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SECTION I

Enzymology

FIRST SESSION. CHAIRMAN: H. BLASCHKO

A. INTRODUCTORY REMARKS

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Today's sessions are devoted to the enzymes that take part in the formation of the catecholamines and in their breakdown. We hope to learn of all the progress that has been made in this field in recent years.

I should like to discuss a few aspects of enzyme chemistry, because it is safe to predict that this is a field of knowledge that will be greatly developed in the near future.

Actually this development began many years ago. It began with a study of the inhibitor and substrate specificities of the enzymes involved. Our ideas on the pathway of formation of the catecholamines began with the finding that the enzyme dopa decarboxylase did not act upon N-methyl-dopa. It was only after the discovery of pyridoxal-5-phosphate and its identification as codecarboxylase that the inability of these enzymes to act on N-methyl amino-acids could be interpreted in chemical terms: the unsubstituted amino group of the substrate is essential in the interaction of the substrate and the pyridoxal moiety of the enzyme.

A simple way to demonstrate the pyridoxal nature of dopa decarboxylase is to prepare a liver extract from a rat reared on a diet deficient in vitamin B_6. The enzymic activity of such an extract is abnormally low but it can be brought up to a normal level by adding pyridoxal-5-phosphate *in vitro*.

Recently a new group of enzymes has been shown to contain pyridoxal. This is the group of the cyanide-sensitive amine oxidases. That these enzymes contain pyridoxal was first proposed by Werle and von Pechmann in 1949 (11). In Oxford we have been interested for some time in one enzyme that belongs to this group, the so-called benzylamine oxidase of mammalian blood plasma. Two years ago the enzyme from pig plasma, which will also oxidize dopamine, mescaline and histamine, was crystallized and shown to be a copper-containing protein (4). In order to establish the presence of pyridoxal, my colleague, Professor Franca Buffoni, hydrolysed the enzyme crystals, and she obtained hydrolysis products that were able to activate an apoenzyme that requires pyridoxal-5-phosphate, the L-tyrosine apodecarboxylase of *Streptococcus faecalis* R (2). From this work, it appears that there are 3 or 4 moles of pyridoxal phosphate per mole of protein. We had already reported that there are about 3 or 4 atoms of copper per mole of protein. It seems, therefore, that the prosthetic group is a copper-pyridoxal complex.

It would be interesting to know what happens to these enzymes in B_6 deficiency. Last year we have found, with the help of our colleagues from Salt Lake City, that in copper-deficient pigs the plasma oxidase is absent; the enzyme makes its appearance when copper is added to the diet (3).

That the amine oxidases of this group are pyridoxal enzymes accounts for a feature of their substrate specificity which has been known for some time: all these enzymes are unable to act on secondary amines (1). Here we have a striking analogy to the amino acid decarboxylases.

The part played by these oxidases in the degradation of the catecholamines is still unknown. This group of enzymes appears to have a great variety of physiologic functions. The most recent work suggests that an amine oxidase is essential in the formation of elastic tissue. In copper-deficient pigs (9) and chicks (8) there occur cardiovascular changes due to a malformation of elastic fibers, and it has been suggested that, in the biosynthesis of "desmosine," an amine oxidase of the type that we have studied is essential in the oxidative removal of the ϵ-amino groups of lysine (7).

I see no reason for assuming that the classical intracellular monoamine oxidase (MAO) contains pyridoxal. This enzyme first aroused interest when it was shown about 30 years ago that it acted on epinephrine (E) as well as on norepinephrine (NE). Generally, a lack of discrimination between primary amines and their N-methylated derivatives is a very characteristic property of this enzyme. I might add that it has been suggested that this enzyme contains copper (5).

If in a search for enzymes related to the intracellular MAO, we make the ability to act on a secondary amino group our criterion, then I should like to draw your attention to a microbial enzyme, the spermidine oxidase (or dehydrogenase) of *Serratia marcescens*, an enzyme that forms Δ^1-pyrroline and 1,3-diaminopropane from spermidine (10). This enzyme, we must believe, attacks its substrate at a secondary amino group. To react with oxygen, the Serratia enzyme requires the presence of flavin adenine dinucleotide and an additional electron carrier. I should like to suggest that this enzyme may serve as a prototype of the intracellular MAO. Some time ago Miss Joyce Hawkins tried to find out if flavin was an essential constituent of amine oxidase, by testing for MAO in riboflavin-deficient rat liver; the enzymic activity was low, but the question as to the nature of the electron carrier remained open (6). It will remain for the future to decide whether MAO is a flavin enzyme.

As the enzymes involved in amine metabolism are being purified, we begin to study the enzyme-substrate interaction on the molecular level. Of the gains already made, we hope to learn in these sessions.

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