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 &