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J. SUMMARY OF DISCUSSION AND COMMENTARY

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In connection with Falck's lecture, Hamberger reported that the pericytes of monoamine oxidase (MAO)-inhibited brain slices accumulate norepinephrine (NE). He suggested that these cells may function in the blood-brain barrier by inactivating catecholamines. By means of a lantern slide of a brain section he showed that after injury of the blood-brain barrier by cold or mercuric chloride exogenous catecholamines accumulated in nerve fibers.

Dahlström agreed with Grillo that the amine-containing granules are probably formed in the neighborhood of the nucleus and then move into the axon. She described a marked accumulation of catecholamines proximal to a ligature on a rat sciatic nerve. Data obtained with Häggendal at Göteborg indicate that the granules are transported distally 0.5 cm per hr, a rate very different from Paul Weiss' estimate of 1 mm per day for axoplasmatic flow in myelinated motor nerves. Preliminary observations have indicated that dopa decarboxylase accumulates above the ligature at a slower rate than catecholamines.

The question whether NE in nerves is stored in dense-core vesicles was discussed by a number of participants. Chidsey stated that, in work carried out with Michaelson, Cooper, and Richardson, heart microsomal preparations contained only small numbers of such particles, a finding which could be attributed in part to the great dilution of the vesicles by other kinds of particle. Whittaker commented that the electron micrographs of Potter's highly purified (1500 times) preparation of heart vesicles contained disappointingly few dense-core vesicles and could not be regarded as convincing evidence that NE is specifically contained in such particles. He suggested that it would be interesting to subject the preparation to the bead-tagging procedure (J. Neurochem. **12:** 363, 1965) to estimate the number of molecules of NE per vesicle. It would be instructive to compare the results of such an experiment with data on adrenal medullary granules. De Robertis noted that in order to observe granulated vesicles in the anterior hypothalamus the tissue must be fixed by perfusion. He cited two papers (Arch. Hist. Jap. **24:** 489, 1964; Experientia **21:** 121, 1965) in which NE-releasing drugs were shown to reduce the number of dense-core vesicles. Fuxe reported, however, that several brain areas rich in monoamines (*e.g.*, *Nuc. tr. solitarii*) show few granular vesicles when the tissue is perfused with glutaraldehyde-chromate; moreover, the large granular vesicles showed no obvious changes after reserpine alone or in combination with α-methyltyrosine and desmethylimipramine. Whittaker stated that his observations on negatively-stained preparations suggest that all vesicles may contain a dense core.

Sharman observed that the differences between De Robertis' and Whittaker's findings on dopamine-containing vesicles could perhaps be explained on the basis of the different brain parts employed in the two studies. Sharman has detected

dopamine in various parts of the brain associated with high concentrations of NE. It is thus possible that in whole brain preparations free dopamine is accumulated by the NE vesicles. Whittaker's work was done with the caudate nucleus, a tissue containing very little NE. Michaelson thought it more likely that the dopamine-containing vesicles observed by De Robertis were in fact unruptured nerve ending particles.

The likelihood that the NE-synthesizing enzymes occur in close proximity, or as a unit, was discussed by Udenfriend. The accumulation of tyrosine appears to be followed rapidly by oxidation, decarboxylation, and hydroxylation. Similar sequences of enzymatic reactions are recognized in connection with the biosynthesis of fatty acids. The high K_m values of dopa and dopamine for their respective enzymes (*ca.* 10^{-3}) also make it unlikely that trace quantities of these catechols can be concentrated enough to permit adequate and continuous synthesis of the hormone. Creveling reported that particles prepared by sedimenting guinea pig brain homogenates at 20,000 $\times$ *g* contain tyrosine hydroxylase, dopa decarboxylase and dopamine-β-hydroxylase.

Additional observations on differences between adrenal medullary and nerve granules were contributed by Schümann. First, calcium ions release catecholamines and ATP only from the medullary particles. Secondly, the uptake of tyramine by the medullary granules is not inhibited by reserpine. L. D. Carlson noted that similarities certainly outweigh differences between the two kinds of particles and inquired whether any differences have been observed *in vivo*. In reply, Vogt pointed out the much greater susceptibility of sympathetic nerve endings to reserpine. The adrenal medullary granules are not released through a direct action on chromaffin cells unless excessively large doses of the drug are administered.

Weiner reported that reserpine pretreatment reduced greatly the formation of NE, dopamine, vanilylmandelic acid (VMA), and dihydroxymandelic acid from tyrosine or dopa by perfused rabbit heart or adrenal medullary slices. Less reduction was seen in homovanillic acid and dihydroxyphenylacetic acid. These results are in harmony with the data presented by Stjärne suggesting an impaired formation of dopamine and NE by isolated nerve granules. Weiner regarded Schümann's values for the Mg and Ca content of bovine adrenal granules as extremely high. He suggested the possibilities of post mortem diffusion of ions into granules and the presence of mitochondria in Schümann's preparations.

In connection with Banks' report De Schaepdryver recalled two of his papers on medullo-adrenalectomy in the dog and bilateral total adrenalectomy in man. In the dog E disappeared from the urine almost completely, although it could be detected after the injection of histamine. In comparison, only 3 of the 10 adrenalectomized patients showed a significant decrease in the urinary excretion of E. These observations suggest species differences in extramedullary chromaffin tissue.