Monoamine Oxidases–New Vistas

Edited by E. Costa and M. Sandler

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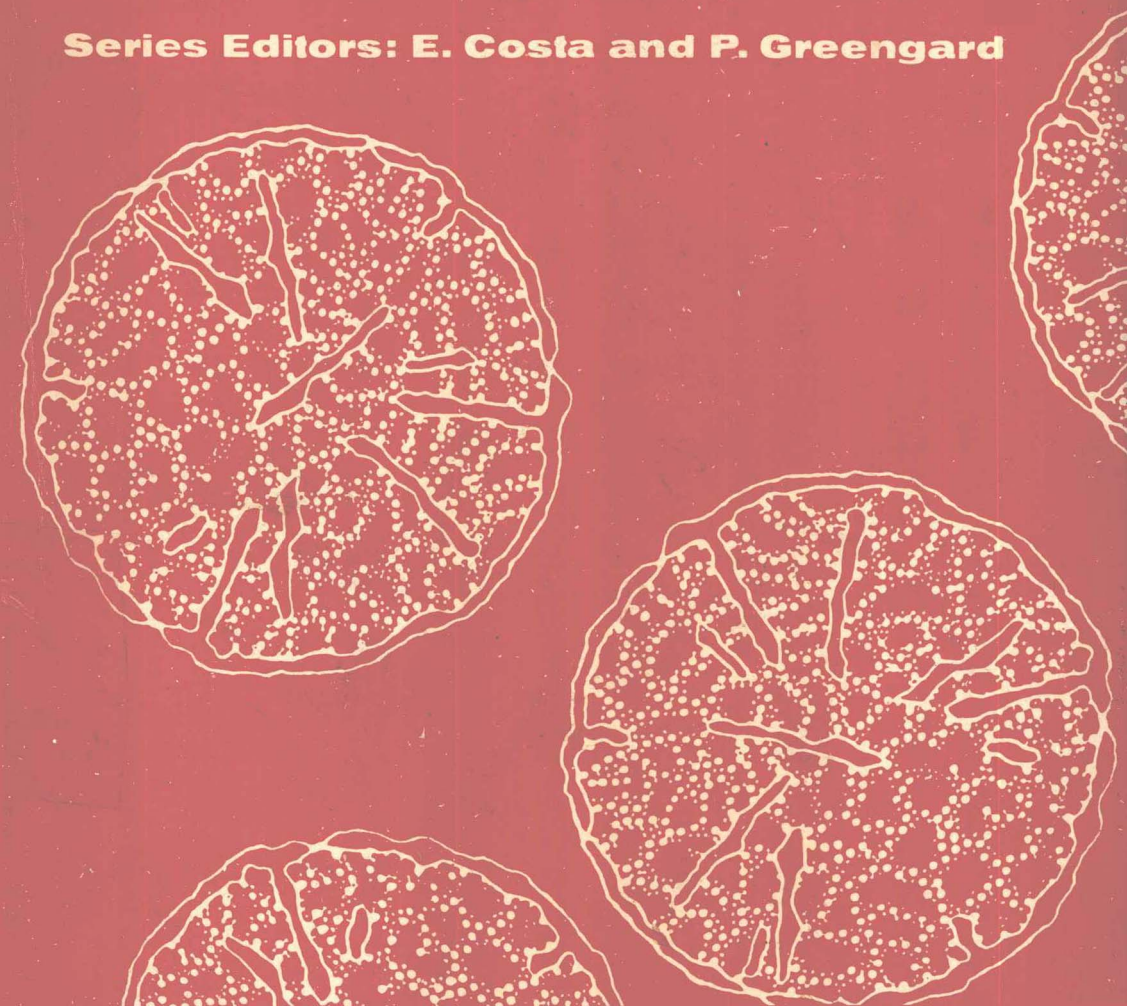

MONOAMINE OXIDASES— NEW VISTAS

Advances in Biochemical Psychopharmacology Volume 5

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Preface

Although more than forty years have passed since the presence of monoamine oxidase in tissue was first described by Hare-Bernheim, our knowledge of the physicochemical mechanisms by which it acts is still lamentably incomplete. Some puzzling inconsistencies in its reaction with substrates, for instance, were only recently resolved, if only in part, by the direct demonstration of multiple forms. This finding has, in turn, generated more questions than answers. Nevertheless, its physiological and clinical implications have given a new impetus to the topic and triggered a profusion of articles scattered through many different journals.

