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

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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

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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

resembling human variegate porphyria in that protoporphyrin is the main pigment that accumulates in the liver (De Matteis, 1967). It is now found that rats given a relatively small dose of this drug exhibit a hepatic porphyria closely resembling variegate porphyria in its latent phase; they become very sensitive to barbiturates and other drugs that can exacerbate the metabolic disorder, changing its biochemical picture to that typical of the human acute attack.

Starved rats were given 3,5-diethoxycarbonyl-1,4-dihydrocollidine (100mg/kg) intraperitoneally and at the same time either phenobarbitone or another drug (50–100mg/kg) orally and killed 15h later. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine given on its own increased the activity of liver 5-aminolaevulinate synthetase (threefold above normal) and the concentration of porphyrins (tenfold) without altering the concentration of either 5-aminolaevulinate or porphobilinogen. When phenobarbitone was given together with 3,5-diethoxycarbonyl-1,4-dihydrocollidine the following increases were observed in the liver: 5-aminolaevulinate synthetase (tenfold), porphyrins (60-fold), porphobilinogen (80-fold) and 5-aminolaevulinate (at least threefold). Like phenobarbitone, thiopentone, phenylbutazone, lindane, dicophane, 2-isopropyl-2-propylacetamide and ethynyloestradiol could all potentiate the porphyria caused by 3,5-diethoxycarbonyl-1,4-dihydrocollidine, but none of these when given alone increased the concentration of porphyrins in the liver. The mechanism by which this potentiation takes place will be discussed in the light of the rapid inhibition of liver ferrochelatase activity caused by 3,5-diethoxycarbonyl-1,4-dihydrocollidine in the rat (De Matteis & Gibbs, 1972) and of the suggestion that inhibition at some point in the pathway of liver haem synthesis may be the underlying defect common to all varieties of human genetic porphyria (Kaufman & Marver, 1970).

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Glycogen Synthetase and Hexokinase Activities in the Perfused Langendorff and Working Heart of Normal and Streptozotocin-Diabetic Rats

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It was shown in a previous study (Chain *et al.*, 1969) that, in the perfused working rat heart rendered insulin-deficient by injection of anti-insulin serum or the diabetogenic antibiotic streptozotocin into the intact animal before excision of the heart, glycogen synthesis from [U - ^{14}C]glucose was greatly decreased, to half or less than half the normal value, and could be restored to normality by addition of insulin (2 munits/ml) to the perfusion liquid, whereas glucose oxidation to $^{14}CO_2$ was not affected.

It was decided to ascertain whether the diminished glycogen synthesis in the insulin-deficient rat heart would reflect itself in decreased activities of some of the enzymes known to be involved in glycogen synthesis. The activities of two such enzymes have now been assayed in the perfused hearts in working and non-working conditions from normal and streptozotocin-diabetic rats in the presence and in the absence of insulin: the glucose 6-phosphate-dependent and -independent glycogen synthetases D and I, catalysing the final step in glycogen synthesis from glucose, and the hexokinases, bringing about the first step of glucose metabolism, which are known to occur in heart tissue as two main soluble isoenzymes (Katzen *et al.*, 1970). Glycogen synthetase was determined by the method of Villar-Palasi *et al.* (1966). Total and soluble hexokinase activities were determined spectrophotometrically, types I and II being distinguished by their different thermal stabilities (Grossbard & Schimke, 1966).

No decrease in total glycogen synthetase activity was found in the diabetic perfused heart, nor were changes in the I/D ratio observed, and the activities of these enzymes were not increased by addition of insulin (10 munits/ml) to the perfusion liquid.

The total hexokinase activity was found to be decreased in the diabetic heart, confirming the results of Katzen *et al.* (1970), and this decrease was shown to be due to a loss of activity of about 34% specifically of the type II isoenzyme; the activity of isoenzyme type I was not affected. However, the decreased activity of type II hexokinase could not be restored to normality by the addition of insulin (10 munits/ml) to the perfusion liquid.

Thus the marked stimulation of glycogen synthesis from glucose observed to occur in the perfused insulin-deficient heart after addition of insulin cannot be explained in terms of a stimulation of the