gether with the various test substances. The glucagon content of the incubation media was then determined by radioimmunoassay. The release of glucagon from the islets in these conditions has been shown to respond to glucose and to amino acids in a similar manner to glucagon release from isolated rat islets and from perfused dog pancreas (Edwards, Howell & Taylor, 1969). Glucagon secretion from guinea-pig islets *in vitro* was found to be inhibited in the presence of low concentrations of fatty acids and of β -hydroxybutyrate. A very small inhibition was also found with high concentrations of glucose (Edwards *et al.* 1969).

More recently the effects of metabolic inhibitors have been studied. Cyanide (1mM), 2,4-dinitrophenol (0.25mM) and iodoacetate (0.4mM) all stimulated the release of glucagon from guinea-pig islets incubated in the presence of glucose alone. Inhibition of release by fatty acids was abolished by 2,4-dinitrophenol but not by iodoacetate.

Release of glucagon therefore is stimulated by a number of cell poisons and agents that disrupt the supply of intracellular energy. It is suggested that, in contradistinction to the release of insulin from the β -cell, expenditure of energy is necessary to retain glucagon within the α -cell.

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Glucose and Glycogen Metabolism in Hypertrophied Denervated Rat Hemidiaphragm

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Section of the phrenic nerve to one half of the rat diaphragm initiates a pronounced, although transient, hypertrophy of the muscle on that side. Feng & Lu (1965), from histological studies, found that the hypertrophy was solely a function of the 'red' fibres of the tissue, the 'white' fibres apparently only being capable of atrophy. Since red fibres and white fibres differ characteristically in their capacity to phosphorylate glucose and to participate in glycogen metabolism (Bär & Blanchaer, 1965), the activities of enzymes associated with these processes were investigated as a function of time after denervation to see if they reflect changing proportions of red cells and white cells in the fibre population.

Expressed per unit weight of tissue, the extractable activity of hexokinase remained largely unchanged for up to 15 days after denervation. During the hypertrophy phase, however, the tissue mass increases by some 30–40%, so that it appears that net synthesis of hexokinase is taking place. The concentration of glycogen in the tissue taken from fed rats was found to fall markedly as a result of denervation: after 3 days the value was 50% of that in the control tissues. Since Buse & Buse (1959) have established that glucose uptake is unaltered during denervation hypertrophy, it seems that glucose entering the cell tends to be metabolized rather than be stored as glycogen. Glycogen phosphorylase activity decreases significantly in the hypertrophied tissue; this decrease is reflected in the higher glycogen contents present in denervated muscle taken from rats starved for 16h before assay. Similarly activities of phosphoglucoisomerase and phosphoglucomutase were both found to have decreased significantly in concentration as soon as 3 days after denervation. These changes in enzyme activities, and especially in the decrease of the phosphorylase/hexokinase activity ratio, are considered to be indicative of a decreasing proportion of glycogenolytic, i.e. white-fibre, metabolism in the hypertrophying tissue.

Activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are found to increase immediately after phrenicectomy, reaching a maximum after 3 days and thereafter declining; this behaviour is compatible with the observed increase in nucleic acid content of the denervated muscle (Manchester & Harris, 1968), and is suggestive of a reorientation of metabolism to direct glucose 6-phosphate towards pentose synthesis.

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Glycerol Metabolism in Perfused Rat Liver

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Livers from starved female albino rats weighing about 200g were perfused by the Miller technique with haemoglobin-free Ringer solution. The preparations were stable for at least 2h. It was found that glycerol was metabolized at a constant rate by each liver, but that the rates varied from liver to liver for reasons as yet unknown. To achieve a steady perfusate concentration glycerol was added to the medium at the beginning of the perfusion, and from its rate of disappearance a rate of constant infusion of 33% glycerol was calculated that would

maintain a steady concentration of about 10mM in the perfusate during the second hour. A 'spike' of [^{14}C]glycerol was injected at the beginning of this period, when necessary.