It thus seemed a fitting time to try to summarize this recent information within the covers of a single volume and, in the process, to honor the scientist whose seminal observations have formed the foundation on which these latterday observations are built.

The early studies of Dr. Hermann Blaschko and his colleagues on the inactivation of epinephrine, followed through the years by a series of classical papers on the nature and taxonomy of the enzyme, are an object lesson to us all. And, indeed, it is hard to think of any aspect of the monoamine field which has not been fertilized by Blaschko's penetrating insight. The character and content of the chromaffin granule and the biosynthetic pathway of norepinephrine are in addition to monoamine oxidase, the two contributions which spring most readily to mind; however, at a time when one important substrate of monoamine oxidase, dopamine, has assumed such clinical pre-eminence, it should not be forgotten that it was Dr. Blaschko who first suggested that this amine might possess a physiological role in its own right and not exist solely as a precursor of norepinephrine. Even the study of that neglected biologically active monoamine, *m*-tyramine, which is only now receiving belated attention, is one more area in which the ground work was laid by Dr. Blaschko and his co-workers.

To prepare for this *Festschrift* his disciples and admirers met in Cagliari, Sardinia on June 7–9, 1971, with Professors G. L. Gessa and G. C. Pepeu as hosts. Through the energetic efforts of all, the manuscripts were ready by early fall.

There remain many problems, seen perhaps more clearly as a result of the

deliberations presented here. The study of monoamine oxidase has reached an exciting stage and we have much for which to thank Dr. Blaschko. We dedicate this volume to this gentle, cultured scholar with gratitude, admiration, and warm personal regards.

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January 10, 1972

Contents

1 Introduction and Historical Background
H. Blaschko

SECTION I. PROPERTIES OF MONOAMINE OXIDASE AND SOME RELATED ENZYMES

11 Some Properties of Monoamine Oxidase
K. F. Tipton

25 Purification and Properties of Amine Oxidases in Brain and Connective Tissue (Dental Pulp)
Toshiharu Nagatsu, Gôjiro Nakano, Kimiko Mizutani, and Minoru Harada

37 Some Properties of Pig Liver Mitochondrial Monoamine Oxidase
Lars Oreland

45 The Flavin Prosthetic Group of Purified Rat Liver Mitochondrial Monoamine Oxidase
M. B. H. Youdim and T. L. Sourkes

55 Qualitative Alterations in Enzymatic Properties of Amine Oxidases
V. Z. Gorkin

67 Multiple Forms of Monoamine Oxidase and Their Properties
Moussa B. H. Youdim

79 Multiple Forms of Mitochondrial Monoamine Oxidase
J. M. Diaz-Borges and A. D'Iorio

91 Mechanistic Aspects of the Bovine Hepatic Monoamine Oxidase Reaction
Kerry T. Yasunobu and Susumu Oi

107 Recent Investigations on the Prosthetic Group of Beef Plasma Amine Oxidase
Kazuho Watanabe, Richard A. Smith, Melvin Inamasu, and Kerry T. Yasunobu

119 The Use of Immunological Techniques for the Characterization of Bovine Monoamine Oxidase from Liver and Brain
Boyd K. Hartman and S. Udenfriend

129 Summary of Section I
Godfrey G. S. Collins

SECTION II. PROPERTIES OF SOME RELATED ENZYMES

133 Pig Plasma Benzylamine Oxidase
F. Buffoni and L. Della Corte

151 The Soluble Monoamine Oxidase of Human Plasma and Sera
Charles M. McEwen, Jr.

167 Diamine Oxidase: Specificity and Mechanism
E. Albert Zeller

181 On the Mechanism of Amine Oxidation by Diamine Oxidases
Bruno Mondovì

185 Amine Oxidases from Microorganisms: Stoichiometry of the Reaction Catalyzed by Amine Oxidase of *Aspergillus niger*
Hideaki Yamada, Haruo Suzuki, and Yasuyuki Ogura

