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Report on the Discussion of the Fourth Session

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THE knowledge of hormonal interactions has increased considerably in recent years and it is still expanding rapidly as pointed out by von Euler in his introductory remarks. This field of research which tackles the most fundamental cellular mechanisms will require, for further development, the combined effort of pharmacologists, biochemists, molecular biologists, physicists, electron microscopists and immunologists. Concepts which have been used for many years to explain the actions of hormones have to be modified as techniques of study become more sophisticated.

Rall's presentation emphasized the need to review present concepts of the role of cyclic adenosine monophosphate (AMP) in hormonal interactions. He suggested that parallel sequences of events could accompany the changes in cyclic AMP after hormone-receptor interaction. He also pointed out that large amounts of adenyl cyclase could be found outside of plasma membranes and that small molecules such as catecholamines entering into cells could influence the activity of intracellular adenyl cyclase. Finally, he indicated that one now needs to consider the possibility of various pools for cyclic AMP. The measurement of total tissue levels of cyclic AMP does not necessarily give information relevant to cell function. It is likely that only a small functional pool of cyclic AMP would be related to the action of hormones, and there is a need to characterize this pool before reaching final conclusions on hormone mechanisms. On that latter point, Pletscher (Hoffmann-La Roche, Basel) drew attention to the fact that cyclic AMP may exist in an inactive form in some subcellular compartments. He recalled previous studies in his laboratory, which showed the presence of cyclic AMP in the serotonin containing granules of blood platelets. Rall (Case Western Reserve, Cleveland) replied that the segregation of cyclic AMP in small particles might not be sufficient to account for a functional pool of cyclic AMP unless one postulates the existence of pools in the precursors of cyclic AMP. Rall also pointed out that variations in the total cyclic AMP content of a tissue such as the brain may be difficult to interpret in view of the large variety of cells present. For instance changes in brain cyclic AMP might take place in glial cells rather than nerve cells. A comment by Richardson (NIH, Bethesda) emphasized the difficulties in interpreting changes in the whole tissue content of cyclic AMP. Richardson quoted the studies of Gilman and Nirenberg (2) which show that in uncloned cells derived from neuroblastoma and glioma the addition of prostaglandin E_1 induced the formation of intracellular cyclic AMP, whereas in experiments performed with cloned adrenergic cells he had found no effect of prostaglandin E_1 on the levels of cyclic AMP.

Kirshner presented evidence for a mechanism of exocytosis in the release of catecholamine from the adrenal medulla. He reported studies in which he used dopamine-β-hydroxylase (DBH) to demonstrate the quantal release of the total vesicular content during insulin stimulation. He also showed that after insulin-induced depletion, the adrenal DBH returned to normal within 48 hr whereas the endogenous norepinephrine was restored only a few days later. Stitzel (West Virginia University, Morgantown) wondered whether the synthesis of adenosine triphosphate (ATP), which binds catecholamines into granules, might be the limiting factor in the recovery of norepinephrine levels. Kirshner answered that there was no indication that the slow norepinephrine recovery was related to the synthesis of ATP in the present experiment. Cohen asked whether dopamine had accumulated in the adrenal at the beginning of recovery when DBH levels were still depleted. Kirshner said that the levels of dopamine had not been measured during the period of recovery. Udenfriend (Roche Institute of Molecular Biology, Nutley, N. J.) asked why phenylethanolamine-N-methyl transferase (PNMT) was not released simultaneously with catecholamines in the process of liberation. Kirshner replied that this might be due to the fact that PNMT, being a soluble enzyme, is probably localized outside the granules. Udenfriend expressed some doubts concerning such a hypothesis, since it implied a mechanism whereby norepinephrine would have to come out of its storage sites into the cytoplasm to be converted into epinephrine and then return into its storage sites.

After Kirshner's presentation, a lively discussion was raised by Engelman (University of Pennsylvania, Philadelphia) concerning the validity of plasma DBH levels as an index of sympathetic activity. In other sessions of the present symposium, the question had been evoked on several occasions. During Axelrod's presentation it appeared that plasma DBH level could be closely correlated with norepinephrine release in various acute experimental conditions, thus suggesting that DBH levels could be an index of sympathetic activity. Engelman pointed out that although this correlation was found in certain conditions it did not seem to hold true in others. For instance during exercise, a condition which is known to raise plasma catecholamine levels manyfold, two studies, one by Cardon (NIMH, Bethesda) and the other by Lovenberg (NIH, Bethesda), had failed to show significant increases in plasma DBH levels. He concluded his remarks by stating that it was difficult to consider plasma DBH levels as a good index of sympathetic activity. It was pointed out by Goldstein (New York University, New York) that the range of DBH levels is rather large in normal subjects and that the range of response to exercise also varied from one individual to another. Goldstein agreed with Engelman that results concerning DBH levels had to be interpreted with caution as long as the uptake, turnover and degradation processes related to DBH were not elucidated. Kopin (NIMH, Bethesda) added that the turnover of circulating catecholamines is very rapid whereas the turnover of plasma DBH is much slower. This difference in the turnover of both substances, would make a correlation rather difficult to establish in various physiological and pathological conditions. Kopin also pointed out to Goldstein that studies made in his section at NIMH had failed to reveal any abnormality of plasma