The mean rate of glycerol utilization (six experiments) was $1.66\mu\text{mol/min per g}$ of liver. There was no sign of the accumulation of glycerol 1-phosphate described by Schimassek (1968); on the contrary, glucose was produced. The mean net rate of accumulation was $0.60\mu\text{mol/min per g}$, which is about twice that reported by Müllhofer, Hesse, Kunzen & Hechtel (1968), and close to the value of $0.48\mu\text{mol/min per g}$ reported by Ross, Hems & Krebs (1967). However, experiments with [^{14}C]glucose showed that there was a distinct turnover of the glucose pool. About 87% of the ^{14}C lost from labelled glycerol was found in glucose, in agreement with Müllhofer *et al.* (1968).

As reported by McCraw, Yarnell & Ashmore (1969), 4mM-octanoate (+ 4% albumin) had no effect on gluconeogenesis from glycerol, but 2mM-palmitate suppressed glucose formation almost completely. Glycerol uptake was only slightly depressed (average $1.45\mu\text{mol/min per g}$), and was considerably larger than one-third of the rate of fatty acid disappearance from the perfusate, suggesting that a switch from gluconeogenesis to triglyceride synthesis was not the complete reason for the failure to synthesize glucose. Substantial quantities of glucose were synthesized from lactate in the presence of palmitate (and glycerol), showing that the anabolic pathway itself is not blocked by the fatty acid.

These results suggest that the long-held view that glycerol carbon supplies a part of the glucose formed by the liver *in vivo* during starvation may need revision.

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The Use of Skin Biopsy in the Diagnosis of the Glycogen-Storage Diseases

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The differential diagnosis of the genetically determined glycogen-storage diseases is usually

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achieved by enzymic assays on liver or muscle biopsies. Dancis, Hutzler, Lynfield & Cox (1969) have found that acid maltase (α -1,4-glucan glucosylhydrolase, EC 3.2.1.3) is absent from tissue cultures of skin fibroblasts from a patient with type II glycogen-storage disease (Pompe's disease). We are in the process of examining some of the enzymes of glycogen metabolism in epidermis and here we report the results of acid maltase and phosphorylase (α -1,4-glucan-orthophosphate glucosyltransferase, EC 2.4.1.1) assays on normal skin and skin from two patients with confirmed type II glycogenosis and one with type V glycogenosis (Gruener, McArdle, Ryman & Weller, 1968).

Samples of skin were obtained either from the edge of a surgical incision or by a vacuum blistering technique (Kiistala, 1968). Acid maltase and phosphorylase activities were assayed by modifications of the methods of Hers (1964).

Samples of normal skin contained both acid maltase (0.32 – $0.79\text{nmol/min per mg wet wt. of tissue}$) and phosphorylase (0.33 – $1.7\text{nmol/min per mg wet wt. of tissue}$) activity. Both the patients with type II glycogen-storage disease showed skin phosphorylase activity within the normal range (0.89 and $0.80\text{nmol/min per mg wet wt. of tissue}$) whereas acid maltase activity could not be detected. The patient with type V glycogenosis, which is characterized by a specific lack of muscle phosphorylase activity, showed skin phosphorylase within the normal range ($0.62\text{nmol/min per mg of tissue}$).

These results suggest that examination of enzyme activity in skin biopsies can be used to replace the more hazardous liver and muscle biopsies for the specific diagnosis of type II glycogenosis.

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Studies on the Structure of Horse Spleen Apoferritin: the Products of Cyanogen Bromide Cleavage

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Apoferritin from horse spleen was prepared from ferritin by the method of Granick & Michaelis (1943) as modified by Crichton (1969b). This protein of molecular weight 460 000 is thought to be made up of 20 identical subunits arranged at the vertices of a pentagonal dodecabedron with a small central space in each pentagonal face (Harrison, 1963; Harrison &