The characteristics of ferritin from human tissues, serum and blood cells

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Summary

1. The properties of ferritin in serum have been compared with those of ferritin from a number of tissues including blood cells. On anion-exchange chromatography with DEAE-Sephadex, the behaviour of human heart ferritin is different from that of liver, kidney or spleen ferritin. Reticulocyte ferritin appears to have similar characteristics to heart ferritin.

2. Serum ferritin from normal subjects and patients with various degrees of iron load, leukaemia or liver disease all have a much lower affinity for the anion-exchange column than any tissue ferritin, suggesting a difference in isoelectric point. The elution point of serum ferritin from patients with acute myeloblastic leukaemia is significantly different from normal.

3. Density gradient centrifugation in sucrose showed that ferritin in leucocyte extracts and partially purified ferritin from the serum of two patients with iron overload behaved as apoferritin rather than the iron-rich protein.

4. The results suggest that ferritin is modified during its entry into the plasma and that even in cases of iron overload the iron content of serum ferritin may be low. The findings are of importance in considering the origin of plasma ferritin, its clearance from plasma and its role in iron metabolism.

Key words: ferritin, haemochromatosis, ion exchange, iron, leucocytes, reticulocytes, leukaemia.

Introduction

Ferritin is the major iron-storage protein of the body and is found in all tissues, but in particularly high concentrations in the liver, spleen and bone marrow. Horse spleen ferritin has been most thoroughly investigated (Harrison, Hoare, Hoy & Macara, 1974) and consists of an outer protein shell (apoferritin) of molecular weight 450 000 and an inner core of hydrous, ferric oxide-phosphate. Ferritin isolated from various organs of the same animal has different electrophoretic properties, which may reflect structural differences (Harrison et al., 1974).

In 1956, Reissmann & Dietrich (1956) detected iron-containing ferritin in the plasma of patients with acute hepatocellular disease and they suggested that this circulating ferritin was released from dying liver cells. Since then, the development of a sensitive immunoradiometric assay for ferritin protein (Addison, Beamish, Hales, Hodgkins, Jacobs & Llewellin, 1972) has made it possible to estimate the concentration of ferritin not only in such pathological sera but also in normal serum. In normal subjects (Walters, Miller & Worwood, 1973) and in patients with iron overload (Jacobs, Miller, Worwood, Beamish & Wardrop, 1972) the serum ferritin concentration is related to the amount of storage iron in the body. Increased concentrations of ferritin occur also in patients with leukaemia, particularly acute myeloblastic leukaemia (Jones, Miller, Worwood & Jacobs, 1973; Worwood, Summers, Miller, Jacobs & Whittaker, 1974), and, in this condition, the serum ferritin may be partly derived from the leukaemic cells, which not only
contain increased concentrations of ferritin (Worwood et al., 1974) but also incorporate increased amounts of [14C]leucine into ferritin protein (White, Worwood, Parry & Jacobs, 1974). In addition, increased serum ferritin concentrations occur in some patients with liver disease (Lipschitz, Cook & Finch, 1974), possibly owing to hepatocellular damage (Prieto, Barry & Sherlock, 1974). In order to obtain more information about the nature of serum ferritin, it has been compared with ferritin from various human tissues and with ferritin in erythrocyte and leucocyte extracts by use of the techniques of anion exchange chromatography and density gradient centrifugation.

**Materials and methods**

**Tissues**

Human spleen ferritin was prepared from spleens removed at operation from two patients with thalassaemia and another spleen was obtained post mortem from a patient with aplastic anaemia. These patients had received many blood transfusions and were loaded with iron. Post-mortem specimens of heart, kidney and liver were obtained from one patient with a cerebral haemorrhage and another with a cerebral tumour. Horse spleen ferritin was either obtained commercially (Koch-Light Laboratories Ltd, Colnbrook, Bucks.) or prepared from the spleen of a freshly slaughtered horse.

