that the control may be effected via phosphorylation of a membrane protein. We have therefore investigated the phosphorylation of the erythrocyte membrane and its stimulation by cyclic AMP on isolated membranes, erythrocyte 'ghosts' and intact erythrocytes.

Membrane phosphorylation in the presence or in the absence of cyclic AMP was measured by the transfer of ^{32}P from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ to membrane components. Erythrocyte membranes were prepared by the method of Hoozeven *et al.* (1970). Cyclic AMP and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ were incorporated into erythrocyte 'ghosts' by the reversed-haemolysis method of Whittam (1962). Intact erythrocytes were allowed to synthesize endogenous $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ by incubating fresh citrated blood with $[\text{P}^{32}]\text{P}_i$ for 45 min. Dibutyl cyclic AMP (6-*N*,2'-*O*-dibutyladenosine 3':5'-cyclic monophosphate) was used in experiments with intact erythrocytes.

In all experiments phosphorylation of the membrane was stimulated by cyclic AMP in intact erythrocytes, 'ghosts' or isolated membranes. Noradrenaline and prostaglandin, which had been shown to enhance the synthesis of cyclic AMP in rat erythrocytes (Sheppard & Burghardt, 1970), also stimulated the incorporation of ^{32}P into components of the membrane. Inhibition of phosphodiesterase enhanced the cyclic-AMP-stimulated phosphorylation.

Separation of the membrane lipid and protein (Boyer & Bieber, 1967) showed that 80% of the bound ^{32}P was in the latter fraction and that the stimulatory effect of cyclic AMP on ^{32}P incorporation was confined to the protein. Hydrolysis of the membrane protein, followed by high-voltage paper electrophoresis and radioautography, yielded phosphoserine and phosphothreonine as the only amino acids containing ^{32}P .

On fractionation of the membrane proteins by electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulphate, six or seven discrete bands were obtained. The three bands representing the highest-molecular-weight proteins contained most of the ^{32}P , and the additional label resulting from stimulation by cyclic AMP was mainly in band 3 (mol.wt. approx. 100000).

Our findings indicate that human erythrocytes are capable of responding to cyclic AMP by increased incorporation of ^{32}P in membrane protein. Whether this observation is of physiological significance and whether it is a property of mature erythrocytes or of reticulocytes remains to be established.

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Mode of Action of Adenosine 3':5'-Cyclic Monophosphate in Insulin Secretion

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It has been shown that the intracellular concentration of cyclic AMP (adenosine 3':5'-cyclic monophosphate) may play a role in determining the rate of insulin release from islets of Langerhans (Montague & Cook, 1971). Since the effects of cyclic AMP in a variety of tissues appear to be related to its binding to a specific receptor protein, which may lead directly to the activation of a protein phosphokinase, we have investigated the occurrence of cyclic-AMP-binding protein in islets of Langerhans and its relationship to cyclic-AMP-dependent protein phosphokinase activity in the same tissue.

Islets of Langerhans prepared by collagenase digestion of rat or guinea-pig pancreas were homogenized at 4°C in 10mM-sodium phosphate buffer, pH 6.5, containing 0.3M-sucrose, and the homogenate was separated by centrifugation into nuclear, mitochondrial, granule, microsomal and supernatant fractions (Howell *et al.*, 1969). Cyclic-AMP-binding and protein phosphokinase activities in the fractions were determined by the methods of Walton & Garren (1970) and Miyamoto *et al.* (1969). Over 90% of the total of both activities present in the homogenate was recovered in the supernatant fraction. Purification of this fraction by ammonium sulphate precipitation followed by polyacrylamide-gel electrophoresis resulted in a single component possessing both cyclic-AMP-binding and cyclic-AMP-dependent protein phosphokinase activities. The cyclic-AMP-dependent protein phosphokinase was capable of phosphorylating not only exogenous histone but also soluble endogenous islet proteins.

Colchicine is known to bind specifically to microtubular protein, to interfere with microtubular function and in islets of Langerhans to inhibit the secretion of insulin. These observations have led to the suggestion that contraction of a microtubular system may provide the mechanism of translocation of insulin-storage granules through the cell during their release (Lacy *et al.*, 1968). It was of considerable interest therefore to find that the cyclic-AMP-stimulated phosphorylation of endogenous islet proteins was significantly inhibited in the presence of colchicine (0.5mM), although phosphorylation of exogenous histone was unaffected under these conditions. It seems possible that the mode of action

of cyclic AMP in stimulating insulin release may be to promote microtubular function by increasing the phosphorylation of microtubular proteins.

