increase the rate of efflux of pregnenolone from the mitochondrion.

Besides acutely stimulating steroidogenesis adrenocorticotrophin also causes adrenocortical growth. Grahame-Smith *et al.* (1967) showed in the rat that at a time after hypophysectomy when adrenal weight had fallen and the acute steroidogenic response to adrenocorticotrophin had disappeared the sensitivity of the adrenal cyclic AMP response to adrenocorticotrophin was undiminished. R. L. Ney (1969) has since shown that cyclic AMP is capable of maintaining adrenal weight in hypophysectomized rats. These experiments indicate that cyclic AMP may also be the intracellular mediator of this action of adrenocorticotrophin.

Davis, W. W. & Garren, L. D. (1966). *Biochem. biophys. Res. Commun.* **24**, 805.

Davoren, P. R. & Sutherland, E. W. (1963). *J. biol. Chem.* **238**, 3016.

Dexter, R. N., Fishman, L. M., Ney, R. L. & Liddle, G. W. (1967). *Endocrinology*, **81**, 1185.

Farese, R. V. (1967). *Biochemistry, Easton*, **6**, 2052.

Ferguson, J. J., jun. (1963). *J. biol. Chem.* **238**, 2754.

Garren, L. D. (1968). *Vitams Horm.* **26**, 119.

Garren, L. D., Ney, R. L. & Davis, W. W. (1965). *Proc. natn. Acad. Sci. U.S.A.* **53**, 1443.

Gill, G. N. & Garren, L. D. (1969). *Proc. natn. Acad. Sci. U.S.A.* **63**, 512.

Grahame-Smith, D. G., Butcher, R. W., Ney, R. L. & Sutherland, E. W. (1967). *J. biol. Chem.* **242**, 5535.

Halkerston, I. D. K., Eichhorn, J. & Hechter, O. (1961). *J. biol. Chem.* **236**, 374.

Haynes, R. C. (1958). *J. biol. Chem.* **233**, 1220.

Haynes, R. C., Koritz, S. B. & Peron, F. G. (1959). *J. biol. Chem.* **234**, 1421.

Imura, H., Matsukara, S., Matsuyama, H., Sitsuda, T. & Miyake, T. (1965). *Endocrinology*, **76**, 933.

Karaboyas, G. C. & Koritz, S. B. (1965). *Biochemistry, Easton*, **4**, 462.

Koritz, S. B. & Kumar, A. M. (1970). *J. biol. Chem.* **245**, 152.

Lefkowitz, R. J., Roth, J., Pricer, W. & Pastan, I. (1970). *Proc. natn. Acad. Sci. U.S.A.* **65**, 745.

Ney, R. L. (1969). *Endocrinology*, **84**, 168.

Stone, D. & Hechter, O. (1954). *Archs Biochem. Biophys.* **51**, 457.

Sutherland, E. W., Oye, I. & Butcher, R. W. (1965). *Recent Prog. Horm. Res.* **21**, 263.

Taunton, O. D., Roth, J. & Pastan, I. (1967). *Biochem. biophys. Res. Commun.* **29**, 1.

Tchen, T. T. (1968). In *Function of the Adrenal Cortex*, vol. 1, p. 3. Ed. by McKerns, K. W. New York: Appleton.

The Role of 3':5'-Cyclic Nucleotide Phosphodiesterase in Regulating Tissue Concentrations of Adenosine 3':5'-Cyclic Monophosphate

By J. I. DAVIES. (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns., U.K.*)

Specificity studies on 3':5'-cyclic nucleotide phosphodiesterases of various tissues (Nair, 1962; Butcher & Sutherland, 1962; Drummond & Perrott-Yee, 1961) suggest that their sole function is to regulate concentrations of 3':5'-cyclic nucleotides. Since cyclic GMP (guanosine 3':5'-cyclic monophosphate) has been found to occur widely (Goldberg, Dietz & O'Toole, 1969; Ishikawa, Ishikawa, Davis & Sutherland, 1969), however, it cannot be assumed that they regulate the concentration of cyclic AMP (adenosine 3':5'-cyclic monophosphate) exclusively. No phosphodiesterase has been shown to be absolutely specific to a single cyclic nucleotide, though enzymes with distinct preferences for either purine nucleotides or pyrimidine nucleotides are known (Hardman & Sutherland, 1965).

Although the subject has not been systematically studied it appears that the phosphodiesterases of various tissues are not identical. Since the relative activity of ox heart phosphodiesterase towards cyclic UMP (uridine 3':5'-cyclic monophosphate) gradually changes during purification (Hardman & Sutherland, 1965), we may conclude that the enzyme multiplicity extends to individual organs. It is not clear whether in this instance the enzymes are derived from different cells within the organ. Certainly in epididymal adipose tissue only about one-quarter of the phosphodiesterase activity resides in the fat-cells (Hepp, Menahan, Wieland & Williams, 1969), the remainder being found in the stromal-vascular fraction (J. I. Davies, unpublished work).

The possibility still remains that individual cells contain more than one enzyme species. Alternatively, differences in its physical environment, as reflected by its soluble or particulate distribution, may affect its properties.

The problems posed by possible heterogeneity in enzyme preparations are illustrated by the existing confusion over the K_m values of various phosphodiesterases. Using an enzyme preparation from rat brain and concentrations of cyclic AMP within the range 0.1–1.0 mM, Brooker, Thomas & Appleman (1968), like many others, obtained a K_m value of the order of 10^{-4} M. With lower concentrations of cyclic AMP, however, the same enzyme preparation yielded a K_m value of 10^{-6} M. This anomaly can be interpreted in several ways. First, the preparation may contain two distinct enzymes or, alternatively, a single enzyme in different

physical environments. It is also possible that the non-linearity of the Lineweaver-Burk plot, which is responsible for the discrepancy, is due to some kinetic property of this enzyme.