**Purification of tissue ferritin**

The method of purification was modified from that of Drysdale & Munro (1965). The tissue was homogenized in distilled water in a Waring Blender and diluted with 4 ml of water per g of tissue. The homogenate was heated in a water bath with stirring until the temperature reached 70°C, and was maintained at 70–75°C for 10 min before cooling in ice. After centrifugation at 1500 g for 15 min, the supernatant was adjusted to pH 4·8 with acetic acid (1 mol/l) and allowed to stand overnight in the refrigerator. The supernatant was collected after centrifugation at 1500 g for 15 min and the pH was adjusted to 5·5 with acetic acid (1 mol/l) after solid sodium acetate was added to a concentration of 50 mmol/l. Ferritin was precipitated from this solution with (NH4)2SO4 (50% saturated, 2·35 mol/l) and the precipitated protein was dissolved in a small volume of NaCl (154 mmol/l) containing sodium azide (3·1 mmol/l) and centrifuged at 10 000 g for 10 min. The supernatant was then centrifuged at 100 000 g for 2 h (MSE Superspeed 50 centrifuge; 8 x 25 ml rotor) and the pellet obtained was dissolved in veronal buffer (pH 7·5; 50 mmol/l) containing sodium azide (3·1 mmol/l). This solution was subjected to gel filtration on Sephadex G-200 [Pharmacia (Great Britain) Ltd, London, W.5]. The fractions at the void volume were collected and concentrated by ultracentrifugation (Chelab C. 50 ultrafiltration cell, Chelab Instruments Ltd, Ilford, Essex). For kidney and heart ferritin, gel filtration was carried out on Sepharose 6B and E350 was measured in the eluted fractions. The fractions comprising the major peak (corresponding to the elution position of purified spleen ferritin) were collected and concentrated. Preparations of ferritin were examined by polyacrylamide gel electrophoresis at pH 8·5.

**Serum and blood cells**

Serum was collected and stored at −20°C before chromatography.

Extracts of erythrocytes and leucocytes were prepared from human blood by the method of Worwood et al. (1974) and stored at −20°C.

**Purification of serum ferritin**

Serum ferritin was partially purified from two batches of 500 ml of serum, one from a patient with primary haemochromatosis (approx. 5 mg of ferritin/l) and the other from a patient with transfusion siderosis (approx. 10 mg of ferritin/l). The sera were diluted with 3 volumes of NaCl (154 mmol/l) and subjected to heat treatment, acidification, precipitation with (NH4)2SO4 and gel filtration on Sephadex G-200 as described above. On occasions it was necessary to acidify to pH 4·2 and to repeat the heat treatment. After gel filtration, the fractions near the void volume containing ferritin were detected by immunoradiometric assay and were concentrated to 1 ml by ultrafiltration. This solution was subjected to anion-exchange chromatography on Sephadex A-50 and the fractions containing ferritin were again concentrated.

**Preparation of apoferritin**

Apoferitin was prepared from horse or human
Anion-exchange chromatography

This was carried out at 10°C on 150 mm x 10 mm columns of Sephadex A-50 equilibrated with veronal buffer (pH 6.8; 25 mmol/l), prepared by mixing appropriate volumes of sodium barbitone (50 mmol/l) and HCl (50 mmol/l). Proteins, sera or cell extracts were dialysed against the buffer and loaded on to the column in a volume of 1 ml. Up to 10 mg of purified protein or 1 ml of serum was loaded on to the column. Erythrocyte extracts contained up to 50 mg of haemoglobin in 1 ml and leucocyte extracts a maximum of 2 mg of protein. Proteins were eluted with increasing concentrations of sodium chloride in the starting buffer and fifty fractions of 5 ml were collected at a rate of 7.5 ml/h. The chloride ion concentration in the eluted fractions was determined by titration with silver nitrate (Kolthoff & Sandell, 1952) and ferritin was estimated either by measuring $E_{280}$ (purified proteins only) or by immunoradiometric assay.

Density gradient centrifugation

Sucrose-density-gradient fractionation of ferritin was carried out by the method of Drysdale & Munro (1966) in 12 ml polypropylene tubes in the 3 x 23 ml swing-out rotor of the MSE Superspeed 50 centrifuge. The tubes were centrifuged at 80 000 g for 2 h and twenty fractions of 0.5 ml were collected by piercing the bottom of the tube and by displacing the contents (through a nozzle in a cap fitted to the top of the tubes) with 30% sucrose solution. The refractive index of each fraction was measured with a refractometer and the concentrations of ferritin were determined either by extinction at 280 nm (purified ferritin only) or by immunoradiometric assay.

