The Purification and Properties of Ferritin from Human Serum

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1. Ferritin has been isolated from the serum of four patients with iron overload by using two methods. 2. In method A, the serum was adjusted to pH 4.8 and heated to 70°C. After removal of denatured protein, ferritin was concentrated and further purified by ion-exchange chromatography and gel filtration. In most cases, only a partial purification was achieved. 3. In method B, ferritin was extracted from the serum with a column of immuno-adsorbent [anti-(human ferritin)] and released from the column with 3 M-KSCN. Further purification was achieved by anion-exchange chromatography followed by the removal of remaining contaminating serum proteins by means of a second immuno-adsorbent. Purifications of up to 31 000-fold were achieved, and the homogeneity of the final preparations was demonstrated by polyacrylamide-gel electrophoresis. 4. Serum ferritin purified by either method has the same elution volume as human spleen ferritin on gel filtration on Sephadex G-200. Serum ferritin has a relatively low iron content and iron/protein ratios of 0.023 and 0.067 (μg of Fe/ μg of protein) were found in two pure preparations. On anion-exchange chromatography serum ferritin has a low affinity for the column when compared with various tissue ferritins. Isoelectric focusing has demonstrated the presence of a high proportion of isoferritins of relatively high pI. 5. Possible mechanisms for the release of ferritin into the circulation are briefly discussed.

The use of an immunoradiometric assay for ferritin (Addison *et al.*, 1972) has made it possible to detect circulating ferritin and to show that its concentration is generally related to the amount of storage iron in the body (Jacobs & Worwood, 1975). Present knowledge about the origin and clearance of plasma ferritin is derived from clinical studies and animal experiments (Jacobs & Worwood, 1975). The demonstration of differences in the isoelectric-focusing patterns of ferritin preparations from various human tissues (Powell *et al.*, 1975a) suggested that a similar study of serum ferritin might give information about the nature of the circulating protein and about its origin. On anion-exchange chromatography (Worwood *et al.*, 1975a), serum ferritin has a lower affinity for the column than human heart, kidney, liver or spleen ferritin and on density-gradient centrifugation behaves as ferritin of low iron content (Worwood *et al.*, 1975a). These findings were confirmed with highly purified serum ferritin prepared by heat treatment of serum at pH 4.8 (Worwood *et al.*, 1975b), but the procedure was lengthy and the overall recovery was low. In the present paper heat treatment of serum was compared with the extraction of serum ferritin with an immuno-adsorbent. Ferritin was purified from the serum of iron-overloaded patients and its properties were investigated.

Experimental

Patients and collection of serum

Serum was collected from four patients with iron overload. Three patients (Le, Oc and St) were receiving regular blood transfusions for the treatment of aplastic anaemia, and plasmapheresis was performed together with some of the transfusions. The fourth patient (Po) with thalassaemia intermedia had received many units of blood, but after splenectomy had maintained a haemoglobin concentration of about 10 g/dl without transfusion. This patient was undergoing a course of venesection for the removal of excess storage iron.

Preparation of spleen liver and heart ferritin

Spleens were obtained at operation from patients with iron overload and normal livers and hearts were obtained *post mortem*. Iron-rich ferritin was prepared as described by Worwood *et al.* (1975a). The inclusion of the high-speed-centrifugation stage provides an effective way of obtaining ferritin free from contaminating proteins. The purified ferritin from each tissue was examined by polyacrylamide-gel electrophoresis and showed only monomer and polymer bands, which stained for both protein and iron. Heart ferritin was additionally chromatographed on

Sephadex A-50 (see below), when the ferritin eluted at a chloride-ion concentration of 0.28M (Fig. 3). Fractions comprising the major peak were concentrated.

Preparation of antisera

High-affinity antiserum for immunoprecipitation and immunoassay. A rabbit was given three intramuscular injections of 1mg of human spleen ferritin with Freund's complete adjuvant at intervals of 2 weeks. After a further 2 weeks the rabbit was bled, and serum was collected. The antiserum was tested for specificity by immunoelectrophoresis on agar plates against normal human serum and the same serum mixed with spleen ferritin. In the absence of the ferritin, no precipitin lines were seen. In addition, the antiserum was incubated with normal human serum (undiluted, and at dilutions up to 1:256) on Ouchterlony plates, and increasing dilutions of antiserum were incubated with normal serum in a similar way. No precipitin lines were seen after washing and staining.

