Assimilation of Glucose Carbon by Various Protein Fractions of Rat Brain and Other Organs

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It has been shown previously that after injection of [U-¹⁴C]glucose into intact rat or mouse, the proteins isolated from the whole body, from various organs or from the subcellular components of organ homogenates become rapidly labelled (Vrba, 1962, 1966, 1967; Vrba, Gaitonde & Richter, 1962). The present experiments have shown that a particular group of brain proteins shows a higher rate of incorporation of ¹⁴C from [U-¹⁴C]glucose than any other group of proteins isolated from brain and liver. Rats were injected with [U-¹⁴C]glucose and the content of ¹⁴C in proteins of brain, liver, heart and blood was estimated at 30, 60 and 120 min. after the injection. The rate of labelling of brain and liver proteins was similar, and was several times higher than the rate of labelling of heart and blood proteins. The proteins of brain and liver were fractionated on the basis of their solubility in water, in sodium deoxycholate and in ammonium sulphate at 72% saturation at pH 7.1. The highest content of ¹⁴C was found in brain proteins that were soluble in water and precipitated by 72% saturated ammonium sulphate. These results indicate that virtually all proteins of brain are in a dynamic state of equilibrium, and that the continuous conversion of glucose into protein is an important part of the maintenance of this equilibrium. The metabolic activity of various brain proteins varies significantly; however, no evidence was obtained to indicate the presence of a significant amount of 'metabolically inert protein' in the brain.

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Gel Filtration of [U-¹⁴C]Glucose-Labelled High-Speed Supernatants of Rat Brains

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A column of Sephadex G-200 (5 cm. wide, 1750 ml. total volume) was equilibrated with 0.9% NaCl (flow rate approx. 25 ml./hr.) and calibrated with a set of eight non-enzymic protein molecular weight markers (mol. wt. 1450–480 000; Mann

Research Laboratories Inc., New York, N.Y., U.S.A.). Cube roots of the distribution coefficient were calculated and plotted against the square roots of mol. wt. (Porath, 1963). A satisfactory linear relationship was observed. Six growing rats (60 g.) were injected subcutaneously with [U-¹⁴C]glucose (The Radiochemical Centre, Amersham, Bucks.; Batch 141, 2.8 mc/m-mole; 56 μ C in 0.7 ml. of 0.9% NaCl/rat). The brains were excised 22 hr. later, homogenized in 20 ml. of 0.9% NaCl in a Potter–Elvehjem homogenizer for 5 min. at 500 rev./min. and centrifuged at 38 000 rev./min. in a Spinco model L, ultracentrifuge with head 40, and the supernatant was subjected to gel filtration on the above column. Blue dextran and D-glucose were added as markers of the void and elution volumes. Then 400 fractions (5 ml.) were collected. Protein and ¹⁴C were estimated in the eluate as described by Vrba (1967); 99% recovery of ¹⁴C was achieved. The temperature during the entire experiment was maintained at 0–2°. The eluate contained a continuous range of proteins with molecular weights ranging from less than 1000 up to more than 800 000, and 22 hr. after the injection of [U-¹⁴C]glucose the labelling of the various proteins separated by gel filtration was nearly uniform.

Similar experiments were performed with intervals of $\frac{1}{4}$, 4, 8, 12 and 92 hr. after injection of [¹⁴C]glucose, and the specific radioactivities of groups of proteins of various ranges of molecular weights were compared. The proteins eluted from the column were first subjected to a purification procedure for the purpose of determining their specific radioactivities as described by Vrba (1967). Our experiments indicated a metabolic instability of cerebral proteins, and supported similar conclusions by Lajtha & Toth (1966), who used ¹⁴C-labelled L-lysine.

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Insulin Secretion and the Intracellular Concentrations of Glucose 6-Phosphate and 6-Phosphogluconate in Isolated Islets of Langerhans

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Evidence has been accumulating that the metabolism of glucose through the pentose phos-

phate pathway in pancreatic β -cells may be related to the secretion of insulin. Thus a high activity of the enzymes of this pathway has been demonstrated in islet cells (Kissane, Lacy, Brolin & Smith, 1964). Further studies from this Laboratory (Montague, Howell & Taylor, 1967) have shown that certain pentoses and pentose derivatives, known to be metabolized through the pentose phosphate pathway, are as effective as glucose in promoting insulin secretion.

