Glucose and the Survival and Recovery of the Anoxic Myocardium

By D. J. HEARSE and E. B. CHAIN (*Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AY, U.K.*)

The mammalian heart is particularly sensitive to periods of O₂ deprivation. Under hypoxic stress, induced either clinically or pathologically, the survival and subsequent recovery of the heart depends on the ability of the myocardium to maintain its biochemical and morphological integrity. An isolated perfused working rat heart preparation (Chain *et al.*, 1969) has been used to investigate the biochemical changes associated with complete anoxia and post-reoxygenation recovery, and also the role played by glucose, K⁺ and insulin in aiding the survival and recovery of the anoxic myocardium.

Within 30s from the onset of anoxia the heart is unable to perform any external work, the creatine phosphate content of the heart falls to less than 50% of its aerobic control value, the ATP content falls less rapidly but after 20min is decreased to less than 10%, the P_i content increases accordingly and there is a great increase in lactate production. Under certain circumstances, even after prolonged anoxia (as long as 30min), after reoxygenation the heart is able to recover almost complete mechanical activity [as monitored by aortic output against a 0.13 MN/m² (100cmH₂O) pressure head]. Recovery is paralleled by a very rapid increase in the creatine phosphate content, a progressive rise in ATP content, a corresponding fall in ADP and AMP contents and a sharp fall in lactate production. It was found that the presence of glucose and insulin in the perfusion fluid during anoxia was essential for complete recovery, and that a high concentration of K⁺ increased not only the rate of recovery but also the final extent of recovery. Glucose could not be replaced by pyruvate or lactate, but could be replaced by high concentrations of glucose 1-phosphate (Beloff-Chain *et al.*, 1953). The ability of the heart to recover is proportional to the myocardial ATP content at the time of reoxygenation. The presence of glucose (11.1mM) during the anoxic period results in a striking diminution in the rate of decrease of ATP and creatine phosphate contents. It is proposed that glucose aids in the survival and recovery of the anoxic heart by serving as an exogenous source of substrate for anaerobic glycolysis, and thereby facilitating the maintenance of higher ATP and creatine phosphate contents, which in turn promote survival and recovery of the heart.

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Formation of Glucose from Glucose 6-Phosphate by Rat Brain Slices

By P. R. DODD, H. F. BRADFORD and E. B. CHAIN (*Medical Research Council Metabolic Reactions Research Unit, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AY, U.K.*)

During investigations into the metabolism of glucose 6-phosphate by rat cerebral-cortex slices it became clear that one of the metabolites formed was glucose (Balliano *et al.*, 1969; Dodd *et al.*, 1971). Since glucose is much preferred to glucose 6-phosphate as a substrate for brain slices, it was decided to investigate the extent of glucose formation from glucose 6-phosphate *in vitro*, as this could be an obligatory first stage in its metabolism.

Rat cerebral-cortex slices were maintained for 1h at 38°C in bicarbonate- or tris-buffered saline in the presence of 25mM-glucose 6-phosphate as substrate, 0.25% (w/v) inulin and 1μCi of [*carboxy*-¹⁴C]inulin. Perchloric acid extracts of tissue and medium were prepared and ¹⁴C radioactivity and glucose determined. The tissue/medium ratio of glucose was approx. 10 times the tissue/medium ratio of ¹⁴C, indicating the presence of intracellular glucose. In contrast, no evidence for the existence of an intracellular glucose pool was found with 10mM-glucose substrate. Evidence for such a pool was found with 25mM-fructose 6-phosphate or 10mM-fructose as substrate, but not with 25mM-fructose 1,6-diphosphate or 25mM-glycerol 2-phosphate as substrate, nor when no substrate was present.

When O₂ uptake and lactate formation were inhibited with CN⁻ and iodoacetate, glucose formation from glucose 6-phosphate continued: approx. 12μmol of glucose/g initial wt. and 15μmol of P_i/g initial wt. accumulated in 1h in the medium from brain slices incubated at 38°C with 25mM-glucose 6-phosphate in tris-buffered saline (a similar amount of P_i was also formed in the absence of the poisons). This apparent glucose 6-phosphatase activity in the medium was inhibited by HCO₃⁻ and P_i ions, but not by glucose. A number of phosphate esters (*p*-nitrophenyl phosphate, glycerol 2-phosphate, fructose 6-phosphate, glucose 1-phosphate) were lysed under similar conditions to those used for glucose 6-phosphate.