Antisera for Sepharose-based immunoabsorbents. A rabbit was given a single intramuscular injection of 1 mg of human spleen ferritin with Freund's complete adjuvant. After 10 days the rabbit was bled and serum was collected. A similar 'first-bleed' antiserum was prepared by injection of human placental ferritin into a sheep. Sheep anti-(whole human serum) was purchased from Wellcome Reagents Limited, Beckenham, Kent.

Other materials

Sephadex A-50, Sephadex G-200, Sepharose 6B, Blue Dextran and CNBr-activated Sepharose 4B were supplied by Pharmacia (G.B.) Ltd., London W5, U.K. Bovine serum albumin (fraction V powder), and catalase (ox liver, crystalline suspension) by Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K., and CM52 cellulose cation-exchanger was supplied by Whatman Biochemicals Ltd., Maidstone, Kent, U.K.

Isoelectric focusing

Isoelectric focusing was carried out by the method of Righetti & Drysdale (1973) using the MRA disc-gel apparatus (MRA Corp., Boston, MA, U.S.A.). Polyacrylamide gels (100mm×3mm) containing 4% acrylamide, 10% glycerol and 2% Ampholine (pH range 5-7, LKB Instruments Ltd., South Croydon, Surrey, U.K.) were loaded with 10 or 50µg samples of ferritin contained in a volume of not more than 50µl. Before focusing, the purified preparations of ferritin were concentrated by vacuum ultrafiltration to 200-1000µg/ml. A current (maximum power 3W) was passed for 17-18h while the temperature was

maintained at 0-2°C with circulating refrigerated water. The gels were expressed from their supporting tubes, fixed for 15 min at 90°C in 5% (w/v) trichloroacetic acid and the ampholyte removed by transverse electrophoresis (MRA transverse-electrophoresis tank) in 5% acetic acid for 1 h at 60mA/disc. Gels containing the larger samples were stained for iron [with 2% (w/v) potassium ferrocyanide in 0.2M-HCl] or for protein in 0.2% Coomassie Blue in water/ethanol/acetic acid (6:3:1, by vol.). In the gels containing the smaller samples, ferritin was detected by immunoprecipitation. The unfixed gels were agitated for 24h in 10% rabbit antiserum to human spleen ferritin in 0.05M phosphate buffer, pH7.4. After washing for 48h in several changes of the same buffer to remove unprecipitated protein, the gels were stained for protein as described above.

Gels containing 50µg of normal human serum protein contained no bands after incubation with the antiserum to spleen ferritin. The isoelectric points of heart and liver ferritin were determined in the same focusing apparatus fitted with preparative gel tubes (140mm×12mm). The ferritin bands were cut out and ampholyte was eluted with 1-2 volumes of boiled water for 6h. The pH measurements were made with a microelectrode at the same temperature as the water in the cooling jacket of the focusing apparatus.

Polyacrylamide-gel electrophoresis

Electrophoresis was carried out at pH7.5 (0.1M-Tris mixed with the appropriate volume of 0.1M-HCl) by using the MRA slab isoelectric-focusing apparatus which was modified for electrophoresis. The gels (175mm×90mm; 0.75 or 1.5mm depth) incorporated 10% glycerol and were run for 3-4h at 20mA at 0-2°C. Samples (0.5 or 2µg of ferritin) were applied in a volume of 5µl. Staining for iron and protein was performed as described above, after fixing the gels for 30min in 5% trichloroacetic acid at room temperature (20°C). Ferritin was precipitated in the unfixed gels containing the smaller samples with 10% antiserum as described above, but the washing time was decreased to 24h.

Protein concentrations

Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. The values obtained were not corrected for differences between the readings given by ferritin and albumin standards (per mg of N), as such correction has not been applied in the standardization of ferritin for immunoassays (Addison *et al.*, 1972; Miles *et al.*, 1974).

Immunoradiometric assay of ferritin

The assay was carried out as described by Addison *et al.* (1972), with iron-rich human spleen ferritin for

production of the rabbit antiserum, and as the standard. The protein content of the standard was determined by the method of Lowry *et al.* (1951).

Anion-exchange chromatography

This was carried out at 4–6°C on 150mm×10mm columns of Sephadex A-50 [Pharmacia (G.B.) Ltd., London W.5, U.K.] equilibrated with veronal buffer, pH 6.8, prepared by mixing appropriate volumes of sodium barbitone (0.05 M) and HCl (0.05 M). Proteins were dialysed against the buffer before being loaded on to the column and were eluted from the column with increasing concentrations of NaCl in the starting buffer. Fractions (50×5ml) were collected at a rate of 15ml/h. The chloride-ion concentration in the fractions was determined by titration with silver nitrate, and ferritin was estimated either by measuring E_{280} (with buffer in the reference cell) or by immunoradiometric assay.