203 Summary of Section II
Sabit Gabay

SECTION III. LOCATION AND PHYSIOLOGY OF MONOAMINE OXIDASE

207 Localization of Monoamine Oxidase in Rat Liver Mitochondria
John W. Greenawalt

227 On the Extraneuronal Nature of Cardiac Monoamine Oxidase in the Rat
A. Horita and M. C. Lowe

243 Cytochemical Differentiation Between Monoamine Oxidase and Other Neuronal Oxidases
F. E. Bloom, K. L. Sims, H. A. Weitsen, G. A. Davis, and J. S. Hanker

263 Endometrial Monoamine Oxidase: The Effect of Sex Steroids
Jennifer Southgate

271 Ontogenetic Development of Monoamine Oxidase
Samuel Eiduson

289 Limitation in the Use of Tetrazolium Salts for the Detection of Multiple Forms of Monoamine Oxidase
M. B. Youdim and J. R. Lagnado

293 The Axonal Transport of Monoamine Oxidases
Annica Dahlström

307 Neuronal Monoamine Oxidase: Specific Enzyme Types and Their Rates of Formation
N. H. Neff and C. Goridis

325 Summary of Section III
E. Costa

SECTION IV. PHARMACOLOGY OF MONOAMINE OXIDASE INHIBITORS

327 Approaches to the Catalytic Mechanism of Mitochondrial Monoamine Oxidase
Leslie Hellerman, Hanson Y. K. Chuang, and Donald C. De Luca

339 Selective Inhibition of Monoamine Oxidase
Ray W. Fuller

355 Multiple Forms of Monoamine Oxidase in Intact Mitochondria as Characterized by Selective Inhibitors and Thermal Stability: A Comparison of Eight Mammalian Species
Richard F. Squires

371 The Influence of Intraneuronal Monoamine Oxidase on Neuronal Net Uptake of Noradrenaline and on Sensitivity to Noradrenaline
U. Trendelenburg, P. R. Draskóczy, and K. H. Graefe

379 The Role of Monoamine Oxidase in Determining the Amount of Monoamines Released by Drugs in the Central Nervous System
C. O. Rutledge, A. J. Azzaro, and R. J. Ziance

393 Some Puzzling Pharmacological Effects of Monoamine Oxidase Inhibitors
J. Knoll and K. Magyar

409 The Role of Intraneuronal Monoamine Oxidase in the Regulation of Norepinephrine Synthesis
Norman Weiner and Richard Bjur

421 Is Metaraminol a Selective Monoamine Oxidase Inhibitor?
L. Manara and P. Carminati

423 Rapid Effects of Monoamine Oxidase Inhibitors on Synthesis and Release of Central Monoamines
J. Glowinski, M. Hamon, F. Javoy, and Y. Morot-Gaudry

441 Clinical Implications of Monoamine Oxidase Inhibition
C. M. B. Pare

445 Summary of Section IV
J. H. Biel

447 Index

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Introduction and Historical Background

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I. INTRODUCTION

One year separates the discovery of the enzyme that we now call monoamine oxidase (MAO) (Hare, 1928) from that of histaminase (Best, 1929). Histamine, the chief substrate of histaminase, was at that time of great interest to students of what has come to be called "autopharmacology." On the other hand, tyramine, the first substrate of MAO to be described, had not then been convincingly shown to occur in mammals. Miss Hare tested adrenaline as a possible substrate, but met with no success. The oxidation of the catecholamines by MAO was only discovered after we had learned to exclude the so-called "autoxidation" of adrenaline in aqueous solutions (Blaschko, Richter, and Schlossmann, 1937*a*, *b*).

Since 1937, the area in which catecholamines are involved has enormously expanded, and there has been a parallel increase in interest in MAO. Moreover, we now know that MAO also takes an important part in the biological inactivation of 5-hydroxytryptamine.