The mechanism by which glucose may release insulin has now been investigated by measuring the concentrations of glucose 6-phosphate and 6-phosphogluconate in islet cells actively secreting in response to various agents.

Isolated islets of Langerhans prepared by collagenase digestion of rat pancreas (Howell & Taylor, 1968) were incubated with various agents known to affect insulin secretion. After 30 min. incubation, the medium was removed from the islets and its insulin content determined by immunoassay. The islets were rapidly cooled to -76° and then disrupted ultrasonically after the addition of perchloric acid. The concentrations of glucose 6-phosphate and 6-phosphogluconate in the perchloric extracts were estimated by using the enzymic cycling procedure described by Lowry, Passonneau, Hasselberger & Schultz (1964).

When the glucose concentration of the incubation medium was raised from 2 to 20 mM, the concentrations of glucose 6-phosphate and 6-phosphogluconate in the islets were markedly increased. In addition, a number of other agents also raised the concentration of 6-phosphogluconate. These included tolbutamide, citrate, glucagon and theophylline, all agents known to promote insulin secretion. In addition, adrenaline ($1\mu\text{M}$) as well as inhibiting the secretion of insulin in response to 20 mM-glucose, also caused a significant decrease in the concentration of 6-phosphogluconate. Citrate and tolbutamide were also found to increase the concentration of glucose 6-phosphate in the islets.

Thus there seems to be a close parallelism between the concentration of 6-phosphogluconate and the secretion of insulin by islets. It is suggested that glucose is normally metabolized through the pentose phosphate pathway in islet cells in order to stimulate the secretion of insulin.

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Insulin Biosynthesis: Studies of Ribonucleic Acid Metabolism *in vitro* in Isolated Surviving Mammalian and Fish Islets

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The present studies were designed to elucidate the role of RNA in insulin biosynthesis in rat islets prepared by microdissection after the pancreatic ducts had been ligated and atrophy of the acinar tissue had been allowed to occur (Keen, Sells & Jarrett, 1965), and in cod islets prepared by excision of the distinct islet organ (Grant & Reid, 1968).

Glucose concentration has a stimulatory effect on the incorporation of labelled precursors into rat islet RNA (Jarrett, Keen & Track, 1967); this effect was not found in cod islet experiments with various glucose concentrations. Rat but not cod islet RNA synthesis was inhibited by actinomycin D. Sucrose-gradient-ultracentrifugation analysis of the rat islet RNA demonstrated that incorporated radioactivity was present in the low-molecular-weight fraction. To establish the *S* value of this labelled material samples of both rat- and cod-islet RNA were separated on polyacrylamide gels. The labelled RNA had a mobility on the gels that indicated that the corresponding *S* value had a range 3.6–4.0S.

The structure of insulin permits calculation of the hypothetical molecular weight of the messenger-RNA (m-RNA) that would 'code' for either the two chains separately or one single polypeptide chain. The isolation (Steiner & Oyer, 1967; Steiner, Cunningham, Spigelman & Aten, 1967; Tung & Yip, 1968; Grant & Reid, 1968) and sequence determination (Chance & Ellis, 1968) of an insulin precursor molecule support the single-polypeptide-chain mode of synthesis, which would require only one m-RNA. As the precursor seems to be composed of approximately 80 amino acids, it would require a m-RNA with an *S* value larger than that of the labelled material found in both rat and cod experiments.

These results suggest that the insulin m-RNA may have a long half-life and that transfer-RNA (t-RNA) modulation (Stent, 1964) may be one of the factors regulating insulin biosynthesis; this would explain the observed *S* values.

Monjardino & MacGillivray (1968) have shown that this type of mechanism seems to be operating in human lymphocytes.

Experiments are in progress with [^3H]methyl-methionine in an attempt to determine whether it is in fact t-RNA that is being labelled in the incubations.

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