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 – 0.79nmol/min per mg wet wt. of tissue) and phosphorylase (0.33 – 1.7nmol/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 0.80nmol/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 (0.62nmol/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 &

Hofman, 1962; Crichton, 1969b). Amino acid analysis of the protein indicates that there are four methionine residues/22 500-dalton subunit (Crichton, 1969b; Williams & Harrison, 1968). Thus on cleavage with cyanogen bromide five unique peptides would be expected, four of which should have C-terminal homoserine and one C-terminal arginine (Mainwaring & Hofmann, 1968).

The dissociated subunits of horse spleen apoferritin were cleaved with a 240 molar excess of cyanogen bromide in 90% formic acid, and the products fractionated on columns of Sephadex G-75. Three major peaks were observed and these fractions were designated I, II and III. The use of an LKB Uvicord II to monitor the column effluent at 280nm while small portions of the effluent were continuously fed to the hydrolysed and unhydrolysed lines of a Technicon peptide analyser gave information both about the size of the peptides and their elution characteristics.

Fractionation of components I and III on Sephadex G-75 enabled essentially homogeneous products to be isolated. Fraction II was rechromatographed on Sephadex G-50 and gave three major components, designated II₁, II₂ and II₃. These were purified by rechromatography on Sephadex G-50 and G-75.

Amino acid analyses on these fractions were carried out, and the results will be presented and discussed in relation to the nature of the subunit structure of apoferritin. A preliminary report of this work has been published (Crichton, 1969a,c; Crichton & Barbiroli, 1969).

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The Organ Specificity of Ferritin in Human and Horse Liver and Spleen

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Ferritin, the major iron storage protein of mammalian spleen and liver, has been shown by a number of groups to be organ-specific in humans. Thus Allfrey, Lynch & Whitley (1967) were able to demonstrate by electrophoresis on cellulose acetate and agar gels that human liver, spleen and reticulocytes each contain an electrophoretically distinct ferritin. Variations in the protein rather than differences in the iron content were responsible for this, since apoferritins of spleen or liver had electrophoretic mobility identical with that of the particular ferritin from which they were prepared. Two electrophoretically distinct ferritins have been found in haemolysates of human bone marrow both by Allfrey *et al.* (1967) and by Gabuzda & Gardner (1967).

We have isolated ferritin from human autopsy liver and spleen and from horse liver and spleen by a procedure essentially similar to that of Granick (1942), but with the introduction of a gel-filtration step on Sephadex G-200 subsequent to the ammonium sulphate precipitation step. The protein was concentrated by ultrafiltration (Diaflo) and any traces of bacterial contamination were removed with a Millipore filtration apparatus.

The ferritins were characterized by their electrophoresis on starch blocks and polyacrylamide gels. Apoferritin prepared as described by Crichton (1969) was also analysed by polyacrylamide-gel disc electrophoresis and in the analytical ultracentrifuge at 130 000g. Amino acid analysis of the ferritin and apoferritin samples was carried out on a Locarte amino acid analyser (Locarte Co. Ltd., London S.W.7, U.K.) and 'fingerprints' of overnight tryptic digests of the apoferritins were carried out as described by Crichton (1969).

The results of Allfrey *et al.* (1967) were confirmed and the organ-specific character of horse ferritin was also established. The fact that there are distinctly different ferritins in liver and spleen of a single species will be discussed in the context of the inherited condition haemochromatosis, in which considerable accumulations of ferritin in both spleen and liver are found.

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