Our present nomenclature is still based on that proposed by Zeller (1938) who found that the classical histaminase of pig kidney also acts on straight-chain diamines. Pugh and Quastel (1937) had already described the oxidation of alphatic monoamines by MAO.

II. MONOAMINE OXIDASE (MAO)

One of the reasons why the discovery of the action of MAO on adrenaline aroused the interest of pharmacologists was the search for a catalyst that played in the adrenergic system a role similar to that held by acetylcholinesterase in the cholinergic system. This was the theme of an article by Burn (1952) entitled: "The Enzyme at Sympathetic Nerve Endings."

The specific association of MAO and the adrenergic neurons has been supported by more recent observations (see, e.g., Jarrott and Iversen, 1971), but we have also learned to appreciate the differences between the fates of the adrenergic and the cholinergic mediator. Burn's article was written before the discovery of the enzyme catechol-O-methyl transferase by Axelrod (1957), a discovery that added two new important substrates of MAO to those already known: metanephrine and normetanephrine.

The biochemists did not readily accept the idea of a full functional parallelism between acetylcholinesterase and MAO. The finer localization of these two catalysts was known to be entirely different. One of the first studies of acetylcholinesterase by centrifugation techniques was carried out at Oxford by Paul Hagen (1955) who showed that in the adrenal medulla the enzyme was located chiefly in the microsomal fraction. This association of acetylocholinesterase with the cell fractions that contain the membranes has been fully corroborated by later studies. On the other hand, MAO is a chiefly mitochondrial enzyme.

The intracellular localization of MAO fits in well with known physiological facts concerning intracellular site of amine degradation and the related phenomena of amine uptake. These two sets of facts make sense of a number of observations that seemed at first difficult to understand and that led some observers to the idea that MAO plays no significant part in catecholamine inactivation (Bacq, 1949).

It is not surprising that in recent years the presence of MAO in adrenergic neurons has been particularly stressed, probably because so much of the released adrenergic mediator re-enters the neuron. However, I believe that our knowledge is presently being supplemented by a study of amine entry into the effector cell. The precise sites of action of the catecholamines (and of 5-hydroxytryptamine) are unknown at present. Much of the enzyme adenyl cyclase appears to be located at the level of the cell membrane. However, it might be well to keep one's mind open to the possibility that some of the sites of action of the amines are intracellular. It is with regard to this possibility that the mitochondrial location of MAO in the different cells is of particular significance. This view is supported by observations which demonstrate an uptake of catecholamines into the cell's interior (see Avakian and Gillespie, 1968; Lightman and Iversen, 1969; Kalsner and Nickerson, 1969*a*, *b*).

Our old work on MAO was carried out using preparations modelled on Otto Warburg's "Körnchensuspension" first described in 1913 (see Blaschko, 1936). Today the outer mitochondrial membrane has been recognized as the main site of MAO. What is still discussed is the existence of true extramitochondrial MAO. In her early studies my colleague Miss Hawkins (1952) discussed the possibility that enzyme not sedimenting with intact mitochondria might be located in mitochondrial debris produced in the breaking up of the tissues. This is a possibility still under discussion. The whole question seems to me to be bound up with that of the origin of the outer mitochondrial membrane:

we do not know if the enzyme is added to this membrane at the exact moment at which the latter envelopes the mitochondrion. We remember here the observations by Weissbach, Redfield and Udenfriend (1957) on soluble liver MAO; this might be an enzyme detached from mitochondria.

As far as amine entry into cells is concerned, I believe we may have to be prepared to find differences from one cell type to another. Pharmacological evidence suggests that 5-hydroxytryptamine enters the smooth muscle cell of the mouse stomach very slowly; on the other hand, this amine is readily taken up by blood platelets (see Born, 1970).

