genase (0.3U) were then added in a volume of 200 μ l. The increase in fluorescence due to NADPH formed in the coupled assay of phosphorylase α at 35°C is continuously recorded after the reaction has been started by the addition of 100 μ l of 1% purified shell-fish glycogen. The rates after 5 min are compared with those obtained with cyclic AMP standards and an extraction blank, and are directly proportional to the logarithm of the cyclic AMP concentrations in the range 1–100 pmol/assay mixture.

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The Assay of Adenosine 3':5'-Cyclic Monophosphate by Saturation Analysis

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The principle of saturation analysis, first applied to the measurement of thyroxine (Ekins, 1960) and vitamin B₁₂ (Barakat & Ekins, 1961), has been used to develop a simple and sensitive assay for cyclic AMP (adenosine 3':5'-cyclic monophosphate). The assay depends upon the specific cyclic AMP-binding protein in adrenal tissue described by Gill & Garren (1969), the specificity and reaction-energy characteristics of which suggested its possible use as a specific reagent for assay purposes (Brown & Ekins, 1970).

Bovine adrenal cortices were homogenized in a medium comprising 0.25M-sucrose, 50mM-tris, pH 7.4, 25mM-potassium chloride and 5mM-magnesium chloride. After centrifugation at 4°C at 2000g for 5 min, the supernatant was respun at 5000g for 15 min. The resultant supernatant was stored at -20°C in 0.5ml portions. Dilutions of this preparation in 50mM-tris buffer, pH 7.4 (containing 8mM-theophylline and 6mM-2-mercaptoethanol), were used as the binding agent.

Standard response curves were obtained by

incubating serial dilutions of cyclic AMP (0–15 pmol) with cyclic [³H]AMP and a dilution of the binding agent at 4°C. After 1.5 h a suspension of 10 mg of charcoal (Norit GSX) in tris buffer containing bovine serum albumin (2%, w/v) was added to each tube to separate the free and bound moieties. The radioactivity of portions of the supernatant obtained after centrifugation (i.e. the bound fraction) was counted in a liquid-scintillation spectrometer and the radioactivity observed was related to the total initial ³H radioactivity present. In the determination of cyclic AMP in biological material cyclic [³H]AMP was added to the sample before extraction and the radioactivity served in the determination both of extraction recovery and the distribution ratio. The appropriate controls, blanks and standards were included in each run.

Investigations of the specificity characteristics of the binding protein have indicated that, of all the nucleotides and nucleosides studied, only cyclic GMP (guanosine 3':5'-cyclic monophosphate) demonstrated measurable cross-reaction (0.3% under normal assay conditions). The lower limit of detection of cyclic AMP in this system is approx. 0.2 pmol/incubation tube.

The application of this assay system to biological extracts will be discussed.

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The Effects of Secretin, Pancreozymin and Acetylcholine on the Concentration of Adenosine 3':5'-Cyclic Monophosphate in Cat Pancreas

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The effect of secretin, but not that of pancreozymin, can be mimicked by the dibutyl derivative of cyclic AMP (adenosine 3':5'-cyclic monophosphate) in the isolated cat pancreas (Case, Laundry & Scratcherd, 1969). By contrast cyclic AMP and its dibutyl derivative have been reported to increase enzyme extrusion by isolated mouse and rabbit pancreas (Kulka & Sternlicht, 1968; Ridderstap & Bonting, 1969; Knodell, Toskes, Heber & Brooks, 1970). The present study was designed to assess more accurately the role of cyclic AMP in the action of secretin and pancreozymin.

Young lean cats, starved for 18 h, were anaesthetized with Nembutal and their pancreatic ducts were

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.