Gel filtration

Gel filtration was carried out at room temperature on Sephadex G-200 or Sepharose 6B in a 900 mm x 15 mm column. Fractions of 3 ml were collected.

Polyacrylamide gel electrophoresis

Electrophoresis was carried out at pH 8.5 with 3.75 or 7.5% acrylamide using the Shandon analytical polyacrylamide electrophoresis apparatus (Shandon Southern Instruments Ltd, Camberley, Surrey). The gels were stained for iron with potassium ferrocyanide (23.7 mmol/l) in HCl (100 mmol/l) or for protein (Amido Black in 7% acetic acid).

Isoelectric focussing

The LKB 8101 Ampholine Electrofocussing column with a volume of 110 ml (LKB Instruments Ltd, S. Croydon, Surrey) was loaded with Ampholine solution (20 g/l; pH range 4–6) in a continuous sucrose gradient. A solution of 2–10 mg of ferritin was applied halfway through the column and current was passed for 48 h with a maximum power of 1 W. The column was maintained at 5°C with water pumped from a refrigeration unit. At the end of the run, fractions of approx 2 ml were collected at a rate of 1 ml/min and the pH of each fraction was measured immediately. The extinction at 280 nm and 400 nm was later measured.

Immunoradiometric assay of ferritin

Ferritin was determined by the method of Addison et al. (1972). Where there was sufficient ferritin, samples were diluted with at least 4 volumes of veronal buffer (pH 8.0, 50 mmol/l) containing bovine serum albumin (5 g/l). In anion-exchange chromatography of normal serum, erythrocyte and leucocyte extracts, the fractions were diluted with an equal volume of the veronal buffer by placing 1 ml of each fraction in a tube and using the fraction-collector to add 1 ml of buffer with a reverse gradient of sodium chloride, giving a final volume of 2 ml/fraction. Thus each tube contained sodium chloride at a concentration of approximately 200 mmol/l. The assay was carried out in veronal buffer (pH 6.8) containing sodium chloride (200 mmol/l). In this way, interference with the assay of ferritin by various concentrations of sodium chloride was avoided.

Results

Anion-exchange chromatography

During anion-exchange chromatography of milligram amounts of purified proteins, horse spleen ferritin and human liver, spleen and kidney ferritins...
TABLE 1. Anion-exchange chromatography of purified ferritin
Results are expressed as mean values ± sd, except where n < 3.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>No. of separations</th>
<th>[Cl⁻] in fraction with max. ferritin concn. (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse spleen ferritin</td>
<td>3</td>
<td>227 ± 3</td>
</tr>
<tr>
<td>Horse spleen ferritin (CdSO₄)</td>
<td>3</td>
<td>240 ± 18</td>
</tr>
<tr>
<td>Horse spleen apoferritin (CdSO₄)</td>
<td>2</td>
<td>235, 238</td>
</tr>
<tr>
<td>Human spleen ferritin</td>
<td>5</td>
<td>204 ± 6</td>
</tr>
<tr>
<td>Human liver ferritin</td>
<td>5</td>
<td>201 ± 19</td>
</tr>
<tr>
<td>Human kidney ferritin</td>
<td>2</td>
<td>210, 224</td>
</tr>
<tr>
<td>Human heart ferritin</td>
<td>2</td>
<td>296, 302</td>
</tr>
</tbody>
</table>

(Righetti & Drysdale, 1973), these proteins and human liver ferritin were examined by both isoelectric focussing and polyacrylamide gel electrophoresis. On isoelectric focussing in sucrose solution, multiple bands could be seen but these were not easily resolved after elution of the column contents (Fig. 2). The results obtained (Table 2) confirm previously published findings. On polyacrylamide electrophoresis in 7.5% acrylamide at both pH 8.5 and 6.6, human and horse spleen ferritin appeared

![Diagram](image1.png)

**Fig. 1. Anion-exchange chromatography of ferritin.** (a) Chloride ion gradient. (a) Chromatography of purified human spleen ferritin (——) and purified human heart ferritin (—). (b) Chromatography of an extract of spleen with ferritin concentrations determined by immunoradiometric assay.

were eluted from the column with a maximum chloride ion concentration of 200–240 mmol/l (Fig. 1a; Table 1). Apoferritin prepared from the commercially produced horse spleen ferritin was eluted at the same concentration as the iron-rich ferritin. Ferritin from human heart eluted at a chloride concentration of 300 mmol/l.

