cannulated. Cyclic AMP was determined in samples of pancreatic tissue (Johnson & Sherratt, 1970). After an intravenous injection of natural or synthetic secretin, pancreatic cyclic AMP concentrations trebled in 30s and reached near-maximal concentration after 1min. Secretion, however, began only after 45s. Cyclic AMP concentrations fell with decline in secretion. However, after 50min, when secretion had ceased, the concentration again rose, reaching a maximum at 80min, returning to the basal value within 140min. During secretin infusion, the pattern of changes in cyclic AMP concentration was identical, except that the initial rise was maintained as long as secretin was infused.

After pancreozymin infusion, the cyclic AMP concentration rose ninefold in 30s, and then approached the basal value within 1min, coinciding with the time-course of enzyme release (Case, Harper & Scratcherd, 1969); subsequently the concentration of cyclic AMP rose and fell again, exactly as for secretin. The same result was obtained if pancreozymin infusion was superimposed on secretin infusion. The possibility that other organ systems caused these changes indirectly was eliminated by repeating the experiments with the perfused cat pancreas (Case, Harper & Scratcherd, 1968). Since vagal stimulation also causes enzyme secretion, close intra-arterial injections of acetylcholine were also tested. Their effects were identical with those of pancreozymin. However, although enzyme secretion was abolished by prior atropine treatment of the animal, the pattern of changes in cyclic AMP concentration persisted.

These observations suggest that, although the initial rise in pancreatic cyclic AMP concentration produced by secretin infusion may be linked to electrolyte secretion, that after acetylcholine infusion, and probably therefore that after pancreozymin infusion, may not be linked to enzyme secretion. The large secondary rises after pancreozymin, secretin and acetylcholine may be associated with protein synthesis within the gland.

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Adenosine 3':5'-Cyclic Monophosphate and Insulin Release

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Many authors have suggested that cyclic AMP (adenosine 3':5'-cyclic monophosphate) may play an important role in the regulation of insulin secretion from the mammalian pancreas, since agents such as theophylline, caffeine and glucagon, which are known to increase cyclic AMP concentrations in a variety of tissues, potentiate the effect of glucose on insulin release. Further, Turtle & Kipnis (1967) were able to correlate the concentrations of cyclic AMP in islet cells with the effects of glucagon and theophylline on insulin release. It was decided to determine whether the effect of glucose on insulin release was paralleled by changes in either the intracellular concentration of cyclic AMP or the amount released into the medium by islets of Langerhans incubated *in vitro*.

Islets of Langerhans prepared by collagenase digestion of rat pancreas were incubated in physiological medium containing glucose alone or glucose plus the agent under study. At the end of various incubation periods the islets were separated from the incubation medium by centrifugation, rapidly frozen in liquid nitrogen and extracted with acid. The incubation medium was acidified and sampled for the determination of its insulin content by radio-immunoassay. The remainder of the medium and the islet extracts were subjected to ion-exchange chromatography before determination of cyclic AMP by the method of Butcher, Ho, Meng & Sutherland (1965). The concentration of cyclic AMP found in islet cells was in the range 40–80 pmol/mg of protein. There was no detectable change in the concentration of cyclic AMP in the islet cells or in the incubation medium when the glucose concentration of the medium was raised from 5mM to 10 or 20mM, even though there was a marked increase in insulin release under these conditions.

Caffeine, 3-isobutyl-1-methylxanthine and glucagon all stimulated insulin release and increased both the intracellular and the extracellular concentrations of cyclic AMP when islets were incubated in the presence of 10mM-glucose. Adrenaline inhibited insulin release under these conditions and also decreased the concentration of cyclic AMP.

It is suggested that, although agents such as glucagon and caffeine may potentiate the effect of glucose on insulin release by increasing the concentration of cyclic AMP in islet cells, glucose does not stimulate insulin release by this mechanism.

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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*.

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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 &