The Mechanism of Hepatic Iron Uptake from Native and Denatured Transferrin and its Subcellular Metabolism in the Liver Cell

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Hepatic iron uptake and metabolism were studied by subcellular fractionation of rat liver homogenates after injection of rats with a purified preparation of either native or denatured rat transferrin labelled with ^{125}I and ^{59}Fe . (1) With native transferrin, hepatic ^{125}I content was maximal 5 min after injection and then fell. Hepatic ^{59}Fe content reached maximum by 16 h after injection and remained constant for 14 days. Neither label appeared in the mitochondrial or lysosomal fractions. ^{59}Fe appeared first in the supernatant and, with time, was detectable as ferritin in fractions sedimented with increasingly lower g forces. (2) With denatured transferrin, hepatic content of both ^{125}I and ^{59}Fe reached maximum by 30 min. Both appeared initially in the lysosomal fraction. With time, they passed into the supernatant and ^{59}Fe became incorporated into ferritin. The study suggests that hepatic iron uptake from native transferrin does not involve endocytosis. However, endocytosis of denatured transferrin does occur. After the uptake process, iron is gradually incorporated into ferritin molecules, which subsequently polymerize; there is no incorporation into other structures over 14 days.

The liver is the major depot for storage iron, but the actual mechanism of hepatic iron uptake remains unclear. Studies with cultured rat liver slices suggest that the process involves binding of transferrin to liver cell membranes followed by an energy-dependent transfer of iron to the cell (Beamish *et al.*, 1975; Gardiner & Morgan, 1974). It is not known, however, whether transferrin enters the hepatocyte in a process akin to that occurring in the immature erythrocyte or whether iron is removed from transferrin at the hepatocyte membrane. Studies from several groups indicate that erythrocyte iron enters the cell bound to transferrin, by endocytosis, and that the protein carrier is subsequently released from the cell (Lane, 1973; Morgan, 1964; Baker & Morgan, 1969; Hemmaplardh & Morgan, 1977; Sullivan *et al.*, 1976). A similar process could occur in the liver.

The mechanism whereby hepatic iron is incorporated into ferritin after uptake is also uncertain. The possibility that iron first enters a chelatable pool (Bailey-Wood *et al.*, 1975) or becomes bound to a non-transferrin non-ferritin carrier protein (Huebers *et al.*, 1971) cannot be discounted.

Previous work on hepatic iron metabolism has utilized a variety of animal models (e.g. Fletcher, 1971; Van Wyk *et al.*, 1971; Drysdale & Munro, 1966). A variety of iron carriers, including dextran and haemoglobin (Van Wyk *et al.*, 1971), have been

used, whose bound iron may be expected to have an alternative metabolic pathway to that of transferrin-bound iron. Studies with ^{59}Fe -labelled serum (Fletcher, 1971; Beamish *et al.*, 1975) may be criticized for their failure to recognize that a small but significant proportion of the iron is not bound to transferrin but may contribute towards observed hepatic iron uptake.

The object of the work now reported was to determine the mechanism of hepatic iron uptake and of its subsequent subcellular route over a period of time, by using an experimental model designed to approach normal physiological conditions as closely as possible. Rats were injected with tracer doses of purified rat transferrin labelled with ^{59}Fe and ^{125}I , so that the fate of both the iron and the protein carrier could be ascertained. In control experiments hepatic iron uptake from denatured dual-labelled rat transferrin was also studied.

A preliminary report of this work has been presented (Milsom & Batey, 1978).

Experimental Methods

Preparation and purification of labelled rat transferrin

Purified rat transferrin was prepared from pooled rat serum samples by the method of Sutton & Karp (1965). After rivanol (Ethodin; Sigma Chemical Co., Poole, Dorset, U.K.) extraction on potato starch (BDH Chemicals, Poole, Dorset, U.K.), the transferrin-containing material was concentrated to a

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volume of 5 ml in an Amicon ultrafiltration cell with a PM 30 membrane. The concentrate was applied to a 90cm $\times$ 1.5cm column of Sephadex G-150 (Pharmacia, London W.5, U.K.). Transferrin-containing fractions were eluted with 0.05M-Tris buffer, pH8.0, and then applied to a DEAE-Sephadex A-50 ion-exchange column containing 3g of Sephadex. Transferrin elution was achieved with a continuous gradient of 0.05–0.5M-Tris, pH8.0. Application and re-elution of the transferrin fractions was then repeated with the same column and conditions. The final transferrin sample was concentrated to a protein content of 5mg/ml in an Amicon ultrafiltration cell. The preparation was tested on an LC Partigen plate for haemopexin (Hoechst, Hounslow, Middx., U.K.).