In summary, one can say that the role of MAO in the biological inactivation of the catecholamines and 5-hydroxytryptamine is now fairly well understood. This statement is true not only for the vertebrates but also for many invertebrates. However, there are species in which the physiological role of MAO is less clear. We have recently reported that in *Petromyzon marinus* liver, the enzyme acts on many substrates not attacked by MAO in most other vertebrate species (Blaschko, Boadle, and Strich, 1969). Patterns of substrate specificity even more divergent from the normal pattern can be found in some invertebrates. The finding of MAO in the Malpighian tubules of *Periplaneta americana* (Blaschko, Colhoun, and Frontali, 1961) was followed by the observation that this enzyme rapidly acts on short-chain diamines such as cadaverine and 1,7-diaminopeptane, and also on agmatine. An even more deviant oxidase was found in another species of cockroach, *Blaberus discoidalis*, where, in the manometric experiment, the homogenate prepared from the Malpighian tubules is without action on the amines normally oxidized by MAO, but will readily oxidize the same diamines, and also agmatine (Boadle and Blaschko, 1968). The question arises: What is the function of this enzyme? It is difficult to tell at present. Also, in the renal appendages, the pancreas, and the optic ganglia of a cephalopod, *Eledone cirrhosa*, there occurs a MAO that readily oxidizes histamine, a substrate not acted upon by the *Eledone* liver enzyme (Boadle, 1969).

III. OTHER MONOAMINE OXIDASES

The enzymes discussed so far may display a great variety in their pattern of substrate specificities, but they can all be considered as belonging to one main group. On the other hand, much new information has become available in recent years on an entirely different family of monoamine oxidases which are in many ways closely related to Zeller's diamine oxidase.

A. Plasma monoamine oxidases

The monoamine oxidases that fall into this second group have only recently attracted much attention. The best-studied members of this group are the

oxidases of mammalian blood plasma. The first of these, spermine oxidase, was discovered almost 20 years ago (Hirsch, 1953); this enzyme occurs in all ruminants and in a small number of non-ruminant species. The rapid action on spermine and spermidine, of course, is not reminiscent of MAO, but spermine oxidase acts in just the same way as the mitochondrial oxidase on many other typical substrates of MAO.

A second plasma oxidase is even more reminiscent of MAO in its substrate specificity, for it does not act on the polyamines. In our laboratory jargon we used to call this enzyme the "nonspermine oxidase," but its proper name is now benzylamine oxidase (Bergeret, Blaschko, and Hawes, 1957; Blaschko, Friedman, Hawes, and Nilsson, 1959).

Both spermine oxidase and benzylamine oxidase have been crystallized (Yamada and Yasunobu, 1962; Buffoni and Blaschko, 1964). Buffoni and I purified the benzylamine oxidase of pig plasma because this enzyme is of interest to the pharmacologist: it acts rapidly on histamine, on mescaline, and on dopamine.

The functional significance of these enzymes is still obscure. Some time ago, I suggested that the polyamines are the physiological substrates of spermine oxidase (Blaschko, 1962), and I saw its biological significance in terms of the removal of spermine and other polyamines. Normally, these polyamines have an intracellular existence, but I thought that in animals like the ruminants there might be a constant absorption of polyamines from the surface of the digestive tract when decaying micro-organisms, e.g., in the rumen, release their soluble contents. Such an interpretation leaves us without any satisfactory ideas on the function of benzylamine oxidase.

The successful crystallization of the diamine oxidase of pig's kidney, the classical source of histaminase, by Yamada, Kumagai, Kawasaki, Matsui, and Ogata (1967) has established beyond doubt the close relationship between diamine oxidase and the plasma enzymes.

The recent work just quoted has made it possible to establish the chemical relationship between the plasma oxidases and pig kidney oxidase (see Blaschko and Buffoni, 1965; Mondovì, Costa, Finazzi Agrò, and Rotilio, 1967). These enzymes all contain copper, and for benzylamine oxidase and pig kidney enzyme the formation of a Schiff base between a pyridoxal group in the enzyme and the amino group of histamine has been made extremely likely (Buffoni, 1968; Kumagai, Nagata, Yamada, and Fukami, 1969).

B. Connective tissue monoamine oxidases

Whereas the function of the MAO's of blood plasma is still obscure, there exists an enzyme, or a group of enzymes, which clearly belong to the same family of copper-containing catalysts and are chiefly defined by their function. This is the amine oxidase (or the amine oxidases) responsible for bringing about the cross-linking in elastic and collagen fibers.