DBH levels in Parkinson patients. Commenting on Engelman's question, Kopin added that although Cardon was unable to detect changes in DBH levels during exercise, he was able to demonstrate a significant increase in plasma DBH levels during cold pressor test, a condition which is also known to increase plasma catecholamine levels. He raised the possibility that the increase in DBH during the cold pressor test and not during exercise might be explained by a release from different sources in each of these conditions.

Wurtman presented his studies on the control of adrenal PNMT under the influence of corticosteroids secreted by the adrenal cortex. He provided evidence that steroids have the ability to induce the synthesis of enzyme rather than produce only a change in enzyme activity. He also showed that adenyl cyclase activity of the adrenal gland was markedly reduced in hypophysectomized animals. Treatment with dexamethasone caused a marked increase in adenyl cyclase activity whereby ATP levels were markedly reduced within minutes after administration of the steroid. Rall inquired whether the adenyl cyclase had been measured in presence of fluoride. After a positive answer on that point, Rall expressed surprise that it was possible to detect so large an increase in adenyl cyclase activity after addition of fluoride since the blank is markedly increased with that procedure. Sandler (Queen Charlotte's and Chelsea Hospital of London) asked whether extramedullary PNMT was also influenced by steroids. Previous studies made by Wurtman have not shown an influence of steroids on the activity of PNMT in frog brain tissue.

Carlsson presented his views on the regulation of tyrosine hydroxylase in the central nervous system. Most of his conclusions were based on studies in which decarboxylase inhibitors were used to get a relative estimate of the synthesis rate of serotonin and catecholamines. After inhibition of decarboxylation, Carlsson reported that the precursors dopa and 5-hydroxytryptophan (5-HTP) accumulated in various regions of the brain in proportion to the endogenous content of the end-product in these regions. Since the accumulation of the precursors was linear for 30 to 60 min after inhibition, Carlsson suggested that the kinetics of the accumulation of the precursor, in the linear portion of the curve, might provide a good estimate for the synthesis rate. Costa (NIMH, Bethesda) asked how turnover rates had been calculated in these studies. Carlsson answered that the absolute synthesis rate had not been calculated in these experiments but that the relative synthesis rate was estimated by calculating the reciprocal of the K_m. Weiner (University of Colorado Medical Center) wondered whether the technique proposed by Carlsson lent itself to the above interpretation since he suspected that this model represents an open-system in which a continuous leakage of dopa might take place. Carlsson replied that in his studies the accumulation of dopa was linear in the first 30 to 60 min after inhibition and that a bending of the curve would have occurred earlier if there had been a continuous leakage of dopa. Although he agreed that there exists no data to prove that this precursor of catecholamines is retained in nerve terminals, it is his feeling that not too much of the precursor leaks out *in vivo*. Someone asked whether the decarboxylase inhibitors had any effect on tissue and plasma levels of tryptophan (TP) and

tyrosine. Carlsson answered that R04-4602 did in fact produce a slight increase in tissue TP but that did not occur with NSD-1015. As for tyrosine levels, they were not affected by either of these two decarboxylase inhibitors. However, the precursors had not been measured in the plasma after enzymatic inhibition. Mandel suggested that decarboxylase inhibition might affect the uptake of the precursors into the cell as a result of the accumulation of dopa and 5-HTP. Carlsson agreed upon the possibility that the uptake of precursors might be affected by such treatment. Kopin wondered whether the decarboxylase was completely inhibited in these experiments since Carlsson had reported that the levels of catecholamines were increased in the brain after inhibition. Carlsson answered that the decarboxylase had been completely inhibited. He added that the changes in catecholamine levels differed with the type of inhibitor used. NSD-1015 also inhibited monoamine oxidase and this might account for the absence of change in catecholamine levels. On the other hand, R04-4602 does not inhibit monoamine oxidase and endogenous catecholamine levels were lowered after administration of that compound. Wurtman (MIT, Cambridge, Massachusetts) added to this discussion two comments: 1) He was able to confirm Carlsson's observation that giving R04-4602 increases brain dopa (3), and 2) there was a similarity between the curve relating brain 5-HTP to brain TP as reported by Carlsson and his own data relating brain serotonin to brain TP after tryptophan administration (1).

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