Polyacrylamide-gel (5% acrylamide) electrophoresis was performed on the final concentrate. The gels were run for 1h in 0.3M-Tris/15mM-Temed (NNN'-tetramethylethylenediamine) buffer, pH9.5, with a current of 2.5mA/gel. A standard preparation of transferrin was run with all new samples for comparison.

Labelling of transferrin

Transferrin was iodinated by the lactoperoxidase method of Kuenzle & Dobelli (1973). After iodination the protein solution was adjusted to pH5.6, and a final concentration of 0.3M-EDTA/0.6M-acetate was achieved to remove iron from the molecule, indicated by decolorization of the protein solution (Jandl & Katz, 1963). After 1h the solution was dialysed overnight at 4°C against two changes of 0.2M-NaHCO₃, and the final solution was adjusted to 0.6M-NaHCO₃ by the addition of solid NaHCO₃. The transferrin was labelled with ⁵⁹Fe in the presence of a 100-fold molar excess of citrate. This was used to prevent non-specific binding of iron complexes to the transferrin molecule. The specific radioactivity of the ⁵⁹Fe was 10 μ Ci/ μ g of Fe, and approx. 0.5 μ g of Fe was added/mg of transferrin protein present. Incubation was carried out for 1h at 37°C, and unbound iron was removed by dialysis overnight against two changes of 0.2M-NaHCO₃.

Purity and specificity of the labelling of the final solution was assessed by polyacrylamide-gel electrophoresis. No free iron was found in any of the preparations injected. The final saturation of the transferrin was 30–40%.

Denaturation of transferrin

Purified dual-labelled transferrin was dialysed overnight against 8M-urea at 25°C. Denaturation was associated with a 50–60% loss of ⁵⁹Fe radioactivity but with no loss of ¹²⁵I. The denatured preparation was dialysed against three changes of 0.9% NaCl before injection into rats.

Injection of animals

Male Sprague-Dawley rats of body wt. 150–200g were used. They had been fed on a standard rat chow from weaning, were caged in plastic cages and were allowed water *ad libitum*. Animals were injected with labelled native transferrin or labelled denatured transferrin via the femoral vein, under light diethyl ether anaesthesia. The dose given was 1 μ g of protein/g body wt. in a total volume of 0.8ml. Specific radioactivity of the labelling was 20nCi of ¹²⁵I and 2nCi of ⁵⁹Fe per μ g of denatured transferrin. The animals were left for periods ranging from 5 min to 14 days, being allowed, with the exception of the 5 min period, to recover from the anaesthetic.

Preparation of liver homogenates

After being deprived of food overnight, animals were killed by a blow on the head. The livers were perfused with ice-cold 0.9% NaCl and removed. Subsequent manipulations were all performed at 4°C. After passage through a coarse wire mesh, each liver was diluted to a total volume of 12ml with 0.25M-sucrose and homogenized with nine up-and-down strokes in a Dounce homogeniser (Kontes Glass Co., Vineland, NJ, U.S.A.).

Differential centrifugation

The homogenate was centrifuged at 600g for 10min (r_{av} 16.5cm) in an MSE Mistral 2L centrifuge. After removal of the supernatant the pellet was resuspended in 0.25M-sucrose, rehomogenized as described above and then re-centrifuged at 600g for 10min, the resulting pellet being designated fraction I. All subsequent centrifugations were carried out in the 8 $\times$ 14ml rotor (r_{av} 5.6cm) of an MSE Superspeed 65 centrifuge. The combined supernatants were centrifuged at 10000g for 5min, and after removal of the supernatant the pellet was washed by resuspension in 0.25M-sucrose and re-centrifugation at the same g force, and designated fraction II. The combined supernatants were then centrifuged at 40000g for 10min, and the resulting pellet was washed as before and designated fraction III. Centrifugation at 78000g for 90min and 260000g for 60min yielded pellets designated fractions IV and V respectively, no washing step being carried out, and the final supernatant was fraction VI. Each pellet was resuspended in 5ml of 0.25M-sucrose.

