43:1305-1318, 1965.

Youdim, M.B.H. and Woods, H.F.: The influence of tissue environment on the rates of metabolic processes and the properites of enzymes. Biochem. Pharmacol. 24:317-323, 1975.

Youdim, M.B.H., Woods, H.F., Mitchell, B., Grahame-Smith, D.G. and Callender, S.: Human platelet monoamine oxidase activity in iron-deficiency anaemia. Clin. Sci. Mol. Med. 48:289-295, 1975.

Zeller, E.A.: Oxidation of amines. In: The Enzymes, Chemistry and Mechanism of Action. Ed. by J.B. Summer and K. Myrback, Vol. 2, Academic Press, New York, 1951, pp. 536-556.

Zile, M.H.: The effect of thyroxine and related compounds on monoamine oxidase activity. Endocrin. 66:311-312, 1960.