There can now be little doubt that in the cross-linking occurring in the formation of elastin, a MAO participates. It is required for the oxidative deamination of the ε-amino group of lysyl residues that occurs in the formation of the desmosines (Partridge, 1966, 1970) and of lysinonorleucine (Franzblau, Faris, Lent, Salcedo, Smith, Jaffé, and Crombie, 1970). The catalyst (or catalysts) at work resemble the copper-containing enzymes of blood plasma: they contain copper and probably pyridoxal phosphate (see Carnes, 1968). In fact, it was in copper-deficient piglets reared for the study of the malformation of elastin in the aorta and other great arteries that the absence of benzylamine oxidase activity was first noted (Blaschko, Buffoni, Weissman, Coulson, and Carnes, 1965).

Our knowledge of the participation of an amine oxidase in the biosynthesis of collagen is even more recent. In collagen fibers there occur both intramolecular and intermolecular cross-links. The former are links between individual alpha-chains of the tropocollagen molecule (Bornstein and Piez, 1966); the latter are formed between different tropocollagen molecules (see Bailey, Peach, and Fowler, 1970*a*). There is evidence both of reversible aldimino bonds (Bailey, Peach, and Fowler 1970*b*; Bailey and Peach, 1971) and of more stable cross-links due to aldol formation between two deaminated lysyl (and hydroxylysyl) residues which have been called syndesine (Bailey et al., 1970*a*; see also Gallop, Blumenfeld, and Paz, 1970). It seems as if in soluble collagen (e.g., rat tail collagen) there occur only the reversible aldimine type of cross-links, whereas in less soluble collagen (Achilles' tendon or bone) the irreversible aldol type of link seems to be preponderant.

For the amine oxidase(s) responsible for the deamination, the term "lysyl oxidase" has been used (Martin, Pinnell, Siegal, and Goldstein, 1970). This seems to be an apt name, as far as connective tissue enzyme is concerned. In fact, the enzyme selects the lysyl (and hydroxylysyl) groups present in the alpha chain of rat collagen for the intramolecular cross-linking of the lysyl residue near the N-terminal end of the chain, in position 9; this is in the non-helical part of the chain, and the reactive lysyl group is probably the only one exposed to the action of the oxidase (Piez, Miller, Lane, and Butler, 1970).

Although it is likely that in its action in connective tissue the oxidase is highly selective, in its general substrate specificity it seems to resemble the plasma enzymes. Rucker and O'Dell (1971) have recently reported observations on a partly purified preparation from bovine aorta, and it is interesting that this preparation acts on spermine, spermidine, benzylamine, and all the other typical substrates of the bovine plasma enzyme. The only exception is that the aorta enzyme, in addition, also oxidizes the lysyl group in lysine vasopressin. This compound is not oxidized by the bovine plasma oxidase. Rucker and O'Dell discussed the possibility that the plasma enzyme is derived from the connective tissue oxidase.

The copper content of the connective tissue enzymes accounts for the

weakness of the aorta in copper deficiency. Another pathological condition in which a malfunction of the enzyme is postulated occurs in lathyrism. It seems likely that it is the carbonyl group in the oxidase that interacts with the lathyrogenic agents (see Levene, 1971). It might be mentioned here that there are also reports of a defective elastin formation in pyridoxine-deficient chicks (Starcher, 1969).

IV. SUBSTRATE SPECIFICITY

There are good reasons to support the view that MAO is a flavoprotein. On the other hand, diamine oxidase and related monoamine oxidases are probably copper-pyridoxal-phosphate proteins. This difference accounts for the fact that the latter enzymes will accept only primary amines as substrates whereas MAO oxidizes secondary and tertiary amines also.

Substrate specificity is a complex phenomenon. Apart from the one difference just mentioned, there is a considerable overlap in specificity between the two groups. For instance, benzylamine oxidase shares many of its substrates with classical MAO, and the enzyme of the Malpighian tubules of *Blaberus* acts on many substrates of diamine oxidase. We account for all these facts by saying that specificity is mainly determined by the enzyme protein. Thus there are two independent factors that determine specificity: cofactor requirements and protein requirements. Both have to be satisfied.