Analysis of fractions

(a) *Measurement of radioactivity.* ⁵⁹Fe and ¹²⁵I radioactivities were measured in triplicate 1ml samples of each fraction by using an LKB Wallac 1280 Ultragamma counter, programmed to correct for cross-over.

(b) *Marker enzyme assays.* All fractions were diluted 1:10 (v/v) with 0.25M-sucrose, except fraction VI, which was diluted 1:2 (v/v). All reagents were

from Sigma Chemical Co., except where stipulated.

Glutamate dehydrogenase was determined spectrophotometrically from the oxidation of NADH in the presence of α -oxoglutarate and $(\text{NH}_4)_2\text{SO}_4$ (Ellis & Goldberg, 1972). Alkaline phosphatase, *N*-acetyl- β -D-glucosaminidase and neutral α -glucosidase were determined by the fluorimetric methods of Seymour & Peters (1977), with the appropriate methylumbelliferyl derivatives (Koch-Light Chemicals, Colnbrook, Bucks., U.K.) as substrates.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

(c) *Identification of ^{125}I and ^{59}Fe in subcellular fractions.* Fractions IV and V were studied without further preparation. Supernatant fractions were concentrated from an initial volume of 26 ml to a final volume of 5 ml in an Amicon ultrafiltration cell. Fractions I, II and III were first incubated with constant mixing with 1% Triton X-100 for 1 h at 37°C, and then centrifuged to sediment the particulate matter. Studies were carried out on the pellet and Triton extract.

(d) *Polyacrylamide-gel electrophoresis.* All samples were studied on polyacrylamide gels (5% acrylamide) with 0.3 M-Tris/15 mM-Temed buffer, pH 9.5. Gels were subjected to a current of 2.5 mA/gel and the marker used was Bromophenol Blue. Samples of purified ferritin and transferrin were run simultaneously to allow the identification of these proteins in the subcellular fractions. All samples were run in duplicate, one gel being stained for protein with Coomassie Blue and one being cut into 5 mm sections for counting of both ^{125}I and ^{59}Fe radioactivity in a Packard Autogamma spectrometer.

When radioisotope and protein bands coincided on gels it was assumed that a labelled protein was present in that sample. If ^{125}I and ^{59}Fe radioactivities corresponded to an R_f of transferrin it was assumed that the radiolabelled injected transferrin was present in that fraction. In certain fractions ^{59}Fe and ^{125}I radioactivities were found to migrate more rapidly than any of the known proteins.

(e) *Immunodiffusion studies.* All samples, including the Triton extracts, were run against anti-(rat transferrin) and anti-(human ferritin) antibodies on Ouchterlony plates. The plates were made of 0.9% agarose (Sigma Chemical Co.) in 0.2 M-Tris buffer, pH 8.0. Previous experiments have shown that anti-(human ferritin) reacts with rat liver ferritin.

Precipitin lines were observed after 36 h incubation at room temperature in all studies.

Results

Purity of transferrin preparations

All dual-labelled samples used in these experiments were studied before injection by immunodiffusion and gave a single line of identity with anti-(rat transferrin)

antibody. Gel electrophoresis demonstrated that all ^{125}I was protein bound; 97–100% of ^{59}Fe was also bound to transferrin, but a small percentage was found in most samples to migrate more rapidly. Passage of a dual-labelled sample of transferrin through an IRA-400 ion-exchange column to remove free ^{59}Fe did not prevent the appearance of this small fraction of non-transferrin-bound iron, which probably represents an iron-glycine complex (Brown & Rother, 1963).

Hepatic concentration of transferrin

The appearance of ^{125}I and ^{59}Fe in the liver homogenate over a period of time after injection of native transferrin is shown in Fig. 1(a). The hepatic content of ^{125}I rapidly reached a maximum of 3–5% of the initial dose by 5 min after injection, and stayed at this value for approx. 2 h. It had decreased to 2% of dose by 3 h and less than 1% by 2 days.

The hepatic content of ^{59}Fe rose linearly for the first hour and continued to rise more slowly for the next few hours. A maximum of approx. 10% of dose was reached by 16 h after injection, and this remained constant over the ensuing 14 days.