Because of the large differences in the reported isoelectric points of horse and human spleen ferritin

![Diagram](image2.png)

**Fig. 2. Isoelectric focussing of purified ferritin.** Details are given in the Materials and Methods section. (a) Ferritin concentrations; (——), pH gradient. (a) Human spleen ferritin (6 mg). (b) Horse spleen ferritin (10 mg).
Characteristics of human ferritin

**Table 2. Isoelectric focusing**

A number of bands were usually visible (Fig. 2). The range given includes all peaks detected in the eluted fractions.

<table>
<thead>
<tr>
<th>Protein</th>
<th>No. of fractionations</th>
<th>pI (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse spleen ferritin</td>
<td>3</td>
<td>4.39-4.59</td>
</tr>
<tr>
<td>Human spleen ferritin</td>
<td>4</td>
<td>5.15-5.55</td>
</tr>
<tr>
<td>Human liver ferritin</td>
<td>4</td>
<td>5.44-5.60</td>
</tr>
</tbody>
</table>

*to have slightly different mobilities. Human liver ferritin had a similar mobility to human spleen ferritin. In 3.75% polyacrylamide at pH 8.5, horse spleen ferritin again appeared to migrate slightly faster than human spleen ferritin and this was confirmed by co-electrophoresis, where the dimers of horse and human spleen ferritin were clearly separated (Fig. 3).*

The results of anion-exchange chromatography were obtained by loading 1-10 mg of ferritin on to each column. The effect of decreasing the amount of ferritin is summarized in Table 3. There is a tendency for the elution maximum to become slightly earlier and for the single symmetrical peak to become several peaks in the same region. This is particularly marked at very low concentrations of ferritin (1-2 µg) in the absence of other protein. When 8.6 mg of human spleen ferritin was incubated with 1 ml of normal human serum for 24 h at 37°C and then dialysed and subjected to anion-exchange chromatography, ferritin (measured at 400 nm) was eluted from the column with a symmetrical peak at a chloride ion concentration of 210 mmol/l. The mean value obtained in four separations in which 1-5 µg of human spleen ferritin was incubated with 1 ml of normal serum was 188 ± 9 (SD) mmol/l. In both these cases, the elution position was not significantly different from that of pure human spleen ferritin applied in comparable amounts. Preincubation with serum did not therefore appear to change the behaviour of purified ferritin on anion-exchange chromatography.

The behaviour of ferritin in tissue extracts was examined and compared with that of purified ferritin. An extract of human spleen obtained after splenectomy from an iron-loaded patient with thalassaemia was prepared by homogenizing 16 g of tissue in 130 ml of distilled water and centrifuging at 10 000 g for 20 min. The supernatant was diluted with 3 volumes of veronal buffer (pH 6.8, see the Materials and Methods section) and dialysed against the same buffer. A portion (1 ml), containing approximately 100 µg of ferritin, was loaded on to a column of Sephadex A-50 and the fractions were collected and assayed for ferritin. The chloride ion concentration at the maximum concentration of ferritin was 210 mmol/l (Fig. 1b). Five reticulocyte-rich erythrocyte extracts were subjected to anion-exchange chromatography and the elution maximum was 286 ± 11 (SD) mmol/l (Fig. 4). The extracts were prepared from blood samples from five patients with pernicious anaemia responding to vitamin B_{12} and their reticulocyte counts varied from 20 to 25%.

![Fig. 3. Polyacrylamide gel electrophoresis of purified ferritin (3.75% polyacrylamide at pH 8.5). Proteins applied were: A, human spleen ferritin, B, horse spleen ferritin (prepared as described in the text) and C, a mixture of human and horse spleen ferritins.](image-url)
leukaemia the pattern was similar but there were two
distinct peaks and concentrations of ferritin were
higher (Fig. 5b). In seven patients with acute leukaemia
one of two patterns was seen, either an elution
pattern similar to serum ferritin (Fig. 5c), or one
corresponding to tissue ferritin (Fig. 5d).