A variety of substances have been shown to alter the activity of phosphodiesterases. The reducing agents 2-mercaptoethanol and dithioerythritol at 5mM concentrations double the activity of dialysed rat brain enzyme. Their action is distinct from that of imidazole. The effect of imidazole, which is maximal at 15mM, is primarily to elevate $V_{\max}$, though at higher concentrations K_m is also increased.

The nucleosides and nucleotides of guanine, hypoxanthine and adenine inhibit rat brain phosphodiesterase with potencies comparable with those of the methylxanthines. Since the inhibitions are strictly competitive (with cyclic AMP concentrations about 0.1–1.0mM) it appears that their actions reflect a relatively broad specificity in the active site of phosphodiesterase. Although Cheung (1967) found that a variety of nucleoside triphosphates, inorganic pyrophosphate and citrate were also inhibitory, it appears likely that their Mg^{2+} -sequestering properties were at least partially responsible.

The primary value of investigating the properties of isolated preparations of phosphodiesterase is to provide an understanding of its function in the intact cell. Emphasis is placed here on the situation that exists in adipose tissue, where a clear relationship has been established between cyclic AMP concentrations and the rate of lipolysis. In this system it appears that cyclic AMP concentrations can be adequately interpreted in terms of the relative activities of adenylate cyclase and phosphodiesterase. Intracellular compartmentation of cyclic AMP has been suggested as a complicating factor by Kuo & De Renzo (1969), but, though it seems likely to have some influence, there is no convincing evidence of its importance.

In an attempt to quantify the relationship between cyclic AMP and the activities of adenylate cyclase and phosphodiesterase, Davies & Williams (1970) derived the relationship:

$$[\text{Cyclic AMP}] = \frac{K_2 \mathcal{V}_{11}}{V_2 - \mathcal{V}_{11}} + \frac{K_2 V_2 \left(\frac{\mathcal{V}_{12} - \mathcal{V}_{11}}{V_2 - \mathcal{V}_{11}} \right) [H]}{cK_H(V_2 - \mathcal{V}_{11}) - (\mathcal{V}_{12} - V_2)[H]}$$

K_2 and V_2 are the K_m and $V_{\max}$ values for phosphodiesterase. $\mathcal{V}_{11}$ and $\mathcal{V}_{12}$ represent the maximum rates of cyclic AMP formation at any specified ATP concentration when adenylate cyclase is in the non-activated and the hormone-activated form respectively. $[H]$ is the hormone concentration and

K_H is the dissociation constant of the hormone-adenylate cyclase interaction. The constant c differs from unity only if the interaction of hormone with adenylate cyclase alters the affinity of the latter for ATP.

The relationship between [cyclic AMP] and $[H]$ predicted takes the form of a rectangular hyperbola with the expected maximum in cyclic AMP if $V_2 > \mathcal{V}_{12}$, i.e. if the capacity of phosphodiesterase to destroy cyclic AMP exceeds the capacity of adenylate cyclase to bring about its synthesis. Efforts to demonstrate the validity of the relationship are in a preliminary stage. For these purposes the crude approximation has been made that over a limited range cyclic AMP concentrations in adipose tissue can be equated to the rate of lipolysis. The predicted effects of manipulating K_2 (with methylxanthines) and K_H (with catecholamine analogues and β -adrenergic blocking agents) are in good agreement with experimental findings.

On the basis of the above equation it is possible to predict the effect of changing the parameters of phosphodiesterase. The results serve to emphasize the profound effect on cyclic AMP concentrations made possible by altering the activity of this enzyme.

Brooker, G., Thomas, L. J. & Appleman, M. M. (1968). *Biochemistry, Easton*, **7**, 4177.

Butcher, R. W. & Sutherland, E. W. (1962). *J. biol. Chem.* **237**, 1244.

Cheung, W. Y. (1967). *Biochemistry, Easton*, **6**, 1079.

Davies, J. I. & Williams, P. A. (1970). *J. theor. Biol.* (in the Press).

Drummond, G. I. & Perrott-Yee, S. (1961). *J. biol. Chem.* **236**, 1126.

Goldberg, N. D., Dietz, S. B. & O'Toole, A. G. (1969). *J. biol. Chem.* **244**, 4458.

Hardman, J. G. & Sutherland, E. W. (1965). *J. biol. Chem.* **240**, 3704.

Hepp, K. D., Menahan, L. A., Wieland, O. & Williams, R. H. (1969). *Biochim. biophys. Acta*, **184**, 554.

Ishikawa, E., Ishikawa, S., Davis, J. W. & Sutherland, E. W. (1969). *J. biol. Chem.* **244**, 6371.

Kuo, J. F. & De Renzo, E. C. (1969). *J. biol. Chem.* **244**, 2252.

Nair, K. G. (1962). *Biochemistry, Easton*, **5**, 150.

Adenylate Cyclase and 'Adenosine Triphosphatase' in Corpus Luteum

By D. A. STANSFIELD and D. J. FRANKS. (*Department of Biochemistry, University of Dundee, Dundee, U.K.*)

Cyclic AMP (adenosine 3':5'-cyclic monophosphate) is believed to be the mediator of many hormonal effects (see Robison, Butcher & Sutherland, 1968; Sutherland, Hardman, Butcher & Broadus, 1969). The formation of cyclic AMP is catalysed by adenylate cyclase, an enzyme present in