As far as the proteins are concerned, if we find so much similarity between the specificity patterns of benzylamine oxidase and of MAO, can we say there is any similarity between these proteins? Are there common properties? At present we cannot tell. The study of the enzyme proteins would require full purification of both in amounts adequate for chemical study. Thus, one is still allowed to speculate. Possibly the enzyme proteins of the two oxidases are similar; they may have evolved from a common ancestor or developed from a common precursor. At present nothing is known either to support or to refute such a possibility.

And what is it that makes a monoamine oxidase different from a diamine oxidase? Zeller has proposed that diamine oxidase has two points at which the two amino groups attach themselves, and he has also produced good evidence that these two points of attachment are not equivalent. We would say the first amino group, the one that is removed in the enzymic reaction, interacts with the carbonyl group in the active center of the enzyme. The second basic group probably interacts with the enzyme protein. Such an interpretation is well supported by experimental evidence, chiefly from Zeller's laboratory.

I have always been interested in the fact that the aliphatic monoamines must have a minimum chain length in order to have significant affinity for MAO. On the other hand, the short-chain aliphatic diamines are not oxidized;

it is the long-chain aliphatic diamines that are oxidized by MAO (Blaschko and Duthie, 1945; Blaschko and Hawkins, 1950). The same general rule is valid for the copper-containing MAO's. (It should be noted, however, that some time ago N. J. Kuhn at Oxford found that methylamine is a substrate of benzylamine oxidase.)

These facts seem to me to indicate that the monoamines not only interact with their substrates through their prosthetic groups, but that they also interact with the hydrophobic region of the polymethylene chain, probably through a hydrophobic region in the enzyme protein.

Superimposed upon these general similarities, we find smaller differences; these are responsible for the minor variations in substrate specificity between different forms of one enzyme, e.g., species differences or differences between isoenzymes of MAO. Similarly, there are the differences between benzylamine oxidase, spermine oxidase, and "lysyl" oxidase among the copper-containing oxidases.

V. CONCLUSION

Last September, when I was a Visiting Professor at Bergen University, I made a few observations on the MAO of the hedgehog. This was just 35 years after I began my work on this enzyme, in the Physiological Laboratory at Cambridge. I have already once told how that came about (Blaschko, 1957). Joseph Barcroft asked me if I knew what the fate of adrenaline was. I went to the Biochemistry Library, and when I could not find the answer to his question there, I decided to do a few experiments.

There were two practical reasons for the decision. Firstly, I then shared a room in the Laboratory with Hans Schlossmann, who had just written a long review article on methods of bioassay (Schlossmann, 1935). Secondly, it became clear that one of the difficulties in the manometric study of adrenaline metabolism was that the amine was "autoxidizable." Now, one of my earliest tasks in Otto Meyerhof's laboratory was a study of autoxidation reactions (Blaschko, 1925). You may remember that Meyerhof had begun his research as a pupil of Otto Warburg, and when I joined him in 1925, he still occasionally worked on problems that related to Warburg's interests.

I thought, therefore, that our combined skills might be useful in the study of adrenaline. A few weeks later we joined forces with Dr. Derek Richter, who was then working in the Cambridge Biochemical Laboratory under Gowland Hopkins.

I was little aware of the general implications of this subject. I had always been interested in the field that lay on the borderline of biochemistry, physiology, and pharmacology.

In retrospect, I feel that I made a most fortunate choice. We shall read in this volume how the subject has grown and expanded during the past 35 years,

and although we believe that we have today some answers to the questions that we then asked ourselves, there is much that remains unresolved.

There are, in addition, all the new lines of enquiry that are opening up: I have already referred to problems relating to the formation of connective tissue. And then there are the intriguing questions as to the origin and location of the oxidases and the way in which they fit into specific sites. Also, there are the molecular problems. In 1935 we were restricted to studying the catalytic effects of the enzymes. Today we can purify them and study them as molecules, in their interactions with substrates.

I have no doubt that this field will continue to be a rewarding one for those who enter it now. My hope is it will give them as much satisfaction as it has given me and my colleagues over the years.

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