In contrast, the hepatic content of ^{59}Fe achieved after injection of denatured transferrin increased much more rapidly to a maximum of approx. 15% of dose by 30 min, remaining approximately constant from this time. The increase in hepatic content of ^{125}I from denatured transferrin was also initially more rapid, reaching a concentration of 9–10% in 5 min and 17% by 30 min. However, after this time the radioactivity present decreased rapidly, to 8% by 1 h and 3% by 3 h.

Fractionation studies

(a) *Enzyme distributions.* These are indicated in Fig. 2. Fractions I and II contained most of the mitochondria, as shown from the activity of glutamate dehydrogenase. *N*-Acetyl- β -D-glucosaminidase activity showed lysosomes to predominate in fractions II and III. Endoplasmic reticulum as judged from the activity of α -glucosidase was present in fractions III and IV, and alkaline phosphatase activity showed plasma membrane to be localized in fractions I, III and IV. Fraction V contained little enzyme activity, and the supernatant, fraction VI, also had little activity, suggesting rupture of organelles to be minimal. Injection of either native or denatured labelled transferrin had no effect on organelle distribution as judged from the activities of marker enzymes.

(b) *Native transferrin injection.* (i) *Distribution of ^{125}I .* This is indicated in Fig. 3: 5 min after injection, most of the radioactivity was present in fraction VI and in fraction IV, the membrane-containing fraction. The amount of label in fractions I, II and III,

Fig. 1. Appearance of ^{59}Fe and ^{125}I within rat liver homogenates after injection of (a) labelled native transferrin and (b) labelled denatured transferrin

Experimental details are given in the text. Results are expressed as percentages of the initial dose. Each point represents a mean $\pm$ s.e.m., $n = 6$ (native transferrin) or $n = 4$ (denatured transferrin).

Fig. 2. Distribution of marker enzymes in fractions I-VI obtained by differential centrifugation of rat liver homogenates. Experimental details are given in the text. The mean relative specific activity $\pm$ s.e.m. ($n = 12$) is indicated for each fraction. The abscissa shows the percentage protein in each fraction.

which contain most of the lysosomes and mitochondria, was not significantly high 30min after injection. ^{125}I radioactivity was still largely in fractions III, IV or VI, but had become more diffused and non-specific and became increasingly so with further time.

(ii) Distribution of ^{59}Fe . This is indicated in Fig. 4: 5 min after injection ^{59}Fe radioactivity was present

Fig. 3. Distribution of ^{125}I in fractions I–VI obtained by differential centrifugation of liver homogenates from rats injected with tracer quantities of labelled native transferrin. Experimental details are given in the text. Times indicated are periods after injection. The mean relative specific radioactivity $\pm$ S.E.M. ($n = 6$) is indicated for each fraction. The abscissa shows the percentage protein in each fraction.

Fig. 4. Distribution of ^{59}Fe in fractions I–VI obtained by differential centrifugation of liver homogenates from rats injected with tracer quantities of labelled native transferrin. Experimental details are given in the text. Times indicated are periods after injection. The mean relative specific radioactivity $\pm$ S.E.M. ($n = 6$) is indicated for each fraction. The abscissa shows the percentage protein in each fraction.

largely in fraction VI, and there were insignificant amounts only in fractions I, II and III.

Some 30 min after injection there was also no evidence for the association of ^{59}Fe with fractions I, II and III. Most remained in fraction VI, although the relative specific radioactivity of fraction V had increased and also, marginally, that of fraction IV.

Fig. 4 also shows the distribution of ^{59}Fe 1 h and 2 h after injection. The relative specific radioactivity of fraction VI fell markedly, accompanied by further increases in fractions V and IV. By 16 h the subcellular distribution pattern of ^{59}Fe had stabilized and no further change was observed up to 14 days. In particular, no incorporation of ^{59}Fe into fractions I, II and III, the lysosomal and mitochondrial fractions, was seen.

In Fig. 5 the percentage of ^{59}Fe in fractions IV, V and VI is plotted as a function of time. The percentage in fraction VI was initially high, approx. 65% of the total radioactivity in the liver, but fell rapidly over the first 30 min to 45% and then more slowly to less than 10% over the next 1–2 days. The percentage in fraction V was initially low, but rose to approx. 20% over the first 2 h and then remained constant. The percentage in fraction IV rose almost linearly for the first few hours to approx. 40% by 16 h and then remained constant.

