W. M. is in receipt of a Wellcome Trust Travel Fellowship.

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Effects of Theophylline and Adenosine 3':5'-Cyclic Monophosphate on Growth Hormone Release from the Isolated Rat Anterior Pituitary Gland

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Recent evidence suggests that cyclic AMP (adenosine 3':5'-cyclic monophosphate) may be involved as a mediator of releasing-factor-induced growth hormone secretion (Schofield, 1967; Müller, Pecile, Kabir Naimzada & Ferrario, 1969). A direct effect of the cyclic nucleotide on growth hormone release from an anterior-pituitary preparation *in vitro* has, however, not yet been reported. During an extended study being undertaken in this laboratory into the regulation of growth hormone secretion, the effects of theophylline and of cyclic AMP on the rate of growth hormone release *in vitro* from a preparation of rat anterior pituitary were examined together with certain of the characteristics of the response to theophylline.

Rat hemipituitaries were incubated singly in a bicarbonate-buffered medium containing glucose (5.5mM) together with the test substances, paired controls being employed throughout. The viability of the tissue under the conditions of incubation was established by metabolic and ultrastructural criteria. After incubation, the growth hormone content of the media was measured by a double-antibody radioimmunoassay (with reagents kindly supplied by Dr A. F. Parlow under the auspices of the Rat Pituitary Hormone Program of the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md., U.S.A.). The assay, which is specific and highly reproducible, is sensitive to 120pg of added rat growth hormone.

In the presence of theophylline (5mM and 0.5mM) growth hormone release was increased to 280% and 180% respectively of the control value. Similarly the dibutyryl derivative of cyclic AMP (1mM and 0.2mM) led to an increase in hormone release to 220% and 134% respectively of the control value. In further investigations it was found that the response to theophylline was abolished by 2,4-dinitrophenol (0.2mM), but unaffected by a concentration of cycloheximide (20µg/ml) previously

shown to produce a 90% inhibition of pituitary protein synthesis. These observations indicate that the secretory response to theophylline is an energy-requiring process but that it is independent of protein synthesis *de novo*.

We are grateful to the Medical Research Council for research funds and for the award of a Clinical Research Fellowship to R. B. L. E.

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Role of Adenosine 3':5'-Cyclic Monophosphate in the Action of Octapeptide Hormones

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Studies of vasopressin-stimulated sodium and water transport across toad (*Bufo marinus*) bladder have shown that maximum sodium transport may be stimulated by 0.0022i.u. of vasopressin/ml and maximum water transport by 0.022i.u. of vasopressin/ml. Measurement of oxygen consumption in toad bladder gave a Q_{O_2} value of 1.09 (± 0.05 S.E.M.) µl/h per mg dry wt. Vasopressin increased the oxygen consumption over a 6h period, reaching a maximum 2h after the addition of the hormone. The maximum corresponds to a Q_{O_2} of 2.66 with 0.100i.u. of vasopressin/ml and to 1.82 with 0.0022i.u. of vasopressin/ml. In the presence of theophylline (1.0mM) the oxygen consumption measured with 0.100 and 0.0022i.u. of vasopressin/ml gave Q_{O_2} values of 1.48 and 1.37 respectively. Theophylline (1.0mM) caused a decrease in oxygen consumption in the absence of hormone, with a minimum Q_{O_2} value of 0.71 at 2h after addition of the theophylline. These results are taken to indicate that vasopressin at 0.0022i.u./ml gives rise to an increased respiration that is insensitive to addition of theophylline, whereas vasopressin at 0.100i.u./ml causes a further increase in respiration that is entirely theophylline-sensitive.