Iron content of ferritin

The iron content of the final preparations was determined by a modification of the International Committee for Standardization in Haematology (1971) reference method for the assay of serum iron, in which the ferritin containing solutions were heated to 56°C for 1h with 10%(w/v) trichloroacetic acid/3%(v/v) thioglycolic acid in 1M-HCl before addition of the chromagen solution.

Determination of elution volume on gel filtration

Gel filtration was carried out on columns (800mm×15mm) of Sephadex G-200 calibrated with Blue Dextran and standard proteins. The relative elution volumes (the ratio of the elution volume of the protein to that of Blue Dextran) were: for human spleen ferritin, 1.17; for catalase, 1.48; for bovine serum albumin, 1.82.

Density-gradient centrifugation

This was performed with a sucrose gradient as described by Worwood *et al.* (1975a).

Preparation of apoferritin

Human spleen ferritin was reduced with 1% thioglycolic acid at 4°C. This was followed by extensive dialysis against 0.05M-sodium phosphate buffer, pH 7.4.

Isolation of serum ferritin

Method A: heat treatment. Batches (250ml) of serum were diluted with an equal volume of 0.05M-sodium

acetate and adjusted to pH 4.8 by stirring the solution and adding 1M-acetic acid dropwise. The batch was divided into portions of about 150ml, heated rapidly to 70°C in a water bath and maintained at 70–75°C for a total time of 10min. After cooling in water, and centrifugation at 25000g for 30min (High-Speed 25 centrifuge; MSE Ltd., Crawley, Sussex, U.K.) the supernatant obtained was carefully adjusted to pH 6.8 by dropwise addition of 1M-NaOH and concentrated to approx. 10ml by ultrafiltration (XM-100A membrane; Amicon Ltd., High Wycombe, Bucks., U.K.). The concentrated solution was loaded on to a column (900mm×25mm) of Sepharose 6B, and proteins were eluted with veronal buffer, pH 6.8, prepared by mixing appropriate volumes of sodium barbitone (0.05M) and hydrochloric acid (0.05M). Fractions (5ml) were collected, and those containing ferritin (detected by assay) were combined and concentrated. The concentrate was subjected to anion-exchange chromatography on Sephadex A-50 in veronal buffer, pH 6.8. Fractions containing ferritin were combined, concentrated (by using an Amicon UM-10 ultrafiltration membrane) and dialysed against 0.02M-sodium acetate solution adjusted to pH 5.0 with acetic acid. The solution was loaded on to a column (150mm×10mm) of carboxymethyl-cellulose (CM52), equilibrated with the same buffer, and ferritin was eluted with a gradient of NaCl from 0 to 0.4M. The fractions (3ml) containing ferritin were collected, concentrated to a volume of about 5ml by ultrafiltration, dialysed against 0.05M-veronal buffer, pH 7.5 (0.05M-barbitone sodium adjusted to pH 7.5 with 5M-HCl and containing 0.02% Na₂N₃) and loaded on to a column (900mm×15mm) of Sephadex G-200 [Pharmacia (G.B.) Ltd.] in the same buffer. The fractions containing ferritin were combined and concentrated.

Isolation of serum ferritin

Method B: use of immunoabsorbents. (1) Preparation of immunoabsorbents. Globulin fractions of the 'first bleed' antisera and the anti-(whole serum) were prepared by mixing the antisera with an equal volume of ice-cold, saturated ammonium sulphate and allowing the suspension to stand in ice for 1h. The precipitated protein was collected after centrifugation at 10000g for 15min and dissolved in sodium citrate buffer, pH 6.5, (5.3vol. of 0.2M-citric acid in 0.4M-NaOH, mixed with 4.7vol. of 0.2M-NaOH), followed by dialysis against the same buffer. CNBr-activated Sepharose 4B was swollen and washed with water adjusted to pH 3 with HCl, and the globulin fraction of an antiserum coupled to the Sepharose (25mg of protein in 5ml of citrate buffer/g of Sepharose) by incubation at room temperature for 2h on a roller mixer. After washing with citrate buffer, the immunoabsorbent was incubated with 1M-ethanolamine

(adjusted to pH 8.0 with HCl) for 2h at room temperature, and finally washed with a large volume of phosphate buffer, pH 7.4, containing 0.02% NaN₃.