An increase in K⁺ concentration in the medium to 55mM, which caused an increase in slice hourly

lactate accumulation rate in tris- or bicarbonate-buffered saline (10 and 30% respectively) with 25 mM-glucose 6-phosphate as substrate, caused a 37% decrease in the internal tissue glucose concentration in bicarbonate-buffered medium. Tissue glycogen concentration was unaffected. Insulin (0.01 i.u./slice) had no effect on tissue glucose concentration in bicarbonate-buffered saline. Since isolated synaptosomes, incubated for 1 h at 38°C in tris-buffered saline with 25 mM-glucose 6-phosphate, had a tissue glucose concentration (per mg of protein) some 10 times that found in slices under similar incubation conditions (glucose concentrations in the medium were similar), it is possible that a large part of the intracellular glucose pool may be located in nerve endings.

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Synaptosome Beds: a Method for the Study *in vitro* of the Metabolism and Function of Nerve Endings

By J. S. DE BELLEROCHÉ and H. F. BRADFORD (*Medical Research Council Metabolic Reactions Research Unit, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AY, U.K.*)

The functional unit of chemical transmission in the nervous system is the nerve ending. When cortex tissue is homogenized in iso-osmotic sucrose, the nerve endings seal off from the axon and may be prepared by sucrose-density-gradient centrifugation (Gray & Whittaker, 1962). Suspensions of nerve endings or synaptosomes exhibit the metabolic properties of cellular material, being able to carry out active glycolysis and oxidation of added glucose, linked to ATP and phosphocreatine production (Bradford, 1969).

When applied iontophoretically to the mammalian cerebral cortex, small concentrations of glutamate and aspartate cause excitation and γ -aminobutyrate causes inhibition of neuronal membranes (Krnjević & Phillis, 1963). Treatment of suspensions of synaptosomes with electrical pulses or elevated K^+ concentrations leads to a specific release of these three physiologically active amino acids (Bradford, 1970).

To decrease the large fluid/tissue ratio of the suspension to something more closely resembling that *in situ*, synaptosomes were sedimented on to nylon gauze, and a sandwich was formed by placing another piece of gauze on top. The resulting bed of synaptosomes could be easily manipulated and rapidly fixed at the end of incubation. This preparation

showed similar metabolic properties to synaptosome suspensions, glycolysing and oxidizing glucose with parallel formation of ATP and phosphocreatine. Application of electrical pulses stimulated O_2 uptake and glycolysis by 40% and oxidation of glucose to amino acids. Both electrical stimulation and elevated K^+ concentrations in the medium led to a significant increase in the release of glutamate, γ -aminobutyrate and aspartate into the medium, whereas the concentrations of other amino acids were not greatly affected.

On the inclusion of amino acid analogues of glutamate or γ -aminobutyrate in the incubation medium, increased concentrations of these amino acids were found in the medium, which indicated that uptake of these amino acids had occurred under normal conditions when the analogues were not present.

In the absence of Ca^{2+} , the release of glutamate, γ -aminobutyrate and aspartate in response to depolarizing influences was greatly decreased. When ethanedioxybis(ethylamine)tetra-acetate was included in the incubation medium containing no Ca^{2+} , the K^+ -stimulated release was totally inhibited. The presence of Na^+ at a concentration greater than 40 mM was found to be essential for maintaining the normal concentration of γ -aminobutyrate found in the synaptosome bed.

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Drug Interactions in Experimental Hepatic Porphyrin: a Model for the Exacerbation by Drugs of Human Variegate Porphyrin

By F. DE MATTEIS (*Biochemical Mechanisms Section, Medical Research Council Toxicology Unit, Woodmansterne Road, Carshalton, Surrey*)

Human variegate porphyria is a genetically transmitted disorder characterized by excessive formation of porphyrins in the liver. There are latent phases of the disease during which porphyrins are excreted in excess and phases of exacerbation (or acute attacks) when excessive amounts of the porphyrin precursors 5-aminolaevulinic acid and uroporphobilinogen are also excreted together with even larger amounts of porphyrins (Dean & Barnes, 1959). The exacerbation of the metabolic disorder is usually brought about by barbiturates and other drugs (Eales, 1971), which do not alter significantly the porphyrin metabolism of normal individuals.

3,5-Diethoxycarbonyl-1,4-dihydrocollidine produces in experimental animals a hepatic porphyria