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Report on the Discussion of the Third Session

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THIS has been an outstanding series of papers, all of which deal, in ultimate analysis, with some basic aspect of catecholamine synthesis and metabolism. We will summarize these reports and we will stress all the significant points. We will also refer briefly to some important enzymological aspects of catecholamine biosynthesis which have not been discussed today.

Goldstein presented an interesting study on the distribution of aromatic-L-amino acid decarboxylase, dopamine-β-hydroxylase (DβH) and phenylethanolamine-N-methyltransferase (PNMT). He and his collaborators purified the three enzymes and prepared the corresponding antienzymes which they utilized for several different experiments. They were able to show that aromatic-L-amino acid decarboxylase is found in cells that contain biogenic amines; on the other hand, PNMT was found only in adrenal gland cells which contain epinephrine, but not in the cells which contain only norepinephrine. With similar immunochemical techniques, they were able to show that DβH and PNMT are immunologically heterogeneous in different animal species. Goldstein also presented studies on human serum DβH which were similar to those that Axelrod presented yesterday (1); both investigators agreed that there is a certain correlation between the degree of sympathetic activity and the levels of serum DβH. As Kopin (NIH, Bethesda) pointed out during the discussion period, the lack of correlation that is sometimes found between the blood levels of catecholamines and DβH may be due to marked differences between the serum half-life of catecholamines and the enzyme. Kirshner (Duke University, Durham, North Carolina) suggested that these differences may be explained because the DβH molecule is large and may be delayed in getting incorporated into the general circulation through the lymphatic vessels.

In order to solve these problems, experiments should be performed to measure the half-life of serum DβH and to determine the time course of the transient elevations of enzyme levels in response to sympathetic hyperactivity. It will also be interesing to determine whether there is a circadian rhythm in the serum levels of this enzyme.

Hartman and Udenfriend presented their studies on a very refined immunological technique which they used to localize DβH in several tissues and to determine different immunological forms of monoamine oxidase (MAO). Their immunofluorescent technique allows the visualization of cell bodies, axons and terminals of DβH-containing neurons without the use of precursor load, MAO inhibitors or surgical procedures; on the other hand, the fluorescence of the nerve

terminals is not as intense as the norepinephrine fluorescence. One definite advantage of the immunofluorescent technique is that serial tissue sections can be obtained which permit mapping of the distribution of noradrenergic cell systems and following of individual axons through considerable extension. The high specificity of this technique has advantages but also some disadvantages since, obviously, it cannot be used to study dopaminergic or serotonergic systems. With this technique, the authors have observed that DβH-containing cells originating in the central nervous system innervate their vessels directly. This is quite an interesting observation with important physiological and theoretical implications. The authors propose that part of the central noradrenergic system has functions analogous to those of the peripheral sympathetic system. As Kety (Harvard University Medical School, Cambridge, Massachusetts) pointed out in the discussion, these findings may explain the old observation that after cervical sympathectomy, some central nervous system vessels retain their adrenergic innervation. In addition, Hartman and Udenfriend determined that the bulk of brain MAO is immunologically very similar, if not identical to, liver MAO; curiously enough, the remaining 20% is immunologically different and does not react with the liver MAO antigen.

Sandler and Youdim have presented an extensive and very critical review of MAO. They have examined very carefully the question of the nature of the multiplicity of molecular forms of MAO. They concluded that the different sensitivities to inhibitors, the different substrate specificities and the fact that the same different forms are obtained when using various isolation procedures, are clear evidence that the multiplicity of forms is not artifactual. Sandler and Youdim made a very lucid analysis of the possible functional significance of the different forms; they indicated that the high incidence of tyramine-induced hypertensive crisis in patients receiving tranylcypromine may be correlated with the high potency of this drug to inhibit the deamination of tyramine by a characteristic form of MAO. They also pointed out that the extreme sensitivity of some patients treated with MAO inhibitors may be related to their inability to conjugate tyramine. It is obvious from their analysis that we should refer not to different enzyme forms, but to different enzymes, different monoamine oxidases. It will be most useful to develop immunofluorescent techniques as for DβB, or histochemical reactions with the use of different inhibitors or substrates, to study the tissue distribution and the cellular localization of the different monoamine oxidases.

Sourkes presented his work on the effect of specific nutrient deficiencies on the synthesis and degradation of catecholamines and on the activity of the purified enzymes. In pyridoxine-deficient rats, they were not able to detect any change in biogenic amine metabolism, not even after an L-dopa load. However, after a D-dopa load, they found a pronounced defect in the conversion of D-dopa to dopamine because the deamination of D-dopa to 3,4-dihydroxyphenylpyruvic acid is partially blocked due to the high sensitivity of transaminases to pyridoxine deficiency. Rats made deficient in riboflavin metabolized N-pentylamine-^{14}C at a much lower rate, which was correlated with a decrease in hepatic MAO activity.

When copper-deficient rats were injected with labeled dopamine, they synthetized less norepinephrine; the administration of 50 μg of copper restored the activity of DβH. The nutritional deficiency of iron produced a marked decrease in MAO activity; the nature of this decrease is being investigated.

All the contributions to this session have been quite interesting, but the time available has not been sufficient to cover some crucial aspects of the enzymology of the first step of catecholamine biosynthesis. Since the last catecholamine symposium, there have been several reports on tyrosine hydroxylase (2, 6–9) but the enzyme has not yet been purified and there is a great deal of controversy on several of its characteristics.

An enzyme which has not yet received as much attention as it should is dihydropteridine reductase. We have found an enzyme in beef adrenal glands and in the brain which is capable of reducing the pteridine cofactor oxidized in the hydroxylation of tyrosine to dopa. This enzyme seems to be similar to the liver dihydropteridine reductase described by Kaufman and Levenberg (3) in the sheep liver.

Dihydropteridine reductase is an essential enzyme for the biosynthesis of catecholamines since it is obvious that without tetrahydropteridines, tyrosine hydroxylase cannot hydroxylate tyrosine to dopa. In addition, this enzyme may have an important role in the regulation of catecholamine biosynthesis; the antagonism between the catecholamine feedback inhibition of tyrosine hydroxylase and the concentration of reduced pteridines suggests that dihydropteridine reductase, by increasing the concentration of reduced pteridines, may antagonize the catecholamine feedback inhibition. This phenomenon is known to happen *in vitro* (5) but still there is no evidence which indicates that dihydropteridine reductase activity may change *in vivo*. The competitive nature of the antagonism between catecholamines and reduced pteridines indicates that the concentrations of reduced cofactor sets the level at which catecholamines will inhibit tyrosine hydroxylase; with low levels of reduced cofactor, tyrosine hydroxylase will be inhibited by low levels of cytoplasmic catecholamines, and *vice versa*.

The concentration of the pteridine cofactor for tyrosine hydroxylase is unknown in most tissues. We know from the work of Lloyd and Weiner (4) that the concentration of biopterin in the adrenal gland is low, in the order of 10^{-6} M. This figure is 100-fold less than what is generally used *in vitro* for the assay of tyrosine hydroxylase; this indicates that *in vivo*, adrenal medulla tyrosine hydroxylase will be very sensitive to feedback inhibition by catecholamines. It should be noted that the concentration of 1×10^{-5} M refers to total unconjugated pteridine. The actual amounts of active cofactor, that is, tetrahydrobiopterin, may be much less, and it will depend on the activity of dihydropteridine reductase. All these considerations suggest that dihydropteridine reductase may have an important role in the regulation of catecholamine biosynthesis.

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