(2) Isolation of serum ferritin with the immuno-adsorbent column. Solid NaCl was added to the serum in small amounts, with stirring, until the concentration was 0.5M. NaN₃ was added to give a 0.02% solution. An immuno-adsorbent column [anti-(human ferritin)] was prepared in a 10ml disposable syringe with a bed volume of 8ml, and was washed with phosphate buffer, pH 7.4, containing 0.5M-NaCl and 0.02% NaN₃. Batches of serum (150ml) were passed through a glass-wool filter and through the immuno-adsorbent column at a rate of 25ml/h at room temperature. The column was washed with the phosphate buffer until no further protein was eluted (determined from E_{280}). Ferritin was eluted with 3M-KSCN in 0.01M-phosphate buffer, pH 7.6, containing 0.02% NaN₃. Ferritin is stable in the presence of 3M-KSCN at this pH (Kist *et al.*, 1975). The eluate was diluted with an equal volume of 0.05M-phosphate buffer and decreased to a volume of approx. 5ml by ultrafiltration. This solution was dialysed against veronal buffer, pH 6.8, and chromatographed on Sephadex A-50. Fractions containing ferritin (determined by immunoradiometric assay) were combined and concentrated to about 10ml. After addition of solid NaCl (0.5M) and NaN₃ (0.02%), the solution was passed through a second immuno-adsorbent column [anti-(human serum protein)], prepared as described above, and the eluate containing un-adsorbed proteins was collected and concentrated to 4-5ml. After gel filtration on Sephadex G-200 in 0.05M-phosphate buffer (containing 0.02% NaN₃) the fractions containing ferritin were combined and concentrated.

Results

Effect of heat treatment at pH 4.8 on liver and spleen ferritin

There was no change in E_{420} or in the isoelectric-focusing profile when purified human spleen ferritin was heated to 75°C at pH 4.8. Spleen ferritin (1 mg) was added to 100ml of serum with a low ferritin concentration and the serum was acidified to pH 4.8, subjected to heating, centrifugation at 25000g, neutralization and concentration. On isoelectric focusing the pattern for this preparation was identical with that of the original spleen ferritin. Finally, ferritin was extracted from human liver by acidifying the homogenate (4ml of water per g of liver) and subjecting this to the serum ferritin preparation [method A (up to and including gel filtration of Sepharose 6B)]. On isoelectric focusing this preparation gave the same bands as liver ferritin prepared in the conventional manner.

Purification of serum ferritin

Method A was applied to serum from all four patients, but only in one case was a highly purified preparation obtained. In all cases recoveries were very low. The results for two preparations are summarized in Table 1. Further details of preparation 1 have been given previously (Worwood *et al.*, 1975b). With immuno-adsorbents, pure preparations were obtained, and recoveries were considerably higher (preparations 3 and 4, Table 1). Details of one purification are given in Table 2. These preparations were examined by polyacrylamide-gel electrophoresis, and in both cases a single line was obtained with direct staining and after immunoprecipitation

Table 1. *Serum-ferritin preparation*

Ferritin concentration, measured by the immunoradiometric assay against a standard of human spleen ferritin. Preparations 1 and 2 were obtained by Method A (heat treatment) and 3 and 4 by Method B (immuno-adsorbent extraction).

	Preparation no. ...	1	2	3*	4†
Patient		Le	Oc	Po	St
Volume of serum (ml)		725	555	620	540
Ferritin concn. (μ g/ml)		13	8.7	3.6	4.3
Recovery (%)		6	8	20	31
Purification (fold)		7000	4200	31 000	24 000
Protein content of preparation (μ g/ml)		60	130	75	18
Ferritin content of preparation (μ g/ml)		86	65	106	29
Iron content of preparation (μ g/ml)		—	—	1.7	1.2
Iron/protein ratio		—	—	0.023	0.067

* Using rabbit antiserum for the first immuno-adsorbent.

† Using sheep antiserum for the first immuno-adsorbent.

EXPLANATION OF PLATE I

Polyacrylamide-gel electrophoresis of human ferritin

For details, see the text. (a) Serum ferritin, preparation 4. (b) Liver ferritin; a band of ferritin dimers is also seen. The gel was stained with Coomassie Blue.