A dose-response characteristic (response against logarithm of the dose) obtained for oxytocin-stimulated respiration in frog (*Rana temporaria*) skin was found to have a double sigmoid form. The effect of addition of theophylline (1.0mM) has shown that the respiration stimulated at lower doses of oxytocin (0.0001–0.003i.u./ml) was insensitive to the presence of theophylline, but at higher concentrations of the hormone (0.003–0.100i.u./ml) the further increased oxygen consumption was entirely theophylline-sensitive. The lower concentrations of hormone (Jard, Bastide &

Morel, 1968) correspond to the stimulation of sodium transport across frog skin, whereas the higher concentrations of hormone correspond to the stimulation of water transport. If the effect of theophylline on the Q_{O_2} may be taken as an indication of the extent of the turnover of cyclic AMP in the tissue, these results suggest that the hormonal effect on sodium transport is independent of any effect in stimulating the adenylate cyclase system.

The activation energy for water transport has been determined as 8.9 kcal/mol across control toad bladder and 9.7 kcal/mol in the presence of cyclic AMP (1.0 mM) plus theophylline (1.0 mM). In the presence of vasopressin (0.022 i.u./ml) the activation energy was decreased to 4.8 kcal/mol, a result that confirms the observation by Hays & Leaf (1962). Although cyclic AMP (1.0 mM) plus theophylline (1.0 mM) increase water transport at 22°C by an amount similar to that produced by vasopressin (0.022 i.u./ml), there is no similar decrease in the permeability activation energy.

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The Co-ordinated Inhibition of Protein and Lipid Biosynthesis by Adenosine 3':5'-Cyclic Monophosphate

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In growth or development of a tissue the simultaneous increase in protein and lipid synthesis is well documented (Tata, 1970). In starvation it has been shown that there is a rapid depletion of liver protein, and furthermore there is a degeneration of the endoplasmic reticulum (Munro, 1968). Either starvation or the administration of adrenaline or glucagon increases the gluconeogenic capacity of the liver. The action of both adrenaline and glucagon is probably mediated by cyclic AMP (adenosine 3':5'-cyclic monophosphate) (Robison, Butcher & Sutherland, 1968), and so we have examined whether cyclic AMP will produce effects on protein synthesis which mimic the changes that occur in starvation.

Liver slices from fed male Wistar Albino rats were incubated for 1 h in phosphate-saline buffer and the incorporation of [3 H]leucine into protein and [14 C]acetate into protein, total lipid, cholesterol and free fatty acids was measured by a modification of the method of Hector, Yoshinaga, Halkerston & Birchall (1967). Cyclic AMP decreased the incorporation into all of these fractions

in a dose-dependent response. The maximum inhibitions by cyclic AMP (2 mM) were as follows: protein synthesis, 50%; total lipid synthesis, 70%; cholesterol synthesis, 40%; fatty acids, 70%. Under the conditions when [14 C]acetate incorporation into lipid and protein was inhibited, its oxidation as measured by the liberation of $^{14}\text{CO}_2$ was unaffected by cyclic AMP, indicating that the decrease in incorporation could not have been due solely to isotopic dilution. This point was further evaluated by measuring the incorporation of tritium from T_2O into cholesterol. It was found that this incorporation was inhibited by cyclic AMP.

Since in starvation there are marked changes in the structure of the endoplasmic reticulum we have examined whether cyclic AMP causes an inhibition of the synthesis of membrane components. After the incubation of rat liver slices, the microsomal fraction was isolated and the incorporation of labelled precursors into the protein and lipid fractions was measured. This was found to be inhibited in a dose-dependent fashion by cyclic AMP. The pattern of inhibition was similar to that noted above for the incorporation into the total cell material.

The observation that cyclic AMP causes an inhibition of protein and lipid biosynthesis could be of some importance in the control of gluconeogenesis. First, the inhibition of energy-dependent pathways such as protein and lipid biosynthesis by cyclic AMP might increase the availability of ATP for gluconeogenesis. Secondly, the inhibition of protein synthesis should spare amino acids that may act as precursors for glucose synthesis.

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Adenosine 3':5'-Cyclic Monophosphate Phosphodiesterase: Kinetic and Inhibitor Studies

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This enzyme has previously been assayed by measuring the liberated phosphate colorimetrically (Butcher & Sutherland, 1962) or by measuring the AMP formed by using an enzymic recycling method (Cheung, 1966). Measurement of cyclic AMP