When serum from normal subjects and from
patients with a variety of diseases was subjected to
anion-exchange chromatography the maximum
ferritin concentrations were found to be at a much
lower chloride ion concentration than for the tissue
ferritins (Table 4; Fig. 6). The mean chloride ion
concentration for the elution of serum ferritin
from patients with acute myeloblastic leukaemia is
significantly lower \( (P < 0.01) \) than the mean value in
normal subjects. A single elution peak for serum
ferritin was found in all subjects except those with
chronic myeloid leukaemia, when two peaks were
present (Fig. 6d). The volume of serum loaded
varied from 0.1 ml to a maximum of 1 ml and there
was no correlation between the amount of serum
loaded and the elution position. Similar results were

![Fig. 4. Anion-exchange chromatography of an extract of human erythrocytes containing approximately 20% reticu­
leocytes. ---, Ferritin concentration; -----, chloride ion concentration.](image)

On chromatography of leucocyte extracts from
normal subjects, a complex pattern was seen which
appeared to contain components similar to both
serum and tissue ferritin (Fig. 5a). In chronic myeloid

![Fig. 5. Anion-exchange chromatography of extracts of human leucocytes. ---, Ferritin concentrations; -----, chloride ion concentration. (a) Normal leucocytes. (b) Leucocytes from a patient with chronic myeloid leukaemia. (c) and (d) Leucocytes from patients with acute myeloblastic leukaemia.](image)
Characteristics of human ferritin

Table 4. Anion-exchange chromatography of serum

Chloride concentrations are expressed as mean values ± SD.

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of separations</th>
<th>Conc. of ferritin (µg/l)</th>
<th>[Cl(^-)] in fraction with max. ferritin concn. (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>185–375</td>
<td>138 ± 8</td>
</tr>
<tr>
<td>Secondary iron overload</td>
<td>4</td>
<td>1440–5040</td>
<td>128 ± 10</td>
</tr>
<tr>
<td>Haemochromatosis</td>
<td>6</td>
<td>1140–4400</td>
<td>141 ± 13</td>
</tr>
<tr>
<td>Miscellaneous liver disease</td>
<td>4</td>
<td>738–27900</td>
<td>133 ± 15</td>
</tr>
<tr>
<td>Acute myeloblastic leukaemia</td>
<td>8</td>
<td>401–4150</td>
<td>123 ± 9</td>
</tr>
<tr>
<td>Chronic myeloid leukaemia</td>
<td>6</td>
<td>395–1800</td>
<td>130 ± 8</td>
</tr>
</tbody>
</table>

obtained when fresh serum was chromatographed instead of frozen serum.

Density gradient centrifugation

Sucrose-density-gradient centrifugation has been carried out with purified ferritin and apoferritin as well as cell and tissue extracts. In each centrifugation, a preparation of purified human spleen ferritin was layered on the gradient in one of the three tubes and the sucrose gradient in each tube was determined by measuring the refractive index of the fractions collected after centrifugation. In fifteen centrifugations, human spleen ferritin sedimented about halfway down the tube with a peak concentration between fractions 8 and 11 (Fig. 7a). Apoferritin prepared from human spleen ferritin remained at the top of the tubes in two centrifugations (Fig. 7b). The proteins behaved in a similar way whether milligram amounts were layered on top of the gradients, and the distribution was determined by measuring $E_{350}$, or whether nanogram or

![Fig. 6. Anion-exchange chromatography of human serum. — Ferritin concentrations; —— —— —— ——, chloride ion concentrations. (a) Normal serum. (b) Serum from a patient with secondary iron overload. (c) Serum from a patient with acute myeloblastic leukaemia. (d) Serum from a patient with chronic myeloid leukaemia.](image-url)
microgram amounts were centrifuged and ferritin concentrations determined by the immunoradiometric assay.

An extract of spleen was subjected to density gradient centrifugation (Fig. 7c) and ferritin (determined by immunoradiometric assay) sedimented in a similar way to purified human ferritin from the same spleen (Fig. 7a). Extracts of leucocytes from three normal subjects (Fig. 7d), five patients with acute myeloblastic leukaemia (Fig. 7e), four patients with haemochromatosis (Fig. 7f) and one patient with chronic myeloid leukaemia all sedimented as apoferritin.