EXPLANATION OF PLATE 2

Isoelectric focusing of human ferritin in polyacrylamide gel

For details, see the text. (a) (1) Serum ferritin, preparation 4; (2) serum ferritin, preparation 3; (3) liver ferritin; (4) heart ferritin, see Fig. 2. The gels were incubated with an antiserum to human spleen ferritin, washed and stained with Coomassie Blue. (b) (1) Serum ferritin, preparation 3, direct staining with Coomassie Blue; (2) serum ferritin, preparation 3, immunoprecipitation; (3) serum ferritin from patient Po prepared by method A, immunoprecipitation; (4) liver ferritin, direct staining with Coomassie Blue. The most alkaline band of serum ferritin is marked 'S'.

Table 2. *Purification of serum ferritin*

Stage	Volume (ml)	Total protein (mg)	Total ferritin (μ g)	Recovery of ferritin (%)	Purification (fold)
Serum	620	48300	2260	100	1
After elution from anti-ferritin immuno-adsorbent	4.5	13.9	1070	47	1600
After Sephadex A-50 chromatography	8.0	2.5	667	30	5800
After passage through anti-(whole serum) column and gel filtration	4.3	0.32	458	20	31000

Fig. 1. *Sucrose-density-gradient centrifugation of human ferritin*

For details, see the text. ○, Spleen apoferritin; □, spleen ferritin, 0.25 μ g of Fe/ μ g of protein; △, serum ferritin, preparation 3, 0.023 μ g of Fe/ μ g of protein.

(Plate 1). In preparation no. 4, iron was present in this band. In both cases the serum ferritin appeared to have a slower mobility than the liver ferritin preparations running alongside. In three highly purified preparations (nos. 1, 3 and 4 in Table 1) the ferritin concentration determined by the immunoradiometric assay was higher than that of the protein concentration (Lowry method).

Gel filtration

On Sephadex G-200 the elution volume of serum ferritin from the four patients was identical with that of human spleen ferritin.

Iron content of serum ferritin

All the preparations were examined by density-gradient centrifugation, and in each case the serum ferritin (detected by the assay) behaved like a ferritin of relatively low iron content (Fig. 1). Determination of the iron content of the two pure preparations (3 and 4) showed iron/protein ratios of 0.023 and 0.067 (μ g of Fe/ μ g of protein). The iron/protein ratio of the purified spleen and liver ferritin was 0.25.

Anion-exchange chromatography

Chromatography was carried out on the serum from each patient during the purification (Fig. 2) and also on a purified serum ferritin. Ferritin was detected with the immunoradiometric assay and in each case the maximum ferritin concentration was found at a chloride-ion concentration of 0.12–0.13 M.

Isoelectric focusing

Purified liver, spleen and heart ferritin were examined, after focusing, by direct staining with Coomassie Blue and by immunoprecipitation. Both staining methods gave the same results; spleen and liver ferritin having identical focusing patterns (pI 5.27–5.55) and heart ferritin giving a band with pI 5.05 (Plate 2a). Each preparation of serum ferritin was examined by isoelectric focusing, and ferritin was detected by immunoprecipitation (Plate 2a). Preparations 3 and 4 were also stained directly with Coomassie Blue, and the same pattern was observed for direct staining and immunoprecipitation (Plate 2b). Each preparation of serum ferritin showed a prominent band which appeared to correspond to the most alkaline component of liver ferritin (present only in trace amounts). This band is marked 'S' in Plates 2(a) and 2(b). On focusing preparations 1, 2 and 3, more acidic bands were observed, which corresponded to bands seen on focusing liver and heart ferritin, although in preparation 4 only the 'S' band was observed (Plate 2a). Ferritin was also partially purified from the serum of patient Po by method A. The isoelectric-focusing pattern was very similar to that of preparation 3 (Plate 2b).

Fig. 2. Anion-exchange chromatography of human ferritin

Fractions of 5 ml were collected. For further details, see the text. (a) Serum ferritin after elution from the anti-ferritin immunoadsorbent, preparation 4. (b) Purified liver ferritin. (c) Heart ferritin, fractions 30–41 were combined and concentrated for isoelectric focusing.

Discussion

Heat treatment of serum at pH 4.8 leads to a considerable purification of ferritin with a high recovery, and studies carried out with liver and with purified spleen ferritin show that this does not lead to changes in the iron content of ferritin or in its isoelectric-focusing pattern. This method provides a useful technique for obtaining partially purified preparations. However the steps necessary for further purification are lengthy and cause large losses of ferritin. Method B, using immunoadsorbents, is rapid and effective, but selection of suitable antisera is difficult. The antiserum must have a high-enough affinity to bind ferritin but a low-enough affinity to allow its subsequent release. With only four stages, purifications of up to 31 000-fold have been achieved, and the purity of the preparations obtained was demonstrated by polyacrylamide-gel electrophoresis. The properties of serum ferritin do not appear to be affected by the method of purification.

