PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 490th Meeting of the Society was held at the University of Manchester on Thursday and Friday, and 10 January 1969, when the following papers were presented:

COLLOQUIUM ON 'CONTROL OF CARBOHYDRATE METABOLISM'

Carbohydrate Metabolism in Pancreatic Islets and the Release of Insulin

By P. J. RANDLE and S. J. H. ASHCROFT. (*Department of Biochemistry, University of Bristol*)

After synthesis within the pancreatic β -cell insulin is stored mainly in secretion granules. The secretion of insulin may involve discharge of granules (emiocytosis) and the mechanism may involve Na^+ -dependent uptake of Ca^{2+} (Lacy, 1961; Milner & Hales, 1968). The secretion process is regulated by chemoreceptors in the β -cell sensitive to such major physiological effectors as glucose and amino acids. Hormones other than insulin may also influence secretion and their effects may be mediated by adenylyl cyclase and 3'5'-(cyclic)-AMP.

It has been suggested that an intracellular metabolite of glucose may mediate the effect of glucose on release, since metabolism of sugars appear to correlate with effects of release of insulin (Coore & Randle, 1964; Grodsky *et al.* 1963). Mannoheptulose suppresses the effect of glucose on release and almost totally inhibits oxidation of glucose by mouse pancreatic islets prepared by microdissection or collagenase digestion, whereas phlorrhizin has no effect on either parameter (Coore & Randle, 1964; Ashcroft & Randle, 1968a).

The most detailed studies of quantitative aspects of the glucose effect have been made with rat pancreas pieces *in vitro* (Malaisse, Malaisse-Lagae & Wright, 1967). The rate curve was sigmoid, i.e. it showed a threshold at about 5mM and tended towards a maximum at about 30mM-glucose. Half-maximum rates of release (K_m) were seen with about 10mM-glucose. Rate curves for glucose oxidation by mouse islets were similar with a threshold of about 5mM-glucose, a K_m of approx. 7mM and tending to a maximum at 12mM-glucose (Ashcroft & Randle, 1968a). Since mannoheptulose (an inhibitor of ATP-D-glucose 6-phosphotransferases) inhibits the effect of glucose on release and the oxidation of glucose by mouse islets it has been suggested that the glucoreceptor is an enzyme or enzymes concerned with glucose phosphorylation.

Homogenates of normal mouse islets contain activities corresponding to those of hexokinase, glucokinase and glucose 6-phosphatase. At physio-

logical ATP concentrations (2.5mM) the hexokinase activity has a low K_m value for glucose (60 μM) and is inhibited competitively by mannoheptulose (K_i 0.2mM) and non-competitively by glucose 6-phosphate (K_i 0.2mM). The total activity is 0.54 unit/g. of islet. The enzyme activity is unusual in that the glucose K_m is markedly dependent on ATP concentration, being 5.3mM at 0.1mM-ATP (Ashcroft & Randle, 1968b). The glucokinase activity is much lower and totals only 0.17 unit/g. of islet. Glucokinase was not detected in an earlier study and it has only been readily detected in more recent studies by inhibiting hexokinase with glucose 6-phosphate. The K_m (for glucose) is of the order of 20mM. The glucose 6-phosphatase activity totals 0.7 unit/g. of islet. The K_m for glucose 6-phosphate was 1mM and it was inhibited by glucose (mixed inhibition), half-maximal inhibition occurring at approx. 10mM-glucose. Mannose was not an inhibitor of the phosphatase, and since mannose stimulates insulin release and is oxidized (like glucose) in a concentration-dependent manner it seems doubtful whether the phosphatase contributes to the glucoreceptor mechanism. It has not been possible to demonstrate activity of the phosphatase in the intact islet by measuring the conversion of mannose into glucose (Ashcroft & Randle, 1968c). In studies with islets from obese-hyperglycaemic mice, Matschinsky & Ellerman (1968) found that glucose phosphorylation is rate-limiting for metabolism of the sugar. Hexokinase and glucokinase were detected at activities comparable to those given above for normal mouse islets.