**Partial purification of serum ferritin**

The properties of serum ferritin from two patients have been further investigated. One was an untreated patient with haemochromatosis and the other patient with aplastic anaemia had received many blood transfusions. Purification was carried out by the method described above. During the final stage of anion-exchange chromatography, the ferritin was eluted at a chloride ion concentration of 130 mmol/l in both cases. After concentration of the fractions containing ferritin from the patient with secondary iron overload the preparation contained 120 µg of protein and 46 µg of ferritin (assay) per ml. This represents a purification of 1800-fold compared with the original serum. In the preparation from the patient with haemochromatosis a purification of 1330-fold was achieved. In four preparations, the recovery of ferritin was from 10 to 40%. On Sephadex G-200 the serum ferritin eluted slightly later than the purified spleen ferritin. On density gradient centrifugation the partially purified serum ferritin sedimented as apoferritin rather than the iron-rich protein (Fig. 7g).

**Discussion**

There is considerable controversy about the proper-
ties of ferritin from various tissues. On cellulose acetate electrophoresis, Alfrey, Lynch & Whitley (1967) found differences in electrophoretic mobility between human liver and spleen ferritin and showed that extracts of bone marrow and of reticulocytes contained a faster-moving component than either liver or spleen. Linder-Horowitz, Ruettinger & Munro (1970) found minor but significant differences between the mobility of ferritin from liver and kidney on polyacrylamide gel electrophoresis and demonstrated that rat heart contained two ferritin components, one of similar mobility to liver ferritin and one migrating considerably faster. Some workers have considered that the minor electrophoretic differences between, for example, liver and spleen ferritin are not significant (Lee & Richter, 1971; Crichton, Millar, Cumming & Bryce, 1973), although Crichton et al. (1973) have found differences in the amino acid composition of human liver and spleen ferritin. With anion-exchange chromatography at pH 6-6, Gabuzda & Pearson (1969) found evidence that rabbit bone marrow contains two types of ferritin. One, which was called 'catabolic ferritin', became labelled after the administration of $^{59}$Fe-labelled heat-damaged erythrocytes. The other, called 'anabolic ferritin', was labelled when $[^{59}$Fe]FeCl$_3$ was injected intravenously.

Another form of heterogeneity of ferritin molecules has been described recently. This is 'micro-heterogeneity' of ferritin from a single organ. On isoelectric focussing human ferritin from various tissues may be split into a number of bands (Righetti & Drysdale, 1973). Drysdale (1974) considers that this micro-heterogeneity represents a structural heterogeneity of the ferritin molecules within the tissue and suggests that this conclusion is supported by the heterogeneity observed on DEAE-Sephadex with horse spleen ferritin (Drysdale, 1974), and, with rat liver ferritin, on CM-cellulose (Urushizaki, Ishitani & Niitsu, 1973).

For the present study anion-exchange chromatography appeared to be most suitable for the study of ferritin in serum and cell extracts where the low concentrations present require the use of the immunoradiometric assay of ferritin. With milligram amounts of several preparations of liver and spleen ferritin, ferritin was eluted in a single symmetrical peak, although sometimes a small protein component was present at a chloride ion concentration of about 70 mmol/l (Fig. 1). This component was particularly noticeable after chromatography of horse spleen apoferritin and human kidney ferritin. In the latter case, this fraction did not contain any ferritin measurable by the immunoradiometric assay. In common with Alfrey et al. (1967) and Linder-Horowitz et al. (1970), we found minor differences between liver, spleen and kidney ferritins but heart ferritin was quite different. The finding of only a single peak for heart ferritin may be contrasted with the two components demonstrated on electrophoresis by other workers. This may be due to the method of purification which selects only iron-rich ferritin molecules. The symmetrical elution peaks observed with anion-exchange chromatography of purified horse spleen and human spleen or liver ferritin provide no evidence for heterogeneity.

One unexplained finding is the behaviour of horse spleen ferritin on anion-exchange chromatography. By isoelectric focussing, horse spleen ferritin has an isoelectric point of approximately 4-4 (see also Righetti & Drysdale, 1973). Human spleen and liver ferritins have pI approximately 5-6 and human heart ferritin pI 5-0-5-6 (Righetti & Drysdale, 1973). Despite these large differences horse and human spleen ferritin are not easily separated by electrophoresis on polyacrylamide gel (see also Crichton et al., 1973), and on anion-exchange chromatography they are both eluted from the column before human heart ferritin.

