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 [$2\text{-}^{14}\text{C}$]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 [$2\text{-}^{14}\text{C}$]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

(adenosine 3':5'-cyclic monophosphate) before and after incubation has also been used (Butcher & Sutherland, 1962). These methods are time-consuming and subject to interference from inhibitors. Liberation of [^{32}P]P_i from cyclic [^{32}P]AMP has been used (Schönhöfer, Skidmore, Krishna & Bourne, 1970), but this substrate was not readily available until recently.

The present method, which is adapted from the cyclic AMP assay method of Brooker, Thomas & Appleman (1968), is rapid and well suited to measuring inhibitor kinetics. A 50 μl portion of enzyme is incubated with 50 μl of inhibitor and 50 μl of cyclic [^3H]AMP as substrate, and the reaction is stopped by adding a suspension of Dowex 1. Excess of snake-venom nucleotidase is present to convert the AMP formed into adenosine. The resin adsorbs unchanged cyclic AMP, leaving adenosine in solution, and on the addition of scintillation fluid only the radioactivity in the adenosine is counted as the radiation from the cyclic AMP is quenched by the resin. Blanks are about 10% of the total counts.

A feature of this method is that very low substrate concentrations can be employed, and by using concentrations in the range 0.03–0.60 μM the K_m for ox heart enzyme prepared by the method of Butcher & Sutherland (1962) to the heat-denaturation stage was found to be $8.25 (\pm 1.63) \times 10^{-7} \text{M}$ (11). However, if higher substrate concentrations were used (3–300 μM) the K_m was $2 \times 10^{-5} \text{M}$. These findings are further discussed by Skidmore & Hindley (1970).

Kinetic studies were made with theophylline and I.C.I. 58301 (3-acetamido-6-methyl-8-*n*-propylsyn-triazolo[4,3-*a*]pyrazine), a new compound with bronchodilator properties in animals. Owing to the presence of two enzyme activities the kinetic pattern is complex, but both substances appear to be mixed inhibitors.

The tricyclic antidepressants imipramine, desmethylinipramine, amitriptyline and nortriptyline were also tested as phosphodiesterase inhibitors. Only amitriptyline was found to have significant activity, but it was much less potent than caffeine, contrary to the report by Abdulla & Hamadah (1970).

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3':5'-Cyclic Nucleotide Phosphodiesterase Activity in Mammalian Tissues

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The introduction of sensitive methods for measuring the activity of 3':5'-cyclic nucleotide phosphodiesterases (Somerville, Rabouhans & Smith, 1970; Schönhöfer, Skidmore, Krishna & Bourne, 1970) has resulted in a re-evaluation of the activity of these enzymes. In many mammalian tissues at least two enzymic activities are present that differ in their affinities for the substrate cyclic AMP (adenosine 3':5'-cyclic monophosphate). K_m values of both $8 \times 10^{-7} \text{M}$ and 5×10^{-6} – 10^{-5}M have been obtained in soluble preparations from guinea-pig heart and lung, and in some preparations there is evidence of a further phosphodiesterase activity with low affinity for the substrate: in ox heart K_m $5 \times 10^{-4} \text{M}$, and in rat fat-cell K_m 10^{-3}M (Schönhöfer *et al.*, 1970). In many tissues cyclic GMP is also hydrolysed rapidly.

In view of the low concentrations of cyclic AMP present in most tissues under normal conditions it seems likely that the activities of phosphodiesterase with high affinity for this nucleotide are important in regulating the concentration of the nucleotide within the cell.

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A Kinetic Study of the Enzymic Hydrolysis of Adenosine 3':5'-Cyclic Monophosphate

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3':5'-Cyclic nucleotide phosphodiesterase was extracted from ox heart by the method of Butcher & Sutherland (1962), and the kinetics of the enzymic hydrolysis of cyclic AMP (adenosine 3':5'-cyclic phosphate) were studied by using the method reported by Nair (1966).

A study of the effect of pH on the values of V_{max} and K_m at 37°C has revealed the presence of two kinetically significant ionizing groups in the enzyme. One has pK 6.8, which is unchanged on formation of the enzyme-substrate complex; the other has pK 9.8, which increases to 10.5 on formation of the enzyme-substrate complex, showing that