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Adenosine 3':5'-Cyclic Monophosphate and Adenylate Cyclase in Subcellular Fractions of Rat Brain

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A cyclic-AMP (adenosine 3':5'-cyclic monophosphate)-binding protein prepared from bovine adrenal cortex has been used to measure cyclic AMP by saturation analysis (Brown *et al.*, 1971).

Rat brains were homogenized and fractionated as described by Gray & Whittaker (1962). Crude mitochondrial fractions from 1g wet wt. of brain contained 3pmol of cyclic AMP, all of which could be hydrolysed by incubation with a preparation of rat brain phosphodiesterase. No cyclic AMP was found in the high-speed supernatant. Subfractionation of crude brain mitochondrial fractions on a discontinuous Ficoll gradient revealed the following distribution of cyclic AMP: myelin fraction, 11.5%; synaptosomal fractions, 77.2%; mitochondrial fraction, 11.3%.

The action of sodium fluoride (10mM), in the presence of theophylline, Mg^{2+} and ATP, to stimulate adenylate cyclase activity was used to study the distribution of this enzyme activity in the same fractions. The following distribution was obtained: myelin fraction, 37.3%; synaptosomal fractions, 49.8%; mitochondrial fraction, 12.9%.

There have been several reports that brain monoamines variably increase the production of cyclic AMP in brain slices. Production of cyclic AMP by crude mitochondrial fractions has therefore been studied in the presence of noradrenaline, dopamine (3,4-dihydroxyphenethylamine), 5-hydroxytryptamine and histamine (10mM). No significant change in the production of cyclic AMP could be demonstrated, although sodium fluoride reliably produced a tenfold increase. Adenosine and acetylcholine were also inactive in crude mitochondrial

fractions. We conclude that these monoamines do not stimulate synaptosomal adenylate cyclase and that their action on brain slices may be either post-synaptic or on non-neuronal tissue. However, one cannot exclude a loss of hormonal response engendered by the tissue preparation.

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The Effect of Putative Transmitters and other Agents on Phosphoprotein Turnover in Respiring Slices of Guinea-Pig Cerebral Cortex

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The application of electrical pulses to respiring slices of cerebral cortex increases the rate of incorporation of [^{32}P]orthophosphate into protein-bound phosphoserine residues, probably located in a membrane protein (Heald, 1957; Trevor & Rodnight, 1965; Jones & Rodnight, 1971). Electrical stimulation of brain slices under similar conditions also increases the cyclic AMP (adenosine 3':5'-cyclic monophosphate) content of the tissue (Kakiuchi *et al.*, 1969). Since membrane fragments prepared from cortical tissue contain a bound protein kinase, stimulated by cyclic AMP and mediating the phosphorylation of a membrane protein (Weller & Rodnight, 1970, 1971), it appears possible that the increased turnover of protein-bound phosphoserine on stimulation results from an increased content of cyclic AMP in the tissue. If this interpretation is correct other agents, such as biogenic amines and adenine derivatives, which increase the cyclic AMP content of respiring cortical slices (Shimizu *et al.* 1970; Sattin & Rall, 1970), might also be expected to have a similar effect on phosphoprotein turnover.

To investigate this hypothesis slices of guinea-pig cerebral cortex were cut and incubated with [^{32}P]orthophosphate as described by Jones & Rodnight (1971). Incorporation of ^{32}P into protein-bound phosphoserine was taken as the radioactivity (c.p.m.) in the alkali-labile P of the tissue proteins relative to the radioactivity (c.p.m.) in the acid-soluble P of the tissue. Exposure of slices to noradrenaline (0.5mM), histamine (0.1mM) or serotonin (5-hydroxytryptamine) (1 μ M) for 10min increased the rate of incorporation of ^{32}P into protein by 10-15% ($P < 0.005$), but had no significant effect on the relative specific radioactivity of the γ -P of ATP. In a comparable series of experiments electrical stimulation for 10s increased incorporation by 15-20% ($P < 0.0025$)