(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