(c) *Denatured transferrin injection.* When rats were injected with denatured transferrin, after 30 min the subcellular distribution of ^{125}I and ^{59}Fe was that shown in Fig. 6. Most of both labels was found in fractions II and III, the lysosomal fractions, with a trace only of ^{59}Fe in fraction VI.

Fig. 6 also shows the distribution of ^{59}Fe found 3 h after the injection of denatured transferrin. This resembled the distribution found after the injection

of native transferrin in that the highest relative specific radioactivity was found in fractions IV and V. By this period of time, a proportion of ^{125}I was also present in fraction VI, the remainder being associated with fractions II and III.

(d) *Immunodiffusion studies.* Experiments with purified antibodies documented the presence of transferrin in all fractions produced by ultracentrifugation. Ferritin was shown consistently to be present in fractions IV, V and VI. This method was not able to quantify accurately the protein content of each fraction. However, fraction VI consistently gave a more pronounced precipitin line with anti-transferrin antibody and the line appeared earlier than with other fractions, suggesting that fraction VI contained a greater quantity of transferrin protein.

Similarly, fractions IV and V contained more ferritin than did fraction VI.

(e) *Polyacrylamide-gel electrophoresis.* (i) After the injection of native transferrin, ^{125}I -labelled transferrin was found in fraction I in all animals, in small quantities, probably representing serum protein trapped during the initial centrifugation of the homogenate.

The supernatant, although containing transferrin in all animals, contained ^{125}I -labelled transferrin only in animals studied 5, 30 and 60 min after injection. ^{59}Fe -labelled transferrin was detectable only in animals studied 5 min after injection.

^{59}Fe was found to be ferritin-associated in fractions IV, V and VI of all animals from 30 min after injection, indicating a rapid transfer of iron from circulating transferrin to the storage pool. The ferritin present in fraction IV demonstrated a different mobility from that present in fractions V and VI. Its major band was more slowly moving, suggesting that a polymer was present.

Fig. 5. Distribution of ^{59}Fe in fractions IV, V and VI at periods after injection of tracer quantities of labelled native transferrin. Experimental details are given in the text. Results are expressed as a percentage of that present in the whole homogenate. Each point represents a mean $\pm$ S.E.M. ($n = 6$).

Fig. 6. Distribution of ^{59}Fe and ^{125}I in fractions I–VI obtained by differential centrifugation of liver homogenates from rats injected with tracer quantities of labelled denatured transferrin

Experimental details are given in the text. Times indicated are periods after injection. The mean relative specific radioactivity $\pm$ S.E.M. ($n = 4$) is indicated for each fraction. The abscissa shows the percentage protein in each fraction.

There was no evidence for any alteration in the amount of total ferritin protein present over the period studied, or of an increase in the polymerized form. A form of ^{59}Fe that was non-transferrin and non-ferritin-bound was also found in animals studied. Up to 30 min after injection, this form of ^{59}Fe accounted for up to 20% of the total iron in fraction VI and it migrated as a single band ahead of labelled transferrin. The identity of the non-ferritin non-transferrin iron was not defined by these experiments. The possibility that it represents an iron chelate of some sort has not been excluded. Traces of non-protein-bound iron were also detectable in fractions I, II, III and IV up to 1 h after injection, and occasionally in fractions II and III after this time.

(ii) After injection of denatured material, ^{59}Fe and ^{125}I were found bound to transferrin in fractions II and III in all studies. In animals studied up to 18 h

after injection, a low-molecular-weight form of ^{125}I was found in fraction VI. In addition, 30 min–3 h after injection ^{59}Fe was found as ferritin or as low-molecular-weight complexes in fractions IV, V and VI, whereas 3–18 h after injection ^{59}Fe was only associated with ferritin.

Discussion

The results presented provide new information on normal hepatic iron uptake and its subsequent metabolic fate, by using an experimental model designed to approach normal physiological conditions as closely as possible.

Hepatic ^{59}Fe uptake from native homologous transferrin increases from 5 min after injection, reaching a maximum of approx. 10% of dose by 16 h. The hepatic ^{125}I content is initially 4% of the dose

and then decreases to less than 2% by 3 h. The lack of a close correlation between hepatic ^{59}Fe and ^{125}I contents, even immediately after injection, suggests that normal iron uptake by hepatocytes occurs independently of the uptake of the transferrin carrier molecule. The process cannot therefore involve endocytosis, and, indeed, the failure to detect significant amounts of either label in the lysosomal fractions of the homogenate supports this belief.