The glucoreceptor mechanism may thus involve hexokinase and glucokinase but is incompletely understood. The kinetic properties of the enzymes may not account for the shape of the rate curves for release or for glucose oxidation, nor may they account for the total inhibition by mannoheptulose of the effect of glucose on release and the oxidation of glucose. Possible glucoreceptor mechanisms will be discussed in detail.

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Regulation of Glycolysis and Gluconeogenesis at the Enzyme Level

By A. SOLS. (*Departamento de Enzymologia, Centro de Investigaciones Biológicas, C.S.I.C., Madrid, Spain*)

In contrast with the very wide occurrence of glycolysis as a major metabolic pathway, gluconeogenesis is restricted to certain types of cells. In most cases where capacity for gluconeogenesis does occur, it coexists with that for glycolysis. In fact, the former shares many reversible steps with the latter. The precise number and kind of physiologically irreversible steps to be by-passed by antagonistic enzymes can vary among different tissues and organisms, particularly at the initial and final steps.

The understanding at the enzyme level of the mechanisms involved in the regulation of each of these major metabolic pathways has recently made great progress in the case of glycolysis (Sols, 1967a). This has led to a coherent interpretation of the mechanism of the Pasteur effect as the result of the integration of several feedback allosteric mechanisms operating in series (Sols, 1967a,b). In contrast with this progress, the interpretation of the regulation of gluconeogenesis is still largely unsolved.

The study of the potentially key enzymes involved at the metabolic 'cross-roads' in cells that can oscillate between glycolysis and gluconeogenesis according to circumstances is a challenging problem in itself, and can also help towards a better understanding of the intrinsic regulation of each of these potentially antagonistic pathways. This dual purpose is involved in recent and current work in the author's Laboratory with two types of cells, those of liver and yeast, that have in common a noted ability to carry on readily either glycolysis or gluconeogenesis according to circumstances. Occasional comparison of the occurrence of certain key enzymes or isoenzymes in related tissues is proving to be of considerable help for a deeper physiological understanding of potentially regulatory mechanisms. A clear case is the identification of the

allosteric activation of certain pyruvate kinases as a control mechanism related to the potential switch-over to gluconeogenesis rather than intrinsic to the regulation of glycolysis (Gancedo, Gancedo & Sols, 1967b).

Adaptive changes involving the amount or the activity of certain key enzymes in liver (Sillero, Sillero & Sols, 1968) and yeast (Gancedo, Gancedo & Sols, 1967a) will be shown, and their likely physiological significance will be discussed. In both systems it has been found that the concentrations of irreversible enzymes at the initial and final stages of the glycolytic and gluconeogenic pathways can be controlled independently of each other, a fact that affords a valuable metabolic flexibility. Moreover, it will be shown that in liver some of the reported changes in the activities of irreversible enzymes are irrelevant in terms of the switch-over from glycolysis to gluconeogenesis.

Emphasis will be placed in the discussion on the enzyme-regulation problems in the key metabolic triangle involving the oxaloacetate-phosphoenolpyruvate-pyruvate 'cross-roads' in rat liver (Marco, Pestaña, Pérez-Díaz & Sols, 1968), currently under study in the author's Laboratory. The fact that at this level carbohydrate, lipid, protein and energy metabolisms are variously interconnected in liver, gives a very wide significance to the understanding of the regulation of this metabolic triangle.

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The Control of Glycolysis in Rat Epididymal Adipose Tissue

By R. M. DENTON, M. L. HALPERIN* and C. I. POGSON. (*Department of Biochemistry, University of Bristol*)

The mechanisms of glycolytic regulation in adipose tissue may not necessarily be similar to those in muscle and brain. Glycolysis in adipose tissue is primarily involved in the provision of L-glycerol 3-phosphate and acetyl-CoA for lipid

* Present address: Department of Medicine, University of Toronto, Ont., Canada.