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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