It appears then that hepatic iron uptake in normal conditions proceeds via a transferrin–hepatocyte plasma membrane interaction in which iron is removed from the carrier molecule and passed through the membrane. Specific receptor proteins have been described for erythrocyte precursors (Sullivan & Weintraub, 1978), and it is presumed that similar proteins exist in the hepatocyte membrane.

The native transferrin used in this study was randomly labelled with iron and therefore may only be capable of donating 50% of its bound ^{59}Fe to the liver cell (Fletcher & Huehns, 1968; Awai *et al.*, 1975). This may account partially for the plateau reached for iron uptake after 16 h. The fall in ^{125}I values after 1 h may be due to reloading of ^{125}I -labelled transferrin with newly absorbed iron, followed by the preferential donation of this to bone-marrow cells. Further, the decrease may reflect disappearance of transferrin from the circulation into cells with a higher affinity for the protein, such as erythrocyte precursors.

Conversely, after injection of denatured transferrin, hepatic contents of ^{125}I and ^{59}Fe are equal for 30 min and most of the radioactivity of both labels is found in the lysosomal fractions. This suggests that the denatured transferrin molecule does not undergo the same specific interaction with the membrane receptors. Instead, iron enters the liver by a relatively non-specific and more rapid process of endocytosis, in which the whole transferrin molecule is taken up in a manner similar to that described for labelled denatured albumin (Mego & McQueen, 1965*a,b*).

After 5 min from the injection of native transferrin, most of the ^{59}Fe and ^{125}I is present in the supernatant, probably reflecting transferrin bound to plasma membrane at the time of removal of the liver and subsequently dislodged by the homogenization and fractionation processes. Up to 1 h after injection, 20% of the ^{59}Fe in the cell supernatant appeared on gel electrophoresis as low-molecular-weight iron compounds. This finding may reflect artifacts caused by the fractionation techniques, but alternatively may indicate the existence of a chelatable iron pool within hepatocytes. Such an intermediate substance has been described in cultured Chang cells (Bailey-Wood *et al.*, 1975). Definition of the properties of this form of iron would require the use of a very highly specifically labelled transferrin preparation, and at present this is not readily available. The use of

free iron for injection must be avoided if the physiological nature of the model is to be maintained.

These experiments have confirmed that ferritin is the major hepatic iron acceptor after uptake. After a short delay iron is incorporated into ferritin molecules that are sedimented only at 260000*g*. With time, polymerization of these molecules apparently occurs, forming larger ferritin complexes that are more readily sedimented. Similar findings have been reported by other workers (Harrison, 1977; Munro & Linder, 1978), though their significance is unclear. That this model represents a tracer study of normal physiological iron turnover, rather than an induction of ferritin synthesis by increased iron concentrations, is suggested by the failure to detect quantitative changes with time in the ferritin content of the subcellular fractions.

Of particular interest, in the studies with denatured transferrin, was the finding that transferrin, having entered the lysosomal fraction, does not remain there. Both ^{59}Fe and ^{125}I appear in the post-lysosomal fractions in the post-injection period. The distribution of enzyme markers remains unchanged, suggesting the release of ^{125}I and ^{59}Fe is not a result of generalized lysosomal lysis. The ability of the lysosome to permit free diffusion of amino acids is well documented (Lloyd, 1971). Preliminary studies with Sephadex G-50 gel chromatography suggest that the released ^{125}I is partly in the form of iodo-tyrosines, resulting from lysosomal enzyme degradation of the denatured protein. The mode of transport of iron across the lysosomal membrane is not clear. However, the released iron is incorporated into ferritin in a manner indistinguishable from that after injection of normal transferrin.

After its appearance within ferritin complexes, iron appears to be comparatively stable. Over 14 days there is no evidence for its further incorporation into mitochondrial proteins or for its appearance within lysosomes as a result of autophagocytosis.

These experiments have clarified some of the problems relating to normal hepatic iron metabolism. In particular, further elucidation of both the mechanism of hepatic iron uptake and of the subsequent subcellular pathway of the iron has been provided.

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