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 dibutyryl derivative of cyclic AMP (adenosine 3':5'-cyclic monophosphate) in the isolated cat pancreas (Case, Laundry & Scratcherd, 1969). By contrast cyclic AMP and its dibutyryl 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