It is worth mentioning that the chemical nature of the fat varies during the year[7] as does the ash composition resulting from selective mineral accumulation[4]. This relation is also affected by the kind of muscle; for example, red muscle from cod gives a strong water × fat correlation, in contrast to white muscle[6].

A knowledge of fat, ash and water content in fish muscle allows the prediction of protein content if the glycogen content is assumed to be negligible. In figure 4 the changes in the components of the muscle are shown on the basis of (A) wet and (B) dry matter. As a percentage of wet weight, protein decreases and water increases. However, expressed on a dry basis the reverse picture emerges which is opposite to the assumption made in the specific gravity calculation for non-fish animals, i.e., that non-fat substances are constant[3]. Assuming a specific gravity for fish fat (0.985 g/cm^3) and ash (4.37 g/cm^3), as well as Kleiber's[3] value for protein, the theoretical curve relating density and chemical composition was calculated. The density of pollan muscles calculated this way increases from 1.320 to 1.606 as the fat content decreases from 8.43 to 0.12% wet weight.

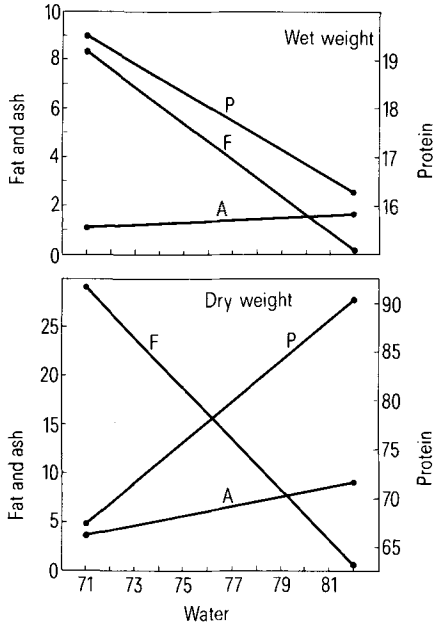

Fig. 4. The changes in water, fat and protein content in fish muscle expressed in wet (A) and dry (B) weight.

It has been reported[4] that cell size decreases and extracellular space increases as the relative water content rises during starvation of several fish species. These changes are most likely linked with the water bound to protein which can affect protein density itself. At the moment no explanation of the discrepancy between the experiment and theoretical figures can be offered, although more extensive studies elucidate the problem.

A recent report on salmon alevins[11] suggested a decrease in fish density from 1.067 at hatching to 1.044 at terminal yolk resorption. These changes correlated with the water content increase which is consistent with the idea that density is inversely proportional to fat content but the method of density measurement using glycerol creates a problem of fish drying out which the authors clearly recognized in their paper.

1 This work was supported by a grant from the Fishmongers' Company of London and The Honourable Irish Society.
2 Acknowledgment. I wish to thank Professor R.B. Wood for critically reading the manuscript and Dr T. Pitcher for help with the calculations.
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Purification of acidic Z protein from human liver

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Summary. Preparation of Z protein from human liver is described. Z protein consists of 2 forms which have different isoelectric points, pI 5.8 and pI 8.7, respectively. The acidic Z protein has a molecular weight of about 11,000 and has binding affinity for BSP using gel filtration.

One of the major functions of the liver is the transfer of organic anions such as bilirubin, sulfobromophthalein, several steroids and drugs from the plasma into the parenchymal cells where the acceptor proteins, such as ligandin and Z protein localized in the supernatant fractions, are important for the intracellular transport of these substances[1-4]. Rat Z protein was firstly described by Levi et al.[1] and Ketterer et al.[5] who designated it as aminoazodye-binding protein A. This protein has been further studied by Mishkin[6,7], Ockner[8,9], Ketterer[10,11] and others[12,13]. The aim of the present work was to determine the existence of the Z protein in the human liver and to characterize further its chemical and physical properties.

Materials and methods. Liver was taken from an autopsy specimen without hepatic involvement within 6 h after death and stored at −70 °C. After homogenization of the liver with 50% 0.25 M sucrose-phosphate buffer 0.01 M, pH 7.4, the solution was centrifuged for 90 min at 110,000 × g. The supernatant fraction was then applied to a DEAE column (Whatman DE-52, 5 × 40 cm) equilibrated with pH 7.5, 0.01 M phosphate buffer, and the eluted protein solution was concentrated and applied to a

Sephadex G-75 column (5×90 cm) with BSP which had been equilibrated with 10 mM phosphate buffer pH 7.4. The low molecular protein solution which bound with BSP was collected. After concentration, the solution was applied to an ampholine electrofocusing column.

Estimations of mol.wt were obtained by gel filtration of 100 µg of purified protein on a column (1.2×90 cm) of Sephadex G-50 equilibrated with 0.01 M phosphate buffer at pH 7.4. Cytochrome C and myoglobin were used as standards. The mol.wt was also estimated by polyacrylamide gel electrophoresis in 0.2% sodium dodecyl sulfate by the method of Weber and Osborn[14] with ovalbumin, chymotrypsinogen, and cytochrome C as standards.