The results described above demonstrate that, on anion-exchange chromatography, the various human tissue ferritin preparations behave in a way which in general corresponds to their mobility on polyacrylamide gel electrophoresis. Ferritin in a crude extract of spleen behaves in the same way as purified ferritin from the same spleen and, provided that it is realized that small shifts in elution position and the appearance of multiple peaks may be seen at very low concentrations of ferritin (particularly in the absence of other protein), the method may be used over a range of ferritin concentrations from a few micrograms to 10 mg.

Reticulocytes contain ferritin which has a greater affinity for the Sephadex A-50 column than liver or spleen ferritin, thus confirming the findings of Yamada & Gabuzda (1974b). Ferritin was detected by the immunoradiometric assay and not by the estimation of iron or by labelling with radioactive iron. Although reticulocyte-rich erythrocytes were studied the ferritin content of the erythrocyte is
very much less than that of the reticulocyte (Worwood et al., 1974).

In extracts of normal leucocytes there are components corresponding to both tissue and serum ferritin. The heterogeneous nature of the peaks in the tissue ferritin region may be due to the various types of leucocytes present and also to the small quantities of both ferritin and protein loaded on to the column. The finding in acute myeloblastic leukaemia of either a 'serum-type' ferritin or a 'tissue-type' ferritin remains to be explained. In all the cases studied by White et al. (1974) increased incorporation of [14C]leucine into both total protein and ferritin was found. The correlation between leucocyte ferritin concentrations and serum ferritin concentrations in patients with leukaemia (Worwood et al., 1974) suggests that some of the serum ferritin may have been released from leukaemic cells and there is a significant difference on anion-exchange chromatography between ferritin in serum from patients with acute myeloblastic leukaemia and ferritin in serum from normal subjects or those with haemochromatosis. The results of density gradient centrifugation suggest that in all cases the iron content of ferritin from leucocytes is low.

The properties of serum ferritin do not correspond to any of the tissue ferritins so far examined. Incubation of small amounts of spleen ferritin with serum failed to change the elution of spleen ferritin to a concentration characteristic of serum ferritin, although Yamada & Gabuzda (1974a) have claimed that rabbit plasma contains naturally occurring antibodies to ferritin and that the properties of the complex of erythroblast ferritin and antibody on starch granule electrophoresis are quite different from those of erythroblast ferritin alone.

It is possible that ferritin destined for the plasma may be synthesized on 'bound' polysomes as opposed to 'free' polysomes (Puro & Richter, 1971) and may be subjected to modification—perhaps by the addition of carbohydrate residues before reaching the plasma. Puro & Richter (1971) noted that although ferritin synthesis by membrane-bound polysomes could be demonstrated no iron-containing ferritin could be seen within the cisternae of the rough and smooth endoplasmic reticulum. This finding is of interest in view of the apparently low iron content of serum ferritin. Even in a patient with haemochromatosis much of the protein is of relatively low density compared with iron-rich ferritin. An alternative explanation for the different properties of serum and tissue ferritin is possible enzymic modification on its release from cells. Existing clinical and experimental evidence suggests that circulating ferritin in the peripheral blood is normally derived from iron stores in the reticuloendothelial cells of the body (Jacobs & Worwood, 1975). In acute liver damage, release from damaged hepatocytes is likely and in acute myeloblastic leukaemia the ferritin may be largely derived from leukaemic cells. The ferritin in serum from patients with these conditions behaves in a similar way on anion-exchange chromatography and this suggests that whatever the origin of the protein the same modification is taking place possibly during its release from cells.

The present paper indicates some of the problems in determining the nature of circulating ferritin. Fuller clarification awaits a more detailed characterization of both tissue and serum ferritin together with increased information regarding its synthesis and secretion. However, these first results do explain some of the differences between the early work of Reissmann & Dietrich (1956), who measured the iron content of serum ferritin, and later measurements with the immunoradiometric assay. Reissmann & Dietrich (1956) found iron-containing ferritin in cases of acute hepatocellular disease but not in patients with aplastic anaemia and haemochromatosis. The immunoradiometric assay demonstrates very high concentrations of ferritin protein in these conditions. The results presented in this paper show that the iron content of circulating ferritin may be low and this must be taken into account when considering the assay of serum ferritin or its role in iron metabolism.

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References


Characteristics of human ferritin