Density-gradient centrifugation of serum ferritin as well as measurement of the iron content of purified preparations shows that the iron content is low. This

is surprising, since the sera were collected from patients with gross iron overload. There is little information about the iron content of ferritin in human tissues, but density-gradient centrifugation of a crude spleen extract from an iron-loaded patient (Worwood *et al.*, 1975a) suggested that the iron/protein ratio was little different from that in a purified preparation of ferritin from the same spleen (about 0.25).

On anion-exchange chromatography, serum ferritin has a lower affinity for the column of Sephadex A-50 than liver, spleen, kidney or heart ferritin (Worwood *et al.*, 1975a). This is unlikely to be caused by interaction with other serum proteins, as incubation of spleen ferritin with serum does not produce the characteristic behaviour of serum ferritin during anion-exchange chromatography (Worwood *et al.*, 1975a). Binding to other serum proteins may also result in changes in the molecular size of ferritin, but on Sephadex G-200 gel filtration serum ferritin has the same elution volume as human spleen ferritin. Both ferritin and apoferritin elute at the same volume under such conditions (Williams & Harrison, 1968) although small differences in size would not be detectable on Sephadex G-200.

On isoelectric focusing, preparations of human ferritin can be resolved into a number of isoferritins (Drysdale, 1974; Powell *et al.*, 1975a), and although the ferritin of human serum has a low affinity for the anion-exchange column, there are isoferritins present with a wide range of isoelectric points. It seems likely that the more alkaline isoferritins are responsible for the low affinity for Sephadex A-50, as in some preparations they account for almost all the ferritin. Preliminary results of Powell *et al.* (1975b) also suggest that on isoelectric focusing, normal serum ferritin contains a prominent alkaline band which they describe as having a pI of 5.75.

One curious finding was the apparently high ferritin content of the purified serum ferritin in the immunoradiometric assay (compared with the protein content measured by the Lowry method). We have shown that, although heart and spleen ferritin cannot be distinguished on Ouchterlony plates by antiserum to spleen ferritin, the apparent ferritin content of heart ferritin in the immunoradiometric assay was only 5% of the protein content (Worwood *et al.*, 1976). The immunoradiometric assay may therefore underestimate the concentration of more acidic, circulating, isoferritins and overestimate the more alkaline ones.

Clinical and experimental evidence suggests that serum ferritin is normally derived from reticulo-endothelial cells (Jacobs & Worwood, 1975), although direct release from damaged tissue is to be expected in some cases of liver disease. The mechanism of release from reticuloendothelial cells is not known, but the results presented in the present paper,

together with previous data on anion-exchange chromatography of sera from normal subjects and pathological sera (Worwood *et al.*, 1975a), show that the alkaline isoferritins seen on isoelectric focusing are likely to be common to all sera. These alkaline isoferritins appear to have a similar pI to the iron-poor isoferritins found in uncentrifuged preparations of human liver ferritin by Powell *et al.* (1975a). Presumably the presence of high concentrations of such isoferritins in serum is due to either selective secretion from the tissues or to differences in the clearance rate of iron-rich and iron-poor isoferritins from the plasma. We have suggested that serum ferritin is secreted from reticuloendothelial cells after assembly on ribosomes of the rough endoplasmic reticulum (Jacobs & Worwood, 1975). During such a process, ferritin reaching the plasma might have little opportunity to accumulate iron, and the protein may differ in other ways from ferritin synthesized on free polyribosomes. Ferritin is known to be synthesized on rough endoplasmic reticulum as well as on free polyribosomes (Puro & Richter, 1971). These authors were not able to detect iron-rich ferritin in the cisternae of the endoplasmic reticulum of iron-loaded rats, and suggested that any ferritin present must contain very little iron. Konijn *et al.* (1973) have found evidence that the structure of ferritin synthesized on bound ribosomes differs from that produced on free polyribosomes. An alternative explanation is that enzymic modification of ferritin during its release from cells may be responsible for the presence of the 'S' component of serum ferritin. We do not know whether the more acidic isoferritins are indicative of tissue damage in these iron-overloaded patients or whether such isoferritins are also found in normal subjects.

The present paper describes the purification and properties of the serum ferritin, detected by the immunoradiometric assay, from patients with iron overload. The protein has a low iron content and a low affinity on anion-exchange chromatography when compared with iron-rich ferritin from human

tissues. Serum ferritin contains a high proportion of more alkaline isoferritins.

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