Preparative electrofocusing was performed using LKB 8100 ampholine column by adding 3 ml of a 40% solution pH 3-10 ampholine and a linear 0-40% sucrose density gradient (w/v). The separation was carried out at 400 V for the first 12 h and at 950 V for additional 4 days. At the completion of the procedure, fractions of 3 ml were collected and their pH values and absorbances at 280 nm determined. Gel electrofocusing was carried out with gels, 10 cm in length, as described by Wrigley[15]. Voltage was maintained at 300 V in a room and separate gels were stained for 1-2 h by the methods of Malik and Barrie[16]. During the purification step, BSP bindings were measured by the gel filtration method using a Sephadex G-50 column (1.0×25 cm). Collected solutions were measured spectrophotometrically at 280 nm for protein and 565 nm for BSP after alkalinization with NaOH. Using the same Sephadex G-50 column, specific activities of each step were measured by triangulation of the area under each peak of 565 nm and 280 nm absorbances, and expressed as unit of BSP/mg of protein (specific activity of the fraction 111 (figure 2) was designed as 1).

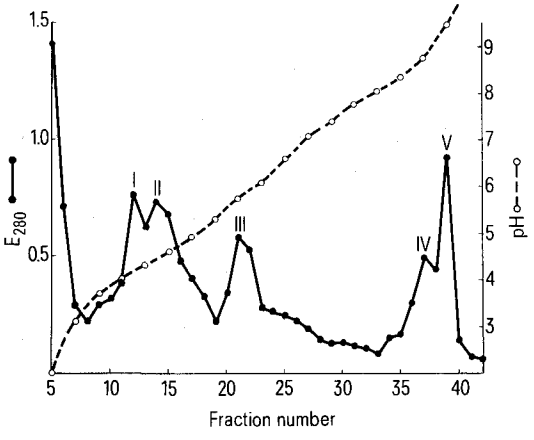

Fig. 1. Preparative electrofocusing of the protein solution after the Sephadex G-75 column procedure. 5 protein peaks were observed and each isoelectric point was 4.1, 4.5, 5.8, 8.7 and 9.5.

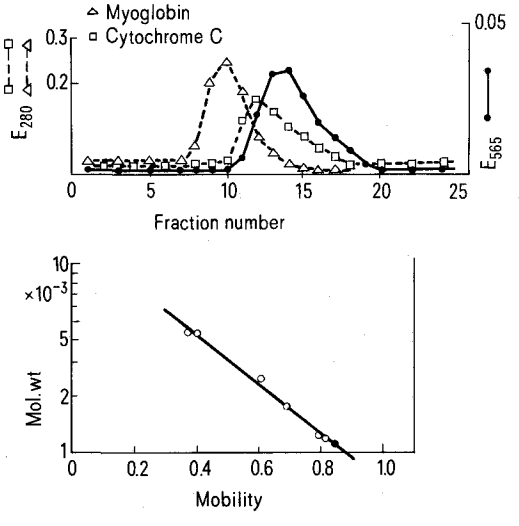

Fig. 2. Upper column shows the elution pattern of Sephadex G-50 column chromatography. Purified protein 111 was applied with BSP and absorbancies at 565 nm were determined. The elution patterns of myoglobin and cytochrome C were measured with absorbancies at 280 nm. Lower column; The mol.wt of protein 111 was compared with different polypeptide chains with their electrophoretic mobilities.

Results and discussion. The specific activities of DEAE step, G-75 Sephadex and electrofocusing, showed 0.02, 0.117 and 1.0, respectively. The yields of each step indicated approximately 40% of the homogenate by DEAE step, 20% by G-75 column and 8% by electrofocusing. The purity of the final protein 111 fraction was homogenous by 10% SDS polyacrylamide gel electrophoresis, as well as single band with gel electrofocusing (pH 3-10). As for fraction IV, the procedure of preparative electrofocusing (pH 7-9) was further need to obtain homogenous protein. BSP was used for the present experiment as a ligand, since this substrate indicates one of the organic anions which is excreted rapidly into the hepatic parenchymal cells and is used clinically for routine liver function test. The presence of Z protein in rat liver has already been implied from binding studies, of which mol.wt has been determined 12,000[6] or 14,000[11]. The mol.wt of purified protein fraction 111 was measured as approximately 11,000, monomer, by gel filtration and 10% SDS polyacrylamide gel electrophoresis (figure 1). We obtained at least 5 protein peaks with preparative electrofocusing. The isoelectric points of each peak revealed 4.1, 4.5, 5.8, 8.7 and 9.5 (figure 2). From our gel filtration data, though qualitative, only 2 (fraction 111 and IV) of the 5 peaks have the binding capacities for BSP as a substrate. Combining these results with those described in the previous report, which stated Z protein has the similar affinity for BSP to that of ligandin in the rat experiment[13], it was speculated that 111 and IV fractions also have the important role as one of the binding proteins in the human liver.

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